Diseases of the Blood

PART XIX

Section 1

The Hematopoietic System

Chapter 495

Development of the Hematopoietic System

Stella T. Chou

HEMATOPOIESIS IN THE HUMAN EMBRYO AND FETUS

Hematopoiesis is the process by which the cellular elements of blood are formed. In the developing human embryo and fetus, hematopoiesis has three developmental waves and is conceptually divided into three anatomic stages: mesoblastic, hepatic, and myeloid. *Mesoblastic* hematopoiesis occurs in extraembryonic structures, principally in the yolk sac, and begins between the 10th and 14th days of gestation. By 6-8 weeks of gestation, the liver replaces the yolk sac as the primary site of blood cell production, and during this time, the placenta also contributes as a hematopoietic site. By 10-12 weeks, extraembryonic hematopoiesis has ceased. *Hepatic* hematopoiesis occurs through the remainder of gestation and then diminishes during the second trimester while bone marrow *(myeloid)* hematopoiesis increases. The liver is the predominant erythropoietic organ through 20-24 weeks of gestation.

Each hematopoietic organ houses distinct populations of cells. The yolk sac predominantly produces erythrocytes, megakaryocytes, and macrophages. The fetal liver is primarily an erythropoietic organ, while the bone marrow produces erythrocytes, megakaryocytes, and leukocytes. The types of leukocytes present in the fetal liver and marrow differ with gestation. Macrophages precede neutrophils in the marrow, and the ratio of macrophages to neutrophils decreases as gestation progresses. Regardless of gestational age or anatomic location, production of all hematopoietic tissues begins with multipotent cells capable of both self-renewal and clonal maturation into all blood cell lineages. Progenitor cells differentiate under the influence of transcription factors and hematopoietic growth factors.

The classical model of hematopoietic differentiation involves differentiation into increasingly lineage-specific progenitors, although there may also be alternate pathways that are used separately or in combination with classical pathways (Fig. 495.1). In the classical pathway, *long-term repopulating hematopoietic stem cells* (LTR-HSCs) are characterized by their ability to self-renew and differentiate into cells that are multipotent. *Multipotent progenitors* (MPPs) have reduced self-renewal capacity and differentiate into *common lymphoid progenitors* (CLPs) or *common myeloid progenitors* (CMPs). The CMP differentiates into all the blood lineages except for lymphoid. The commitment of hematopoietic cells to increasingly lineage-restricted cells requires cytokine stimulation and regulation by transcription factors.

Erythrocytes in the fetus are larger than in adults, and at 22-23 weeks' gestation, the mean corpuscular volume can be as high as 135 femtoliters (fL) (Fig. 495.2A). Similarly, the mean corpuscular hemoglobin is very high at 22-23 weeks and falls relatively linearly with advancing gestation (see Fig. 495.2B). In contrast, the mean corpuscular hemoglobin concentration is constant throughout gestation at 34 ±1 g/dL. While the size and quantity of hemoglobin in erythrocytes diminish during gestation, the hematocrit and blood hemoglobin concentration gradually increase (Fig. 495.3).

Platelet concentration in the blood increases gradually between 22 and 40 weeks' gestation (Fig. 495.4), but the platelet size, assessed by mean platelet volume, remains constant at 8 ±1 fL. No differences are observed between males and females in fetal and neonatal reference ranges for erythrocyte indices, hematocrit, hemoglobin, platelet counts, or mean platelet volume measurements.

FETAL GRANULOCYTOPOIESIS

Neutrophils are first observed in the human fetus about 5 weeks after conception as small clusters of cells around the aorta. The fetal bone marrow space begins to develop around the eighth week, and from 8-10 weeks, the marrow space enlarges, but no neutrophils appear there until 10.5 weeks. From 14 weeks through term, the most common granulocytic cell type in the fetal bone marrow space is the neutrophil. Neutrophils and macrophages originate from a common progenitor cell, but macrophages appear before neutrophils in the fetus, first in the yolk sac, liver, lung, and brain, all before the bone marrow cavity is formed.

Granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) are expressed in developing fetal bone as early as 6 weeks after conception, and both are expressed in the fetal liver as early as 8 weeks. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SCF) also are distributed widely in human fetal tissues. However, no changes in expression of any of these factors, or of their specific receptors, appear to be the signal for fetal production of neutrophils or macrophages.

Fetal blood contains few neutrophils until the third trimester. At 20 weeks' gestation, the blood neutrophil count is 0-500/mm^3. Although mature neutrophils are scarce, progenitor cells with the capacity to generate neutrophil clones are abundant in fetal blood. When cultured in vitro in the presence of recombinant G-CSF, they mature into large colonies of neutrophils. The physiologic role of G-CSF includes upregulating neutrophil production, and the low number of circulating neutrophils in the mid-trimester human fetus may be caused, in part, by low production of G-CSF. Monocytes isolated from the blood of adults produce G-CSF when stimulated with a variety of inflammatory mediators, such as bacterial lipopolysaccharide (LPS) or interleukin (IL)-1. In contrast, monocytes isolated from the blood or organs of fetuses up to 24 weeks' gestation generate only small quantities of G-CSF protein and messenger RNA (mRNA) after LPS or IL-1 stimulation. Despite this, G-CSF receptors on the surface of neutrophils of newborn infants are equal in number and affinity to those on adult neutrophils.

In the fetus, actions of the granulocytic factors (G-CSF, M-CSF, GM-CSF, and SCF) are not limited to hematopoiesis. Receptors for each of

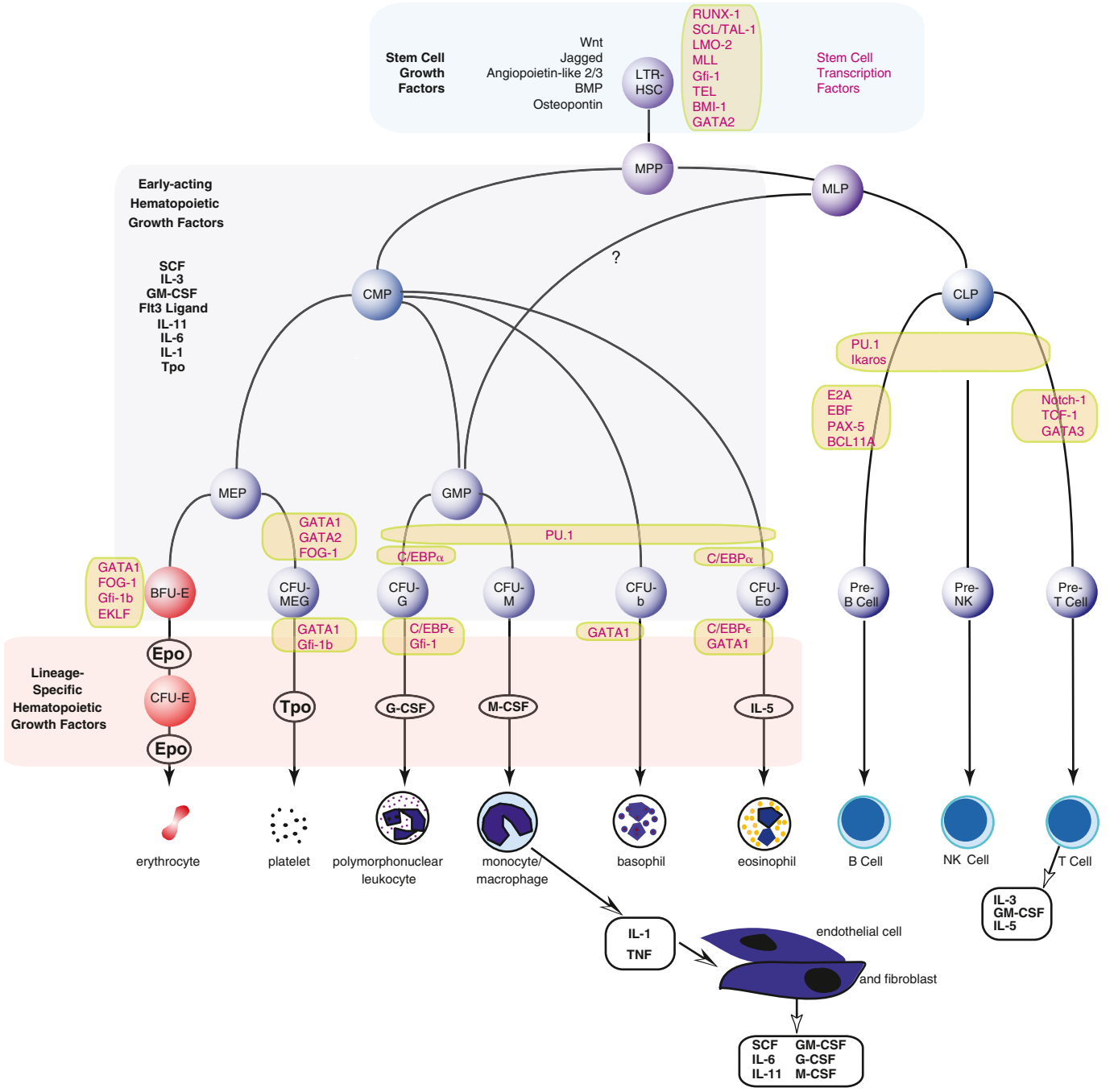

Fig. 495.1 Major cytokine sources and actions to promote hematopoiesis. Cells of the bone marrow microenvironment, such as macrophages, endothelial cells, and reticular fibroblasts, produce macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) after stimulation. These cytokines and others listed in the text have overlapping interactions during hematopoietic differentiation, as indicated; for all lineages, optimal development requires a combination of early- and late-acting factors. BFU, Burst-forming unit; BMP, bone morphogenic protein; CFU, colony-forming unit; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; Epo, erythropoietin; GMP, granulocyte-monocyte progenitor; IL, interleukin; LTR-HSC, long-term repopulating-hematopoietic stem cell; MEP, megakaryocyte-erythroid progenitor; MLP, multipotent lymphoid progenitor; MPP, multipotent progenitor; SCF, stem cell factor; TNF, tumor necrosis factor; Tpo, thrombopoietin. *(From Sieff CA, Daley GO, Zon LI. The anatomy and physiology of hematopoiesis. In Orkin SH, Fisher DE, Ginsburg D, et al., eds. Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th ed. Philadelphia: Elsevier, 2015.)*

these are located in areas of the fetal central nervous system and gastrointestinal tract, where their patterns of expression change with development.

FETAL THROMBOPOIESIS

Several biologic differences exist between fetal, neonatal, and adult megakaryopoiesis and thrombopoiesis. There is a developmentally unique pattern of fetal/neonatal megakaryopoiesis characterized by rapid proliferation, followed by full cytoplasmic maturation without polyploidization. Fetal and neonatal megakaryocytes are significantly smaller, exhibit lower ploidy, and produce fewer platelets. However, fetal and neonatal megakaryocytes have a higher proliferative potential than adult progenitors. These differences allow fetuses and neonates to populate their rapidly expanding bone marrow space and blood volume while maintaining normal platelet counts.

Megakaryocyte progenitors are categorized as *burst-forming unit–megakaryocytes* (BFU-MK), which are primitive megakaryocyte

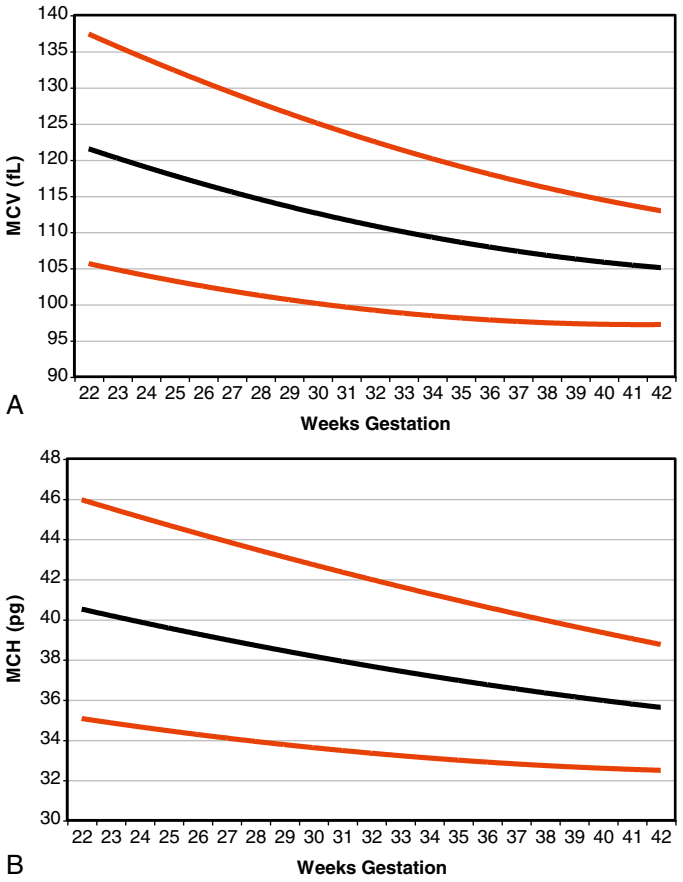

Fig. 495.2 A, Erythrocyte mean corpuscular volume (MCV), and B, mean corpuscular hemoglobin (MCH) from 22 weeks' gestation through term. The *lines* represent the 5th percentile, the mean, and the 95th percentile reference range. *(From Christensen RD, Jopling J, Henry E, et al. The erythrocyte indices of neonates, defined using data from over 12,000 patients in a multihospital healthcare system. J Perinatol 2008;28:24–28.)*

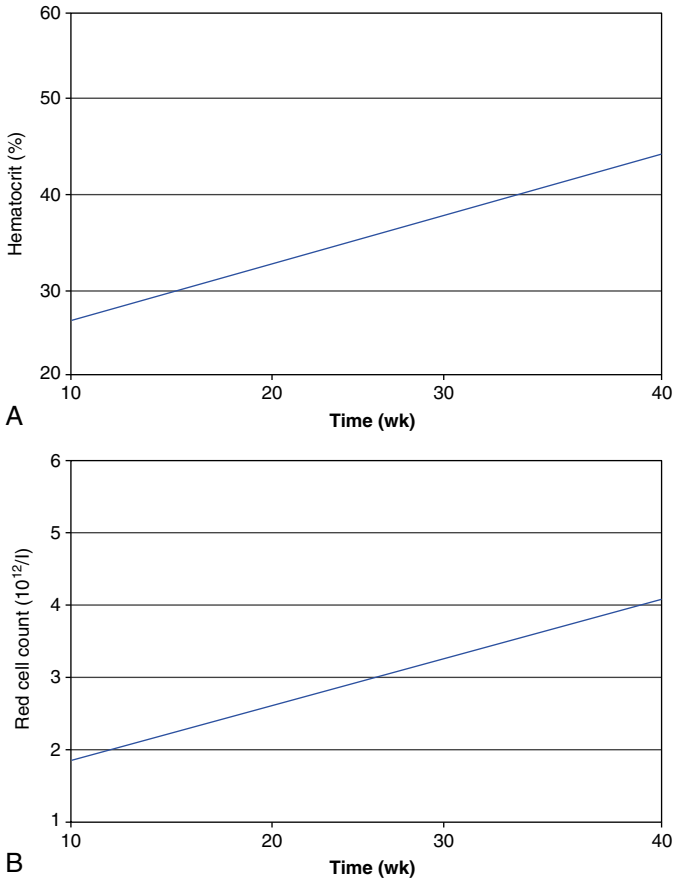

Fig. 495.3 Reference ranges of fetal hematocrit (A) and fetal red blood cell count (B) by cordocentesis throughout gestations.

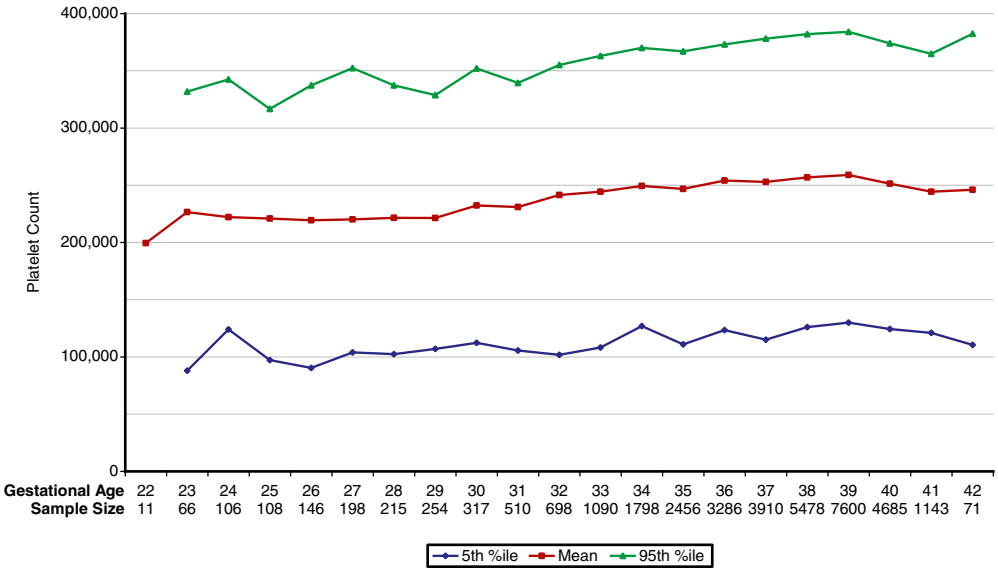

Fig. 495.4 Platelet count from 22 weeks' gestation through term. The *lines* represent the 5th percentile, the mean, and the 95th percentile reference range. *(From Wiedmeier SE, Henry E, Sola-Visner MC, et al. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. J Perinatol 2009;29:130–136.)*

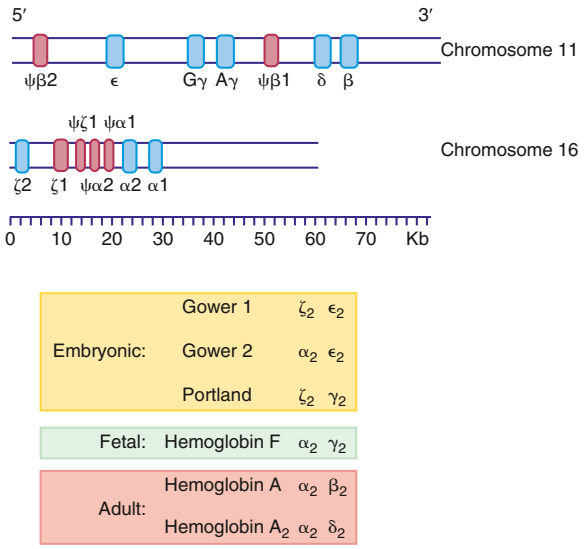

Fig. 495.5 Organization of the globin genes. The *bottom line* reflects the scale in kilobases. The *upper segment* represents the β-like globin genes on chromosome 11, and the *lower segment* the α-like genes on chromosome 16. Regions of the gene that code for primary globin proteins are shown as *blue segments*, and regions that code for pseudogenes ("ψ," nonexpressed remnants) are shown as *pink segments*. The composition of embryonic, fetal, and adult hemoglobins is listed. α, Alpha; β, beta; γ, gamma; δ, delta; ε, epsilon; ζ, zeta.

progenitors, and *colony-forming unit–megakaryocytes* (CFU-MK), which are more differentiated. BFU-MK produce large multifocal colonies containing ≥50 megakaryocytes, whereas CFU-MK generate smaller (3-50 cells/colony) unifocal colonies. Megakaryocytes are identified by their morphologic characteristics as they undergo endoreduplication, which results in large cells with polyploid nuclei. Megakaryocytes, unlike megakaryocyte progenitors, do not have the capacity to generate colonies. Rather, they undergo maturation, progressing from small mononuclear cells to large polyploid cells. The modal megakaryocyte *ploidy* (the number of sets of complete chromosomes) in normal adult marrow is 16N. In the fetus and neonate, ploidy is lower, primarily 2N and 4N, and mature megakaryocyte size is smaller. Large megakaryocytes generate more platelets than do small megakaryocytes; in vitro studies suggest that megakaryocytes of neonates produce fewer platelets than do their adult counterparts.

The exact mechanisms by which megakaryocytes release platelets into the circulation remain incompletely understood. In situ examination of this process suggests that mature megakaryocytes migrate to a perivascular site and extend a process through the endothelium, giving rise to proplatelets, which then release platelets. An alternate mechanism is that platelets are released from megakaryocytes in the lungs as a result of shear forces.

Thrombopoietin (TPO) is the dominant regulator of megakaryocyte development and platelet production. TPO is predominantly produced in the liver from early fetal to adult life but is also expressed by cells in the kidney, and, to a lesser extent, by smooth muscle and marrow cells. TPO concentrations are higher in healthy neonates of any gestational age than in healthy adults. TPO is a primary stimulator of megakaryocyte and platelet production, but SCF, IL-3, IL-11, IL-6, and erythropoietin also stimulate megakaryopoiesis and thrombopoiesis in vitro and in vivo. Importantly, TPO promotes expansion of hematopoietic stem cells (HSCs) and progenitor cells, and the TPO receptor is expressed on HSCs and erythroid progenitors in addition to megakaryocyte progenitors, megakaryocytes, and mature platelets.

FETAL ERYTHROPOIESIS

Similar to hematopoietic production of other cell lineages, fetal erythropoiesis is regulated by growth factors produced by the fetus, not by the mother. Erythropoietin (EPO) does not cross the human placenta. Stimulating maternal EPO production does not enhance fetal erythropoiesis, nor does suppressing maternal erythropoiesis by hypertransfusion.

EPO plays a central regulatory role on the proliferation and maturation of erythroid progenitors. Erythroid-committed progenitors consist of *burst-forming unit–erythroid* (BFU-E) and *colony-forming unit–erythroid* (CFU-E) cells. In colony-forming assays, human BFU-E cells are more proliferative, forming colonies of multiple clusters of erythroblasts, as compared with CFU-E cells, which form one or two clusters, with each containing 8-100 hemoglobinized erythroblasts. EPO is essential for erythrocyte production from CFU-E cells by inducing survival and proliferation of erythroblasts. EPO binds to specific receptors on the surface of committed erythroid precursors, and its expression is regulated by an oxygen-sensing mechanism through the hypoxia-inducible factor (HIF) family of proteins. HIF-1α and HIF-2α are regulated by oxygen tension, whereas HIF-1β is constitutively expressed. Together, HIF proteins maintain oxygen homeostasis and regulate erythropoiesis by inducing EPO under hypoxic conditions.

EPO is produced by monocytes and macrophages in the fetal liver during the first and second trimesters. After birth, the anatomic site of EPO production shifts to the kidney. The specific stimulus for this shift is unknown but may involve the increase in arterial oxygen tension that occurs at birth. Epigenetic modification of gene expression may also play a role because it appears that renal and hepatic EPO genes are methylated to different degrees. Although EPO mRNA and protein can be found in the human fetal kidney, it is not known whether this production is biologically relevant. It appears that renal production of EPO is not essential for normal fetal erythropoiesis, as evidenced by the normal serum EPO concentration and normal hematocrit of anephric fetuses.

Hemoglobins in the Fetus and Neonate

Hemoglobin is a tetramer of four *globin* chains with an iron-containing porphyrin ring called *heme* covalently bound to each chain. A dynamic interaction between heme and globin gives hemoglobin its unique properties in the reversible transport of oxygen. The hemoglobin molecule consists of two alpha (α)-like and two beta (β)-like polypeptide chains, with each chain having a heme group attached (Fig. 495.5). There are two β-globin genes and four α-globin genes. Within erythrocytes of an early embryo, fetus, child, and adult, six different hemoglobins may normally be detected (Fig. 495.6): the **embryonic** hemoglobins (Gower-1, Gower-2, and Portland), **fetal** hemoglobin (HbF), and the **adult** hemoglobins (HbA and HbA_2). The electrophoretic mobilities of hemoglobins vary with their chemical structures.

Expression and quantitative relationships among the hemoglobins are determined by complex developmental processes. Globin chain expression is developmental stage specific and occurs through two hemoglobin switches, mediated primarily through changes of the β-globin genes expressed. There are five functional β-like globin chain genes: embryonic *(HBE1)*, two fetal *(HBG1, HBG2)*, and two adult *(HBD, HBB)*; and three α-like globin chain genes: embryonic *(HBZ)* and two adult *(HBA1, HBA2)*. Primitive erythroid cells primarily express embryonic globins. The first β-globin switch occurs at approximately 6 weeks' gestation to fetal globin *(HBG)*, which coincides with the onset of definitive hematopoiesis. The major hemoglobin in the fetus (HbF) consists of two α and two gamma (γ) globin chains ($\alpha_2\gamma_2$). The second globin switch is responsible for the expression of the major hemoglobin of adults (HbA), consisting of two α and two β polypeptide chains ($\alpha_2\beta_2$) and is first expressed at mid-gestation. A key regulator of the fetal-to-adult hemoglobin switch is the transcription factor **BCL11A**, which binds to the β-globin gene and acts to silence γ-globin expression and thus HbF.

Embryonic Hemoglobins

The blood of early human embryos contains two slowly migrating hemoglobins, *Gower-1* and *Gower-2,* and *Hb Portland,* which has HbF-like mobility. The zeta (ζ) chains of Hb Portland and Gower-1 are structurally quite similar to α chains. Both Gower hemoglobins contain the epsilon (ε) β-like globin polypeptide chain. Hb Gower-1 has the structure $\zeta_2\varepsilon_2$, whereas Gower-2 has $\alpha_2\varepsilon_2$. Hb Portland has the structure $\zeta_2\gamma_2$. In embryos up to 6 weeks' gestation, the Gower hemoglobins predominate but are no longer detectable by 3 months of gestation.

Fetal Hemoglobin

By 6-8 weeks' gestation, HbF ($\alpha_2\gamma_2$) is the predominant hemoglobin; at 24 weeks' gestation, it constitutes 90% of the total hemoglobin. HbF

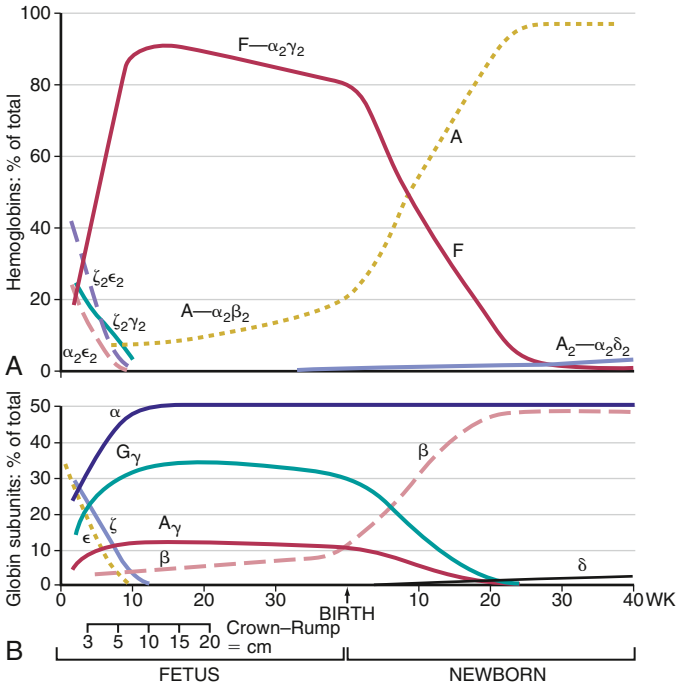

Fig. 495.6 Changes in hemoglobin tetramers (**A**) and in globin subunits (**B**) during human development from embryo to early infancy. *(From Polin RA, Fox WW. Fetal and Neonatal Physiology, 2nd ed. Philadelphia: Saunders, 1998: p. 1769.)*

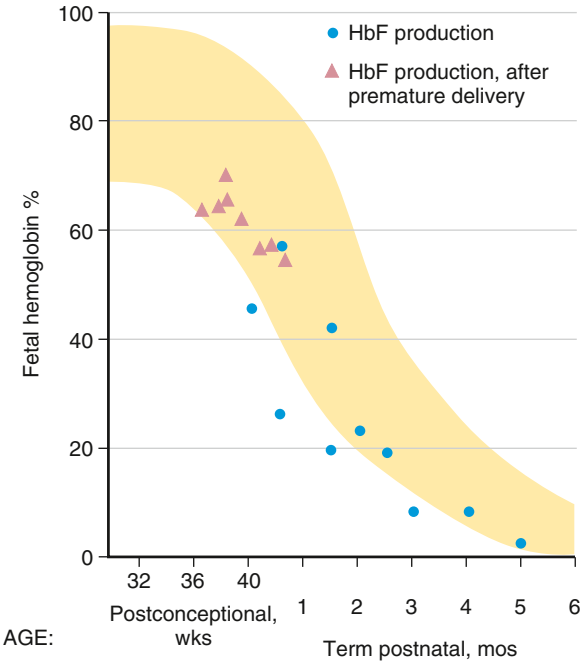

Fig. 495.7 Pre- and postnatal changes in the percentage of total hemoglobin represented by fetal hemoglobin (HbF) *(yellow)*. The *triangles* represent postnatal production by reticulocytes in premature infants, and the *circles* represent cord blood and postnatal reticulocyte production in term infants. *(From Brown MS. Fetal and neonatal erythropoiesis. In: Stockman JA, Pochedly C eds. Developmental and Neonatal Hematology. New York: Raven Press, 1988.)*

declines modestly in the third trimester, such that the HbF comprises 70–80% of the total hemoglobin. HbF production decreases rapidly postnatally (Fig. 495.7), and by 6-12 months of age declines to adult concentrations of <2%. Understanding the molecular basis of the fetal-to-adult hemoglobin switch is of interest because of the therapeutic benefits to patients with β-thalassemia and sickle cell disease, whose clinical severity is improved with modest elevation of HbF. The exact mechanisms by which BCL11A acts to repress HbF are not fully elucidated, but erythroid-specific enhancers of BCL11A have been identified and are potential targets for therapeutic HbF induction.

Adult Hemoglobins

HbA constitutes 5–10% of total hemoglobin at 24 weeks' gestation and steadily increases, so that at term, HbA averages 30% of total hemoglobin. By 6-12 months of age, individuals reach adult concentrations of HbA. The minor adult hemoglobin component, HbA_2, contains delta (δ) chains and has the structure $\alpha_2\delta_2$. At birth, <1.0% of HbA_2 is detected, but by 12 months of age the normal level is 2.0–3.4%. Throughout life, the normal ratio of HbA to HbA_2 is approximately 30:1.

Alterations of Hemoglobins

HbF levels may be elevated with hemoglobinopathies, hereditary persistence of HbF, or bone marrow failure syndromes or may be associated with stress erythropoiesis. Because the HbF level is elevated during the first years of life, knowledge of its normal pattern of decline is important (see Figs. 495.6 and 495.7). Two disorders resulting from pathogenic variants in the β-globin gene (HBB), β-thalassemia and sickle cell disease, become symptomatic postnatally as fetal γ-globin expression decreases and adult β-globin increases. In both these disorders, elevated HbF levels persist in childhood and later. In patients with the most severe type, β^0 thalassemia, except for a small amount of HbA_2, HbF is the only hemoglobin produced. At the other end of the spectrum, in individuals with β-thalassemia trait, the postnatal decrease of HbF is delayed and mildly elevated levels of HbF (>2%) may persist throughout life. Individuals with sickle cell disease, who also have a pathogenic variant in the *HBB* gene, typically demonstrate elevated levels of HbF, ranging from approximately 5% to up to 30%. In contrast, elevated HbF is not characteristic of α-thalassemia syndromes, but tetramers of γ chains (γ_4 or Hb Barts) may be found in the neonatal period. Because α-globin chains are expressed in fetal and adult hemoglobin, four α gene pathogenic variants leading to functional deletions are not compatible with life. Fetuses die in utero or shortly after birth from the severe anemia and hydrops fetalis. Inheritance of only one normal gene of the four (α –/–) results in **hemoglobin H disease**, which is usually associated with a moderate anemia. Inheritance of two or three normal α genes results in α-**thalassemia** trait or carrier status, respectively.

Hereditary persistence of HbF (HPFH) is a benign genetic condition caused by heterozygous deletions or nucleotide substitutions in regions of the β-globin locus that regulate transcription of *HBG1* and *HBG2*, causing persistent pancellular HbF expression levels of approximately 30% of total hemoglobins. Individuals with HPFH do not exhibit anemia.

Preterm infants treated with human recombinant EPO increase HbF production during active erythropoiesis. Moderate elevations of HbF may also occur in many diseases accompanied by hematologic stress, such as hemolytic anemias, leukemia, and bone marrow failure syndromes, such as Diamond Blackfan anemia.

The normal adult level of HbA_2 (2.0–3.4%) is seldom altered. Levels of HbA_2 > 3.4% are found in most persons with the β-thalassemia trait and in those with megaloblastic anemias secondary to vitamin B_{12} and folic acid deficiency. Decreased HbA_2 levels are found in those with iron-deficiency anemia (see Chapter 504) and α-thalassemia (see Chapter 511.10).

RED CELL LIFE SPAN IN THE FETUS AND NEONATE

In general, the highest hematocrit during a person's lifetime occurs at birth, and the lowest hematocrit occurs at the physiologic nadir that occurs 8-10 weeks postnatally. A shortened life span of fetal and neonatal red blood cells (RBCs) has been suggested as an important component. The average erythrocyte life span in normal adults is approximately 120 days. The life span of fetal/neonatal **erythrocytes** was once estimated to be considerably less, with an average of 60-90 days suggested by chromium (^{51}Cr)-labeled erythrocyte studies. However, newer studies indicate that the life span of fetal/neonatal RBCs is similar to that of adults. Neocytolysis is the active removal of young **erythrocytes** that were generated in relatively hypoxic conditions, after normoxic or hyperoxic conditions. This process has also been suggested as an explanation for the physiologic nadir of neonates.

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Chapter 496

Anemias

Courtney D. Thornburg

Anemia is defined as a reduction of the hemoglobin concentration or red blood cell (RBC) volume below the range of values occurring in healthy persons. Normal ranges for hemoglobin and hematocrit (packed red cell volume) vary substantially with age and between males and females (Table 496.1) and by race and ethnicity (Table 496.2). Anemia is a significant global health problem affecting children and reproductive-age females (Figs. 496.1 and 496.2).

Physiologic responses to anemia include increased cardiac output, augmented oxygen extraction (increased arteriovenous oxygen difference), and shunting of blood flow toward vital organs and tissues. In addition, the concentration of 2,3-diphosphoglycerate increases within the RBC. The resultant "rightward shift" of the oxygen dissociation curve reduces the affinity of hemoglobin for oxygen and results in more complete transfer of oxygen to the tissues. This same shift in the oxygen dissociation curve can also occur at high altitude. Higher levels of erythropoietin (EPO) and consequent increased RBC production by the bone marrow further help the body to adapt.

HISTORY AND PHYSICAL EXAMINATION

A detailed history and thorough physical exam are essential when evaluating an anemic child. Important historical facts include demographics, diet, medications, chronic diseases, infections, travel, and exposures. A family history of anemia and associated difficulties (e.g., splenomegaly, jaundice, early-age onset of gallstones) is also important. Often, few physical symptoms or signs result solely from a low hemoglobin, particularly when the anemia develops slowly. Clinical findings generally do not become apparent until the hemoglobin level falls to <7-8 g/dL. Clinical features can include pallor, sleepiness, irritability, and decreased exercise tolerance. Pallor can involve the tongue, nail beds, conjunctiva, palms, or palmar creases. A flow murmur is often present. Ultimately, weakness, tachypnea, shortness of breath on exertion, tachycardia, cardiac dilation, and high-output heart failure results from increasingly severe anemia, regardless of its cause. Unusual physical findings linked to specific underlying disease etiologies are discussed in detail in sections describing the associated disorders and in Table 496.3.

Table 496.1 Normal Mean and Lower Limits of Normal for Hemoglobin, Hematocrit, and Mean Corpuscular Volume

	HEMOGLOBIN (g/dL)		HEMATOCRIT (%)		MEAN CORPUSCULAR VOLUME (fL)	
AGE (yr)	MEAN	LOWER LIMIT	MEAN	LOWER LIMIT	MEAN	LOWER LIMIT
0.5-1.9	12.5	11.0	37	33	77	70
2-4	12.5	11.0	38	34	79	73
5-7	13.0	11.5	39	35	81	75
8-11	13.5	12.0	40	36	83	76
12-14 female	13.5	12.0	41	36	85	78
12-14 male	14.0	12.5	43	37	84	77
15-17 female	14.0	12.0	41	36	87	79
15-17 male	15.0	13.0	46	38	86	78
18-49 female	14.0	12.0	42	37	90	80
18-49 male	16.0	14.0	47	40	90	80

From Brugnara C, Oski FJ, Nathan DG, eds. *Nathan and Oski's Hematology of Infancy and Childhood*, 7th ed. Philadelphia: Saunders, 2009: p. 456.

Table 496.2 NHANES-III Hemoglobin Values for Non-Hispanic Whites and Blacks Ages 2-18 Yr*

	WHITE NON-HISPANIC		BLACK	
AGE (yr)	MEAN	−2 SD	MEAN	−2 SD
2-5	12.21	10.8	11.95	10.37
6-10	12.87	11.31	12.40	10.74
11-15 male	13.76	11.76	13.06	10.88
11-15 female	13.32	11.5	12.61	10.85
16-18 male	15.00	13.24	14.18	12.42
16-18 female	13.39	11.61	12.37	10.37

*Sample size is 5,142 (White, 2,264; Black, 2,878).
NHANES-III, Third National Health and Nutrition Examination Survey; SD, standard deviation.
Adapted from Robbins EB, Blum S. Hematologic reference values for African American children and adolescents. *Am J Hematol* 2007;82:611–614.

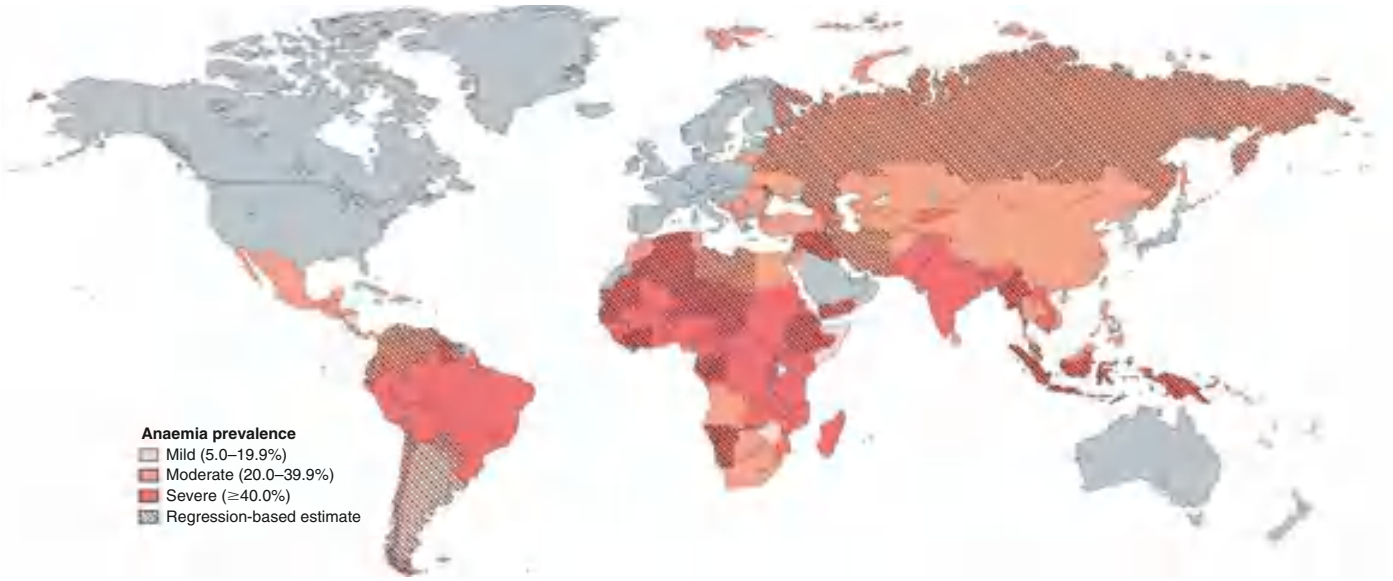

Fig. 496.1 Global prevalence of anemia in children of preschool age (0-5 yr). *(Adapted from Worldwide prevalence of anaemia 1993–2005. In WHO Global Database on Anaemia. Geneva: World Health Organization, 2008.)*

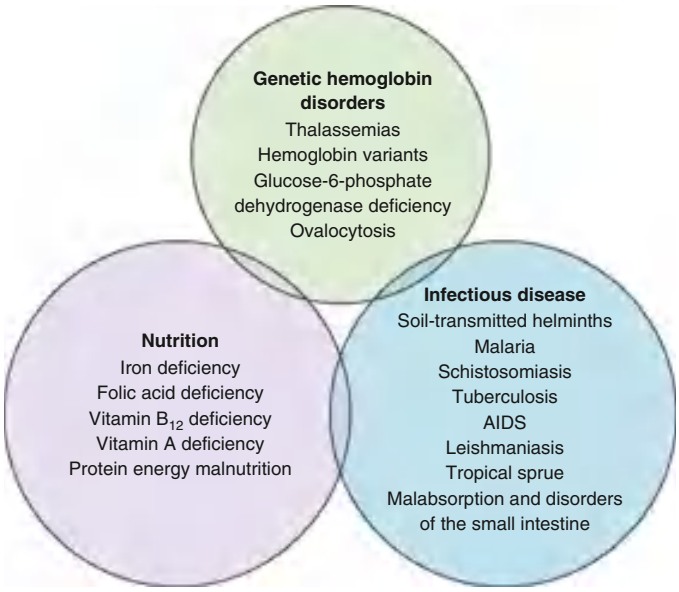

Fig. 496.2 Causes of anemia in countries with low- or middle-income populations. *(From Balarajan Y, Ramakrishnan U, Özaltin E, et al. Anaemia in low-income and middle-income countries. Lancet 2011;378:2123–2134; Fig 3.)*

LABORATORY STUDIES

Initial laboratory testing should include hemoglobin, hematocrit, RBC indices, white blood cell (WBC) count and differential, platelet count, reticulocyte count, and examination of the peripheral blood smear. The need for additional laboratory studies is dictated by the history, physical exam, and initial testing.

DIFFERENTIAL DIAGNOSIS

Anemia can result from many underlying pathologic processes. To narrow the diagnostic possibilities, anemias may be classified based on their morphology and physiology (Fig. 496.3).

Anemias may be morphologically categorized by red cell size (mean corpuscular volume [MCV]) and microscopic appearance. Anemias can be classified as *microcytic, normocytic,* or *macrocytic* based on whether the MCV is low, normal, or high, respectively (Table 496.4). RBC size also changes with age, and normal developmental changes in MCV should be accommodated before a designation is made (see Table 496.1). Examination of a peripheral blood smear often reveals changes in RBC appearance that will help to further narrow the diagnostic categories (Fig. 496.4 and Table 496.5). Details regarding morphologic changes associated with specific disorders are described in subsequent sections.

Anemias may also be further divided based on underlying pathophysiology. The two major categories are **decreased production** and **increased destruction** (or **loss**). These two groups are not always mutually exclusive. Decreased RBC production may be a consequence of either ineffective erythropoiesis or complete failure of erythropoiesis. Increased destruction or loss may be secondary to hemolysis, sequestration, or bleeding. The peripheral blood reticulocyte percentage or absolute number helps to distinguish between the two physiologic categories. The normal reticulocyte percentage of total RBCs during most of childhood is approximately 1%, with an absolute reticulocyte count of 25,000-75,000/mm^3. In the presence of anemia, EPO production and the absolute number of reticulocytes should rise. Low or normal numbers of reticulocytes generally represent an inadequate response to anemia that is associated with relative bone marrow failure or ineffective erythropoiesis. Increased numbers of reticulocytes represent a normal bone marrow response to ongoing RBC destruction (hemolysis), sequestration, or loss (bleeding).

Figure 496.3 presents a useful approach to assessing the common causes of anemia in the pediatric age group. Children with **microcytic anemia** and low or normal reticulocyte counts most often have defects in erythroid maturation or ineffective erythropoiesis. **Iron deficiency** is the most common cause (see Chapter 504). **Thalassemia trait** constitutes the primary differential diagnosis when iron deficiency is suspected (see Chapter 511). Distinctions between these entities are presented in Table 504.2. Chronic disease or inflammation (more often normocytic), lead poisoning, and sideroblastic anemias should also be considered and are discussed in other chapters. Microcytosis and elevated reticulocyte counts are associated with thalassemias and

Table 496.3 Physical Findings in the Evaluation of Anemia

SYSTEM	OBSERVATION	SIGNIFICANCE
Skin	Hyperpigmentation	Fanconi anemia, dyskeratosis congenita
	Café-au-lait spots	Fanconi anemia
	Vitiligo	Vitamin B_{12} deficiency
	Partial oculocutaneous albinism	Chédiak-Higashi syndrome
	Jaundice	Hemolysis, hepatitis
	Petechiae, purpura	Bone marrow infiltration, autoimmune hemolysis with autoimmune thrombocytopenia (Evans syndrome), hemolytic-uremic syndrome
	Erythematous rash	Parvovirus, Epstein-Barr virus
	Butterfly rash	Systemic lupus erythematosus
Head	Frontal bossing	Thalassemia major, severe iron deficiency, chronic subdural hematoma
	Microcephaly	Fanconi anemia Diamond-Blackfan anemia
Eyes	Microphthalmia	Fanconi anemia
	Retinopathy	Sickle cell disease, types SS and SC
	Optic atrophy, blindness	Osteopetrosis
	Blocked lacrimal gland	Dyskeratosis congenita
	Kayser-Fleischer ring	Wilson disease
	Blue sclera	Iron deficiency
Ears	Deafness	Osteopetrosis
Mouth	Glossitis	Vitamin B_{12} deficiency, iron deficiency
	Angular stomatitis	Iron deficiency
	Cleft lip, palate	Diamond-Blackfan anemia
	Pigmentation	Peutz-Jeghers syndrome (intestinal blood loss)
	Telangiectasia	Osler-Weber-Rendu syndrome (blood loss)
	Leukoplakia	Dyskeratosis congenital
Chest	Shield chest or widespread nipples	Diamond-Blackfan anemia
	Murmur	Endocarditis; prosthetic valve hemolysis
Abdomen	Hepatomegaly	Hemolysis, infiltrative tumor, chronic disease, hemangioma, cholecystitis
	Splenomegaly	Acquired hemolytic anemia, inherited hemolytic anemia (hereditary spherocytosis, pyruvate kinase deficiency, sickle cell disease [age of presentation varies based on phenotype]), thalassemia, malaria, leukemia/lymphoma, Epstein-Barr virus, portal hypertension, hemophagocytic syndromes
	Nephromegaly	Fanconi anemia
	Absent kidney	Fanconi anemia
Extremities	Absent thumbs	Fanconi anemia
	Thenar eminence hypoplasia: triphalangeal thumb	Diamond-Blackfan anemia
	Spoon nails	Iron deficiency
	Beau line (nails)	Heavy metal intoxication, severe illness
	Mees line (nails)	Heavy metals, severe illness, sickle cell disease
	Dystrophic nails	Dyskeratosis congenita
	Edema	Milk-induced protein-losing enteropathy with iron deficiency, renal failure
Rectal	Hemorrhoids	Portal hypertension
	Heme-positive stool	Intestinal hemorrhage
Nerves	Irritable, apathy	Iron deficiency
	Peripheral neuropathy	Deficiency of vitamins B_1 and B_{12}, lead poisoning
	Dementia	Deficiency of vitamins B_{12} and E
	Ataxia, posterior column signs	Deficiency of vitamins B_{12} and E
	Stroke	Sickle cell disease, paroxysmal nocturnal hemoglobinuria

Adapted from Scott JP. Hematology. In: Behrman RE, Kliegman RM, eds. *Nelson Essentials of Pediatrics,* 2nd ed. Philadelphia: Saunders, 1994: p. 520.

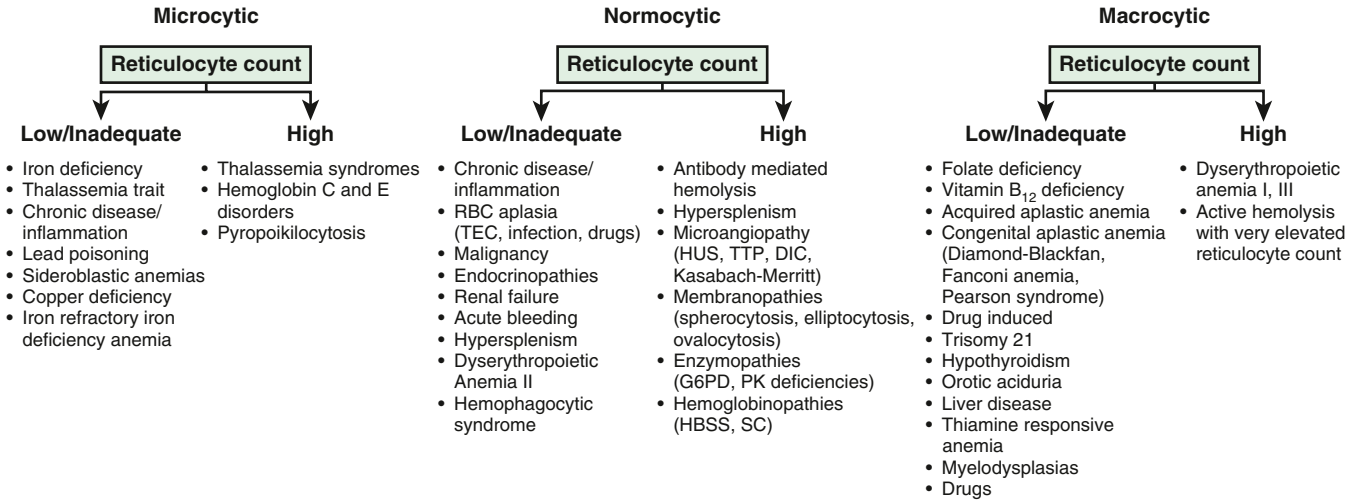

Fig. 496.3 Use of the mean corpuscular volume (MCV) and reticulocyte count in the diagnosis of anemia. *(Adapted from Brunetti M, Cohen J. The Harriet Lane Handbook, 17th ed. Philadelphia: Mosby, 2005. p. 338.)*

Table 496.4 Causes of High or Low Mean Corpuscular Volume

LOW MEAN CORPUSCULAR VOLUME
Iron deficiency
Thalassemias
Lead toxicity
Anemia of chronic disease
Copper deficiency
Sideroblastic anemia
Hemoglobin E
Hereditary pyropoikilocytosis
HIGH MEAN CORPUSCULAR VOLUME
Normal newborn
Elevated reticulocyte count
Vitamin B_{12} or folate deficiency
Diamond-Blackfan anemia (congenital hypoplastic anemia)
Fanconi anemia
Aplastic anemia
Down syndrome
Hypothyroidism (occasionally)
Orotic aciduria
Lesch-Nyhan syndrome
Drugs (zidovudine, chemotherapy)
Chronic liver disease
Paroxysmal nocturnal hemoglobinuria
Thiamine-responsive megaloblastic anemia
Myelodysplasias
Dyserythropoietic anemias

From Kliegman RM, Toth H, Bordini BJ, Basel D, eds. *Nelson Pediatric Symptom-Based Diagnosis: Common Diseases and Their Mimics.* 2nd ed. Philadelphia: Elsevier, 2023: Table 49.5, p. 910.

hemoglobin C and E (see Chapter 511). Notably, thalassemias and hemoglobinopathies are most often seen in patients of Mediterranean, Middle Eastern, African, or Asian descent.

Normocytic anemia and low reticulocyte count characterize many anemias. The **anemia of chronic disease/inflammation** is usually normocytic (see Chapter 504). The anemia associated with renal failure, primarily a result of reduced EPO production, will invariably be associated with clinical and laboratory evidence of significant kidney disease. Decreased or absent RBC production secondary to transient erythroblastopenia of childhood (see Chapter 499), infection, medications, or endocrinopathy usually results in a normocytic anemia, as does bone marrow infiltration by malignancy. Normocytic anemia in combination with leukopenia (or significant leukocytosis with blasts) and/or thrombocytopenia should raise suspicion for malignancy. See Chapters 517 and 518 to review pancytopenias. Acute bleeding, hypersplenism, and congenital dyserythropoietic anemia type II are also normocytic (see Chapter 501).

In children with normocytic anemia and an appropriate (high) reticulocyte response, the anemia is usually caused by bleeding, hypersplenism, or ongoing hemolysis. In hemolytic conditions, reticulocytosis, indirect hyperbilirubinemia, and increased serum lactate dehydrogenase are indicators of accelerated erythrocyte destruction. Many causes of hemolysis result from conditions that are extrinsic (usually acquired) or intrinsic (usually congenital) to the erythrocyte. Abnormal RBC morphology (e.g., spherocytes, dacryocytes or sickle forms, and schistocytes) identified on the peripheral smear is often helpful in ascertaining the cause.

The anemia seen in children with macrocytic blood cells is sometimes megaloblastic, resulting from impaired DNA synthesis and nuclear development (see Chapter 503). The peripheral blood smear in **megaloblastic anemias** contains large macroovalocytes, and the neutrophils often show nuclear hypersegmentation. The major causes of megaloblastic anemia include folate deficiency, vitamin B_{12} deficiency, and rare inborn errors of metabolism. Other **macrocytic anemias** with low or normal reticulocyte counts include acquired and congenital (Diamond-Blackfan anemia and Fanconi anemia) aplastic anemias and hypothyroidism. Patients with trisomy 21 have macrocytic cells, although an accompanying anemia is generally not present. High MCV and reticulocytosis is seen in congenital dyserythropoietic anemias I and III and in situations where hemolysis results in such a large outpouring of young red cells that the MCV is abnormally high.

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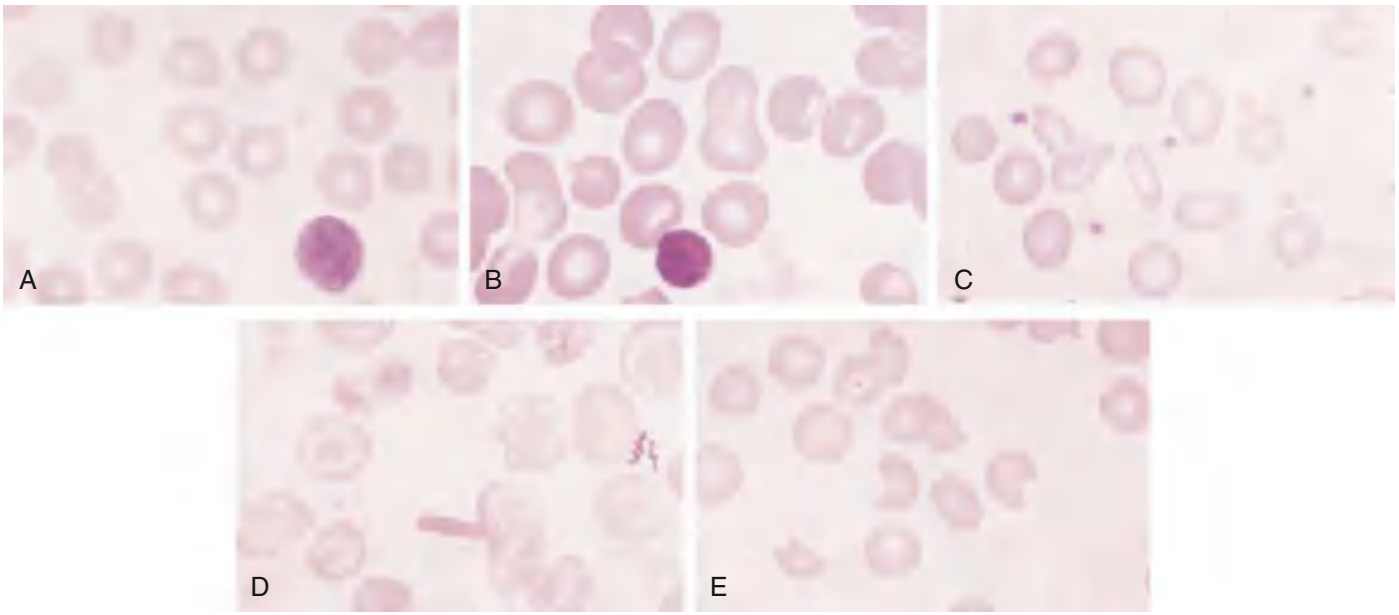

Fig. 496.4 Morphologic abnormalities of the red blood cell. **A,** Normal. **B,** Macrocytes (folic acid or vitamin B_{12} deficiency). **C,** Hypochromic microcytes (iron deficiency). **D,** Target cells (HbCC disease). **E,** Schistocyte (hemolytic-uremic syndrome). *(Courtesy Dr. Elias Schwartz, Children's Hospital of Philadelphia.)*

Table 496.5 Peripheral Blood Morphologic Findings in Various Anemias

MICROCYTES	**BASOPHIL STIPPLING**
Iron deficiency	Thalassemia
Thalassemias	Lead intoxication
Lead toxicity	Myelodysplasia
Anemia of chronic disease	
MACROCYTES	**RED BLOOD CELL FRAGMENTS, HELMET CELLS, BURR CELLS**
Newborns	Disseminated intravascular coagulation
Vitamin B_{12} or folate deficiency	Hemolytic uremic syndrome
Diamond-Blackfan anemia	Thrombotic thrombocytopenic purpura
Fanconi anemia	Kasabach-Merritt syndrome
Aplastic anemia	Waring blender syndrome (artificial heart valve)
Liver disease	Uremia
Down syndrome	Liver disease
Hypothyroidism	
SPHEROCYTES	**HYPERSEGMENTED NEUTROPHILS**
Hereditary spherocytosis	Vitamin B_{12} or folate deficiency
Immune hemolytic anemia (newborn or acquired)	
Hypersplenism	**BLASTS**
	Leukemia (ALL or AML)
SICKLED CELLS	Severe infection (rarely)
Sickle cell anemias (SS disease, SC disease, $S\beta^+$ thalassemia, $S\beta^0$ thalassemia)	**LEUKOPENIA/THROMBOCYTOPENIA**
	Fanconi anemia
ELLIPTOCYTES	Aplastic anemia
Hereditary elliptocytosis	Leukemia
Iron deficiency	Hemophagocytic histiocytosis
Megaloblastic anemia	
TARGET CELLS	**HOWELL-JOLLY BODIES**
Hemoglobinopathies (especially hemoglobin C and SC and thalassemia)	Asplenia, hyposplenia
	Severe iron deficiency
Liver disease	
Xerocytosis	**DACROCYTES (TEARDROP CELLS)**
	Myelodysplasia
	Leukemia
	Neuroblastoma

ALL, Acute lymphocytic leukemia; AML, acute myeloid leukemia; SC, sickle cell C disease; SS, sickle cell S disease.
From Kliegman RM, Toth H, Bordini BJ, Basel D, eds. *Nelson Pediatric Symptom-Based Diagnosis: Common Diseases and Their Mimics.* 2nd ed. Philadelphia: Elsevier, 2023: Table 49.7, p. 910.

Section 2

Anemias of Inadequate Production

Chapter 497

Congenital Hypoplastic Anemia (Diamond-Blackfan Anemia)

Courtney D. Thornburg

Diamond-Blackfan anemia (DBA) is a rare, congenital **bone marrow failure syndrome** that usually becomes symptomatic in early infancy. More than 90% of cases are recognized in the first year of life. The disorder is characterized by anemia, usually normochromic and macrocytic; reticulocytopenia; and insufficient or absent red blood cell (RBC) precursors in an otherwise normally cellular bone marrow. Up to 50% of affected individuals have additional, extrahematopoietic anomalies.

ETIOLOGY

The most common DBA-associated pathogenic variants are in *RPS19* (Fig. 497.1). This gene encodes a component protein of the small 40S ribosomal subunit and pathogenic variants are present in approximately 25–30% of patients with additional ribosomal protein (RP) genes, each encoding a different small (40S) or large (60S) ribosomal subunit protein, implicated as well. All of these pathogenic variants are inherited in an autosomal dominant fashion, with 40–45% of cases inherited from a parent and 55–60% with a de novo pathogenic variant. Less frequently, X-linked inherited cases of DBA involve pathologic genetic variants in *GATA1* or *TSR2*. Patients with *GATA1*-related DBA usually have no extra hematopoietic manifestations (Fig. 497.2). *TSR2*-related DBA is associated with mandibulofacial dysostosis. Approximately 20% of cases do not have an identified genetic variant. Because most causative variants are in ribosomal genes, the disorder is often referred to as a **ribosomopathy**.

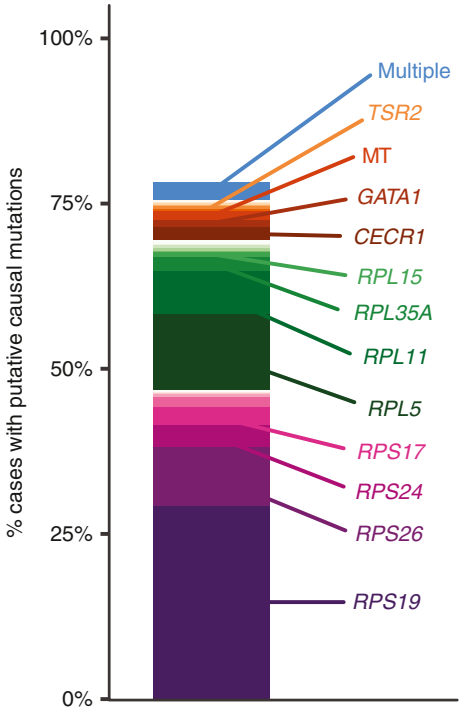

Fig. 497.1 Mutational spectrum of likely pathogenic variants in DBA. Percentage of putative causal mutations in each gene. A total of 78% of case subjects have a putative causal mutation. *(Modified from Ulirsch JC, Verboon JM, Kazerounian S, et al. The genetic landscape of Diamond-Blackfan anemia. Am J Hum Genetics 2018;103:930–947, Fig.1.)*

EPIDEMIOLOGY

DBA affects about 5-10 individuals per million live births. Notably, there is substantial phenotypic diversity in DBA, even in families whose members share the same pathologic genetic variant, suggesting that additional genetic modifiers affect phenotypic expression of the disease.

CLINICAL MANIFESTATIONS

Profound anemia usually becomes evident by 2-6 months of age, occasionally somewhat later. Approximately 25% of patients are anemic at birth, although hydrops fetalis occurs rarely; 92% are diagnosed within the first year of life. Approximately 40–50% of patients have congenital anomalies, and more than one anomaly is found in 25% of DBA patients (Table 497.1). Craniofacial abnormalities are the most common (50% of patients) and include a depressed nasal bridge and high-arched palate. Skeletal anomalies, mostly upper limb and hand, affect 30–40%. This includes thumb abnormalities, including flattening of the thenar eminence and triphalangeal thumb, that may be bilateral or unilateral. The radial pulse may be absent. Genitourinary (39%), cardiac (30%), ophthalmologic, and musculoskeletal anomalies have also been described. Short stature is common, but it is often unclear whether this characteristic results from the disease itself, related therapies, or both.

LABORATORY FINDINGS

The RBCs are usually macrocytic for age, but no hypersegmented neutrophils or other characteristics of megaloblastic anemia are appreciated on the peripheral blood smear. RBC characteristics are like those of a "fetal" RBC population, with increased expression of "i" antigen and elevated fetal hemoglobin (HbF). **Erythrocyte adenosine deaminase** (eADA) activity is increased in most patients with DBA, a finding that helps distinguish congenital RBC aplasia from acquired **transient erythroblastopenia of childhood** (TEC) (see Chapter 499). Because elevated eADA activity is not a fetal RBC feature, measurement of this enzyme may be particularly helpful when diagnosing DBA in very young infants. Thrombocytosis,

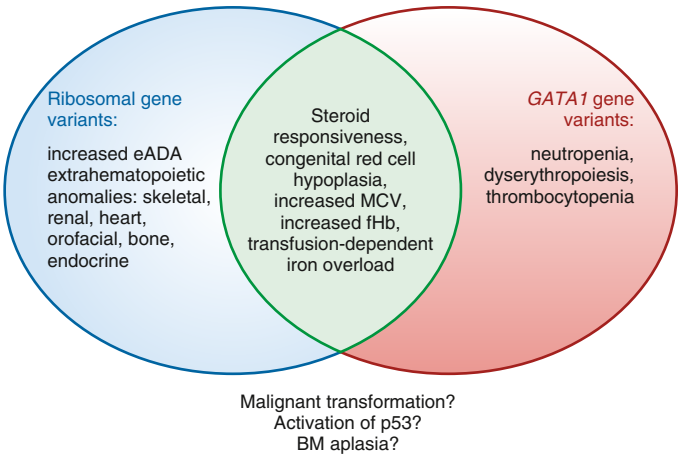

Fig. 497.2 Common and distinct phenotypes in congenital red cell aplasia caused by variants in *RP* genes and in *GATA1*. BM, Bone marrow; eADA, erythrocyte adenosine deaminase activity; fHb, fetal hemoglobin; MCV, mean corpuscular volume. *(Adapted from Weiss MJ, Mason PJ, Bessler M. What's in a name? J Clin Invest 2012;122:2346–2349.)*

or rarely thrombocytopenia, and occasionally neutropenia, may also be present. **Reticulocytopenia** is characteristic despite severe anemia. Bone marrow erythrocyte precursors are greatly reduced in most patients; other marrow elements are usually normal. Serum iron levels are elevated. Unlike Fanconi anemia, there is no increase in chromosomal breaks when lymphocytes are exposed to alkylating agents. Table 497.2 outlines suggested diagnostic criteria for "classical" DBA. Patients may be diagnosed with "nonclassical" DBA or "probable" DBA depending on their individual genetic test results, family history, and major and minor criteria.

Table 497.1 Range of Congenital Anomalies Observed in Diamond-Blackfan Anemia

TYPE/LOCATION	ANOMALIES
Craniofacial	Hypertelorism Broad, flat nasal bridge Cleft palate High-arched palate Microcephaly Micrognathia Microtia Low-set ears Low hairline Ptosis
Ophthalmologic	Congenital glaucoma Strabismus Epicanthal folds Congenital cataract
Neck	Short neck Webbed neck Sprengel deformity Klippel-Feil deformity
Thumbs	Triphalangeal Duplex or bifid Hypoplastic Flat thenar eminence Absent radial artery
Urogenital	Absent kidney Horseshoe kidney Hypospadias
Cardiac	Ventricular septal defect Atrial septal defect Coarctation of the aorta Complex cardiac anomalies
Other	Low birth weight Short stature Syndactyly Learning difficulties

Multiple anomalies, most often including craniofacial, are present in up to 25% of affected individuals. At least one anomaly is present in 40–50%.
From Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond-Blackfan anaemia: Results of an international clinical consensus conference. *Br J Haematol* 2008;142:859–876, Table IV.

DIFFERENTIAL DIAGNOSIS

DBA must be differentiated from other anemias associated with reticulocytopenia. The syndrome of TEC is often the primary alternative diagnosis. Table 499.1 shows a useful comparison of findings in these two disorders (see Chapter 499). TEC often is differentiated from DBA by its relatively late onset, although it occasionally develops in infants younger than 6 months of age. Macrocytosis, congenital anomalies, fetal RBC characteristics, and elevated eADA are generally associated with DBA and not TEC.

Other inherited macrocytic bone marrow failure syndromes, particularly **Fanconi anemia** and **Shwachman-Diamond syndrome** (see Chapter 517), should also be considered, as should myelodysplastic syndrome. **Aase syndrome** includes congenital RBC aplasia with triphalangeal thumb, congenital heart disease, and cleft palate. **Hemolytic disease of the newborn** can also mimic features of DBA because it can have a protracted course and can be coupled with greatly reduced erythropoiesis. The anemia in this disorder usually resolves spontaneously at 5-8 weeks of age. Several types of chronic hemolytic diseases may be complicated by an **aplastic crisis**, characterized by reticulocytopenia and decreased numbers of RBC precursors. This event usually occurs after the first several months of life and is often caused by **parvovirus B19 infection** (see Chapter 499). Infection with parvovirus B19 in utero is also associated with pure RBC aplasia in infancy and even with hydrops fetalis at birth (see Chapter 298). When diagnosing DBA in young infants, it is important to rule out parvovirus B19 infection using a polymerase chain reaction assay because serologic testing may be inaccurate. Other infections, including HIV, as well as drugs, immune processes, and Pearson syndrome (see Chapter 498), should also be ruled out.

TREATMENT

Corticosteroids are a mainstay of therapy, and approximately 80% of patients initially respond. Because corticosteroids impair linear growth as well as physical and neurocognitive development, many hematologists maintain infants on chronic transfusion therapy and delay the start of steroids until after age 1 year. Prednisone or prednisolone in doses totaling 2 mg/kg/day is used as an initial trial. An increase in RBC precursors is usually seen in the bone marrow 1-3 weeks after therapy is begun and is followed by peripheral reticulocytosis. The hemoglobin can reach normal levels in 4-6 weeks, although the rate of response is quite variable. Once it is established that the hemoglobin concentration is increasing, the dose of corticosteroid may be reduced gradually by tapering and then by eliminating all except a single, lowest effective daily dose. This dose may

Table 497.2 Diagnostic Criteria for Diamond-Blackfan Anemia

DIAGNOSTIC CRITERIA	SUPPORTING CRITERIA	
	MAJOR CRITERIA	MINOR CRITERIA
Age younger than 1 yr	Pathogenic variant described in "classical DBA"	Elevated red cell adenosine deaminase
Macrocytic anemia with no other significant cytopenias	Positive family history	Congenital anomalies described in "classical" DBA
Reticulocytopenia		Elevated HbF
Normal marrow cellularity with paucity of bone marrow erythroid precursors		No evidence for another inherited bone marrow failure syndrome

"Classical DBA" diagnosis is made if all the diagnostic criteria are met.
DBA, Diamond Blackfan anemia, HbF, fetal hemoglobin.
From Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond-Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* 2008;142:859–876.

then be doubled, used on alternate days, and tapered still further while maintaining the hemoglobin level at ≥9 g/dL. The target maintenance dose should not exceed 0.5 mg/kg/day or 1 mg/kg every other day. In some patients, very small amounts of prednisone, as low as 2.5 mg twice a week, may be sufficient to sustain adequate erythropoiesis. Scheduled surveillance examinations and testing for corticosteroid side effects should be pursued in all patients, regardless of dose. Appropriate *Pneumocystis jiroveci* prophylaxis should be considered after the first month of high-dose steroids and continued until the patient is on low-dose alternate-day therapy. In the setting of illness, stress steroids should be considered for children on chronic corticosteroids. Many children with DBA stop taking corticosteroids, usually because of unacceptable side effects (i.e., cushingoid features, pathologic fractures, cataracts) or the evolution of corticosteroid refractoriness.

Chronic red cell transfusions are required in approximately 35% of patients, including patients who are never steroid responsive (30%), are steroid refractory (15%), or cannot be weaned to acceptable low dose (50%). Transfusions are given at intervals of 3-5 weeks to maintain a hemoglobin level >8 g/dL. Some younger children may require hemoglobin >9 g/dL to sustain normal growth and activities. Appropriate screening and ultimately the initiation of chelation therapy are required for transfusion-related iron overload.

L-leucine (700 mg/m^2 orally three times per day) has been evaluated in a phase I/II trial patients with DBA 2 years of age and older. In 43 evaluable patients, 16% had erythroid response, 36% had increase in weight, and 44% had an increase in linear growth velocity.

Spontaneous remission of anemia with independence from steroid or red cell transfusion therapy has been reported. The likelihood of remission is 25% by age 25 years, with most of these patients experiencing remission during the first decade. Mild macrocytic anemia and increased erythrocyte ADA levels persist in these circumstances.

Hematopoietic stem cell transplantation (HSCT) can be curative. Indications for HSCT include steroid resistance or unacceptable toxicity and transfusion dependence as well as significant complications of chronic red cell transfusions including iron overload and alloimmunizations. HLA-matched sibling HSCT is recommended for transfusion-dependent children with DBA. One recommendation is for HSCT between ages 3 and 9 years, and some advocate HSCT at a younger age to avoid iron overload and allosensitization from chronic red cell transfusions. It is important that sibling donors be carefully screened, including genotype if known, to ensure that the donor does not carry the pathologic genetic variant. Overall, outcomes are improving for matched-sibling and alternative donor HSCT.

PROGNOSIS

DBA has been identified as a **cancer predisposition syndrome** because of the higher risk of myelodysplastic syndrome, acute myeloid leukemia, colon carcinoma, osteogenic sarcoma, and female genital cancers. Patients are at risk for iron overload-related endocrine abnormalities (diabetes, hypogonadism), especially if transfused. Patients who have undergone HSCT are at risk of associated late effects (see Chapters 179, 180, and 181). The overall actuarial survival of all patients with DBA is approximately 75% at age 40 years, with approximately 87% for those maintained on corticosteroids and approximately 57% for transfusion-dependent patients. Of reported deaths, 67% were treatment related and 22% were DBA related (malignancy and severe aplastic anemia).

Treatment outcome and survival data are collected through the Diamond-Blackfan Anemia Registry (https://www.dbar.org).

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Chapter 498

Pearson Syndrome

Courtney D. Thornburg

Pearson syndrome (PS) is a rare multisystem mitochondrial disorder that presents with a hypoplastic anemia that may be initially confused with Diamond-Blackfan syndrome (anemia) or transient erythroblastopenia of childhood (see Chapter 499). The marrow failure usually appears in the neonatal period and is characterized by a *sideroblastic macrocytic anemia* and, occasionally, neutropenia and thrombocytopenia. There are vacuolated erythroblasts and myeloblasts in the bone marrow (Fig. 498.1). PS is considered a unique variant of congenital sideroblastic anemia because the marrow also contains ringed sideroblasts. The hemoglobin F level is elevated. There is multiorgan involvement manifested by failure to thrive and symptoms of exocrine pancreas dysfunction, liver and renal tubular defects, malabsorption, and myopathy. Endocrine dysfunction (type 1 diabetes, adrenal insufficiency, hypoparathyroidism, and hypothyroidism) has also been reported. Children that survive early childhood typically develop **Kearns-Sayre syndrome**, an early-onset, mitochondrial disorder with lactic acidosis, progressive external ophthalmoplegia (impaired eye movement and ptosis), pigmentary retinitis, deafness, cerebellar ataxia, and heart block. Pearson syndrome is caused by a large heteroplasmic **mitochondrial DNA (mtDNA) deletion** (see Chapter 108) that predominates in the hematopoietic lineage. Subsequently, there is heterogeneity in different tissues and between patients, accounting for the variable clinical picture. The proportion of deleted mtDNA in the bone marrow correlates with the severity of the hematologic disease, and a reduction in the percentage of deleted mtDNA over time may be associated with spontaneous improvement of red blood cell hypoproliferation. PS may be misdiagnosed as Diamond-Blackfan anemia (DBA) based on the overlapping features, including severe anemia starting at a young age. Evaluation for mtDNA deletion differentiates PS from DBA (see Chapter 497).

Therapy for the hematologic manifestations of the disease includes red cell transfusions to correct anemia and granulocyte colony-stimulating factor in the setting of severe neutropenia. The hematologic manifestations may spontaneously resolve within the first few years of life and stem cell transplantation may be considered for persistent cytopenias.

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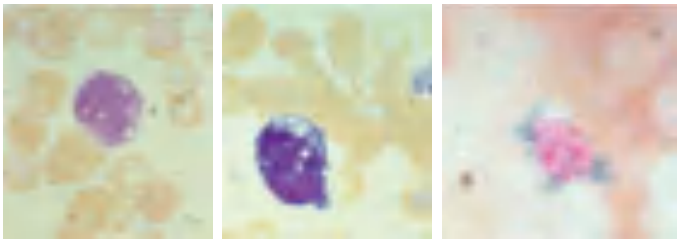

Fig. 498.1 Bone marrow morphology in Pearson syndrome. *Left,* Vacuoles in myeloid precursor. *Center,* Vacuoles in erythroid precursor. *Right,* Ringed sideroblast. (*From Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev 2010;24:101–122; Fig 14.*)

then be doubled, used on alternate days, and tapered still further while maintaining the hemoglobin level at ≥9 g/dL. The target maintenance dose should not exceed 0.5 mg/kg/day or 1 mg/kg every other day. In some patients, very small amounts of prednisone, as low as 2.5 mg twice a week, may be sufficient to sustain adequate erythropoiesis. Scheduled surveillance examinations and testing for corticosteroid side effects should be pursued in all patients, regardless of dose. Appropriate *Pneumocystis jiroveci* prophylaxis should be considered after the first month of high-dose steroids and continued until the patient is on low-dose alternate-day therapy. In the setting of illness, stress steroids should be considered for children on chronic corticosteroids. Many children with DBA stop taking corticosteroids, usually because of unacceptable side effects (i.e., cushingoid features, pathologic fractures, cataracts) or the evolution of corticosteroid refractoriness.

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DBA has been identified as a **cancer predisposition syndrome** because of the higher risk of myelodysplastic syndrome, acute myeloid leukemia, colon carcinoma, osteogenic sarcoma, and female genital cancers. Patients are at risk for iron overload-related endocrine abnormalities (diabetes, hypogonadism), especially if transfused. Patients who have undergone HSCT are at risk of associated late effects (see Chapters 179, 180, and 181). The overall actuarial survival of all patients with DBA is approximately 75% at age 40 years, with approximately 87% for those maintained on corticosteroids and approximately 57% for transfusion-dependent patients. Of reported deaths, 67% were treatment related and 22% were DBA related (malignancy and severe aplastic anemia).

Treatment outcome and survival data are collected through the Diamond-Blackfan Anemia Registry (https://www.dbar.org).

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Chapter 498

Pearson Syndrome

Courtney D. Thornburg

Pearson syndrome (PS) is a rare multisystem mitochondrial disorder that presents with a hypoplastic anemia that may be initially confused with Diamond-Blackfan syndrome (anemia) or transient erythroblastopenia of childhood (see Chapter 499). The marrow failure usually appears in the neonatal period and is characterized by a *sideroblastic macrocytic anemia* and, occasionally, neutropenia and thrombocytopenia. There are vacuolated erythroblasts and myeloblasts in the bone marrow (Fig. 498.1). PS is considered a unique variant of congenital sideroblastic anemia because the marrow also contains ringed sideroblasts. The hemoglobin F level is elevated. There is multiorgan involvement manifested by failure to thrive and symptoms of exocrine pancreas dysfunction, liver and renal tubular defects, malabsorption, and myopathy. Endocrine dysfunction (type 1 diabetes, adrenal insufficiency, hypoparathyroidism, and hypothyroidism) has also been reported. Children that survive early childhood typically develop **Kearns-Sayre syndrome**, an early-onset, mitochondrial disorder with lactic acidosis, progressive external ophthalmoplegia (impaired eye movement and ptosis), pigmentary retinitis, deafness, cerebellar ataxia, and heart block. Pearson syndrome is caused by a large heteroplasmic **mitochondrial DNA (mtDNA) deletion** (see Chapter 108) that predominates in the hematopoietic lineage. Subsequently, there is heterogeneity in different tissues and between patients, accounting for the variable clinical picture. The proportion of deleted mtDNA in the bone marrow correlates with the severity of the hematologic disease, and a reduction in the percentage of deleted mtDNA over time may be associated with spontaneous improvement of red blood cell hypoproliferation. PS may be misdiagnosed as Diamond-Blackfan anemia (DBA) based on the overlapping features, including severe anemia starting at a young age. Evaluation for mtDNA deletion differentiates PS from DBA (see Chapter 497).

Therapy for the hematologic manifestations of the disease includes red cell transfusions to correct anemia and granulocyte colony-stimulating factor in the setting of severe neutropenia. The hematologic manifestations may spontaneously resolve within the first few years of life and stem cell transplantation may be considered for persistent cytopenias.

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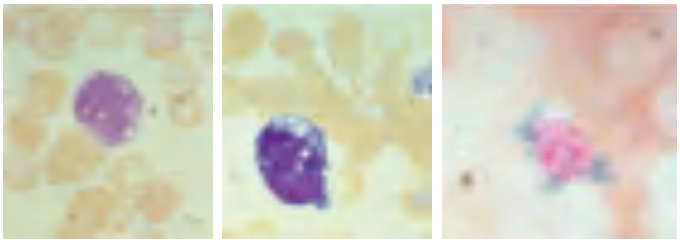

Fig. 498.1 Bone marrow morphology in Pearson syndrome. *Left,* Vacuoles in myeloid precursor. *Center,* Vacuoles in erythroid precursor. *Right,* Ringed sideroblast. (*From Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev 2010;24:101–122; Fig 14.*)

Chapter 499

Acquired Pure Red Blood Cell Anemia

Courtney D. Thornburg

TRANSIENT ERYTHROBLASTOPENIA OF CHILDHOOD

Transient erythroblastopenia of childhood **(TEC)** is the most common *acquired red cell aplasia* occurring in children. It is more prevalent than congenital hypoplastic anemia (Diamond-Blackfan anemia [DBA]). This syndrome of severe *transient hypoplastic anemia* occurs mainly in previously healthy children between 6 months and 3 years of age. Most children are older than 12 months at onset. Only 10% of affected patients are more than 3 years of age. The annual incidence is estimated to be 4.3 cases per 100,000 children, although it is likely higher, because TEC often resolves spontaneously with many cases undiagnosed. The suppression of erythropoiesis has been linked to IgG, IgM, and cell-mediated mechanisms. Familial cases have been reported, suggesting a hereditary component. TEC often follows a viral illness, although no specific virus has been consistently implicated.

The temporary suppression of erythropoiesis results in reticulocytopenia and moderate to severe normocytic anemia. Some degree of neutropenia occurs in up to 20% of cases. Platelet numbers are normal or elevated. Like the situation observed in iron-deficiency anemia and other red blood cell (RBC) hypoplasias, thrombocytosis is presumably caused by increased erythropoietin (EPO), which has homology to thrombopoietin (TPO). Mean corpuscular volume (MCV) is characteristically normal for age, and fetal hemoglobin (HbF) levels are normal before the recovery phase. RBC adenosine deaminase levels are normal in TEC, thus contrasting with the elevation noted in most cases of congenital hypoplastic anemia (Table 499.1). Differentiation from DBA is sometimes difficult, but differences in age at onset and in age-related MCV, HbF, and adenosine deaminase are usually helpful. The peak occurrence of TEC coincides with that of iron-deficiency anemia in infants receiving milk as their main caloric source; differences in MCV should help to distinguish between TEC and DBA.

Virtually all children recover within 1-2 months. RBC transfusions may be necessary for severe anemia in the absence of signs of early recovery. The anemia develops slowly, and significant symptoms usually develop only with severe anemia. *Corticosteroid therapy is of no value in this disorder.* Any child with presumed TEC who requires more than one transfusion should be reevaluated for another possible diagnosis. In rare instances, a prolonged case of apparent TEC may be caused by parvovirus-induced RBC aplasia, occurring in children with *hemolytic anemia* or *congenital* or *acquired immunodeficiencies.*

RED CELL APLASIA ASSOCIATED WITH PARVOVIRUS B19 INFECTION

Parvovirus B19 is a common infectious agent that causes **erythema infectiosum** (fifth disease) (see Chapter 298). It is also the most clearly documented viral cause of RBC aplasia in patients with chronic hemolytic anemia or an immunocompromised state. This single-stranded virus is cytotoxic to marrow erythroid progenitor cells, interacting specifically by binding to the red cell P antigen. In addition to decreased or absent erythroid precursors, characteristic nuclear inclusions in erythroblasts and giant pronormoblasts may be seen under light microscopy in bone marrow specimens. The virus does not cause significant anemia in immunocompetent individuals with a normal RBC life span.

Because parvovirus infection usually lasts less than 2 weeks, anemia may not develop or be appreciated in otherwise normal children whose peripheral RBC life span is 100-120 days. The RBC life span is much shorter in patients with **chronic hemolysis** secondary to conditions such as hereditary spherocytosis, immune hemolytic anemia, or sickle cell disease. In these children, a brief cessation of erythropoiesis can cause severe anemia, known as **aplastic crisis**. When a definitive diagnosis is required, the workup should include

Table 499.1 Comparison of Diamond-Blackfan Anemia and Transient Erythroblastopenia of Childhood

FEATURE	DBA	TEC
Male:female	1:1	1:3
AGE AT DIAGNOSIS, MALE (MO)		
Mean	10	26
Median	2	23
Range	0-408	1-120
AGE AT DIAGNOSIS, FEMALE (MO)		
Mean	14	26
Median	3	23
Range	0-768	1-192
Males >1 yr	9%	82%
Females >1 yr	12%	80%
Etiology	Genetic	Acquired, possibly familial
Antecedent history	None	Viral illness
Physical examination abnormal (congenital anomalies present)	25%	0%
LABORATORY		
Hemoglobin (g/dL)	1.2-14.8	2.2-12.5
WBCs <5,000/μL	15%	20%
Platelets >400,000/μL	20%	45%
Adenosine deaminase	Increased	Normal
MCV increased at diagnosis	80%	5%
MCV increased during recovery	100%	90%
MCV increased in remission	100%	0%
HbF increased at diagnosis	100%	20%
HbF increased during recovery	100%	100%
HbF increased in remission	85%	0%
i Antigen increased	100%	20%
i Antigen increased during recovery	100%	60%
i Antigen increased in remission	90%	0%

DBA, Diamond-Blackfan anemia; HbF, fetal hemoglobin; MCV, mean cell volume; TEC, transient erythroblastopenia of childhood; WBC, white blood cell.

From Nathan DG, Orkin SH, Ginsburg D, et al., eds. *Nathan and Oski's Hematology of Infancy and Childhood.* 6th ed. Philadelphia: Saunders; 2003, p. 329. Adapted from Alter BP. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. *Hematology of Infancy and Childhood.* 3rd ed. Philadelphia: Saunders; 1987. p 159; and Link MP, Alter BP. Fetal erythropoiesis during recovery from transient erythroblastopenia of childhood (TEC). *Pediatr Res* 1981;15:1036–1039.

serum parvovirus IgM and IgG titers. In young infants, a polymerase chain reaction (PCR) assay should be used since serologic testing may be inaccurate. Recovery from moderate to severe anemia is usually spontaneous, heralded by the appearance of nucleated RBCs and subsequent reticulocytosis in the peripheral blood. Nonetheless the parvovirus PCR may remain positive for months after recovery. An RBC transfusion may be necessary if the anemia is associated with significant symptoms. Parvovirus-induced aplastic crisis usually occurs only once in children with chronic hemolysis. In families with more than one child with a hemolytic disorder, parents should be warned that a similar aplastic episode can occur in the other children if they have not been previously infected. During the episode of aplastic crisis, the child is potentially contagious and should be isolated from at-risk patients.

Persistent parvovirus infection may occur in children with congenital immunodeficiency diseases, lymphoproliferative disorders, those being treated with immunosuppressive agents, and those with HIV/AIDS, because these children may be unable to mount an adequate antibody response. The resultant pure RBC aplasia may be severe, and affected children may be thought to have TEC. This type of RBC aplasia differs from TEC in that there is no spontaneous recovery, and more than one transfusion is often needed. The diagnosis of parvovirus infection is made by PCR of peripheral blood or bone marrow DNA because the usual serologic responses, reflected by parvovirus serum IgM or IgG titers, are impaired in immunodeficient children. In chronically infected patients, the disease may be treated with high doses of intravenous immunoglobulin, which contains neutralizing antibody to parvovirus and is effective in the short term.

Parvovirus infection and destruction of erythroid precursors can also occur in utero. Such events are associated with increased rates of fetal loss in the first and second trimesters. Infants may be born with **hydrops fetalis** and anemia (see Chapter 138). The presence of persistent congenital parvovirus infection is detected by PCR of peripheral blood and/or bone marrow DNA because immunologic tolerance to the virus can prevent the usual development of specific antibodies.

OTHER RED CELL APLASIAS IN CHILDREN

Acquired red cell aplasia in adults is usually mediated by a chronic antibody and often associated with disorders such as chronic lymphocytic leukemia, lymphoma, thymoma, lymphoproliferative disorders, and systemic lupus erythematosus. This chronic antibody-mediated type of RBC aplasia, often responsive to immunosuppressive therapy, is quite rare in childhood. Cases of acquired pure RBC aplasia attributable to T-cell suppression have also been described.

Infections other than parvovirus, such as cytomegalovirus, Epstein-Barr virus, and human herpes virus-6, may cause pure RBC aplasia. Certain drugs, such as chloramphenicol, also can inhibit erythropoiesis in a dose-dependent manner. Reticulocytopenia, erythroid hypoplasia, and vacuolated pronormoblasts in the bone marrow are reversible effects of this drug. These effects are distinct from the idiosyncratic and rare development of severe aplastic anemia in chloramphenicol recipients. Acquired antibody-mediated (to erythropoietin) pure RBC aplasia is a rare complication in chronic kidney disease patients treated with erythropoietin. In addition to discontinuing erythropoiesis-stimulating agents, therapy and addressing anemia with red cell transfusions, further treatment may require immunosuppression and eventually renal transplantation.

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Chapter 500

Anemia of Chronic Disease and Renal Disease

500.1 Anemia of Chronic Disease

Courtney D. Thornburg

The anemia of chronic disease **(ACD)**, also referred to as **anemia of inflammation**, is found in conditions where there is ongoing immune activation. It occurs in a wide range of disorders, including infections, malignancies, chronic disease, autoimmunity, and graft-versus-host disease. A similar anemia is associated with chronic kidney disease. ACD is typically a mild to moderate normocytic, normochromic, hypoproliferative anemia associated with a decreased serum iron and low transferrin saturation.

ETIOLOGY

Decreased red cell life span, impaired erythropoiesis, and an increased uptake of iron in the reticuloendothelial system are important mechanisms contributing to ACD.

ACD-associated alterations in iron recycling are characterized by an accumulation of iron in reticuloendothelial macrophages despite low levels of serum iron. The diversion of iron from the circulation into the reticuloendothelial system results in *functional* iron deficiency, which causes the impaired heme synthesis and iron-restricted erythropoiesis that contribute to anemia. These alterations in iron metabolism have been attributed to inflammation-associated excess synthesis of hepcidin, a key regulatory protein that controls intestinal iron absorption and tissue distribution. Hepcidin, although mainly synthesized by hepatocytes, is expressed in other cells, including monocytes and macrophages. It functions by binding to and initiating the degradation of the iron exporter, ferroportin (Fig. 500.1).

CLINICAL MANIFESTATIONS

Although the important symptoms and signs associated with ACD are those of the underlying disease, the mild to moderate anemia can affect the patient's quality of life.

LABORATORY FINDINGS

Hemoglobin concentrations are generally 6-9 g/dL. The anemia is usually normochromic and normocytic, although some patients have modest hypochromia and microcytosis, particularly if there is concomitant iron deficiency. Absolute reticulocyte counts are normal or low, and leukocytosis is common. The serum iron level is low, without the increase in serum transferrin (the iron transport protein) that occurs in iron deficiency. This pattern of low serum iron and low-to-normal serum transferrin is a valuable diagnostic feature. *However, note that the serum ferritin level may be elevated secondary to inflammation*. Soluble transferrin receptor (sTfR) is a useful diagnostic test to distinguish ACD from iron-deficiency anemia (IDA) because sTfR levels are high in IDA and normal in ACD. A bone marrow biopsy typically shows normal cellularity with decreased or adequate red blood cell precursors; marrow hemosiderin may be increased and granulocytic hyperplasia may be present.

serum parvovirus IgM and IgG titers. In young infants, a polymerase chain reaction (PCR) assay should be used since serologic testing may be inaccurate. Recovery from moderate to severe anemia is usually spontaneous, heralded by the appearance of nucleated RBCs and subsequent reticulocytosis in the peripheral blood. Nonetheless the parvovirus PCR may remain positive for months after recovery. An RBC transfusion may be necessary if the anemia is associated with significant symptoms. Parvovirus-induced aplastic crisis usually occurs only once in children with chronic hemolysis. In families with more than one child with a hemolytic disorder, parents should be warned that a similar aplastic episode can occur in the other children if they have not been previously infected. During the episode of aplastic crisis, the child is potentially contagious and should be isolated from at-risk patients.

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Chapter 500

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Courtney D. Thornburg

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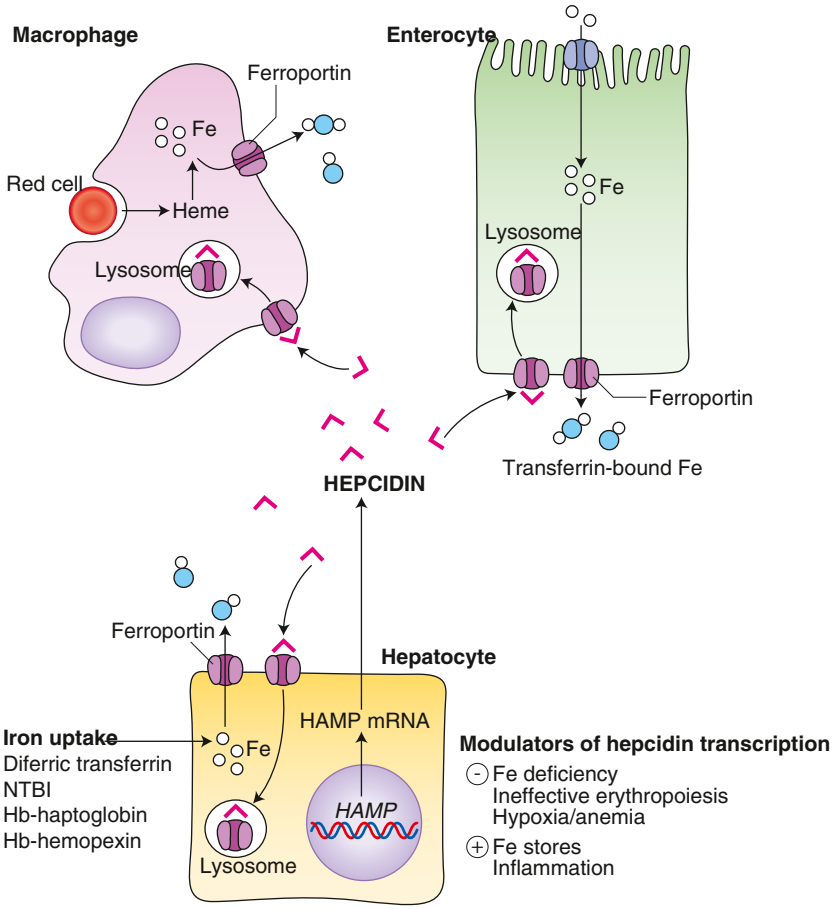

Fig. 500.1 Central role of hepcidin in iron metabolism. Hepcidin, produced by hepatocytes, downregulates iron export to circulating transferrin from iron "donor" cells (hepatocytes, macrophages, and duodenal enterocytes) by promoting the internalization and lysosomal degradation of ferroportin. Hepatocytes take up iron in several forms, whereas enterocytes obtain their iron predominantly from the gut lumen, and macrophages are specialized to deal with the high throughput of iron from senescent red cells. (*From Pippard M. Iron deficiency anemia, anemia of chronic disorders and iron overload. In Porwit A, McCullough J, Erber WN, eds. Blood and Bone Marrow Pathology, 2nd ed. London: Elsevier, 2011; Fig 11-5.*)

TREATMENT

Where possible, the best approach to ACD is the treatment of the underlying disorder. If the associated systemic disease can be controlled, the anemia typically improves or resolves. Transfusions raise the hemoglobin concentration temporarily but are rarely indicated. Erythropoiesis-stimulating agents (ESAs), such as recombinant human erythropoietin (EPO) or related extended half-life formulations, increase the hemoglobin level and improve activity and the sense of well-being. When using ESAs, *treatment with iron* is usually necessary to produce optimal effect. Response to these agents is highly variable, and poorly responsive patients may require high doses to reach target hemoglobin levels. In adults, high doses are associated with a higher incidence of adverse events, such as stroke, cardiovascular events, cancer progression, and death, leading the U.S. Food and Drug Administration (FDA) to require a "black box" warning on labels.

ACD does not respond to iron alone unless there is concomitant deficiency. Unfortunately, it is a common clinical challenge to identify iron deficiency in patients with an inflammatory disease (see Chapters 496 and 504). In this circumstance, a trial of iron therapy might be helpful, although there may be no response because persistent inflammation impairs iron absorption and utilization; intravenous iron may further increase hepcidin production.

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500.2 Anemia of Renal Disease

Courtney D. Thornburg

Anemia is common in children with chronic kidney disease (CKD). The anemia is usually normocytic, and the absolute reticulocyte count is normal or low. Although most patients with end-stage renal disease (ESRD) are anemic, earlier stages of CKD are also associated with a lower prevalence of anemia. In adults, a lower glomerular filtration rate (GFR) has been correlated with lower hemoglobin concentration, and hemoglobin has been reported to decline below a GFR threshold of 40-60 mL/min/1.73 m^2. In children with CKD, hemoglobin levels decline as the GFR decreases below 43 mL/min/1.73 m^2.

Decreased hemoglobin values are linked to increased incidence of left ventricular hypertrophy, impaired physical activity, and a reduced quality of life in pediatric patients with CKD. In those with ESRD on dialysis, anemia is also associated with increased risk of hospitalization and mortality.

ETIOLOGY

Although the anemia of CKD shares many features with anemia of chronic disease, its predominant cause is decreased erythropoietin (EPO) production by diseased kidneys. Other important causes include absolute and/or functional iron deficiency because of chronic blood loss (from blood sampling, surgeries, and dialysis) and disturbances in iron metabolism. Higher hepcidin levels have also been implicated in the anemia of CKD. Hepcidin is filtered by the glomerulus and excreted by the kidney; serum concentrations are increased in patients with decreased GFR. Inflammation may also be a contributing factor in pediatric dialysis patients who have elevated levels of proinflammatory cytokines. Hyperparathyroidism and deficiencies of vitamin B_{12}, folate, and carnitine may also have a role in anemia of CKD.

LABORATORY FINDINGS

Anemia in children with CKD is defined by age: hemoglobin <11.0 g/dL (0.5-5 years), <11.5 g/dL (5-12 years), <12 g/dL (12-15 years), <13.0 g/dL (males >15 years), and <12.0 g/dL (females >15 years). The anemia of CKD is hypoproliferative and usually normocytic and normochromic, unless there is concomitant iron deficiency or vitamin deficiency. The EPO level and absolute reticulocyte count are usually

low. White cell and platelet counts are generally normal. Ferritin will be low if there is accompanying iron deficiency and high if there is associated inflammation.

TREATMENT

Oral iron therapy is recommended for all pediatric CKD patients with anemia. Intravenous (IV) iron therapy may be considered for those receiving maintenance hemodialysis and those who to do not respond to oral iron. Current IV iron preparations (iron-gluconate, iron-sucrose, iron-carboxymaltose, iron-isomaltoside, ferumoxytol) have iron as a core within a carbohydrate stabilizer shell, thus preventing the uncontrolled release of free iron and thus reducing serious side effects.

Erythropoiesis-stimulating agents (ESAs) are the mainstay of therapy and, particularly for children with ESRD, have greatly reduced the need for frequent transfusions, decreasing the incidence of associated iron overload and alloimmunization.

Hemoglobin levels at which ESAs are initiated may be guided by individual patient characteristics, with a goal of 11-12 g/dL for children on maintenance ESA therapy. Dosing varies with age and dialysis modality. Darbepoetin, a synthetic form of EPO, appears to be equally effective as recombinant human EPO and has the benefit of less frequent dosing because of a longer half-life. Iron therapy should be prescribed when using ESAs because treatment demands additional iron for erythropoiesis. Infants and children require higher doses of ESAs.

In the rare case in which anti-EPO antibody-mediated pure red cell aplasia develops, ESA therapy should be stopped, and immunomodulatory therapy may be indicated to suppress the antibody response.

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Chapter 501

Congenital Dyserythropoietic Anemias

Courtney D. Thornburg

The congenital dyserythropoietic anemias **(CDAs)** are a heterogeneous class of inherited disorders resulting from abnormalities of late erythropoiesis. These rare conditions are characterized by variable degrees of anemia, ineffective erythropoiesis, hyperbilirubinemia, gallstones, splenomegaly, and secondary hemochromatosis (iron overload). They may be misdiagnosed as other congenital anemias, such as hereditary spherocytosis or thalassemia. **Dyserythropoiesis** is the major cause of anemia but a shortened half-life of circulating red blood cells (RBCs) may also contribute. The CDAs have been classified into four major types (I, II, III, and IV) and variants based on distinctive bone marrow morphology, clinical features, and genetic mutations. **Genetic testing** is now the key method for achieving a timely accurate diagnosis (Fig. 501.1).

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE I

Pathogenesis

Type I CDA **(CDA I)** is an autosomal recessive disorder. It is caused by pathogenic variants in *CDAN1,* the first gene to be implicated, which encodes Codanin-1, a cell-cycle regulated protein. Pathogenic variants in *CDIN1* (CDAN1-interacting nuclease 1, previously *C15orf41*) also cause CDA I. Proteins encoded by *CDAN1* and *CDIN1* likely contribute to DNA repair and/or chromatin reassembly after DNA replication.

Clinical Manifestations

CDA I may be diagnosed at any age, although most cases are recognized during childhood or adolescence in the setting of moderate to severe macrocytic anemia with relative reticulocytopenia. CDA I is rarely diagnosed in utero with severe anemia resulting in hydrops fetalis. Neonates with CDA 1 may have neonatal jaundice or pulmonary hypertension related to anemia. Type I CDA has been associated with **dysmorphic features** in 4–25% of patients, primarily involving the digits (syndactyly, absence of nails or curved toenails, supernumerary toes, skin pigmentation, café-au-lait spots, macrocephaly, dolichocephaly, spinal fusion, scoliosis, and short stature). Patients have progressive iron overload even in the absence of transfusions.

Laboratory Findings

Hemoglobin concentrations generally range between 7-11 g/dL. The anemia is usually macrocytic (mean corpuscular volume: 100-120 fL), but normocytic indices may be seen during childhood. **Anisopoikilocytosis** is appreciated on the peripheral blood smear. In some cases, normoblasts and basophilic stippling of RBCs may be seen. The reticulocyte count is inadequate for the degree of anemia **(reticulocytopenia)**. Elevated serum ferritin secondary to iron overload may be present. The bone marrow aspirate shows erythroid hyperplasia. Binucleate erythroblasts make up 2.5–10% of late erythroblasts. Incompletely divided cells with thin chromatin bridges between nuclei of pairs of erythrocytes are highly specific for type I CDA. Electron microscopy is the gold standard for clinical diagnosis, revealing erythroblasts with a characteristic "Swiss cheese" heterochromatin pattern.

Treatment

Treatment of this disorder includes transfusions, especially in the neonatal period and early childhood and in association with co-inherited disorders, such as thalassemia or RBC enzymopathy. However, adolescents and adults may require episodic transfusions only during aplastic crises, infection, or pregnancy. For patients with CDAN1 mutations, treatment with PEGylated interferon-α_2 can reduce transfusion requirements. Because of potential side effects of spastic diplegia and peripheral neuropathy, treatment initiation may be reserved for patients beyond early childhood requiring frequent transfusions. Patients do not respond to erythropoietin; splenectomy is generally not recommended. Cholecystectomy is often required for management of pigmented gallstones. Allogeneic stem cell transplantation may also be considered for severe cases.

The most important long-term complication is progressive **iron overload**, caused by increased intestinal absorption of iron and ineffective erythropoiesis and transfusion therapy. Regular phlebotomies is a treatment option as long as there is not significant anemia. If this approach is untenable, chelation therapy should be employed when repeated ferritin levels exceed 1,000 μg/L or liver iron is elevated as determined by hepatic magnetic resonance imaging T2*.

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II

Pathogenesis

CDA type II **(CDA II)**, the most common type of CDA, is an autosomal recessive disorder caused by biallelic pathogenic variants in *SEC23B*. This gene encodes a component of the cytoplasmic coat protein II (COPII) complex that is involved in endoplasmic reticulum vesicle trafficking.

Clinical Manifestations

CDA II presents with varying degrees of normocytic anemia and no or mild reticulocytosis. Approximately 20% of patients are transfusion dependent. CDA II may be initially misdiagnosed as hereditary spherocytosis due to overlapping symptoms of anemia, jaundice, splenomegaly, or hepatomegaly. Extramedullary hematopoiesis may result in

low. White cell and platelet counts are generally normal. Ferritin will be low if there is accompanying iron deficiency and high if there is associated inflammation.

TREATMENT

Oral iron therapy is recommended for all pediatric CKD patients with anemia. Intravenous (IV) iron therapy may be considered for those receiving maintenance hemodialysis and those who to do not respond to oral iron. Current IV iron preparations (iron-gluconate, iron-sucrose, iron-carboxymaltose, iron-isomaltoside, ferumoxytol) have iron as a core within a carbohydrate stabilizer shell, thus preventing the uncontrolled release of free iron and thus reducing serious side effects.

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Chapter 501

Congenital Dyserythropoietic Anemias

Courtney D. Thornburg

The congenital dyserythropoietic anemias **(CDAs)** are a heterogeneous class of inherited disorders resulting from abnormalities of late erythropoiesis. These rare conditions are characterized by variable degrees of anemia, ineffective erythropoiesis, hyperbilirubinemia, gallstones, splenomegaly, and secondary hemochromatosis (iron overload). They may be misdiagnosed as other congenital anemias, such as hereditary spherocytosis or thalassemia. **Dyserythropoiesis** is the major cause of anemia but a shortened half-life of circulating red blood cells (RBCs) may also contribute. The CDAs have been classified into four major types (I, II, III, and IV) and variants based on distinctive bone marrow morphology, clinical features, and genetic mutations. **Genetic testing** is now the key method for achieving a timely accurate diagnosis (Fig. 501.1).

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE I

Pathogenesis

Type I CDA **(CDA I)** is an autosomal recessive disorder. It is caused by pathogenic variants in *CDAN1,* the first gene to be implicated, which encodes Codanin-1, a cell-cycle regulated protein. Pathogenic variants in *CDIN1* (CDAN1-interacting nuclease 1, previously *C15orf41*) also cause CDA I. Proteins encoded by *CDAN1* and *CDIN1* likely contribute to DNA repair and/or chromatin reassembly after DNA replication.

Clinical Manifestations

CDA I may be diagnosed at any age, although most cases are recognized during childhood or adolescence in the setting of moderate to severe macrocytic anemia with relative reticulocytopenia. CDA I is rarely diagnosed in utero with severe anemia resulting in hydrops fetalis. Neonates with CDA 1 may have neonatal jaundice or pulmonary hypertension related to anemia. Type I CDA has been associated with **dysmorphic features** in 4–25% of patients, primarily involving the digits (syndactyly, absence of nails or curved toenails, supernumerary toes, skin pigmentation, café-au-lait spots, macrocephaly, dolichocephaly, spinal fusion, scoliosis, and short stature). Patients have progressive iron overload even in the absence of transfusions.

Laboratory Findings

Hemoglobin concentrations generally range between 7-11 g/dL. The anemia is usually macrocytic (mean corpuscular volume: 100-120 fL), but normocytic indices may be seen during childhood. **Anisopoikilocytosis** is appreciated on the peripheral blood smear. In some cases, normoblasts and basophilic stippling of RBCs may be seen. The reticulocyte count is inadequate for the degree of anemia **(reticulocytopenia)**. Elevated serum ferritin secondary to iron overload may be present. The bone marrow aspirate shows erythroid hyperplasia. Binucleate erythroblasts make up 2.5–10% of late erythroblasts. Incompletely divided cells with thin chromatin bridges between nuclei of pairs of erythrocytes are highly specific for type I CDA. Electron microscopy is the gold standard for clinical diagnosis, revealing erythroblasts with a characteristic "Swiss cheese" heterochromatin pattern.

Treatment

Treatment of this disorder includes transfusions, especially in the neonatal period and early childhood and in association with co-inherited disorders, such as thalassemia or RBC enzymopathy. However, adolescents and adults may require episodic transfusions only during aplastic crises, infection, or pregnancy. For patients with CDAN1 mutations, treatment with PEGylated interferon-α_2 can reduce transfusion requirements. Because of potential side effects of spastic diplegia and peripheral neuropathy, treatment initiation may be reserved for patients beyond early childhood requiring frequent transfusions. Patients do not respond to erythropoietin; splenectomy is generally not recommended. Cholecystectomy is often required for management of pigmented gallstones. Allogeneic stem cell transplantation may also be considered for severe cases.

The most important long-term complication is progressive **iron overload**, caused by increased intestinal absorption of iron and ineffective erythropoiesis and transfusion therapy. Regular phlebotomies is a treatment option as long as there is not significant anemia. If this approach is untenable, chelation therapy should be employed when repeated ferritin levels exceed 1,000 μg/L or liver iron is elevated as determined by hepatic magnetic resonance imaging T2*.

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II

Pathogenesis

CDA type II **(CDA II)**, the most common type of CDA, is an autosomal recessive disorder caused by biallelic pathogenic variants in *SEC23B*. This gene encodes a component of the cytoplasmic coat protein II (COPII) complex that is involved in endoplasmic reticulum vesicle trafficking.

Clinical Manifestations

CDA II presents with varying degrees of normocytic anemia and no or mild reticulocytosis. Approximately 20% of patients are transfusion dependent. CDA II may be initially misdiagnosed as hereditary spherocytosis due to overlapping symptoms of anemia, jaundice, splenomegaly, or hepatomegaly. Extramedullary hematopoiesis may result in

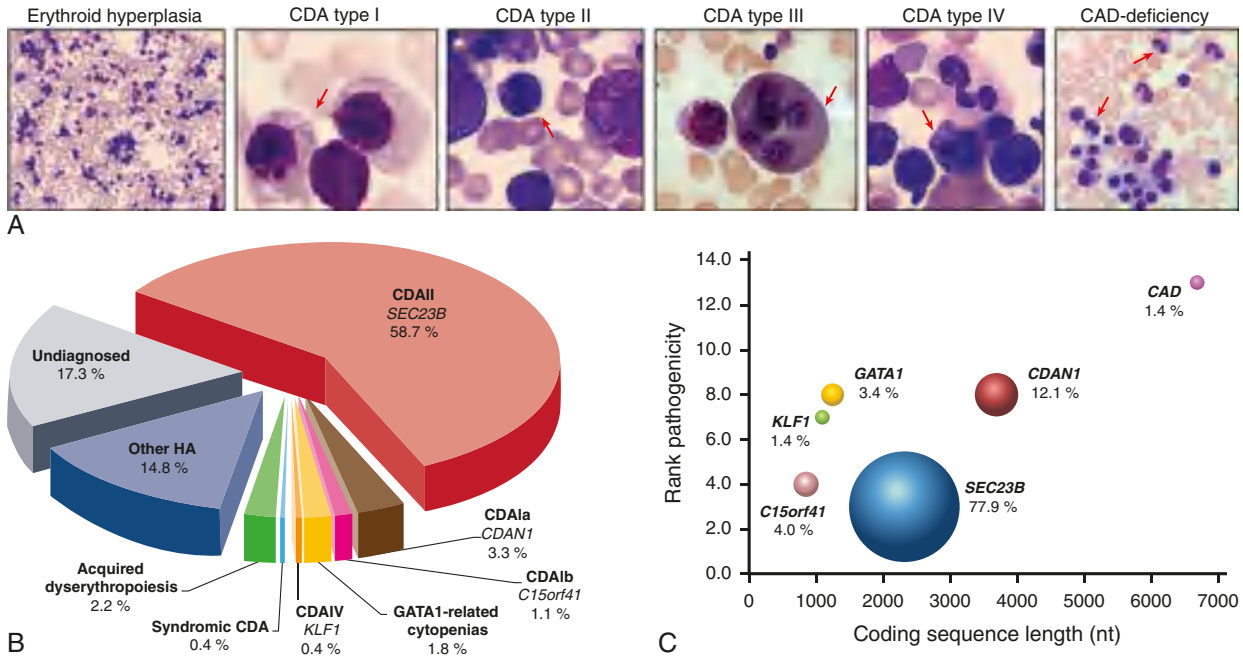

Fig. 501.1 Morphologic and molecular features of patients with congenital dyserythropoietic anemias (CDA). **A,** Light microscopy analysis of the bone marrow from patients with different CDA subtypes. CDA patients generally show erythroid hyperplasia. *Red arrows* indicate typical findings for each CDA subtype: CDAI, internuclear chromatin bridging; CDAII, binucleate erythroid precursors; CDAIII, giant multinucleated erythroblasts; CDAIV, multinucleate erythroblasts; and CAD deficiency, binucleate CDAII-like precursors. **B,** Pie chart showing the frequencies of the different CDA subtypes diagnosed after genetic testing in patients clinically suspected of having CDA. The frequency of each condition was calculated as the ratio between the number of patients in each CDA subtype and the overall count of patients tested (n = 218 patients [those included in our international registry of CDAs from 1995 to 2019]). Six patients originally suspected of CDA showed conclusive diagnosis of acquired dyserythropoiesis: two patients with liver failure, two with iron-deficiency anemia, one with erythrophagocytosis, and one with transient erythroblastopenia. Syndromic CDA refers to one patient with a mutation in the CAD gene. GATA1-related cytopenias include: X-linked thrombocytopenia with or without dyserythropoietic anemia; congenital erythropoietic porphyria; and idiopathic cytopenias of undetermined significance. Other hereditary anemias (HA) include: hereditary spherocytosis; hereditary dehydrated stomatocytosis; red cell enzymatic defects; and sideroblastic anemia. The undiagnosed cases were evaluated by analysis of the CDA gene panel, by extended targeted next-generation sequencing for hereditary anemias, or by whole-exome sequencing. **C,** Bubble chart defining the lengths of the coding sequences of each CDA-causative gene and their relative pathogenicity scores. These scores were calculated by combining the constraint metrics of each gene available at the ExAC database (http://exac.broadinstitute.org/). High pathogenicity scores identify increased constraints (intolerance to variation). The more intolerant to variation a gene is, the less likely it is to be mutated. The size of each bubble represents the frequency of the mutations in each gene, as calculated by the ratio of the number of mutated alleles for each gene and the overall count of disease alleles (n = 149, from 78 patients included in our international registry of CDAs from 2008 to 2019). (*From Iolascon A, Andolfo I, Russo R. Congenital dyserythropoietic anemias. Blood. 2020;136:1274–1281; Fig. 1.*)

posterior mediastinal or paravertebral masses. Iron overload occurs in both transfusion dependent and independent patients.

Laboratory Findings

The anemia is normocytic and is generally mild with inappropriately low reticulocytes. Hemoglobin levels are lower in children than adults and range from 8-11 g/dL. The peripheral blood smear shows anisopoikilocytosis, occasional basophilic stippling, as well as a few, sometimes binucleate, mature erythroblasts. The bone marrow aspirate is normoblastic but hypercellular, with erythroid hyperplasia. Binucleated and multinucleated erythroblasts with equal nuclei size (10–35%) and karyorrhexis (fragmentation of the nuclei) in >2% are characteristic. Electron micrographs demonstrate vesicles laden with endoplasmic reticulum proteins running beneath the plasma membrane (double membranes). Ninety-five percent of patients with CDA II have hypoglycosylated band 3, which has faster migration on the gel, and the diagnosis may be made by analyzing red blood cell membrane proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE).

Treatment

Most patients can lead a normal life and have a normal life expectancy if complications and consequences are managed appropriately. Approximately 10% of patients will require red cell transfusions in infancy and childhood but rarely during adulthood. Splenectomy may provide hematologic improvement and is currently recommended for patients with severe anemia and/or symptomatic splenomegaly. Splenectomy does not prevent further iron overloading, even in those patients whose hemoglobin is normalized, presumably because of persistent ineffective erythropoiesis in the bone marrow. Secondary iron overload is the most prominent long-term complication and should be approached as previously outlined.

Allogeneic stem cell transplantation may also be considered for severe cases.

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE III

CDA type III **(CDA III)** is an extremely rare, poorly defined entity characterized by mild to moderate macrocytic anemia. It is inherited in an autosomal dominant or de novo fashion, although there have been cases that might represent other inheritance patterns. CDA III is caused by pathogenic variants in *KIF23*, which encodes a ubiquitous protein, mitotic kinesin-like protein 1, which regulates daughter cell separation during mitosis. Iron overload is not clinically significant (likely due to hemolysis being predominantly intravascular), and spleen size is generally normal. Patients can present with angioid streaks with macular degeneration. An association with monoclonal gammopathy and multiple myeloma is also reported. The blood smear shows macrocytes, anisopoikilocytosis, and occasional basophilic stippling. The bone marrow is notable for giant erythroid precursors that are often multinucleated, containing up to 12 nuclei per cell. Such multinucleated erythroblasts can also be seen in myelodysplasia and erythroleukemia. Transfusions are usually not required.

TRANSCRIPTION FACTOR–RELATED CDA

Transcription factor–related CDA includes CDA type IV (CDA IV) and X-linked thrombocytopenia with or without dyserythropoietic anemia (XLTDA). CDA IV has an autosomal dominant inheritance due to pathogenic variants in *KLF1*, which encodes an erythroid transcription factor that regulates fetal hemoglobin switching and is necessary for terminal erythroid differentiation. Patients have severe hemolytic anemia with no or mild reticulocytosis and very high fetal hemoglobin. Bone marrow morphology consists of hypercellularity and binucleate or multinucleate erythroblasts. Electron micrographs show immature red cell progenitors that have atypical inclusions within the cytoplasm, nuclear membrane invaginations, and heterochromatin.

XLTDA is an X-linked condition due to pathogenic variants in *GATA1*, a DNA-binding protein with two zinc fingers and a transactivation domain that plays a key role in development and maintenance of red cell and platelet lineages. The laboratory features of this condition include mild to severe anemia and macrothrombocytopenia with poorly granulated platelets. Bone marrow morphology shows dyserythropoiesis and megakaryocytes that are abnormal and reduced in number. Patients present with bleeding and anemia, and management includes transfusion and supportive care.

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Chapter 502

Physiologic Anemia of Infancy

Courtney D. Thornburg

At birth, normal full-term infants have higher hemoglobin (Hb) levels and larger red blood cells (RBCs) than do older children and adults. However, within the first week of life, a progressive decline in Hb level begins and then persists for 6-8 weeks. The resulting anemia is known as the **physiologic anemia of infancy**.

With the onset of respiration at birth, considerably more oxygen becomes available for binding to Hb, and as a result the Hb-oxygen saturation increases from 50–95% or more. There is also a gradual, normal developmental switch from fetal to adult Hb synthesis after birth that results in the replacement of high-oxygen-affinity fetal Hb with lower-affinity adult Hb, capable of delivering more oxygen to tissues. The increase in blood oxygen content and delivery results in the downregulation of erythropoietin (EPO) production, leading to suppression of erythropoiesis. Because there is no erythropoiesis, aged RBCs that are removed from the circulation are not replaced, and the Hb level decreases. The Hb concentration continues to decline until tissue oxygen needs become greater than oxygen delivery. Normally, this point is reached between 8-12 weeks of age, when the Hb concentration is about 11 g/dL. In healthy term infants, the nadir rarely falls below 10 g/dL. At this juncture, EPO production increases and erythropoiesis resumes. The supply of stored reticuloendothelial iron, derived from previously degraded RBCs, remains sufficient for this renewed Hb synthesis, even in the absence of dietary iron intake, until approximately 20 weeks of age. In all, this "anemia" should be viewed as a physiologic adaptation to extrauterine life, reflecting the excess oxygen delivery relative to tissue oxygen requirements. There is no hematologic problem, and no therapy is required unless physiologic anemia of infancy is exacerbated by other ongoing processes including nutritional deficiency and blood loss. The degree of anemia at birth is correlated with maternal hemoglobin.

A late **hyporegenerative anemia**, with reticulocytopenia, can occur in infants with **hemolytic disease of the newborn**. The persistence of maternally derived anti-RBC antibodies in the infant's circulation can lead to an ongoing low-grade hemolytic anemia that can exaggerate the physiologic anemia. Lower-than-expected Hb at the "physiologic" nadir has also been seen in infants after intrauterine or neonatal RBC transfusions. When infants are transfused with adult blood containing HbA, the associated shift of the oxygen dissociation curve facilitates oxygen delivery to the tissues. Accordingly, the definition of anemia and the need for transfusion should be based not only on the infant's Hb level, but also on oxygen requirements and the ability of circulating RBCs to release oxygen to the tissues.

Premature infants also develop a physiologic anemia, known as **anemia of prematurity**. The Hb decline is both more extreme and more rapid. Hb levels of 7-9 g/dL usually are reached by 3-6 weeks of age, and levels may be even lower in very small premature infants (see Chapter 139). The same physiologic factors at play in term infants are exaggerated in preterm infants. In premature infants, the physiologic Hb decline may be intensified by blood loss from repeated phlebotomies obtained to monitor ill neonates. Demands on erythropoiesis are further heightened by the premature infant's presumed shortened RBC life span (40-60 days) and the accelerated expansion of RBC mass that accompanies the premature baby's rapid rate of growth. Nonetheless, plasma EPO levels are lower than would be expected for the degree of anemia, resulting in a suboptimal erythropoietic response. The reason for diminished EPO levels is not fully understood. During fetal life, EPO synthesis is handled primarily by the liver, and the liver's oxygen sensor is less sensitive to hypoxia compared with that of the kidney. The developmental switch from liver to kidney EPO production is not accelerated by early birth, and thus the preterm infant must rely on the liver as the primary site for synthesis, leading to diminished responsiveness to anemia. An additional mechanism thought to contribute to diminished EPO levels may be accelerated EPO metabolism. Because the pronounced decline in Hb that occurs in many very low birthweight infants may be associated with abnormal clinical signs, this "anemia of prematurity" is not considered benign and usually requires transfusions when symptomatic.

TREATMENT

In the **full-term infant**, physiologic anemia requires no therapy beyond ensuring that the infant's diet contains essential nutrients for normal hematopoiesis. For infants who are exclusively breastfed, iron supplementation is indicated beginning at 4 months of age and continued until oral iron intake via food is sufficient. In **premature infants**, an optimal Hb has not been established and is usually dictated by the infant's overall clinical condition. Transfusions may be needed to maintain the Hb at what is considered safe for that child. Premature infants who are feeding well and growing normally rarely need transfusion unless iatrogenic blood loss has been significant. Although factors such as poor weight gain, respiratory difficulties, and abnormal heart rate have prompted transfusion, the beneficial effect has not been documented. Laboratory tests such as blood lactate, EPO, and mixed venous oxygen saturation have poor predictive value. A restrictive strategy does not increase infant morbidity or mortality. Long-term neurodevelopmental outcomes have been found to be poorer in liberally transfused neonates. Exposure to packed RBCs may be related to the development of necrotizing enterocolitis, and early transfusions may be associated with the risk of intraventricular hemorrhage. Iron supplementation is indicated starting at 2 to 6 weeks of age until 6 to 12 months of age; dosing depends on birth weight and infants with smaller birth weights require higher doses.

Strategies to decrease anemia include delayed cord clamping, avoiding unnecessary phlebotomy, and providing adequate iron supplementation. Delayed cord clamping at birth results in fewer transfusions and a reduction in both intraventricular hemorrhage and necrotizing enterocolitis in preterm infants. Given the impact of phlebotomy losses during monitoring in the neonatal intensive care unit, attention to reducing unnecessary blood draws also has been advocated. Iron therapy is indicated for all neonates with anemia of prematurity starting at 1 month of age and continuing until about 1 year of age. Iron is prescribed as mg of elemental iron per kg/day and depends on birthweight.

When transfusions are necessary, an RBC volume of 10-15 mL/kg is recommended. It is good practice to split units derived from a single donor to minimize donor exposure if sequential transfusions are indicated.

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Chapter 503

Megaloblastic Anemias

Courtney D. Thornburg

Megaloblastic anemia describes a group of disorders that are caused by impaired DNA synthesis. Red blood cells (RBCs) are larger than normal at every developmental stage, and there is maturational asynchrony between the nucleus and cytoplasm of erythrocytes. The delayed nuclear development becomes increasingly evident as cell divisions proceed. Myeloid and platelet precursors are also affected, and giant metamyelocytes and neutrophil bands are often present in the bone marrow. *There is often an associated thrombocytopenia and leukopenia*. The peripheral blood smear is notable for large, often oval RBCs with increased mean corpuscular volume (Fig. 503.1). Neutrophils are characteristically hypersegmented, with many having more than five lobes (see Fig. 503.1). Most cases of childhood megaloblastic anemia result from a deficiency of folic acid or vitamin B_{12} (cobalamin), vitamins essential for DNA synthesis (Table 503.1). Rarely, these anemias may be caused by inborn errors of metabolism. Megaloblastic anemias resulting from malnutrition are relatively uncommon in the United States but are important worldwide (see Chapters 62, 64, and 496).

503.1 Folic Acid Deficiency

Courtney D. Thornburg

Folates are essential for DNA replication and cellular proliferation. Humans cannot synthesize folate and must depend on dietary sources, including green vegetables, fruits, and animal organs (e.g., liver, kidney). Folates are heat labile and water soluble; consequently, boiling or heating folate sources leads to decreased amounts of the vitamin. Naturally occurring folates are in a polyglutamated form that is less efficiently absorbed than the monoglutamate species (i.e., folic acid). Dietary folate polyglutamates are hydrolyzed to simple folates that are absorbed primarily in the proximal small intestine by a specific carrier-mediated system. Folates travel in the bloodstream and are taken up in cells, primarily in the form of unconjugated methyltetrahydrofolate, which is subsequently reconjugated (polyglutamated) in the cell. There is an active enterohepatic circulation. Although rare, megaloblastic anemia because of folate deficiency has its peak incidence at 4-7 months of age, somewhat earlier than iron-deficiency anemia, although both conditions may be present concomitantly in infants with poor nutrition.

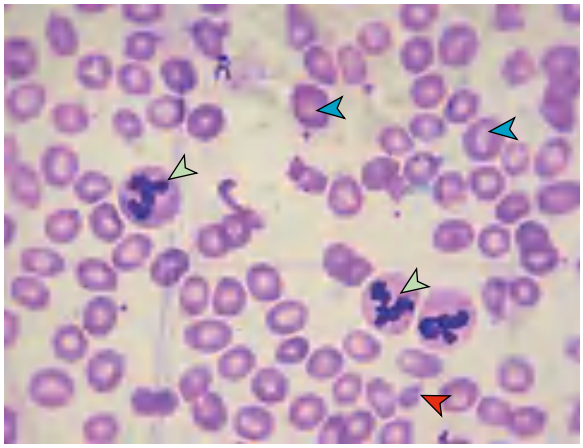

Fig. 503.1 Peripheral blood film shows hypersegmented neutrophils *(green arrowheads)*, macrocytes *(blue arrowheads)*, and a teardrop-shaped red blood cell *(red arrowhead)*. *(From Bawaskar HS, Bawaskar P, Bawaskar PH, Parekh PB. Tropical megaloblastic anemia. Lancet 2019;393:2261–2262.)*

ETIOLOGY

Folic acid deficiency can result from inadequate folate intake, decreased folate absorption, or acquired and congenital disorders of folate metabolism or transport (Table 503.2).

Inadequate Folate Intake

In the United States, anemia caused by insufficient folate intake usually occurs in the context of increased vitamin requirements associated with pregnancy, periods of accelerated growth, and chronic hemolysis (see Chapter 67.6). Folate requirements greatly increase during pregnancy, in part to meet fetal needs, and deficiencies are common in mothers, particularly those who are poor or malnourished. Folate supplementation is recommended from the start of pregnancy to prevent neural tube defects and to meet the needs of the developing fetus. Folate-deficient mothers generally do not give birth to infants with clinical folate deficiency because there is selective transfer of folate

Table 503.1 Causes of Red Blood Cell Macrocytosis

CAUSATIVE CONDITIONS	ACCOMPANYING HEMATOLOGIC FEATURES
MEGALOBLASTIC ANEMIA	
Cobalamin (vitamin B_{12}) deficiency	Megaloblastic changes, including hypersegmented neutrophils
Folate deficiency	Macrocytosis can become severe
Antifolate drugs (e.g., methotrexate)	Mild reticulocytopenia
Cytotoxic drugs (e.g., hydroxyurea, 5-FU)	Pancytopenia (when the megaloblastic process is severe)
Immunosuppressive drugs (e.g., azathioprine)	
Thiamine-responsive anemia	
Hereditary orotic aciduria	
DISORDERS OF ERYTHROID PRODUCTION	
Aplastic anemia, PRCA, Blackfan-Diamond anemia	Nonmegaloblastic
Some sideroblastic anemias	Some disorders feature dyserythropoiesis and sometimes hyposegmented neutrophils
CDA, non-CDA dyserythropoiesis, Fanconi anemia	
Myelodysplasia, myeloproliferative diseases	Macrocytosis can often be severe (e.g., aplastic processes)
	Reticulocytopenia (often severe)
RETICULOCYTOSIS	
Chronic hemolytic anemia	Nonmegaloblastic; no hypersegmented neutrophils
DRUGS AND TOXINS	
Alcohol abuse	Mechanism of macrocytosis is often unknown
Some antiviral drugs (e.g., nucleoside RT inhibitors)	No hypersegmented neutrophils
Some anticonvulsant drugs	
NONHEMATOLOGIC DISEASES	
Chronic liver diseases	Nonmegaloblastic; no hypersegmented neutrophils
Hypothyroidism	
Copper deficiency	Macrocytes are rarely oval
ARTIFACTS	
RBC clumping by cold agglutinins; some warm RBC antibodies	Nonmegaloblastic; no hypersegmented neutrophils
Severe hyperglycemia	Disparity between high MCV and normal morphologic examination
Hyponatremia	

CDA, Congenital dyserythropoietic anemia; MCV, mean corpuscular volume; PRCA, pure red cell aplasia; RBC, red blood cell; RT, reverse transcriptase; 5-FU, 5-fluorouracil.
From Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015; Table 10-3.

Table 503.2 Causes of Folate Deficiency* in Adults and Children

INADEQUATE NUTRITION
Poor diet†
Poor food preparation methods
Exclusive feeding with goat's milk
DEFECTS IN ABSORPTION
Gastric achlorhydria§
Diseases of the upper small intestine
Tropical sprue
Celiac disease
Dermatitis herpetiformis
Inflammatory bowel disease
Oral pancreatic replacement therapy§
Hereditary folate malabsorption
INCREASED REQUIREMENTS OR LOSSES
Pregnancy
Lactation§
Prematurity§,‖
Chronic hemolytic anemia§
Dialysis
Hyperthyroidism§
Lesch-Nyhan syndrome
DISORDERS OF TRANSPORT
Cerebral folate deficiency (genetic or acquired)¶
DISORDERS OF CELLULAR METABOLISM
Drugs inhibiting folate metabolism
Antifolates (e.g., methotrexate)
Pyrimethamine§; trimethoprim§
Sulfasalazine§
Valproic acid§,#
Inherited defects
Methylenetetrahydrofolate reductase (MTHFR) deficiency
Disorders of intracellular cobalamin metabolism
Dihydrofolate reductase deficiency
Methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1 (MTHFD1) deficiency
Others
MULTIFACTORIAL OR UNCERTAIN MECHANISMS
Alcohol use disorder‡
Anticonvulsants§
Oral contraceptives§

*Folate deficiency is often multifactorial.
†Relative dietary insufficiency (i.e., intake that is adequate under usual circumstances) is a particularly important cofactor that can convert borderline folate deficiency to clinically overt deficiency when other conditions coexist, such as increased requirements for folate or mild malabsorption.
§Megaloblastic anemia rarely results unless other limitations of folate status coexist.
‖Neonatal stores are low in premature infants.
¶Cerebral folate deficiency can occur on a genetic basis or an autoimmune basis; megaloblastic anemia does not occur because folate deficiency appears not to exist outside the central nervous system.
#Disrupts mitochondrial folate metabolism in utero.
‡Poor intake is often associated with alcoholism in adults.
From Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015; Box 10-2.

to the fetus via placental folate receptors. Rapid growth after birth increases demands for folic acid, and infants who are premature or ill and those with certain hemolytic disorders will have particularly high folate requirements. Human breast milk, infant formulas, and pasteurized cow's milk provide adequate amounts of folic acid. *Goat's milk is folate deficient, and supplementation must be given when it is the child's main food source.* Unless supplemented, powdered milk may also be a poor source of folic acid.

Malnutrition is the most common cause of folate deficiency in older children. In addition, children with chronic hemolytic anemias, infections, or malabsorption are at increased risk due to increased demand. Because body stores of folate are limited, deficiency can develop quickly in malnourished individuals. On a folate-free diet, megaloblastic anemia will occur after 2-3 months.

Decreased Folate Absorption

Malabsorption caused by chronic diarrheal states or diffuse inflammatory disease can lead to folate deficiency. In both situations, some of the decreased folate absorption may be caused by impaired folate conjugase activity. Chronic diarrhea also interferes with the enterohepatic circulation of folate, thereby enhancing folate losses because of rapid intestinal passage. Megaloblastic anemia caused by folic acid deficiency can occur in celiac disease or chronic infectious enteritis and in association with enteroenteric fistulas. Previous intestinal surgery is another potential cause of decreased folate absorption.

Certain anticonvulsant drugs (e.g., phenytoin, phenobarbital) can impair folic acid absorption, and many patients treated with these drugs have low serum levels. Frank megaloblastic anemia is rare and readily responds to folic acid therapy, even when administration of the offending drug is continued. Alcohol overuse also is associated with folate malabsorption.

Congenital Abnormalities in Folate Transport and Metabolism

Inborn errors of folate transport or metabolism are rare but can be life threatening. Those associated with megaloblastic anemia include hereditary folate malabsorption and certain extremely uncommon enzyme deficiencies.

Hereditary folate malabsorption (HFM) is an autosomal recessive disorder caused by loss-of-function pathogenic variants in the *SLC46A1* gene encoding the protein-coupled folate transporter. HFM is associated with an inability to absorb folic acid, 5-tetrahydrofolate, 5-methyltetrahydrofolate, or 5-formyltetrahydrofolate (folinic acid). It can become apparent at 2-6 months of age with megaloblastic anemia and other deficits, including infections and diarrhea. Neurologic abnormalities attributable to folate deficiency in the central nervous system (CNS) include seizures, developmental delay, and intellectual disability. Folate transport is impaired both in the intestine and at the brain's choroid plexus. Serum and cerebrospinal fluid (CSF) folate levels are very low, with a loss of the normal 3:1 ratio of CSF to serum folate.

Treatment for HFM involves parenteral (intramuscular) folate or high-dose oral 5-formyltetrahydrofolate (5-formylTHF), folinic acid, leucovorin, or the active isomer of 5-formylTHF (Isovorin or Fusilev) targeting normal or near normal trough levels in the CSF to ameliorate both the megaloblastic anemia and neurologic complications of folate deficiency.

Functional methionine synthase deficiency may result from pathologic genetic variants affecting the function of methionine synthase reductase or methionine synthase. These disorders are autosomal recessive and characterized not only by megaloblastic anemia but also by cerebral atrophy, nystagmus, blindness, and altered muscle tone. Both respond to hydroxocobalamin (OHCbl) plus betaine with variable clinical success. **Dihydrofolate reductase (DHFR) deficiency** is extremely rare and is associated with homozygous pathologic genetic variants in the *DHFR* gene. Clinical symptoms include megaloblastic anemia and neurologic manifestations. Although **methylenetetrahydrofolate reductase (MTHFR) deficiency** is the most common inborn error of folate metabolism, and severe cases can produce several neurologic and vascular complications, there is *no* associated megaloblastic anemia.

Drug-Induced Abnormalities in Folate Metabolism

Several drugs have anti–folic acid activity as their primary pharmacologic effect and regularly produce megaloblastic anemia. *Methotrexate* binds to dihydrofolate reductase and prevents formation of tetrahydrofolate, the active form of folate. *Pyrimethamine,* used in the therapy of toxoplasmosis, and *trimethoprim,* used for treatment of various infections, can induce folic acid deficiency and occasionally megaloblastic anemia. Therapy with folinic acid (5-formyltetrahydrofolate) is usually beneficial.

CLINICAL MANIFESTATIONS

In addition to the clinical features associated with anemia, folate-deficient infants and children may manifest irritability, chronic diarrhea, and poor weight gain. Hemorrhages from thrombocytopenia may occur in advanced cases. HFM and other rare etiologies of folate deficiency may be further associated with hypogammaglobulinemia, severe infections, failure to thrive, neurologic abnormalities, and cognitive delays.

LABORATORY FINDINGS

The anemia is macrocytic (mean corpuscular volume >100 fL). Variations in RBC shape and size are common (see Chapter 496, Fig. 496.4B). The reticulocyte count is low, and nucleated RBCs with megaloblastic morphology are often seen in the peripheral blood. Neutropenia and thrombocytopenia may be present, particularly in patients with long-standing and severe deficiencies. The neutrophils are large, some with *hypersegmented nuclei* (see Fig. 503.1). The bone marrow is hypercellular because of erythroid hyperplasia, and megaloblastic changes are prominent. Large, abnormal neutrophilic forms (giant metamyelocytes) with cytoplasmic vacuolation are also seen.

Levels of RBC folate are a better indicator of chronic deficiency than serum folic acid levels. The normal RBC folate level is 150-600 ng/mL of packed cells. Levels of iron and vitamin B_{12} in serum usually are normal or elevated. Serum activity of lactate dehydrogenase, a marker of ineffective erythropoiesis, is markedly elevated.

TREATMENT

When the diagnosis of folate deficiency is established, folic acid may be administered orally or parenterally at 0.5-1.0 mg/day. If the specific diagnosis is in doubt, smaller doses of folate (0.1 mg/day) may be used for 1 week as a diagnostic test because a hematologic response can be expected within 72 hours. Doses of folate >0.1 mg can correct the anemia of vitamin B_{12} deficiency but might aggravate any associated neurologic abnormalities. In most medical settings in developed countries, this therapeutic trial to distinguish the different causes of megaloblastic anemia is rarely necessary because vitamin B_{12} and folate blood levels are usually readily available. Folic acid therapy (0.5-1.0 mg/day) should be continued for 3-4 weeks until a definite hematologic response has occurred. Maintenance therapy with a multivitamin (containing 0.2 mg of folate) is adequate. Parenteral or high doses of specific folate formulations are required in the setting of HFM. Transfusions are indicated only when the anemia is severe, or the child is very ill.

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503.2 Vitamin B_{12} (Cobalamin) Deficiency

Courtney D. Thornburg

Vitamin B_{12}, a generic term encompassing all biologically active cobalamins, is a water-soluble vitamin with a central, functional cobalt atom and a planar corrin ring. Methylcobalamin and adenosyl cobalamin are the metabolically active derivatives, serving as cofactors in two essential metabolic reactions: methylation of homocysteine to methionine (via methionine synthase) and conversion of methyl-malonyl-coenzyme A (CoA) to succinyl CoA (via L-methyl-malonyl-CoA mutase). The products and by-products of these enzymatic reactions are critical to DNA, RNA, and protein synthesis.

Cobalamin (Cbl) is synthesized *exclusively* by microorganisms, and humans must rely on dietary sources (animal products, including meat, eggs, fish, and milk) for their needs (see Chapter 67.7). Unlike folate, older children and adults have sufficient vitamin B_{12} stores to last 3-5 years. In young infants born to mothers with low vitamin B_{12} stores, clinical signs of Cbl deficiency can become apparent in the first 6-18 months of life.

METABOLISM

Under normal circumstances, cobalamin is released from food protein in the stomach through peptic digestion. Cbl then binds to *haptocorrin* (HC), a salivary glycoprotein. This complex moves into the duodenum, where HC is digested by pancreatic proteases and Cbl is liberated. Cbl then binds to *intrinsic factor* (IF), another glycoprotein that is produced by gastric parietal cells. The Cbl-IF complex subsequently enters mucosal cells of the distal ileum by receptor-mediated endocytosis. Through a complex series of protein interactions, Cbl is taken up into the blood stream and taken up into cells. Cobalamins are processed in the cytoplasm to a common intermediate that can be allocated to the methylcobalamin and adenosylcobalamin synthesis pathways to meet cellular needs.

ETIOLOGY

Vitamin B_{12} deficiency can result from inadequate dietary intake of Cbl, lack of IF, impaired intestinal absorption of IF-Cbl, inactivation, or absence of vitamin B_{12} transport protein (see Table 503.1).

Inadequate Vitamin B_{12} Intake

Vitamin B_{12} deficiency in infants is most often nutritional, resulting from low Cbl levels in the breast milk of B_{12}-deficient mothers. Associated **megaloblastic anemia** often appears during the first year of life. Maternal deficiency may be caused by **pernicious anemia** or gastrointestinal disorders such as *Helicobacter pylori* infection, celiac disease, Crohn disease, or pancreatic insufficiency. Previous gastric bypass surgery, treatment with proton pump inhibitors, and inadequate intake from a strict unsupplemented vegetarian diet have also been implicated. Fortunately, because of active placental Cbl transport in utero, most children of B_{12}-deficient mothers maintain Cbl levels sufficient to support adequate prenatal development. Such infants are born with low B_{12} stores, the depletion of which is associated with a gradual onset of clinical manifestations. *Vitamin B_{12} replacement often results in rapid improvement, but the longer the deficient period, the greater the likelihood of permanent disabilities.* Neonatal screening programs may detect maternal-neonatal nutritional B_{12} deficiency because of an increase in propionyl carnitine, but there is higher sensitivity using a measurement of methylmalonic acid. In high-income countries, dietary deficiency during childhood or adolescence is infrequent but can result from strict vegetarian or vegan diet. Daily requirements range from 0.4-2.4 μg.

Impaired Vitamin B_{12} Absorption

Gastric surgery or medications that impair gastric acid secretion may result in IF deficiency, leading to decreased vitamin B_{12} absorption. Pancreatic insufficiency can also lead to Cbl deficiency because of impaired cleavage and IF complex formation. Patients with neonatal necrotizing enterocolitis, inflammatory bowel disease, celiac disease, or surgical removal of the terminal ileum may also have impaired absorption of vitamin B_{12}. An overgrowth of intestinal bacteria within diverticula or duplications of the small intestine can cause vitamin B_{12} deficiency by consumption of (or competition for) the vitamin or by splitting of its complex with IF. In these cases, *hematologic response can follow appropriate antibiotic therapy.* In endemic areas, when the fish tapeworm *Diphyllobothrium latum* infests the upper small intestine, similar mechanisms may be operative. When megaloblastic anemia occurs in such situations, the serum vitamin B_{12} level is low, and the gastric fluid contains IF.

Hereditary intrinsic factor deficiency (HIFD) is a rare autosomal recessive disorder caused by a variety of pathologic genetic variants in the *IF* gene that produce a lack of gastric IF or a functionally abnormal IF. HIFD differs from typical adult pernicious anemia in that gastric acid is secreted normally and the stomach is histologically normal. It is not associated with antibodies or endocrine abnormalities. Unlike Imerslund-Grasbeck syndrome, described next, HIFD is only occasionally associated with proteinuria. Symptoms become prominent at an early age (6-24 months), consistent with exhaustion of vitamin B_{12} stores acquired in utero. As the anemia becomes severe, weakness, irritability, anorexia, and listlessness occur. The tongue is smooth, red, and painful. Neurologic manifestations include ataxia, paresthesias, hyporeflexia, Babinski responses, and clonus. Oral vitamin B_{12} is usually *ineffective*, and lifelong intramuscular (IM) or intranasal Cbl should be used to bypass the absorption defect. The natural form, OHCbl, is believed to be more effective than the synthetic form, cyanocobalamin (CNCbl).

Imerslund-Grasbeck syndrome is a rare, recessively inherited pediatric disorder resulting in selective vitamin B_{12} malabsorption in the ileum and consequent vitamin B_{12} deficiency. It usually becomes clinically apparent within the first 6 years of life. In addition to megaloblastic anemia, the patient may also have neurologic defects (e.g., hypotonia, developmental delay, brain atrophy, movement disorders, dementia) and/or proteinuria. Patients carry pathologic variants in genes *CUBN* or *AMN*, which encode proteins that form the cubam receptor for the ileal IF-Cbl complex. Because CUBN is also a key receptor for protein reabsorption in the kidney, impaired expression at this site results in associated proteinuria. The disease can be fatal if it remains untreated. Early diagnosis and treatment with IM or intranasal Cbl will reverse the hematologic and neurologic abnormalities. Proteinuria does not respond to therapy.

Classic pernicious anemia (autoimmune gastritis) usually occurs in older adults but can rarely affect children. This disorder **(juvenile pernicious anemia)** usually presents during adolescence. In such cases, the disease is associated with various detectable antibodies, including those against IF and the hydrogen-potassium adenosine triphosphatase proton pump in gastric parietal cells. These children can have additional immunologic abnormalities, cutaneous candidiasis, hypoparathyroidism, and other endocrine deficiencies; atrophy of the gastric mucosa and achlorhydria may occur. IM or intranasal vitamin B_{12} should be administered regularly.

Absence of Vitamin B_{12} Transport Protein Transcobalamin

Transcobalamin (TC) deficiency is a rare cause of megaloblastic anemia. A congenital deficiency is inherited as an autosomal recessive condition resulting in a failure to absorb and transport vitamin B_{12}. Most patients lack TC, but some have functionally defective forms. This disorder usually manifests in the first weeks of life. Characteristically, there is failure to thrive, diarrhea, vomiting, glossitis, neurologic abnormalities, and megaloblastic anemia. The diagnosis can be difficult given that total serum vitamin B_{12} levels are often normal because approximately 80% of serum Cbl is bound to HC. The diagnosis is suggested by the presence of severe megaloblastic anemia in the face of normal folate levels and no evidence of another inborn error of metabolism. Plasma homocysteine and methylmalonic acid levels are elevated. A definitive diagnosis is made by measuring plasma TC. The serum vitamin B_{12} levels must be kept high to force enough Cbl into cells and allow normal function using high-dose oral supplementation or IM or intranasal treatment. Symptoms and laboratory studies should be monitored, and doses adjusted as needed.

Disorders of Intracellular Cobalamin Metabolism

Disorders of intracellular cobalamin metabolism are associated with pathologic genetic variants in genes associated with lettered complementation groups. These include *MMACHC (cblC)*, *MMADHC* (*cblD*-combined and *cblD* homocystinuria), *MTRR (cblE), LMBRD1 (cblF), MTR (cblG), ABCD4 (cblJ), THAP11* (*cblX*-like), *ZNF143* (*cblX*-like), or *HCFC1* (*cblX*, which can show a cblC complementation class). The disorders present at a range of ages starting in utero and have a variable clinical presentation including complications of methylmalonic acidemia, homocystinuria, and megaloblastic anemia. Patients require care with a metabolic specialist to guide treatment with OHCbl, avoidance of agents that can cause metabolic decompensation, and surveillance for complications.

Vitamin B_{12} deficiency may develop by inactivation of vitamin B_{12}. This most often occurs in adolescents who chronically use "Whippets," which contain nitrous oxide, as a recreational agent to get high. Conversely, patients with underlying vitamin B_{12} deficiency may have an exacerbation after nitrous oxide anesthesia.

CLINICAL MANIFESTATIONS

Children with Cbl deficiency often present with nonspecific manifestations such as weakness, lethargy, feeding difficulties, failure to thrive, and irritability. Other common findings include pallor, glossitis, vomiting, diarrhea, and icterus. Hyperpigmentation is another feature that may mimic Addison disease. Neurologic symptoms can include paresthesia, sensory deficits, hypotonia, seizures, developmental delay, developmental regression, neuropsychiatric changes, and brain/spine MRI changes. Neurologic problems from vitamin B_{12} deficiency may occur in the *absence* of any hematologic abnormalities. Thrombotic microangiopathy is a rare manifestation.

LABORATORY FINDINGS

The hematologic manifestations of folate and Cbl deficiency are identical. The anemia resulting from Cbl deficiency is macrocytic, with prominent macro-ovalocytosis of the RBCs (see Chapter 496, Fig. 496.2, and Fig. 503.1). The neutrophils may be large and hypersegmented (see Fig. 503.1). In advanced cases, neutropenia and thrombocytopenia can occur, simulating aplastic anemia or leukemia. Macrocytosis may not always be present. Serum vitamin B_{12} levels are low, and the serum concentrations of methylmalonic acid and homocysteine are elevated. Concentrations of serum iron and serum folic acid are normal or elevated. Serum lactate dehydrogenase activity is markedly increased, a reflection of ineffective erythropoiesis. Moderate elevations of serum bilirubin levels (2-3 mg/dL) also may be found. Excessive excretion of methylmalonic acid in the urine (normal: 0-3.5 mg/24 hr) is a reliable and sensitive index of vitamin B_{12} deficiency and is especially helpful when the serum vitamin B_{12} level is in the low-normal range.

DIAGNOSIS

A comprehensive medical history is essential to the clinical recognition of possible Cbl deficiency. Information regarding clinical symptoms, dietary history, diseases, surgeries, or medications and drugs is likely to provide important clues. The physical examination may reveal relevant findings such as irritability, pallor, pigmentation or specific neurologic symptoms. Screening laboratory findings offer important information, but more focused testing will be required to confirm a diagnosis of vitamin B_{12} deficiency and its cause. Cbl deficiency is usually identified by measuring total or TC-bound vitamin B_{12} in the blood. Although an extremely low level is generally diagnostic, this may not be the case because false negatives and false positives are reportedly common using currently available assays. As a result, it is wise not to discount vitamin B_{12} deficiency, particularly in the face of clinical symptoms, macrocytic anemia, an abnormal blood smear, and a normal folate level. In untreated patients, methylmalonic acid and total homocysteine levels are often helpful because they are greatly elevated in the majority of those with clinical signs of B_{12} deficiency. Excessive urinary methylmalonic acid excretion is also a sensitive test of B_{12} deficiency. Although modest increases occur with renal failure, elevated methylmalonic acid is otherwise quite specific for B_{12} deficiency. Notably, however, serum homocysteine is also elevated in folate deficiency, homocystinuria, and renal failure.

If vitamin B_{12} deficiency has been confirmed and there is no evidence of inadequate dietary intake or, in the case of an infant, inadequate maternal B_{12}, malabsorption should be investigated. Anti-IF antibodies and anti–parietal cell antibodies are useful for the diagnosis of pernicious anemia. Measurement of IF and testing from more specialized laboratories may be required for less common disorders.

TREATMENT

Treatment regimens in children have not been well studied. The cause of vitamin B_{12} deficiency should ultimately dictate treatment dosage and route of administration as well as the duration of therapy. Cyanocobalamin is available as a nasal spray as an alternative to parenteral injection. Dose adjustments should be made in response to clinical status and laboratory values. The physiologic requirement for vitamin B_{12} is about 1-3 µg/day. Hematologic responses have been observed with small doses, indicating that a mini-dose may be administered as a therapeutic test when the diagnosis of vitamin B_{12} deficiency is in doubt or in circumstances where the anemia is severe and higher initial doses might result in severe metabolic disturbances.

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503.3 Other Rare Megaloblastic Anemias

Courtney D. Thornburg

Orotic aciduria is a rare autosomal recessive disorder that usually appears in the first year of life and is characterized by growth failure, developmental delay, megaloblastic anemia, and increased urinary excretion of orotic acid (see Chapter 110). Rarely, orotic aciduria occurs without megaloblastic anemia. This defect is the most common metabolic error in the de novo synthesis of pyrimidines and therefore affects nucleic acid synthesis. The usual form of hereditary orotic aciduria is caused by a deficiency (in all body tissues) of orotic phosphoribosyl transferase and orotidine-5-phosphate decarboxylase, two sequential enzymatic steps in pyrimidine nucleotide synthesis. The diagnosis is suggested by the presence of severe megaloblastic anemia with normal serum B_{12} and folate levels and no evidence of TC deficiency. A presumptive diagnosis is made by finding increased urinary orotic acid. However, confirmation of the diagnosis requires assay of the transferase and decarboxylase enzymes in the patient's erythrocytes. Failure to thrive and intellectual disability often accompany this condition. The anemia is refractory to vitamin B_{12} or folic acid, but the anemia responds promptly to administration of uridine.

Thiamine-responsive megaloblastic anemia (Rogers syndrome) is a very rare autosomal recessive disorder characterized by megaloblastic anemia, sensorineural deafness, and diabetes mellitus. Congenital heart defects, arrhythmias, visual problems, short stature, trilineage myelodysplasia, and strokes are also described. Thiamine-responsive megaloblastic anemia usually presents in infancy but may occasionally develop in childhood and adolescence and occurs in several ethnically distinct populations. The bone marrow is characterized not only by megaloblastic changes but also by ringed sideroblasts. The defect is caused by biallelic pathologic genetic variants in *SCL19A2*, which encodes a high-affinity plasma membrane thiamine transporter. Continuous thiamine supplementation usually reverses the anemia and diabetes, but not existing hearing defects.

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Chapter 504
Iron-Deficiency Anemia

Jennifer A. Rothman

Iron deficiency is the most widespread and common nutritional disorder in the world. It is estimated that 30–50% of the global population has iron-deficiency anemia, and most of these individuals live in developing countries. In the United States, 8–14% of children ages 12-36 months are *iron deficient*, and 30% of this group progresses to *iron-deficiency anemia*.

A full-term newborn infant contains about 0.5 grams of iron, compared with 5 grams of iron in adults. This change in quantity of iron from birth to adulthood means that an average of 0.8 mg of iron must be absorbed each day during the first 15 years of life. A small additional amount is necessary to balance normal losses of iron by shedding of cells. It is therefore necessary to absorb approximately 1 mg daily to maintain positive iron balance in childhood. Because <10% of dietary iron is usually absorbed, a dietary intake of 8-10 mg of iron daily is necessary to maintain iron levels. During infancy, when growth is most rapid, the 1 mg/L of iron in cow's and breast milk makes it difficult to maintain body iron. Although breastfed infants have an advantage because they absorb iron 2-3 times more efficiently than infants fed cow's milk, breastfed infants nonetheless are at risk of developing iron deficiency without regular intake of iron-fortified foods by 6 months of age.

ETIOLOGY

Most iron in neonates resides in circulating hemoglobin. As the relatively high hemoglobin concentration of the newborn infant falls during the first 2-3 months of life, considerable iron is recycled. These iron stores are usually sufficient for blood formation in the first 6-9 months of life in term infants. Stores are depleted sooner in premature infants, low birthweight infants, or infants with perinatal blood loss, because their iron stores are smaller. Delayed (1-3 minute) clamping of the umbilical cord can improve iron status and reduce the risk of iron deficiency, whereas early clamping (<30 seconds) puts the infant at risk for iron deficiency. Dietary sources of iron are especially important in these infants. In term infants, anemia caused solely by inadequate dietary iron usually occurs at 9-24 months of age and is less common thereafter. The usual dietary pattern observed in infants and toddlers with nutritional iron-deficiency anemia in developed countries is excessive consumption of cow's milk (low iron content, blood loss from milk protein colitis) in a child who is often overweight or bottle feeding beyond 12 months of age. Worldwide, **undernutrition** is usually responsible for iron deficiency (Table 504.1).

Blood loss must be considered as a possible cause in every case of iron-deficiency anemia. Sources of blood loss, particularly in older children and adolescents, include menstrual losses, recurrent nosebleeds, or intravascular hemolysis with hemoglobinuria, as seen in diseases such as malaria or endurance athletics such as triathletes. Chronic iron-deficiency anemia from occult bleeding may be caused by a lesion of the gastrointestinal (GI) tract, such as peptic ulcer, Meckel diverticulum, polyp, hemangioma, or inflammatory bowel disease. Infants can have chronic intestinal blood loss induced by exposure to cow's milk protein. Involved infants characteristically develop anemia that is more severe and occurs earlier than would be expected simply from an inadequate intake of iron. The ongoing loss of blood in the stools can be prevented either by breastfeeding or by delaying the introduction of whole cow's milk in the first years of life and then limiting the quantity to <24 oz/24 hours. *Infants and toddlers who have excessive raw milk intake with no iron supplementation and limited iron containing foods are at high risk of iron deficiency.* Unrecognized blood loss also can be associated with chronic diarrhea and, rarely, with pulmonary hemosiderosis. In developing countries, infections with hookworm, *Trichuris trichiura,* and *Plasmodium* often contribute to iron deficiency. Since iron is absorbed in the proximal duodenum with the assistance of gastric acid, gastric bypass procedures or *Helicobacter pylori* infection may interfere with iron absorption. Similarly, inflammation of the bowel from celiac disease and giardiasis may also interfere with iron absorption.

Approximately 2% of adolescent females have iron-deficiency anemia, largely as a result of their adolescent growth spurt and menstrual blood loss. The highest risk of iron-deficiency anemia (>30%) is among teenagers who are or have been pregnant.

CLINICAL MANIFESTATIONS

Most children with iron-deficiency anemia are asymptomatic and are identified by routine laboratory screening at 9-12 months of age. Normal hemoglobin values vary according to age, gender, ethnicity, and method of testing, such as capillary versus venous blood. Pallor is the

TREATMENT

Treatment regimens in children have not been well studied. The cause of vitamin B_{12} deficiency should ultimately dictate treatment dosage and route of administration as well as the duration of therapy. Cyanocobalamin is available as a nasal spray as an alternative to parenteral injection. Dose adjustments should be made in response to clinical status and laboratory values. The physiologic requirement for vitamin B_{12} is about 1-3 µg/day. Hematologic responses have been observed with small doses, indicating that a mini-dose may be administered as a therapeutic test when the diagnosis of vitamin B_{12} deficiency is in doubt or in circumstances where the anemia is severe and higher initial doses might result in severe metabolic disturbances.

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CLINICAL MANIFESTATIONS

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serum transferrin levels that are accompanied by the deposition of non–transferrin-bound iron in parenchymal tissues. Because hepcidin production is induced by inflammatory stimuli, hepcidin elevation is also a feature of the **anemia of chronic disease** (ACD). However, in contrast to patients with IRIDA, in whom the hepcidin dysregulation is congenital, patients with ACD generally retain normal to high iron stores because of the acquired nature of their hepcidin elevation (see Chapter 500.1). Rare medical causes that may mimic IRIDA include vascular malformations of the GI tract, Castleman disease (where IL-6 overproduction causes hepcidin elevation), autoimmune gastritis, and pathogenic germline variants in *KCNQ1* (which regulates gastric acid secretion).

TREATMENT

Because of the underlying pathophysiology of IRIDA, *parenteral iron supplementation* is required to correct the anemia. Although parenteral iron therapy raises body iron stores, the hematologic response is usually slow and not completely corrective. This likely results from insufficient export of the processed iron from macrophages into the circulation, an expected consequence of hepcidin elevation. Serum ferritin levels increase with parenteral iron therapy in a dose-dependent manner and may raise concerns for iron overload, which would be expected to exhibit a reticuloendothelial rather than a parenchymal pattern of iron loading. Given the limited number of IRIDA cases reported to date, the optimal formulation and dosing of parenteral iron have not yet been established. Although oral iron supplementation does not appear to have a significant role in treatment of IRIDA, the addition of ascorbic acid to a ferrous sulfate oral supplement has been associated with hematologic responses in isolated cases. Treatment with recombinant erythropoietin has not been found to produce significant clinical benefit in patients with IRIDA.

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Chapter 505
Other Microcytic Anemias

Jennifer A. Rothman

A number of rare microcytic anemias need to be considered when children with microcytic anemia fail to respond to oral iron (Table 504.5). These anemias include thalassemia or thalassemia trait (see Chapter 511), infantile poikilocytosis and hereditary pyropoikilocytosis (Chapter 508), and anemia of chronic disease (Chapter 500.1). Additionally, rare nutritional disorders and disorders of iron metabolism can cause microcytosis.

INFANTILE POIKILOCYTOSIS AND HEREDITARY PYROPOIKILOCYTOSIS

Infants with common **hereditary elliptocytosis** (see Chapter 508) may initially present with hemolytic anemia characterized by marked poikilocytosis with budding and fragmentation of the red blood cells (RBCs). These small RBC fragments reduce the overall mean corpuscular volume (MCV), resulting in microcytosis. By 2 years of age, the findings become typical of hereditary elliptocytosis. **Hereditary pyropoikilocytosis** is a much less common variant of hereditary elliptocytosis, in which the hemolytic anemia and RBC changes are more severe.

COPPER DEFICIENCY

Copper deficiency is a rare cause of microcytic anemia and neurologic dysfunction. Copper is absorbed in the stomach and proximal duodenum. Deficiency is associated with malabsorption; severe malnutrition, often with feeding of milk alone; gastric surgery or feedings that bypass the stomach and duodenum; or parenteral nutrition with inadvertent omission of supplemental copper. Zinc and copper are competitively absorbed from the gastrointestinal (GI) tract, so zinc excess may inadvertently lead to copper deficiency. Diagnosis is made by measuring a serum copper level, serum ceruloplasmin, and possibly a zinc level. Treatment includes either oral or parenteral supplementation, depending on the underlying cause.

DEFECTS OF IRON METABOLISM

Rare microcytic anemias may be associated with defects in iron trafficking and regulation. Most are inherited and usually identified in childhood, including defects of iron absorption, transport, utilization, and recycling. A *defect of iron absorption* is **iron-refractory iron-deficiency anemia** (see Chapter 504.1). *Defects of iron recycling* include aceruloplasminemia and atransferrinemia. **Aceruloplasminemia** is an autosomal recessive disorder in the *CP* gene that encodes ceruloplasmin. Iron cannot be appropriately transported from macrophages to plasma to be available for RBC production but accumulates instead in the brain and visceral organs, which can lead to an adult-onset neurodegenerative disorder. The diagnosis is made by a combination of absence of serum ceruloplasmin, low serum copper and iron, elevated ferritin, and increased liver iron concentration. Hypotransferrinemia or **atransferrinemia** is also an autosomal recessive disorder caused by pathogenic variants in the transferrin *(TF)* gene. Diagnosis is made by low or absent serum transferrin and liver iron overload. Genetic testing can confirm the diagnosis for both disorders. Treatment includes iron chelation therapy, limiting iron supplementation and dietary iron, and possibly fresh-frozen plasma to replace ceruloplasmin and/or transferrin. Purified transferrin (apotransferrin) infusions are available.

Defects of mitochondrial iron utilization are a diverse group of acquired and inherited defects known as **sideroblastic anemias** (Table 505.1). Several genes associated with these disorders have been described. Impaired heme synthesis leads to retention of iron within the mitochondria of marrow RBCs. The perinuclear distribution of mitochondria results in a pattern of iron staining surrounding the nucleus. These are *ringed sideroblasts* (Fig. 505.1), which are distinct from the more diffuse cytoplasmic distribution of iron in normal RBC precursors. The anemia is characterized by hypochromic, microcytic RBCs mixed with normal RBCs, so the complete blood count indicates a very high RBC distribution width. The serum iron concentration usually is elevated, and the transferrin saturation of iron is increased.

Congenital sideroblastic anemia (CSA) can be *syndromic* or *nonsyndromic*. The most common type of nonsyndromic CSA is an X-linked disorder and is most often a result of pathogenic variants in *ALAS2* that encodes erythrocytic isozyme 5-aminolevulinic acid synthetase, the rate-limiting enzyme reaction in heme synthesis. An important cofactor for 5-ALA synthetase is *pyridoxal phosphate,* with several pathogenic variants occurring near its binding site. Severe anemia is recognized in infancy or early childhood, whereas milder cases might not become apparent until early adulthood or later. Clinical findings include pallor, icterus, and moderate splenomegaly and/or hepatomegaly. The severity of the anemia varies such that some patients require no therapy and others need regular RBC transfusions. A subset of patients with hereditary sideroblastic anemia manifest a hematologic response to pyridoxine doses of 50-200 mg/day. Iron overload, as manifested by elevated serum ferritin, elevated serum iron, and increased transferrin saturation, is a major complication of this disorder. Clinical evidence of iron overload (e.g., diabetes mellitus, liver dysfunction) may be found in some patients who have little or no anemia, which may require iron chelation therapy. Stem cell transplantation has been used to treat affected children who are dependent on RBC transfusions.

A unique variant of congenital sideroblastic anemia is **Pearson syndrome** (see Chapter 498), but the anemia is usually *macrocytic* and not microcytic. Another rare variant of sideroblastic anemia is caused by pathogenic variants in *TRNT1* and manifests with developmental

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Heme synthesis defects	XLSA	X-linked	No	*ALAS2*	100s	Infancy to adulthood	Mild to severe	↓ males N/↑ female	Iron overload in the absence of transfusions
	SLC25A38 deficiency	AR	No	*SLC25A38*	40	Infancy	Severe	↓	Transfusional iron overload
	EPP	AR/PSD	No	*FECH*	100s	Childhood	Mild	↓	Acute photosensitivity
Fe-S biogenesis defects	GLRX5 deficiency	AR	No	*GLRX5*	2	Adulthood	Mild to severe	↓	Iron overload
	HSPA9 deficiency	AR/PSD	No	*HSPA9*	12	Childhood	Mild to severe	N/↓	Retinitis pigmentosa
	HSCB deficiency	AR	No	*HSCB*	1	Childhood	Moderate	N	None
	XLSA/A	X-linked	Yes	*ABCB7*	5	Childhood	Mild to moderate	↓	Cerebellar ataxia and hypoplasia, delayed motor development
Mitochondrial protein synthesis defects	PMPS	SP/M	Yes	*mtDNA*	100s	Early childhood	Severe	↑	Lactic acidosis, exocrine pancreatic insufficiency, failure to thrive, hepatic/renal failure
	MLASA1	AR	Yes	*PUS1*	10	Childhood	Mild to severe	N/↑	Myopathy, lactic acidosis, facial dysmorphism
	MLASA2	AR	Yes	*YARS2*	40	Childhood	Mild to severe	N/↑	Myopathy, lactic acidosis, cardiomyopathy
	LARS2 deficiency	AR	Yes	*LARS2*	1	Infancy	Severe	↑	Lactic acidosis, cardiomyopathy, hepatopathy, seizures
	SIFD	AR	Yes	*TRNT1*	30	Infancy	Severe		Immunodeficiency (B.T), aseptic febrile episodes, developmental delay, seizures, cardiomyopathy, retinitis pigmentosa, other
Mitochondrial respiratory protein mutations	MT-ATP6-SA	SP/M	Yes	*MT-ATP6*	4	Infancy to early childhood	Moderate to severe	N/↑	Lactic acidosis, myopathy, neurologic abnormalities
	NDUFB11-SA	X-linked	Yes	*NUDFB11*	5	Early childhood	Moderate	N	Lactic acidosis, myopathy
	Multifactorial TRMA	AR	Yes	*SLC19A2*	50	Early childhood	Mild to severe	↑	Sensorineural deafness, non-type 1 diabetes mellitus, optic atrophy, strokelike episodes

↑, increased; ↓, decreased; AR, autosomal recessive; EPP, erythropoietic protoporphyria; MCV, mean red blood cell volume; MLASA, mitochondrial myopathy lactic acidosis and sideroblastic anemia;. mtDNA, mitochondrial DNA; N, normal; PMPS, Pearson marrow pancreas syndrome; PSD, Pseudodominant; SIFD, sideroblastic anemia, immunodeficiency (B cell), periodic fevers, and developmental delay; SP/M, sporadic/mitochondrial; TRMA, thiamine responsive megaloblastic anemia; XLSA, X-linked sideroblastic anemia.

Adapted from Ducamp S and Fleming D. The molecular genetics of sideroblastic anemia. *Blood* 2019:133(1):59–69.

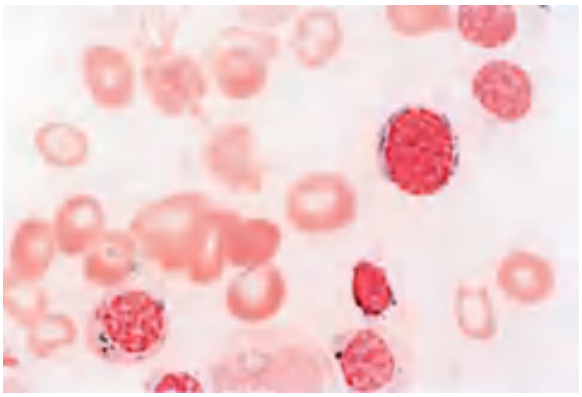

Fig. 505.1 Ring sideroblast in myelodysplastic syndrome (refractory anemia with ring sideroblasts)—iron stain. *(From Ryan DH, Cohen HJ. Bone marrow examination. In: Hoffman R, Benz EJ Jr, Shattil SJ, et al., eds. Hematology, 4th ed. Philadelphia: Churchill Livingstone, 2005.)*

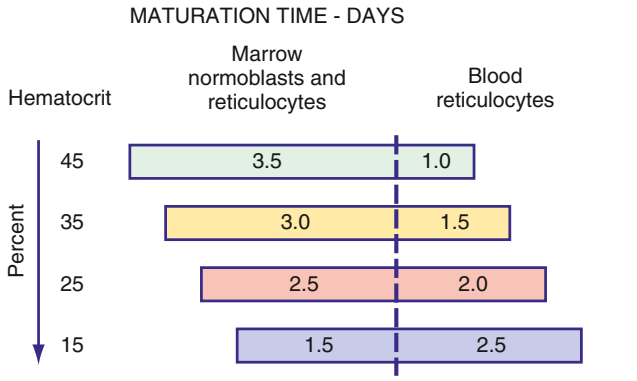

Fig. 506.1 Number of days for maturation of reticulocytes to mature erythrocytes in the marrow and blood. The duration of maturation as blood reticulocytes is taken as μ, which is used in the correction equation in this chapter. *(Modified from Hillman RS, Finch CA. Red cell manual. Philadelphia: FA Davis, 1983.)*

delay, periodic fevers, and B-cell immunodeficiency in addition to sideroblastic anemia.

Acquired sideroblastic anemias can be triggered by copper deficiency or drugs and toxins that disturb mitochondrial iron metabolism, including lead, chloramphenicol, penicillamine, ethanol, and isoniazid. The acquired neoplastic sideroblastic syndromes (myelodysplasias) seen in adults are very rare in children.

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Section 3

Hemolytic Anemias

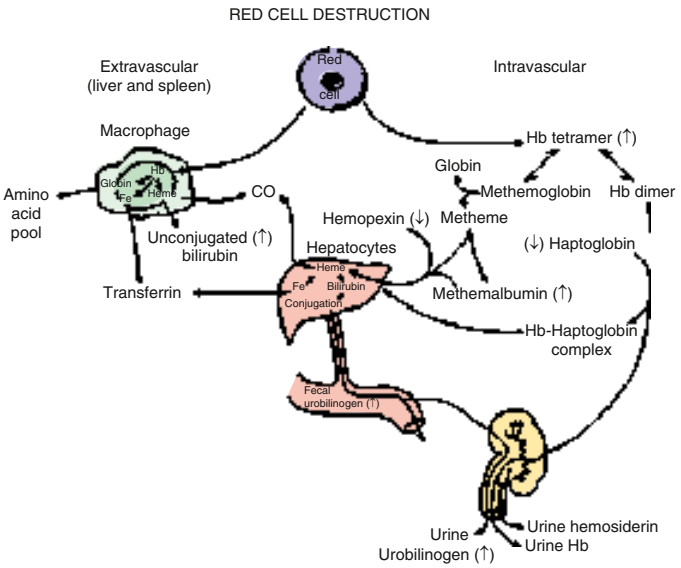
Chapter 506

Definitions and Classification of Hemolytic Anemias

Stephanie Prozora and Patrick G. Gallagher

Hemolysis is defined as the premature destruction of red blood cells (RBCs). Anemia results when the rate of destruction exceeds the capacity of the marrow to produce additional RBCs. Normal RBC survival time is 110-120 days (half-life: 55-60 days), and thus, approximately 0.85% of the most senescent RBCs are removed and replaced each day. During hemolysis, RBC survival is shortened, the RBC count falls, erythropoietin is increased, and marrow erythropoietic activity is stimulated. This leads to compensatory erythroid hyperplasia with increased RBC production, reflected by an increase in the reticulocyte count. The marrow can increase its output twofold to threefold acutely, with a maximum of sixfold to eightfold in chronic hemolysis. The reticulocyte percentage can be corrected to measure the magnitude of marrow production in response to hemolysis as follows:

$$\text{Reticulocyte index} = \text{reticulocyte } \% \times \frac{\text{Observed hematocrit}}{\text{Normal hematocrit}} \times \frac{1}{\mu}$$



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where μ is a maturation factor of 1-3 related to the severity of the anemia (Fig. 506.1). The normal reticulocyte index is 1.0; therefore the index measures the fold increase in erythropoiesis (e.g., twofold, threefold). Because the reticulocyte index is essentially a measure of RBC production per day, the maturation factor μ provides this correction (see Fig. 506.1).

When hemolysis is chronic, compensatory erythroid hyperplasia may lead to significant expansion of the medullary spaces at the expense of cortical bone. This is particularly prominent in children with severe chronic hemolytic anemia such as thalassemia. These changes may be evident on physical examination or on radiographs of the skull and long bones. In severe cases, there is increased propensity for long bone fracture. Hemolysis also leads to increased degradation of hemoglobin. This process can result in indirect hyperbilirubinemia, increased biliary excretion of heme pigment derivatives, and formation of bilirubinate gallstones.

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Chapter 505
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Jennifer A. Rothman

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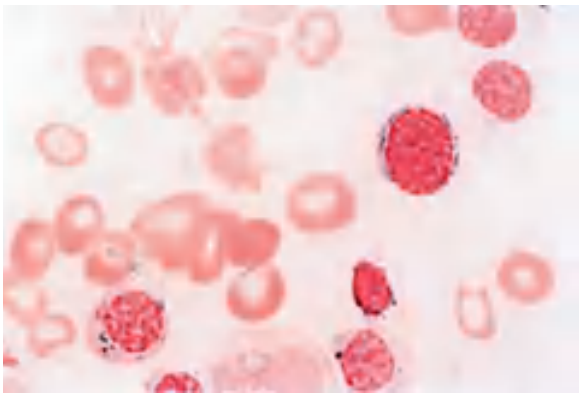

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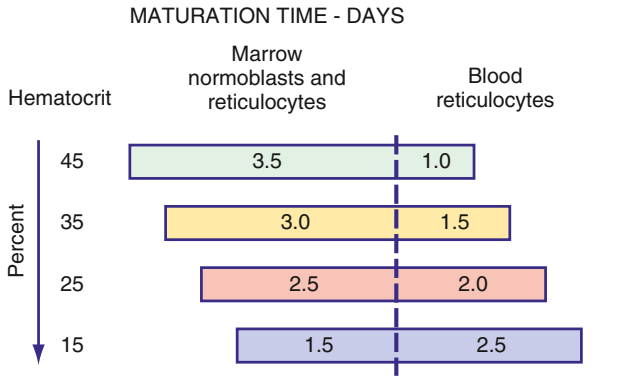

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Section 3

Hemolytic Anemias

Chapter 506

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Stephanie Prozora and Patrick G. Gallagher

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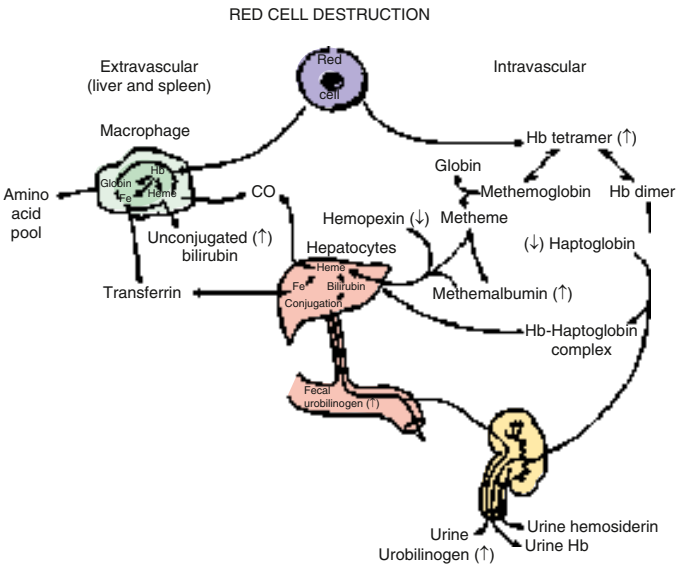

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Table 506.3 Clinical and Laboratory Features Suggestive of Hemolytic Anemia

Pallor	↑ RDW (caused by ↑ reticulocyte count)
Icterus	Abnormal RBC morphology
Splenomegaly	↑ Indirect bilirubin (normal direct bilirubin)
Gallstones	↓ Serum haptoglobin level
History of neonatal icterus	↑ Urinary urobilinogen level
Positive family history of anemia, splenectomy, cholecystectomy	Hemoglobinuria (+ dipstick test result for blood; no RBCs in urine)
↑ Reticulocyte count	↑ LDH level

LDH, Lactate dehydrogenase; RBC, red blood cell; RDW, red cell distribution width.
From Kliegman RM, Lye PS, Bordini BJ, et al., eds. *Nelson Pediatric Symptom-Based Diagnosis.* Philadelphia: Elsevier, 2018: 674; Table 37.12.

Table 506.4 Hemolytic Anemias: Diagnostic Clues Based on Red Blood Cell Shape

Sickle cells: sickle cell disease
Target cells: hemoglobinopathies (HbC, HbS, thalassemia), liver disease
Schistocytes/burr cells/helmet cells/RBC fragments: microangiopathic hemolytic anemia (DIC, HUS, TTP)
Spherocytes: hereditary spherocytosis, autoimmune hemolytic anemia
Cigar-shaped cells: hereditary elliptocytosis
"Bite" cells: G6PD deficiency
Poikilocytosis, microcytosis, fragmented erythrocytes, elliptocytes: hereditary pyropoikilocytosis

DIC, Disseminated intravascular coagulation; G6PD, glucose-6-phosphate dehydrogenase; HbC, hemoglobin C; HbS, sickle hemoglobin; HUS, hemolytic-uremic syndrome; RBC, red blood cell; TTP, thrombotic thrombocytopenia purpura.
From Kliegman RM, Lye PS, Bordini BJ, et al., eds. *Nelson Pediatric Symptom-Based Diagnosis.* Philadelphia: Elsevier, 2018: 674; Table 37.13.

Chapter 507

Hereditary Spherocytosis

Stephanie Prozora and Patrick G. Gallagher

Hereditary spherocytosis (HS) is a common cause of inherited hemolytic anemia, with a prevalence of approximately 1 in 2,000-5,000 persons. Described in patients of all ethnic groups, it is the most common inherited abnormality of the erythrocyte associated with inherited hemolytic anemia in persons of Northern European origin. HS is marked by wide variability in the associated clinical, laboratory, and genetic manifestations. Symptomatology ranges from asymptomatic patients with well-compensated anemia to severely affected patients with hemolytic anemia requiring regular blood transfusions.

ETIOLOGY

The pathophysiology underlying HS is twofold: an intrinsic defect of the erythrocyte membrane and an intact spleen that selectively retains, damages, and removes abnormal HS erythrocytes. Qualitative or quantitative defects of key membrane proteins lead to a multistep process of accelerated HS erythrocyte destruction. Abnormalities of ankyrin or spectrin are the most common molecular defects (Table 507.1). Defects in these membrane proteins result in uncoupling of the "vertical" interactions of the lipid bilayer with the underlying membrane skeleton with subsequent release of membrane microvesicles. The loss of membrane surface area without a proportional loss of cell volume causes decreased erythrocyte deformability. This impairs cell passage from the splenic cords to the splenic sinuses, leading to the trapping and premature destruction of HS erythrocytes by the spleen (Figs. 507.1 and 507.2). Splenectomy markedly improves erythrocyte life span and may be indicated in some patients with HS.

CLINICAL MANIFESTATIONS

Hereditary spherocytosis is usually transmitted as an autosomal dominant trait. However, as many as 25% of patients have no previous family history, representing either recessive inheritance or de novo gene variants.

In the neonatal period, HS is a significant cause of hemolysis and can manifest as anemia and/or hyperbilirubinemia severe enough to require phototherapy, transfusion, or exchange transfusions. Hemolysis may be more prominent in the newborn because hemoglobin F binds 2,3-diphosphoglycerate poorly, and the increased level of free 2,3-diphosphoglycerate destabilizes interactions among spectrin, actin, and protein 4.1 in the red blood cell (RBC) membrane (see Fig. 507.1). The need for transfusions in infancy is not indicative of more severe disease later in life because infants are typically slow to mount an adequate reticulocyte response for the first few months after birth.

Disease severity varies and can be used to clinically classify HS (Table 507.2). Mild cases (20–30% of all HS) are asymptomatic into adulthood and have well-compensated mild anemia where reticulocyte production and erythrocyte destruction are essentially balanced. Cases of moderate or "typical" HS (60–70%) have partially compensated hemolytic anemia with reticulocytosis, frequently with symptoms of fatigue, pallor, and intermittent jaundice. Splenomegaly is common after infancy, and it is present in almost all HS patients by young adulthood. Severe cases of HS (3–5%) have life-threatening anemia and are transfusion dependent.

Bilirubin gallstone formation is a function of age; they can form as early as 4-5 years of age and are present in most adult HS patients.

HS patients are susceptible to **aplastic crises** primarily as a result of parvovirus B19 infection, hypoplastic crises associated with other infections, and megaloblastic crises due to folate deficiency (Fig. 507.3). During these crises, high RBC turnover in the setting of erythroid marrow failure can result in profound anemia (hematocrit <10%), high-output heart failure, cardiovascular collapse, and death. Leukocyte and platelet counts may also fall.

Rare complications associated with HS include splenic sequestration crisis, gout, cardiomyopathy, priapism, leg ulcers, and neurologic or muscular abnormalities, including spinocerebellar degeneration.

Table 507.1 Common Gene Pathogenic Variants in Hereditary Spherocytosis

PROTEIN	GENE	COMMON PATHOGENIC VARIANTS	PREVALENCE	INHERITANCE	DISEASE SEVERITY
Ankyrin-1	*ANK1*	Frameshift Nonsense Splicing Missense Insertion/deletion Promoter region	50–67% 5–10% in Japan	Mostly dominant, rare recessive	Mild to moderate
Band 3	*AE1 (SLC4A1)*	Missense Nonsense	15–20%	Dominant	Mild to moderate
β-Spectrin	*SPTB*	Nonsense Missense Insertion/deletion	15–20%	Dominant	Mild to moderate
α-Spectrin	*SPTA1*	Splicing Nonsense Missense	<5%	Recessive	Severe
Protein 4.2	*EPB42*	Missense Nonsense Splicing Deletion	<5% 45–50% in Japan	Recessive	Mild to moderate

Modified from Bolton-Maggs PHB, Langer JC, Iolascon A, et al. Guidelines for the diagnosis and management of hereditary spherocytosis–2011 update. *Br J Haematol*. 2011;156:37–49.

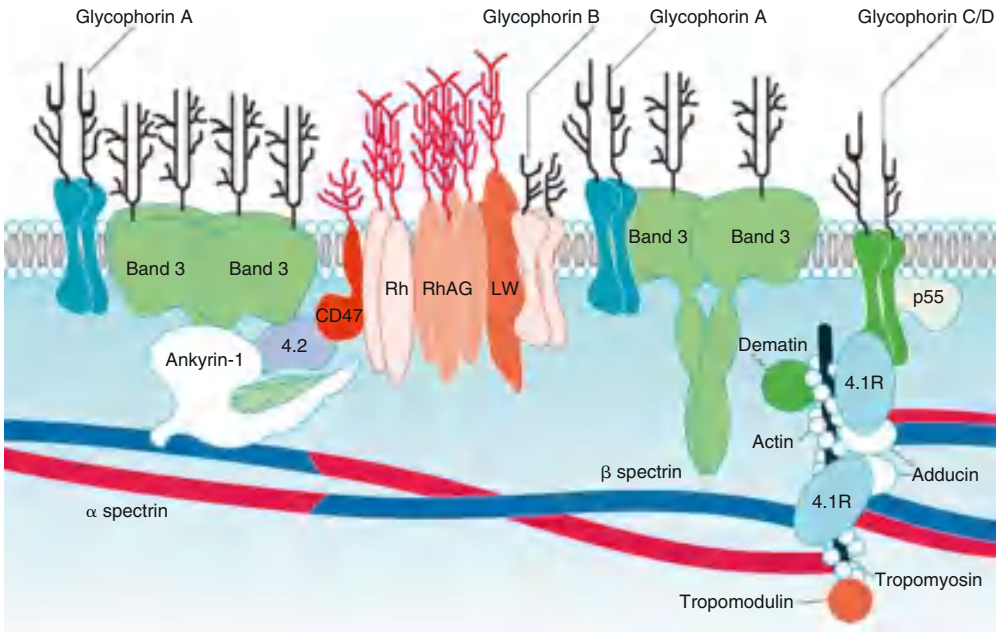

Fig. 507.1 A simplified cross-section of the red blood cell (erythrocyte) membrane. The lipid bilayer forms the equator of the cross-section with its polar heads (small circles) turned outward. 4.1R, protein; 4.2, protein 4.2; LW, Landsteiner-Wiener glycoprotein; Rh, Rhesus polypeptide; RhAG, Rh-associated glycoprotein. *(From Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet 2008;372:1411–1426.)*

DIAGNOSIS

Typically, there is evidence of hemolytic anemia with reticulocytosis and indirect hyperbilirubinemia. The mean corpuscular volume (MCV) of HS erythrocytes is low normal or even slightly decreased and the mean corpuscular hemoglobin concentration (MCHC) is usually increased (>35 g/dL). An MCHC > 35.4 g/dL combined with an RBC distribution width (RDW) <14% has been suggested as a screening test for HS. Erythroid cells on peripheral blood smear vary in size and include spherocytes and polychromatophilic reticulocytes. Spherocytes are smaller in diameter, hyperchromic due to elevated hemoglobin concentration from cellular dehydration, and lacking in central pallor (Fig. 507.4). Numbers of spherocytes are variable, with increased numbers likely reflecting the severity of disease. Other markers of hemolysis include decreased haptoglobin and elevated lactic dehydrogenase.

The diagnosis of HS can be established from a positive family history and the presence of typical clinical and laboratory features of the disease: splenomegaly, spherocytes on the blood smear,

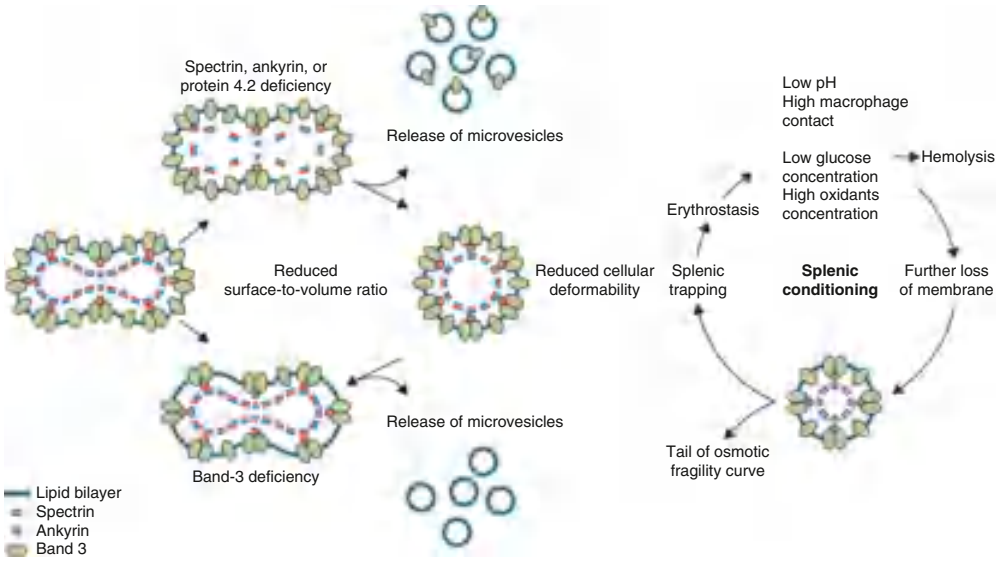

Fig. 507.2 Pathophysiologic effects of hereditary spherocytosis. *(From Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet 2008;372:1411–1426.)*

reticulocytosis, and an elevated MCHC. If these are present, no additional testing is necessary to confirm the diagnosis clinically. If the diagnosis is less certain, additional testing can be performed. Binding of fluorescently labeled eosin-5-maleimide (EMA) to band 3 and other membrane proteins is decreased in HS erythrocytes. This flow cytometry–based screening test is easy to perform and has good diagnostic sensitivity and specificity. In the classic incubated osmotic fragility test, HS erythrocytes are incubated in progressive dilutions of sodium chloride causing the cells to swell and lyse, with spherocytes lysing at a lower dilution due to their lower surface area to volume ratio. This test detects the presence of spherocytes in the blood, but it is not specific to HS and may be abnormal in other anemias with prominent spherocytosis. Osmotic fragility testing has poor sensitivity and may miss cases of mild HS where the numbers of spherocytes are few. Other assays such as the cryohemolysis test, the acidified glycerol lysis test, and the osmotic gradient ektacytometry have been used for diagnosis of HS, but they are not available in many laboratories. Genetic diagnosis is widely available on a commercial basis. The precise role of molecular testing in HS diagnosis and management is evolving. Some experts suggest molecular testing before splenectomy to verify the diagnosis of HS.

Diagnosis in the neonatal period requires a high index of suspicion as the disease presents differently than in older children, particularly in de novo and recessively inherited cases where family history is not available for guidance. Jaundice is frequently observed, and kernicterus can occur. Hemolytic anemia may be severe enough to require blood transfusion. In fact, HS is the leading cause of Coombs-negative hemolytic anemia, requiring transfusion in the first months of life. Splenomegaly is uncommon in neonates.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for spherocytosis on peripheral blood smear includes isoimmune and autoimmune hemolysis. Isoimmune hemolytic disease of the newborn, particularly when there is a result of ABO incompatibility, closely mimics the appearance of HS. The detection of antibody on an infant's RBCs using a direct antiglobulin (Coombs) test should establish the diagnosis of immune hemolysis. Autoimmune hemolytic anemias also are characterized by spherocytosis, but there will typically be evidence of previously normal values for hemoglobin, hematocrit, and reticulocyte count. Rare causes of spherocytosis include thermal injury, hemolytic transfusion reaction, clostridial sepsis, severe hypophosphatemia, Wilson disease, and snake, bee, or wasp envenomation, which all may manifest as transient spherocytic hemolytic anemia.

TREATMENT

General Supportive Care

Infants born to a parent with known HS should be monitored carefully because hyperbilirubinemia may peak several days after birth. Parents should be advised of the risk of neonatal anemia and jaundice and the potential need for transfusion, phototherapy, and/or exchange transfusion to treat anemia or hyperbilirubinemia. A subset of infants will be transfusion-dependent until development of adequate erythropoiesis to compensate for the ongoing hemolysis, usually between 6-12 months of age. Transfusion dependence after this time is rare and is most likely due to recessive HS.

Once the baseline level of disease severity is reached, an annual visit to the hematologist usually is sufficient follow-up. Growth should be monitored, and exercise tolerance and spleen size documented. Vaccinations should be up to date. Screening for gallbladder disease should begin at ~4 years of age, repeated every 3-5 years, or as indicated clinically. Documentation of parvovirus B19 susceptibility or immunity should be obtained in newly diagnosed patients. Similarly, HIV and hepatitis serology should be documented in patients who have received transfusions. Folic acid supplementation is recommended in cases of moderate and severe HS due to the demands of brisk erythropoiesis. Parents should receive anticipatory guidance regarding the risk of aplastic crisis secondary to parvovirus infection and hypoplastic crises with other infections. Parents and patients should be informed of an increased risk for gallstone development.

Guidelines for Splenectomy

Because spherocytes are destroyed almost exclusively in the spleen, splenectomy is curative in most patients because hemolysis, anemia, hyperbilirubinemia, and the incidence of gallstones are significantly lessened, if not completely eradicated, after splenectomy. Thus splenectomy became routine in the care of HS patients. However, splenectomy is associated with *short-term* risks, related to the operative procedure itself, and *long-term* risks, particularly increased lifelong

Table 507.2 Clinical and Laboratory Classification of Hereditary Spherocytosis

	NORMAL	MILD SPHEROCYTOSIS	MODERATE SPHEROCYTOSIS	MODERATELY SEVERE SPHEROCYTOSIS	SEVERE SPHEROCYTOSIS*
Inheritance	—	Autosomal dominant	Autosomal dominant, de novo variant	Autosomal dominant, de novo variant	Autosomal recessive
Proportion of hereditary spherocytosis cases	—	~20–30%	~60–70%	~10%	<5%
Hemoglobin (Hb, g/dL)†	11.5-16‡	10.5-15	8-12	6-8	<6
Reticulocytes (%)†	0.5-1.5	1.5-6	≥6	≥10	≥10
Bilirubin (mg/dL)†,‖	0-1	0.5-2	≥2	≥2	≥3
Peripheral smear*	Normal	Mild spherocytosis	Spherocytosis	Spherocytosis	Spherocytosis ± poikilocytosis
Osmotic fragility (fresh)	Normal	Normal or slightly increased	Increased	Increased	Greatly increased
Osmotic fragility (incubated)	Normal	Usually increased	Increased	Increased	Greatly increased
MCHC (g/dL)§	32-36	34-37	34-38	35-39	
RDW (%)§	11-14	12-19	16-23	20-30	
Hb/MCHC*	0.38-0.41	0.35-0.40	0.29-0.33	0.18-0.28	
Hb/RDW§	0.95-1.05	0.7-1.0	0.48-0.74	0.16-0.35	
Serum transferrin receptor (nmol/L)§	18-25	30-65	80-125	100-150	
Erythropoietin (mIU/mL)§	7-16	9-30	25-90	30-300	
Membrane protein patterns (SDS-PAGE)¶	—	"Normal"# Slight ↓ spectrin Slight ↓ spectrin and ankyrin Slight ↓ band 3 and 4.2 Absent protein 4.2 and ↓ CD47	↓ Spectrin ↓ Spectrin and ankyrin ↓ Band 3 and protein 4.2 Absent protein 4.2 and ↓ CD47	↓ Spectrin ↓ Spectrin and ankyrin ↓ Band 3 and protein 4.2	⇊ Spectrin ⇊ Spectrin and ankyrin ⇊ Band 3 and protein 4.2**
Transfusions	—	No	Sometimes required in infancy or with aplastic crisis	Occasionally with crises	Regular*
Splenectomy	—	Rarely, partial splenectomy††	Sometimes; consider partial splenectomy	Usually (6-9 yr)	Yes (>3 yr)

*Patients with severe disease are transfusion dependent by definition. Values are in untransfused patients or at nadir before transfusion.
†Data modified from Eber SW, Armbrust R, Schröter W. Variable clinical severity of hereditary spherocytosis: relation to erythrocytic spectrin concentration, osmotic fragility and autohemolysis. *J Pediatr.* 1990;177:409–416.
‡Varies with age.
§Ranges shown encompass the majority of individuals in each category. From Rocha S, Costa E, Rocha-Pereira P, et al. Complementary markers for the clinical severity classification of hereditary spherocytosis in unsplenectomized patients. *Blood Cells Mol Dis.* 2011;46:166–170.
‖Multiply by 17.1 to convert to μmol/L.
¶Indicates common patterns observed on SDS gels. Decreased spectrin alone is seen in α-spectrin or β-spectrin defects. Decreased spectrin and ankyrin arc observed with ankyrin defects. Decreased band 3 and protein 4.2 occur with band 3 defects. Absent protein 4.2 and decreased CD47 occur with protein 4.2 defects.
#Patients with mild spherocytosis who appear normal probably have small deficits (10–15%) that cannot be distinguished from normal findings on SDS gels.
**Rare patients with severe spherocytosis who are homozygous or compound heterozygous for band 3 defects.
††Consider in adolescents and adults who require a cholecystectomy or have disfiguring chronic jaundice.
CD, cluster of differentiation; MCHC, Mean corpuscular hemoglobin concentration; RDW, red cell distribution width (measure of variation in shape); SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; ↓, decreased.
From Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood.* 8th ed. Philadelphia: Elsevier, 2015:518; Table 16.3.

risk for sepsis, often caused by encapsulated bacteria. This risk is not eliminated but reduced with the requisite preoperative and postoperative vaccination against pneumococcus, meningococcus, and *Haemophilus influenzae* type b. In addition, there are increasing concerns regarding the emergence of penicillin-resistant pneumococci, as well as increased risk for cardiovascular diseases including thrombosis, pulmonary hypertension, and atherosclerosis, which have tempered the practice of routine splenectomy in HS.

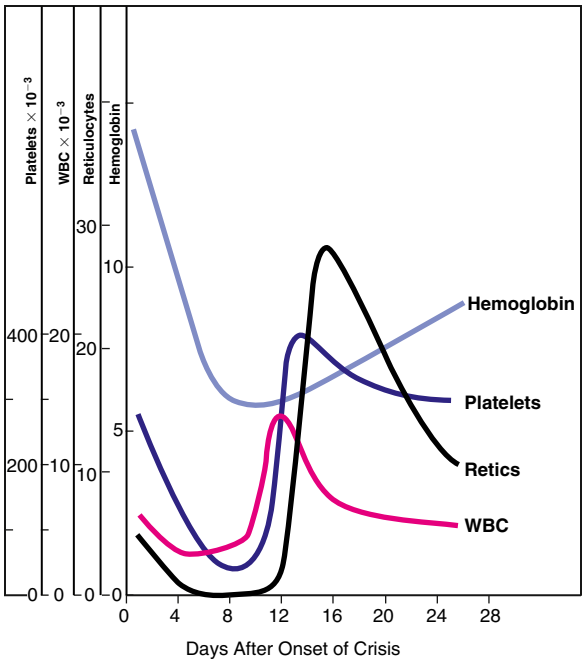

Fig. 507.3 Parvovirus-induced aplastic crisis. Progression of the changes in blood count is shown for a patient with hereditary spherocytosis and infection with parvovirus. Note that the fall in baseline reticulocytosis is associated with a rapid fall in hemoglobin. White blood cells (WBC) and platelets are also affected; Retics, reticulocytes. *(Adapted from Nathan DG, Orkin SH, Ginsburg D, et al., eds. Hematology of Infancy and Childhood, 6th ed. Philadelphia: Saunders, 2003.)*

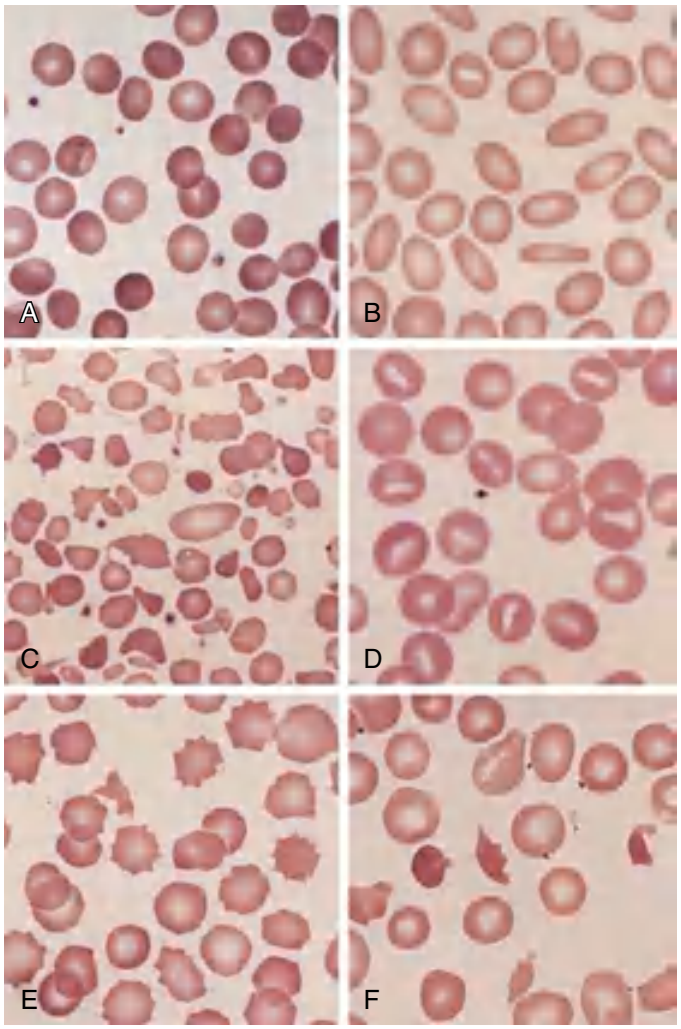

Fig. 507.4 Morphology of abnormal red cells. **A,** Hereditary spherocytosis. **B,** Hereditary elliptocytosis. **C,** Hereditary pyropoikilocytosis. **D,** Hereditary stomatocytosis. **E,** Acanthocytosis. **F,** Fragmentation hemolysis.

When considering splenectomy, the patient and the parents, together with their healthcare providers, should review and consider the risks and benefits. Individual-specific factors may confer additional risk after a splenectomy; time and distance from medical care in case of febrile illness and residence in or travel to areas where parasitic diseases such as malaria or babesiosis occur should be considered.

Most experts recommend splenectomy for patients with severe HS and believe it should be strongly considered for patients with moderate HS and frequent hypoplastic or aplastic crises, poor growth, or cardiomegaly. It is generally not recommended for patients with mild HS. When splenectomy is indicated, it should be performed after the age of 6 years, if possible, to avoid the heightened risk of postsplenectomy sepsis in younger children. The laparoscopic approach has less surgical morbidity and has become the technique of choice. Partial or subtotal splenectomy (removal of 85–95% of spleen volume) has been shown to decrease the hemolytic rate while preserving *some* splenic phagocytic function, although the decrease in hemolysis is less than that achievable with total splenectomy. Partial splenectomy is most attractive in children with severe HS requiring frequent transfusion early in childhood.

In children undergoing splenectomy, a concomitant cholecystectomy should be performed if there are gallstones. It is controversial whether to perform a concomitant splenectomy in less-severely ill patients who are undergoing cholecystectomy for gallstone disease. Postsplenectomy thrombocytosis is commonly observed but requires no treatment and usually resolves spontaneously. The patient should remain current on their vaccinations, as should household and frequent contacts. Prophylactic antibiotics are typically prescribed at least until the patient is 5 years of age or at least 2 years postsplenectomy. Folate supplementation should be continued if the hemoglobin level and reticulocyte count do not normalize.

Splenectomy failure may occur in cases of accessory spleen, accidental autotransplantation of splenic tissue into peritoneum at time of surgery, inaccurate diagnosis, or another co-inherited hemolytic anemia. Clues include return of hemolysis and disappearance of Howell-Jolly bodies on peripheral blood smear. The diagnosis can be made by radionucleotide studies.

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Chapter 508

Hereditary Elliptocytosis, Hereditary Pyropoikilocytosis, and Related Disorders

Stephanie Prozora and Patrick G. Gallagher

Hereditary elliptocytosis (HE), hereditary pyropoikilocytosis (HPP), and related disorders are characterized by the finding of elliptocytes on peripheral blood smear (Table 508.1). Whereas hereditary spherocytosis is thought of as a disorder of the vertical interactions coupling the erythrocyte membrane skeleton to the lipid bilayer, the HE syndromes interfere with the horizontal interactions that link spectrin molecules to each other and to membrane skeleton junctional complexes (see Chapter 507, Fig. 507.1), leaving the cell vulnerable to shear stress.

HE is the prototypical member of the group, in which abnormal shear stress over time results in elliptical deformation of the cell. It is much more common than hereditary spherocytosis, but it is much less likely to cause significant clinical symptomatology. The severity of HE varies markedly, with most patients experiencing little or no symptomatology beyond the finding of elliptocytes on peripheral blood smear. About 10% of patients have hemolytic HE with ongoing hemolysis and anemia. HE is often worse during infancy, with hemolytic anemia and hyperbilirubinemia that evolves to a well-compensated state with anemia that is absent, sporadic, or chronic. Hereditary elliptocytosis occurs worldwide and in all ethnic groups, but it is more common in patients with ancestry linked to areas of endemic malaria.

HPP is a subtype of HE characterized by severe hemolytic anemia with findings on peripheral blood smear reminiscent of a thermal burn (*pyro*, fire). HE and HPP are seen co-segregating in the same families because they involve overlapping pathogenic variants in spectrin. HPP occurs predominantly in patients of African descent.

Table 508.1 Clinical Subtypes of Hereditary Elliptocytosis and Related Disorders

CLINICAL MANIFESTATIONS	LABORATORY MANIFESTATION
TYPICAL HETEROZYGOUS HE	
Asymptomatic Dominant inheritance: one parent with HE No splenomegaly *Variants:* Some neonates have moderately severe hemolytic anemia and HPP-like smear; converts to typical HE by "1 yr." Some patients with typical HE have mild to moderate chronic hemolysis and some poikilocytosis, caused by co-inheritance of the low-expression variant α-spectrin^{LELY}, coexistence of chronic disease producing splenomegaly, or unknown factors.	Blood smear: elliptocytes, rod forms, few or no poikilocytes No anemia, little or no hemolysis (reticulocytes, 1–3%) Normal osmotic fragility Usually a defect in α-spectrin or β-spectrin leading to decreased spectrin self-association, or a defect in protein 4.1 leading to partial deficiency or dysfunction
HOMOZYGOUS HE OR HPP	
Moderate to severe hemolytic anemia Splenomegaly Intermittent jaundice Aplastic crises Recessive inheritance: typically one parent with HE and one with α-spectrin mutation, or both parents with HE Good improvement after splenectomy	Blood smear: bizarre poikilocytes, fragments, ± spherocytes, ± elliptocytes Reticulocytosis Decreased MCV because of red cell fragmentation Increased osmotic fragility, positive EMA test *α-Spectrin defects:* Decreased red cell and spectrin heat stability Marked defect in spectrin self-association In the more severe variants, partial spectrin deficiency (indicated by more spherocytes on the blood smear)
SPHEROCYTIC HE	
Mild to moderate hemolytic anemia Splenomegaly Intermittent jaundice Aplastic crises Dominant inheritance pattern Excellent response to splenectomy	Blood smear: rounded elliptocytes, ± spherocytes; may see variable morphology within a kindred Reticulocytosis Increased osmotic fragility *Variable molecular defects:* C-terminal truncation of β-spectrin Protein 4.2 deficiency (some patients); variable morphology: spherocytes, ovalocytes, stomatocytes, or spiculated cells, sometimes resembling spherocytic HE Glycophorin C deficiency (rare)
SOUTHEAST ASIAN OVALOCYTOSIS	
Anemia and jaundice in neonatal period; then asymptomatic Dominant inheritance Lowland Aboriginal tribes, especially in Melanesia, Malaysia, and the Philippines Very rigid red cells that resist invasion by malarial parasites in vitro and protect against cerebral malaria in vivo	Blood smear: rounded elliptocytes, some with a transverse bar that divides the central clear space (theta cells) No hemolysis or anemia after neonatal period Normal osmotic fragility, positive EMA test Mutant band 3 that lacks anion exchange function and tends to aggregate, leading to a rigid membrane

EMA, Eosin-5′-maleimide; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; MCV, mean cell volume.
Adapted from Wilensky ID, Narla M, Lux SF. Disorders of the red cell membrane. In: Handin RI, Lux SF, Stossel TP, eds. *Blood: Principles and Practice of Hematology*. Philadelphia: Lippincott, 2003.

Southeast Asian ovalocytosis (SAO) is a disorder characterized by the presence of ovalocytes (less elongated and plumper than elliptocytes), some with one or two transverse ridges on peripheral smear. SAO is found in individuals from New Guinea, Malaysia, Indonesia, and the Philippines. Unlike HE and HPP, SAO is due to a variant in a transmembrane protein, band 3, affecting vertical skeletal interactions and leading to increased red cell rigidity. These changes may lead to protection from malaria, particularly cerebral malaria.

ETIOLOGY

Various molecular defects have been described in HE. HE is inherited as an autosomal dominant disorder with occasional de novo cases. Most commonly, there are missense pathogenic variants of α- or β-spectrin that interfere with the formation of spectrin heterodimers into tetramers, the primary structural unit of the membrane skeleton (see Fig. 507.1). Erythrocytes carrying many of these spectrin pathogenic variants are resistant to malaria in vitro, hypothesized to explain the increased prevalence of HE in malaria-endemic areas. Less commonly, elliptocytosis results from pathogenic variants in protein 4.1 or glycophorin C, proteins of the junctional complex that link spectrin tetramers to the actin cytoskeleton. These defects in horizontal membrane skeleton protein interactions leave the cell susceptible to shearing forces, leading to the characteristic elliptical deformation of the cell and potentially membrane fragmentation.

In HPP, two abnormal spectrin alleles are inherited. Frequently, an HPP patient inherits an abnormal spectrin allele carrying a self-association site missense variant from one parent, who has mild or asymptomatic HE, and a production-defective allele that leads to quantitative deficiency of spectrin from the other parent, who is otherwise clinically well.

SAO is an autosomal dominant disorder associated with an in-frame, nine amino acid deletion in band 3.

CLINICAL MANIFESTATIONS

Most HE patients do not have clinically significant hemolysis (see Fig. 507.4B). HE may be an incidental finding on a blood film examination for an unrelated indication. The diagnosis of HE is established by the findings on the peripheral blood smear, the autosomal dominant inheritance pattern, and the absence of other causes of elliptocytosis. The differential diagnosis for other causes of elliptocytosis includes deficiencies of iron, folic acid, and vitamin B_{12}, thalassemia, myelodysplastic syndromes, and pyruvate kinase deficiency.

Interestingly, elliptocytes are not always present on the peripheral blood smear in the first few months of life. Even in hemolytic HE, which may lead to neonatal jaundice and anemia, the peripheral blood smear typically shows bizarre poikilocytes and pyknocytes with rare to no elliptocytes. Hemolysis and anemia are aggravated in the newborn period because of the increased presence of hemoglobin F, which binds poorly to 2,3-diphosphoglycerate. The increased free 2,3-diphosphoglycerate tends to destabilize the spectrin–actin–protein 4.1 complex, leading to membrane instability (see Fig. 507.1). The usual features of a chronic hemolytic process due to hemolytic HE manifest as anemia, jaundice, and splenomegaly. Cholelithiasis may occur in later childhood and aplastic crises have been reported. HPP is characterized by extreme microcytosis (mean corpuscular volume [MCV], 50-65 fL/cell), extraordinary variation in cell size and shape, and microspherocytosis with occasional elliptocytosis (see Fig. 507.4C). Hemolysis is chronic and significant.

SAO is associated with neonatal hyperbilirubinemia, but it is associated with little to no hemolysis later in life.

LABORATORY FINDINGS

Examination of the peripheral blood smear is essential to establish the diagnosis of HE (see Fig. 507.4*B*). HE elliptocytes are normochromic and normocytic with varying degrees of elongation. Because some HE patients may present with relatively low numbers of elliptocytes, there is no cutoff percentage that is useful diagnostically. In hemolytic HE, other abnormal RBC shapes may be present, depending on the severity of hemolysis, including spherocytes, pyknocytes, and other poikilocytes. In HPP, microspherocytes, red cell fragments, and occasional elliptocytes are seen. SAO is suggested when ovalocytes, which in contrast to elliptocytes are less elongated, are observed.

Reticulocyte levels and other markers of hemolysis, such as total bilirubin, lactate dehydrogenase, and haptoglobin, are helpful in establishing the severity of hemolysis, if present. In hemolytic HE and HPP, additional testing may include the eosin-5-maleimide (EMA) binding test, which detects binding to band 3 by flow cytometry, or incubated osmotic fragility testing. In cases of chronic hemolysis, splenomegaly and cholelithiasis can be assessed with abdominal ultrasound.

TREATMENT

If the presentation is that of typical HE (i.e., an isolated peripheral blood smear abnormality without clinically evident hemolysis), no treatment is necessary. For chronic HE and HPP, red blood cell transfusions are occasionally required. Splenectomy decreases the hemolysis and should be considered using criteria similar to that of hereditary spherocytosis. If hemolysis continues after splenectomy, patients should receive folic acid to prevent secondary folic acid deficiency. SAO does not require treatment beyond the newborn period.

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Chapter 509

Hereditary Stomatocytosis Syndromes

Stephanie Prozora and Patrick G. Gallagher

The hereditary stomatocytosis syndromes are a group of heterogeneous, dominantly inherited disorders in which alterations in red cell cation permeability lead to alterations in intracellular water content (Table 509.1). A net increase in sodium and potassium ions allows water to enter the erythrocyte, creating stomatocytes or hydrocytes, whereas a net loss of sodium and potassium ions leads to water loss, creating dehydrated red cells, or xerocytes (Fig. 509.1).

HEREDITARY XEROCYTOSIS

Pathophysiology

Hereditary xerocytosis, the most common type of the hereditary stomatocytosis syndromes, is a dominant disorder of erythrocyte dehydration. The underlying defect is most commonly a missense variant in *PIEZO1*, a mechanosensory transduction protein, associated with delayed channel inactivation. In a few patients, pathogenic variants in the Gardos channel, important in erythrocyte dehydration in sickle cell disease, have been observed. Typically, there is a net loss of intracellular potassium that is not accompanied by a compensatory increase in sodium. Subsequently, the gradual loss of intracellular water leads to erythrocyte dehydration. Hereditary xerocytosis may be associated with a syndrome of hydrops fetalis with perinatal anemia and ascites. These findings are transient and remain unexplained.

Clinical Features

Affected patients exhibit a mild compensated macrocytic hemolytic anemia with variable degrees of splenomegaly and intermittent jaundice. The mean corpuscular hemoglobin concentration (MCHC) and mean cell volume (MCV) are elevated, and erythrocyte osmotic fragility is decreased, as are potassium concentration and total monovalent cation content. There are small numbers of stomatocytes, target cells, and contracted red blood cells (RBCs) with hemoglobin puddled to the side on peripheral blood smear. Treatment is supportive, similar

Southeast Asian ovalocytosis (SAO) is a disorder characterized by the presence of ovalocytes (less elongated and plumper than elliptocytes), some with one or two transverse ridges on peripheral smear. SAO is found in individuals from New Guinea, Malaysia, Indonesia, and the Philippines. Unlike HE and HPP, SAO is due to a variant in a transmembrane protein, band 3, affecting vertical skeletal interactions and leading to increased red cell rigidity. These changes may lead to protection from malaria, particularly cerebral malaria.

ETIOLOGY

Various molecular defects have been described in HE. HE is inherited as an autosomal dominant disorder with occasional de novo cases. Most commonly, there are missense pathogenic variants of α- or β-spectrin that interfere with the formation of spectrin heterodimers into tetramers, the primary structural unit of the membrane skeleton (see Fig. 507.1). Erythrocytes carrying many of these spectrin pathogenic variants are resistant to malaria in vitro, hypothesized to explain the increased prevalence of HE in malaria-endemic areas. Less commonly, elliptocytosis results from pathogenic variants in protein 4.1 or glycophorin C, proteins of the junctional complex that link spectrin tetramers to the actin cytoskeleton. These defects in horizontal membrane skeleton protein interactions leave the cell susceptible to shearing forces, leading to the characteristic elliptical deformation of the cell and potentially membrane fragmentation.

In HPP, two abnormal spectrin alleles are inherited. Frequently, an HPP patient inherits an abnormal spectrin allele carrying a self-association site missense variant from one parent, who has mild or asymptomatic HE, and a production-defective allele that leads to quantitative deficiency of spectrin from the other parent, who is otherwise clinically well.

SAO is an autosomal dominant disorder associated with an in-frame, nine amino acid deletion in band 3.

CLINICAL MANIFESTATIONS

Most HE patients do not have clinically significant hemolysis (see Fig. 507.4B). HE may be an incidental finding on a blood film examination for an unrelated indication. The diagnosis of HE is established by the findings on the peripheral blood smear, the autosomal dominant inheritance pattern, and the absence of other causes of elliptocytosis. The differential diagnosis for other causes of elliptocytosis includes deficiencies of iron, folic acid, and vitamin B_{12}, thalassemia, myelodysplastic syndromes, and pyruvate kinase deficiency.

Interestingly, elliptocytes are not always present on the peripheral blood smear in the first few months of life. Even in hemolytic HE, which may lead to neonatal jaundice and anemia, the peripheral blood smear typically shows bizarre poikilocytes and pyknocytes with rare to no elliptocytes. Hemolysis and anemia are aggravated in the newborn period because of the increased presence of hemoglobin F, which binds poorly to 2,3-diphosphoglycerate. The increased free 2,3-diphosphoglycerate tends to destabilize the spectrin–actin–protein 4.1 complex, leading to membrane instability (see Fig. 507.1). The usual features of a chronic hemolytic process due to hemolytic HE manifest as anemia, jaundice, and splenomegaly. Cholelithiasis may occur in later childhood and aplastic crises have been reported. HPP is characterized by extreme microcytosis (mean corpuscular volume [MCV], 50-65 fL/cell), extraordinary variation in cell size and shape, and microspherocytosis with occasional elliptocytosis (see Fig. 507.4C). Hemolysis is chronic and significant.

SAO is associated with neonatal hyperbilirubinemia, but it is associated with little to no hemolysis later in life.

LABORATORY FINDINGS

Examination of the peripheral blood smear is essential to establish the diagnosis of HE (see Fig. 507.4*B*). HE elliptocytes are normochromic and normocytic with varying degrees of elongation. Because some HE patients may present with relatively low numbers of elliptocytes, there is no cutoff percentage that is useful diagnostically. In hemolytic HE, other abnormal RBC shapes may be present, depending on the severity of hemolysis, including spherocytes, pyknocytes, and other poikilocytes. In HPP, microspherocytes, red cell fragments, and occasional elliptocytes are seen. SAO is suggested when ovalocytes, which in contrast to elliptocytes are less elongated, are observed.

Reticulocyte levels and other markers of hemolysis, such as total bilirubin, lactate dehydrogenase, and haptoglobin, are helpful in establishing the severity of hemolysis, if present. In hemolytic HE and HPP, additional testing may include the eosin-5-maleimide (EMA) binding test, which detects binding to band 3 by flow cytometry, or incubated osmotic fragility testing. In cases of chronic hemolysis, splenomegaly and cholelithiasis can be assessed with abdominal ultrasound.

TREATMENT

If the presentation is that of typical HE (i.e., an isolated peripheral blood smear abnormality without clinically evident hemolysis), no treatment is necessary. For chronic HE and HPP, red blood cell transfusions are occasionally required. Splenectomy decreases the hemolysis and should be considered using criteria similar to that of hereditary spherocytosis. If hemolysis continues after splenectomy, patients should receive folic acid to prevent secondary folic acid deficiency. SAO does not require treatment beyond the newborn period.

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Chapter 509

Hereditary Stomatocytosis Syndromes

Stephanie Prozora and Patrick G. Gallagher

The hereditary stomatocytosis syndromes are a group of heterogeneous, dominantly inherited disorders in which alterations in red cell cation permeability lead to alterations in intracellular water content (Table 509.1). A net increase in sodium and potassium ions allows water to enter the erythrocyte, creating stomatocytes or hydrocytes, whereas a net loss of sodium and potassium ions leads to water loss, creating dehydrated red cells, or xerocytes (Fig. 509.1).

HEREDITARY XEROCYTOSIS

Pathophysiology

Hereditary xerocytosis, the most common type of the hereditary stomatocytosis syndromes, is a dominant disorder of erythrocyte dehydration. The underlying defect is most commonly a missense variant in *PIEZO1*, a mechanosensory transduction protein, associated with delayed channel inactivation. In a few patients, pathogenic variants in the Gardos channel, important in erythrocyte dehydration in sickle cell disease, have been observed. Typically, there is a net loss of intracellular potassium that is not accompanied by a compensatory increase in sodium. Subsequently, the gradual loss of intracellular water leads to erythrocyte dehydration. Hereditary xerocytosis may be associated with a syndrome of hydrops fetalis with perinatal anemia and ascites. These findings are transient and remain unexplained.

Clinical Features

Affected patients exhibit a mild compensated macrocytic hemolytic anemia with variable degrees of splenomegaly and intermittent jaundice. The mean corpuscular hemoglobin concentration (MCHC) and mean cell volume (MCV) are elevated, and erythrocyte osmotic fragility is decreased, as are potassium concentration and total monovalent cation content. There are small numbers of stomatocytes, target cells, and contracted red blood cells (RBCs) with hemoglobin puddled to the side on peripheral blood smear. Treatment is supportive, similar

Table 509.1 Features of Hereditary Stomatocytosis-Xerocytosis Syndromes

FEATURE (NORMAL VALUE)	OTHER HEREDITARY STOMATOCYTOSIS SYNDROMES				HEREDITARY XEROCYTOSIS (DEHYDRATED STOMATOCYTOSIS)	
	OVERHYDRATED HEREDITARY STOMATOCYTOSIS	MILD HEREDITARY STOMATOCYTOSIS	HEREDITARY CRYOHYDROCYTOSIS	SOUTHEAST ASIAN OVALOCYTOSIS	TYPICAL	PSEUDO-HYPERKALEMIA
Hemolysis	Severe	Mild to moderate	Mild to moderate	Mild (neonatal only)	Moderate	Mild or none
Anemia	Moderate to severe	Mild to moderate	Mild to moderate to completely compensated	Mild (neonatal only)	Mild to moderate to completely compensated	None
Blood smear	Stomatocytes; ± spherocytes	Stomatocytes; ± spherocytes	Stomatocytes, sometimes with curved or offset stoma; ± spherocytes; ± target cells	Rounded elliptocytes, some with a double central clearing and a theta (θ) shape; ± stomatocytes	Target cells; sometimes small numbers of echinocytes or stomatocytes	Target cells; few stomatocytes
MCV	Increased	Normal to increased	Normal to increased		High normal to increased	Normal to increased
MCHC	Decreased	Normal to decreased	Normal to increased		Normal to increased	Normal to increased
Unincubated osmotic fragility	Very increased	Variable	Variable	Unknown	Decreased to very decreased	Slightly decreased
RBC Na^+ (5-12 mEq/L)*	60-150	30-60	15-100	Normal	5-30	10-30
RBC K^+ (90-105 mEq/L)*	20-55	40-85	30-100	Normal	60-90	75-100
RBC $Na^+ + K^+$ (95-110 mEq/L)*	110-170	115-145	70-130	Normal	70-100	85-110
RBC passive membrane leak*†	20-40	~3-10	1-6	2-4	2-4	1-2
Cold hemolysis	No	Unknown	Yes	Yes	No	No
Pseudohyperkalemia	Sometimes	Unknown	Yes	Unknown	Sometimes	Yes
Perinatal ascites	No	Unknown	No	No	Sometimes	No
Stomatin markedly decreased	Yes	No	No (type 1) Yes (type 2)	No	No	No
Effect of splenectomy on hemolysis	Some benefit	Some benefit	Minimal or no effect	No significant hemolysis	No effect	No significant hemolysis
Thromboembolism risk after splenectomy	Yes	Unknown	?Yes	Unknown	Yes	Unknown
Genetics	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Defective gene(s)	*KHAG*	Band 3 *(SLC4A1)*	Type 1: Band 3 *(SLC4A1)* Type 2: Glut 1 *(SLC2A1)*	Band 3 *(SLC4A1)* in-frame deletion	*PIEZO1*	*PIEZO1, ABCR6*

*Based on a relatively small number of measurements reported in the literature.
†Times normal. Defined as the ouabain- and bumetanide-resistant $^{86}Rb^+$ influx at 37°C, and expressed as the ratio of patient residual leak to normal residual leak (normal: 0.06-0.10 mmol/L RBC/hr).
MCHC, Mean corpuscular hemoglobin concentration; MCV, mean cell volume; RBC, red blood cell.
From Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015: 561; Table 16.12.

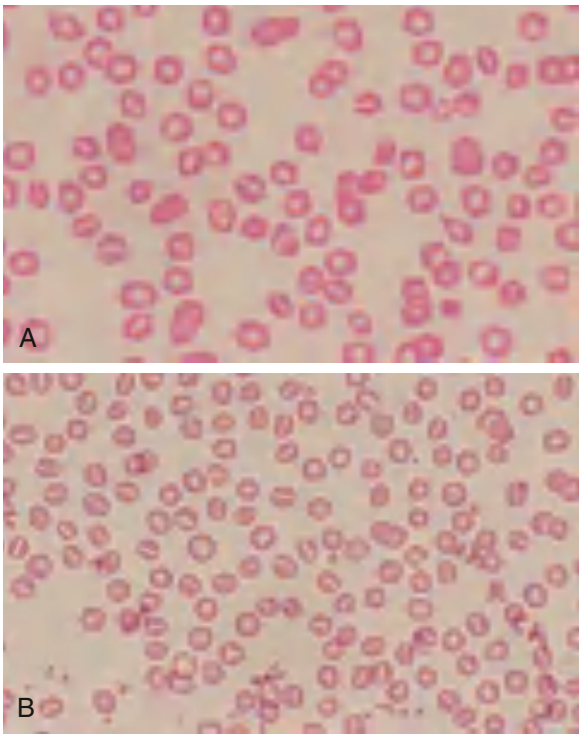

Fig. 509.1 Peripheral blood smears. A, Hereditary xerocytosis. B, Hereditary hydrocytosis.

to other disorders with congenital hemolytic anemia. Because of the apparent predisposition to major thromboses postsplenectomy, *splenectomy is not recommended in hereditary xerocytosis and related disorders.* Another unusual manifestation of hereditary xerocytosis is the propensity for iron overload, independent of transfusion history. Thus iron indices should be monitored at regular intervals.

HEREDITARY HYDROCYTOSIS

Pathophysiology

Hereditary hydrocytosis is a very rare, dominant disorder associated with large, swollen stomatocytic erythrocytes. The principal defect is an increase in Na^+ and K^+ permeability, leading to markedly increased intracellular sodium and water content. The molecular defect is unknown in most cases. In a subset of cases, missense variants in the Rh-associated glycoprotein (RhAG) have been identified.

Clinical Features

Hereditary hydrocytosis is the most clinically severe disorder of altered erythrocyte volume regulation. It is characterized by moderate to severe hemolysis and macrocytosis (110-150 fL), with a low MCHC (24–30%), elevated erythrocyte sodium concentration, reduced potassium concentration, increased total Na^+ and K^+ content, and increased erythrocyte osmotic fragility. There are large numbers (10–30%) of stomatocytes on peripheral blood smear. Patients typically develop jaundice, splenomegaly, and cholelithiasis.

Treatment is supportive. RBC transfusions are occasionally required. Patients should be followed for evidence of hematologic decompensation during acute illness. Interval ultrasonography to detect cholelithiasis should be obtained. When there is significant hemolysis, folate should be prescribed daily. Similar to hereditary xerocytosis, significant postsplenectomy major thromboses have been observed; thus splenectomy is not recommended in hereditary hydrocytosis.

INTERMEDIATE SYNDROMES AND OTHER VARIANTS

Hereditary xerocytosis and hereditary hydrocytosis are at the extremes of disorders with alterations in erythrocyte permeability. Patients with intermediate defects have been described with varying degrees of hemolysis and anemia. One of these intermediate syndromes is **cryohydrocytosis** in which affected patients typically suffer from mild anemia associated with stomatocytes, spherocytes, and sphero-stomatocytes on peripheral blood smear. Cryohydrocytosis erythrocytes are deficient in band 3 and demonstrate a significant cation leak upon cooling to low temperature (*kyros* = cold). This disorder is due to missense variants in band 3 that likely convert band 3 from an anion exchanger to a nonselective cation leak channel.

Rh deficiency syndrome, also known as Rh_{null} syndrome, is mild to moderate hemolytic anemia associated with markedly decreased (Rh_{mod}) or absent (Rh_{null}) Rh antigens on the erythrocyte membrane. Rh_{null} erythrocytes, which lack all Rh antigens, LW, and Fy5 antigens and have decreased reduced expression of Ss, U, and Duclos antigens, are dehydrated with decreased cell cation and water content. Findings on blood smear include reticulocytes, stomatocytes, and spherocytes. In response to immunization during pregnancy or after blood transfusion, Rh_{null} patients produce antibodies varying in specificity from reacting to anti-e or anti-C to reacting with all erythrocytes tested, an antibody called "anti-total Rh."

Familial deficiency of high-density lipoproteins (Tangier disease) is a rare recessive disorder that results from pathogenic variants in the cholesterol and phospholipid transport protein ABCA1, which lead to perturbations of cellular cholesterol transport and result in the accumulation of cholesterol esters in many tissues. Hematologic manifestations include a mild to moderate stomatocytic hemolytic anemia and thrombocytopenia. Affected patients can also have large orange tonsils, hepatosplenomegaly, lymphadenopathy, cloudy corneas, peripheral neuropathy, and premature atherosclerosis.

Sitosterolemia, also known as phytosterolemia, is a recessive disorder in which the absorption of sterols, both cholesterol and its plant-derived relatives (e.g., sitosterol), is unlimited and unselective. Clinical manifestations include early-onset xanthomatosis, short stature, and premature coronary artery disease. Hematologic abnormalities include macrothrombocytopenia and stomatocytic hemolytic anemia. The plasma cholesterol may or may not be abnormal, but mass spectrometry always shows a massive increase in plant sterol levels in the plasma and in the membranes of platelets and erythrocytes. Pathogenic variants in *ABCG5* or *ABCG8*, transporters that actively pump plant sterols out of intestinal cells back into the intestine and out of liver cells into bile ducts, lead to gastrointestinal hyperabsorption and decreased biliary elimination of plant sterols, as well as altered cholesterol metabolism. Treatment involves dietary restriction of cholesterol and plant sterols and prescription of ezetimibe, a sterol absorption inhibitor, and cholestyramine and other related bile acid–sequestering agents.

OTHER DISORDERS ASSOCIATED WITH STOMATOCYTOSIS

Acquired stomatocytosis may be seen with liver disease, alcoholism, malignancy, and cardiovascular disease and after vinca alkaloid administration. Stomatocytes can be seen as a blood smear–processing artifact.

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Chapter 510

Paroxysmal Nocturnal Hemoglobinuria and Acanthocytosis

Stephanie Prozora and Patrick G. Gallagher

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Etiology

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of the cell membranes of multipotent bone marrow stem cells. The underlying somatic pathogenic variant propagates into a clonal population of stem cells so that all blood cells derived from these variant clonal progenitors, especially red blood cells (RBCs), are susceptible to complement-mediated destruction (Fig. 510.1). The pathogenic variant causes cell membranes to be deficient (either partially or completely) in proteins that impede complement-mediated lysis via the constitutively active alternative pathway. These complement regulating proteins include decay-accelerating factor (CD55), the membrane inhibitor of reactive lysis (CD59), and the C8 binding protein. The underlying defect involves the glycolipid anchor that maintains these protective proteins on the cell surface. Various pathogenic variants in the *PIGA* gene that encodes the glycosylphosphatidylinositol anchor protein have been identified in patients with PNH.

Clinical Manifestations

PNH is a rare disorder in children. Approximately 60% of pediatric patients have marrow failure, and the remainder have either intermittent or chronic anemia, often with prominent intravascular hemolysis (Tables 510.1 and 510.2). Nocturnal and morning hemoglobinuria is the classic finding in adults, but only a minority of PNH patients experience this. Most patients experience chronic hemolysis, often with thrombocytopenia and leukopenia. Hemoglobinuria is rarely seen in children compared with adults with PNH. *Thrombosis and thromboembolic phenomena* are serious complications that may be related to altered glycoproteins on the platelet surface and resultant platelet activation and production of procoagulant microparticles. Abdominal venous thrombosis presents as recurrent episodes of abdominal pain, Budd-Chiari syndrome (hepatic veins), or splenomegaly (splenic vein). Released free hemoglobin results in depletion of nitric oxide, fostering vasoconstriction, thrombosis, and pain. Back and head pain may also be prominent. Hypoplastic or aplastic pancytopenia can precede or follow the diagnosis of PNH; rarely, PNH may progress to acute myelogenous leukemia. At the time of presentation, more than 90% of patients with PNH have some blood abnormality (including ~35% with anemia alone, ~15% with anemia and thrombocytopenia, ~7% with anemia and neutropenia, and ~30% with pancytopenia), >10% have abdominal pain, and >5% have thrombosis. The mortality in PNH is related primarily to the development of aplastic anemia or thrombotic complications. The predicted survival rate for children before the development of eculizumab was 80% at 5 years, 60% at 10 years, and 28% at 20 years.

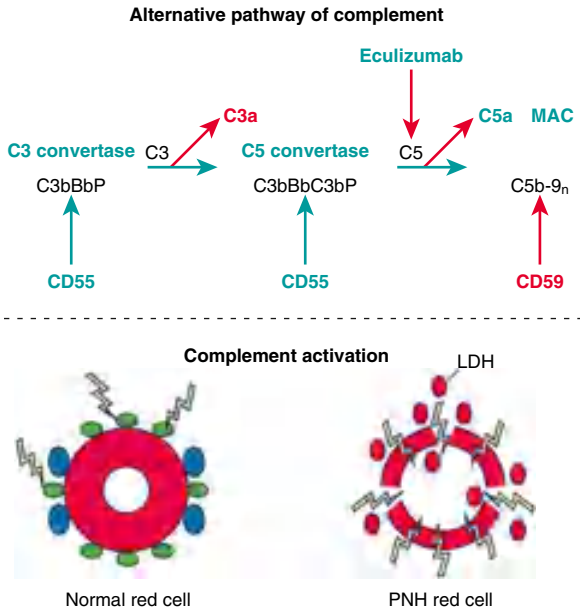


Fig. 510.1 Complement-mediated lysis in paroxysmal nocturnal hemoglobinuria (PNH). *Red circles* are hemoglobin. *Blue circles* are decay accelerating factor (CD55). *Green circles* are membrane inhibitor of reactive lysis (CD59). Bb, activated factor B; C3b, activated C3; C5b, activated C5; MAC, membrane attack complex (consisting of C5b, C6, C7, C8, and several molecules of C9 [9n]). *(From Parker C. Eculizumab for paroxysmal nocturnal haemoglobinuria. Lancet 2009;373:759–767.)*

Laboratory Findings

Hemoglobin levels can range from normal to markedly decreased. Common findings reflect chronic intravascular hemolysis and include hemosiderinuria, an elevated reticulocyte percentage, a low serum haptoglobin, and increased lactic dehydrogenase. Initially, the anemia is normocytic, but if iron deficiency develops, it becomes microcytic. On the blood smear, poikilocytosis and anisocytosis may be present. Markedly reduced levels of RBC acetylcholinesterase activity and decay-accelerating factor are also found. *Flow cytometry is the diagnostic test of choice for PNH*. With the use of anti-CD59 for RBCs and anti-CD55 and anti-CD59 for granulocytes, flow cytometry is more sensitive than the classic RBC lysis (Ham or sucrose) tests in detecting these reduced glycolipid-bound membrane proteins. Fluorescently labeled aerolysin testing can heighten the sensitivity of detection by binding selectively to glycosylphosphatidylinositol anchors.

Treatment

Eculizumab therapy has resulted in sustained survival in the majority of patients. Eculizumab is a monoclonal antibody against complement component C5 that interrupts formation of the membrane attack complex, blocking downstream complement destruction of RBCs and activation of platelets. It decreases the rate of hemolysis, stabilizes hemoglobin levels, reduces the number of transfusions, reduces the risk of thrombosis, and improves quality of life. Eculizumab is an approved and effective treatment for PNH in adults. A recent phase I/II trial demonstrated safety and efficacy in patients 11-17 years of age. Because of the cost and duration of treatment (i.e., lifelong) required, particularly in children, it may be most useful in preventing thrombosis, anemia, and other symptoms while stem cell transplant is considered. Survival in adults with PNH treated with eculizumab may not be different from sex- and age-matched control patients from the general population. However, the medication does not improve the hematopoietic clonal expansion or prevent marrow failure. Before beginning eculizumab, it is recommended to immunize patients with the meningococcal vaccine if the patient hasn't already received this vaccine because a very serious risk of complement inhibition is increased susceptibility to *Neisseria* infections. If eculizumab therapy is desired to begin immediately for clinical reasons, the vaccine can be given at the same time, and prophylactic antibiotics can be given for a 2-week bridge. Headache is a common adverse effect after the first few doses but disappears subsequently. A poor response to eculizumab may be due to polymorphisms in the C5 gene that produce resistance to

Table 510.1 Suggested Criteria as Indications for PNH Testing

CATCH CRITERION	INDICATIONS FOR TESTING	SUPPORTING INFORMATION
Cytopenias	Patients for whom a bone marrow examination is considered for otherwise unexplained cytopenia(s)	Additional features such as elevated LDH, DAT-negative hemolysis, history of unexplained TE, and hemoglobinuria
AA/MDS	All patients with a diagnosis or suspicion of AA Testing should be done at diagnosis and monitored at least q6 months Low or intermediate-1 risk MDS, and especially if hypoplastic	Additional features such as elevated LDH, DAT-negative hemolysis, history of unexplained TE, and hemoglobinuria
Thrombosis	Unprovoked and/or unusual site TE (e.g.,, splenic, hepatic, CNS), especially if recurrent and/or despite anticoagulation	Additional features such as elevated LDH, DAT-negative hemolysis, otherwise-unexplained cytopenias, especially including anemia
Coombs-negative hemolysis	Hemolysis or hemolytic anemia (i.e., elevated LDH and indirect bilirubin, reduced haptoglobin, DAT/Coombs test negative) without other clear cause	Test in all patients unless a clear alternate explanation exists Supportive information may be helpful but is not necessary
Hemoglobinuria	Otherwise-unexplained hemoglobinuria or cases where "hematuria" has been identified without evidence of erythrocytes on microscopy	Test in all patients unless a clear alternate explanation exists Supportive information may be helpful but is not necessary

AA, Aplastic anemia; CNS, central nervous system; DAT, direct antiglobulin test (aka Coombs test); LDH, lactate dehydrogenase; MDS, myelodysplastic syndromes; TE, thromboembolic event.
From Patriquin CJ, Kiss T, Caplan S, et al. How we treat paroxysmal nocturnal hemoglobinuria: A consensus statement of the Canadian PNH Network and review of the national registry. *Eur J Haematol*. 2019;102:36–52: Table 2, p. 39.

Table 510.2 Classification of Paroxysmal Nocturnal Hemoglobinuria

CATEGORY	RATE OF INTRAVASCULAR HEMOLYSIS*	BONE MARROW	FLOW CYTOMETRY	BENEFIT FROM ECULIZUMAB
Classic clinical PNH	Florid (markedly abnormal LDH, often with episodic macroscopic hemoglobinuria)	Cellular marrow caused by erythroid hyperplasia and normal or near-normal morphology†	Large population (>50%) of GPI-AP– PMNs§	Yes
Clinical PNH in the setting of another bone marrow failure syndrome‡	Mild (often with minimal abnormalities of biochemical markers of hemolysis)	Evidence of a concomitant bone marrow failure syndrome‡	Although variable, the percentage of GPI-AP– PMNs is usually relatively small (<50%)	Typically no, but some patients have relatively large clones and clinically significant hemolysis and may benefit from treatment
Subclinical PNH	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant bone marrow failure syndrome‡	Small (<1%) population of GPI-AP– PMNs detected by high-resolution flow cytometry	No

*Based on macroscopic hemoglobinuria, serum LDH concentration and reticulocyte count.
†Karyotypic abnormalities are uncommon.
‡Aplastic anemia or low-risk myelodysplastic syndrome.
§Analysis of PMNs is more informative than analysis of red blood cells because of selective destruction GPI-AP– RBCs.
GPI-AP–, Glycosylphosphatidylinositol–anchored protein–deficient; LDH, lactate dehydrogenase; PMNs, polymorphonuclear leukocytes.
From Parker CJ, Ware RE. Paroxysmal nocturnal hemoglobinuria. In: Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015: Table 14-1.

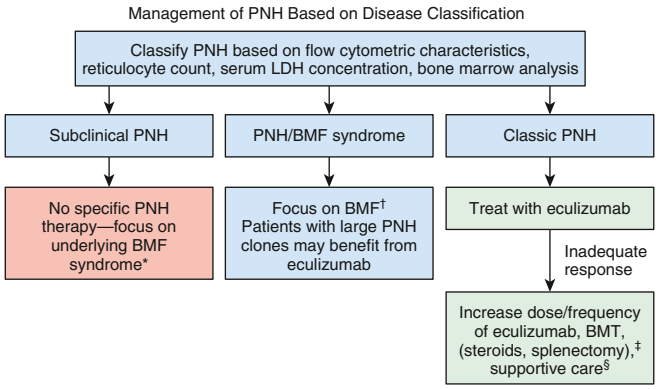
eculizumab blockades. Patients on eculizumab therapy require regular monitoring, including complete blood counts with reticulocytes, lactate dehydrogenase, total bilirubin, and repeat flow cytometry every 6-12 months.

Hematopoietic stem cell transplantation (HSCT) is a key therapeutic consideration if a suitable donor exists, particularly in children. Severe aplastic anemia is a strong indication for transplant in PNH (Fig. 510.2). HSCT is the only potentially curative therapy available for PNH. Nonmyeloablative transplantation (with reduced intensity conditioning regimens) are often used to reduce transplant-related mortality and morbidity; because eradication of only the PNH clones is sought, total myeloablation is not necessary.

Glucocorticoids such as prednisone can be used for acute hemolytic episodes; the dosage should be tapered as soon as the hemolysis abates. Prolonged anticoagulation (heparin or low molecular weight heparin) therapy may be of benefit when thromboses occur. Because of chronic urinary loss of iron as hemosiderin, iron therapy may be necessary. Androgens (e.g., fluoxymesterone), antithymocyte globulin, cyclosporine, and growth factors (e.g., erythropoietin and granulocyte colony-stimulating factor) have been used to treat marrow failure.

ACANTHOCYTOSIS

Acanthocytosis is characterized by RBCs with irregular circumferential pointed projections (also known as spur cells) (see Fig. 507.4E).



*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST).
† BMT eradicates the PNH clone, and typically, treatment with IST does not affect PNH clone size.
‡ Consider for patients with clinically significant extravascular hemolysis.
§ Transfusion.

Fig. 510.2 Management algorithm for paroxysmal nocturnal hemoglobinuria (PNH). A management scheme based on classification of PNH into two categories: subclinical, PNH in the setting of another bone marrow failure syndrome (PNH/BMF), and classic PNH (see Table 510.2 for characteristics of each category.) *LDH*, Lactic dehydrogenase; *BMF*, bone marrow failure (aplastic anemia and low risk MDS); *BMT*, bone marrow transplant. *(From Orkin SH, Fisher DE, Ginsburg D, et al., eds. Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th ed. Philadelphia: Elsevier, 2015. Fig 14-9.)*

Table 510.3 Hereditary Acanthocytosis Syndromes

SYNDROME	INHERITANCE	GENES
Chorea-acanthocytosis	Autosomal recessive	*VPS13A*
McLeod syndrome	X-linked recessive	*XK*
Huntington disease–like 2	Autosomal dominant	*JPH3*
Pantothenate kinase-associated neurodegeneration	Autosomal recessive	*PANK2*
Abetalipoproteinemia	Autosomal recessive	*MTTP*
Hereditary hypobetalipoproteinemia	Autosomal recessive	*APOB, MTP, PCSK9*
Aceruloplasminemia	Autosomal recessive	*CP*

This morphologic finding results from alterations in the membrane ratio of cholesterol to phospholipid, with the morphology attributed to an excess of lipid in the outer layer relative to the inner layer of the membrane bilayer. In liver disease, acanthocytes develop because of increased abundance of free cholesterol, as patients develop splenic congestion, hemolytic anemia, and jaundice. **Abetalipoproteinemia** is an inherited autosomal recessive disease in which acanthocytosis is associated with fat malabsorption, progressive ataxia, and retinitis pigmentosa. The fat malabsorption may become apparent in the first year of life, whereas the ataxia develops at school-age. The anemia is usually mild. **Hypobetalipoproteinemia** is a recessive familial disease that has a similar clinical spectrum, but with milder findings. Aceruloplasminemia leads to anemia, diabetes, ocular problems, tremors, chorea, ataxia, and facial abnormalities due to increased iron in the brain and other organs.

There are four genetically diverse **neuroacanthocytosis syndromes** (Table 510.3). *Chorea-acanthocytosis* is an adult-onset disease without anemia, variable numbers of acanthocytes on peripheral blood smear, with multiple neurologic findings such as limb chorea, tics, and hypotonia. The rare *X-linked McLeod syndrome* (marked by absence of the Kell antigen) presents with mild hemolytic anemia, late-onset myopathy, peripheral neuropathy, chorea, and splenomegaly. There are usually >3% acanthocytes on peripheral smear and caudate atrophy noted on MRI. McLeod syndrome is the only neuroacanthocytosis syndrome likely to present in childhood. Acanthocytes also are seen in *pantothenate kinase-associated neurodegeneration* (with dystonia, rigidity, chorea, dysarthria, spasticity, retinopathy) and *Huntington disease–like 2*.

In contrast to acanthocytes, echinocytes or "burr cells" have a more regular distribution of projections or serrations along the surface of the RBCs and will tend to a more spheroidal cell contour as they age. They are seen often as artifacts (e.g., due to elevated pH, contact with glass, or blood storage) and infrequently in end-stage renal disease, liver disease, uremia, pyruvate-kinase deficiency, long-distance runners, and patients with hypomagnesemia and hypophosphatemia.

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Chapter 511

Hemoglobinopathies

Kim Smith-Whitley and Janet L. Kwiatkowski

HEMOGLOBIN DISORDERS

Hemoglobin is a tetramer consisting of two pairs of globin chains. Abnormalities in these proteins are referred to as **hemoglobinopathies**.

More than 800 variant hemoglobins have been described. The most common and useful clinical classification of hemoglobinopathies is based on nomenclature associated with alteration of the involved *globin chain*. Two hemoglobin (Hb) gene clusters are involved in Hb production and are located at the end of the short arms of chromosomes 16 and 11. Their control is complex, including an upstream locus control region on each respective chromosome and an X-linked control site. On chromosome 16, there are three genes within the alpha (α) gene cluster: zeta (ζ), alpha 1 (α_1), and alpha 2 (α_2). On chromosome 11, there are five genes within the beta (β) gene cluster: epsilon (ε), gamma 1 (γ_1), gamma 2 (γ_2), delta (δ), and beta (β).

The order of gene expression within each cluster roughly follows the order of expression during the embryonic period, fetal period, and eventually childhood. After 8 weeks of fetal life, the embryonic hemoglobins, Gower-1 ($\zeta_2\varepsilon_2$), Gower-2 ($\alpha_2\varepsilon_2$), and Portland ($\zeta_2\gamma_2$), are formed. At 9 weeks of fetal life, the major hemoglobin is hemoglobin F (HbF; $\alpha_2\gamma_2$). Hemoglobin A (HbA; $\alpha_2\beta_2$) first appears at approximately 1 month of fetal life but does not become the dominant hemoglobin until after birth, when HbF levels start to decline. HbA_2 ($\alpha_2\delta_2$) is a minor hemoglobin that appears shortly before birth and remains at a low level after birth. The final hemoglobin distribution pattern that occurs in childhood is not achieved until at least 6 months of age and sometimes later. The normal hemoglobin pattern is ≥95% HbA, ≤3.5% HbA_2, and <2.5% HbF.

511.1 Sickle Cell Disease

Kim Smith-Whitley and Janet L. Kwiatkowski

Children with sickle cell disease should be followed by experts in the management of this disease, most often by pediatric hematologists. Children who receive disease-specific care that focuses on prevention of infectious complications and end-organ damage should have a

higher likelihood of survival to adult age. Medical care provided by a pediatric hematologist is also associated with a decreased frequency of emergency department (ED) visits and length of hospitalization compared to patients who were not seen by a hematologist within the last year.

PATHOPHYSIOLOGY

Hemoglobin S (HbS) is the result of a single base-pair change, thymine for adenine, at the 6th codon of the β-globin gene. This change encodes valine instead of glutamine in the sixth residue in the β-globin molecule. Sickle cell anemia (HbSS), homozygous HbSS, occurs when both β-globin alleles have the sickle cell pathogenic variant (βs). Sickle cell disease refers not only to patients with sickle cell anemia but also to compound heterozygotes where one β-globin allele includes the sickle cell pathogenic variant and the second β-globin allele includes a gene pathogenic variant other than the sickle cell pathogenic variant, such as HbC, β-thalassemia, HbD, and HbO^{Arab}. In sickle cell anemia, HbS is typically as high as 90% of the total hemoglobin, whereas in sickle cell disease, HbS is >50% of all hemoglobin.

In red blood cells (RBCs), the hemoglobin molecule has a highly specified conformation allowing for the transport of oxygen in the body. In the absence of globin-chain pathogenic variants, hemoglobin molecules do not interact with one another. However, the presence of HbS results in a conformational change in the Hb tetramer, and in the deoxygenated state, HbS molecules interact with each other, forming rigid polymers that give the RBC its characteristic "sickled" shape. Intraerythrocytic changes lead to a shortened RBC life span and hemolysis. Hemolysis leads to multiple changes, including altered nitric oxide metabolism and oxidant stress, which contribute to endothelial dysfunction. Intravascular sickling primarily occurs in the postcapillary venules and is a function of both mechanical obstruction by sickled erythrocytes, platelets, and leukocytes and increased adhesion between these elements and the vascular endothelium. Sickle cell disease is also an inflammatory disease based on nonspecific markers of inflammation, including, but not limited to, elevated baseline white blood cell (WBC) count and cytokines.

DIAGNOSIS AND EPIDEMIOLOGY

Every state in the United States has instituted a mandatory newborn screening program for sickle cell disease. Such programs identify newborns with the disease and provide prompt diagnosis and referral to providers with expertise in sickle cell disease for anticipatory guidance and the initiation of penicillin prophylaxis before 4 months of age.

The most commonly used procedures for newborn diagnosis include thin-layer/isoelectric focusing (IEF) and high-performance liquid chromatography (HPLC). Some laboratories perform genetic testing on specimens demonstrating abnormal hemoglobins. A confirmatory step is recommended, with all patients who have initial abnormal screens being retested during the first clinical visit, which helps to account for potential administrative errors in specimen handling. In addition, a complete blood cell count (CBC) and Hb phenotype determination is recommended for both parents to provide an opportunity for genetic counseling and to better characterize disease genotype in the affected offspring if necessary. Infants who may have **HbS-hereditary persistence fetal hemoglobin (HbSHPFH)** but do not have full parental studies should have molecular testing for β-globin genotype before 12 months of age (or sooner if hydroxyurea is initiated). Table 511.1 correlates the initial hemoglobin phenotype at birth with the type of hemoglobinopathy.

In newborn screening programs, the hemoglobin with the greatest quantity is reported first, followed by other hemoglobins in order of decreasing quantity. Some states perform IEF initially on newborn blood samples, then use DNA probes to confirm abnormal hemoglobins found on IEF. In newborns with a hemoglobin analysis result of **HbFS**, the pattern supports HbSS, HbSHPFH, or $HbS\beta^0$-thalassemia. In certain situations, a newborn with a hemoglobin analysis of HbFS may have $HbS\beta^+$-thalassemia and the hemoglobin A may not be demonstrating on the electrophoresis in quantities high enough for detection. In a newborn with a hemoglobin analysis of **FSA**, the pattern is supportive of the diagnosis of $HbS\beta^+$-thalassemia. The diagnosis of $HbS\beta^+$-thalassemia is confirmed if at least 50% of the hemoglobin is HbS, HbA is present, and the amount of HbA_2 is elevated (typically >3.5%), although HbA_2 is not elevated in the newborn period. In newborns with a hemoglobin analysis of **FSC**, the pattern supports a diagnosis of HbSC. In newborns with a hemoglobin analysis of **FAS**, the pattern supports a diagnosis of HbAS (sickle cell trait); however, in this circumstance, care must be taken to confirm that the newborn has not received an RBC transfusion before testing.

A newborn with a hemoglobin analysis of **AFS** either has been transfused with RBCs before collection of the newborn screen to account for the greater amount of HbA than HbF, or there has been an error. The patient may have either sickle cell disease or sickle cell trait and

Table 511.1 Various Newborn Sickle Cell Disease Screening Results with Baseline Hemoglobin

NEWBORN SCREENING RESULTS: SICKLE CELL DISEASE*	POSSIBLE HEMOGLOBIN PHENOTYPE†	BASELINE HEMOGLOBIN RANGE AFTER AGE 5 YR
FS	SCD-SS	6-11 g/dL
	SCD-S β^0 thal	6-10 g/dL
	SCD-S β^+ thal	9-12 g/dL
	SCD-S $\delta\beta^-$ thal	10-12 g/dL
	S HPFH	12-14 g/dL
FSC	SCD-SC	10-15 g/dL
FSA‡	SCD-S β^+ thal	9-12 g/dL
FS other	SCD-S β^0 thal	6-10 g/dL
	SCD-SD, SO^{Arab}, SC^{Harlem}, S^{Lepore}	Variable
AFS‡§	SCD-SS	6-10 g/dL
	SCD-S β^+ thal	6-9 g/dL
	SCD-S β^0 thal	7-13 g/dL variable

*Hemoglobins are reported in order of quantity.
†Requires confirmatory hemoglobin analysis after at least 6 mo of age and, if possible, β-globin gene testing or hemoglobin analysis from both parents for accurate diagnosis of hemoglobin phenotype.
‡Sickle cell trait is another possible diagnosis.
§Impossible to determine the diagnosis because the infant most likely received a blood transfusion before testing.
A, Normal hemoglobin A; C, hemoglobin C; F, fetal hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; O^{Arab}, hemoglobin O^{Arab}; S, sickle hemoglobin; SC, sickle-hemoglobin C; SCD, sickle cell disease; SS, homozygous sickle cell disease; thal, thalassemia.

should be started on penicillin prophylaxis until the final diagnosis can be determined.

Given the implications of a diagnosis of sickle cell disease versus sickle cell trait in a newborn, the importance of repeating the Hb identification analysis in the patient and obtaining a Hb identification analysis and CBC to evaluate the peripheral blood smear and RBC parameters in the parents cannot be overemphasized. Unintended mistakes do occur in state newborn screening programs. Newborns who have the initial phenotype of HbFS but whose final true phenotype is HbSβ$^+$-thalassemia have been described as one of the more common errors identified in newborn screening hemoglobinopathy programs. Determining an accurate phenotype is important for appropriate genetic counseling for the parents. In addition, distinguishing HbSS from HbSHPFH in the newborn period usually requires parental or genetic testing. Infants who maintain HbF percentages above 25% after 12 months of age without evidence of hemolysis should have testing for β-globin gene deletions consistent with HPFH. These children have a much milder clinical course and do not require penicillin prophylaxis or hydroxyurea therapy.

If the parents are tested for sickle cell trait or hemoglobinopathy trait, full disclosure to the parent tested should be provided privately, and in some circumstances, the issue of paternity may be disclosed. For this reason and because of healthcare privacy, common practice is to always seek permission for the genetic testing and to report the hemoglobinopathy trait results back to each parent separately.

In the United States, sickle cell disease is the most common genetic disorder identified through the state-mandated newborn screening program, occurring in 1:2,647 births. The sickle hemoglobin gene is more prevalent in communities of color because descendants of people where malaria has been endemic are more likely to have been protected from the severe manifestations of malaria. Regarding ethnicity in the United States, sickle cell disease occurs in Black persons at a rate of 1:396 births and in Hispanics at a rate of 1:36,000 births. In the United States, an estimated 100,000 people have sickle cell disease, with an ethnic distribution of 90% Black and 10% Hispanic. The U.S. sickle cell disease population represents a fraction of the worldwide burden of the disease, with global estimates of 312,000 neonates born annually with HbSS disease.

CLINICAL MANIFESTATIONS AND TREATMENT OF SICKLE CELL ANEMIA

For a comprehensive discussion of the clinical management of children and adolescents with sickle cell disease, refer to the National Heart, Lung, and Blood Institute (NHLBI) 2014 Expert Panel Report on the Evidence-Based Management of Sickle Cell Disease (https://www.nhlbi.nih.gov/sites/www.nhlbi.nih.gov/files/sickle-cell-disease-report.pdf).

Fever and Bacteremia

Fever in a child with sickle cell anemia is a medical emergency, requiring prompt medical evaluation and delivery of antibiotics because of the increased risk of serious bacterial infection and subsequent high mortality rate. As early as 6 months of age, infants with sickle cell anemia develop abnormal immune function because of splenic dysfunction. By 5 years of age, most children with sickle cell anemia have complete functional asplenia. Regardless of age, all patients with sickle cell anemia are at increased risk of infection and death from bacterial infection, particularly encapsulated organisms such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis,* as well as *Salmonella* spp.

Several clinical strategies have been developed to manage children with sickle cell disease who present with fever. These strategies range from hospital admission for intravenous (IV) antimicrobial therapy to administering a third-generation cephalosporin in an ED or outpatient setting to patients without established risk factors for occult bacteremia (Table 511.2). Given the observation that the average time for a positive blood culture is <20 hours in children with sickle cell anemia, admission for 24 hours is probably the most prudent strategy for children and families who live out of town or who are identified as high risk for poor follow-up.

Table 511.2 Clinical Factors Associated with Increased Risk of Acute Complications in Febrile Children with Sickle Cell Disease

Seriously ill appearance
Hypotension: systolic blood pressure <70 mm Hg at 1 yr of age or <70 mm Hg + 2 × age (yr) for older children
Poor perfusion: capillary refill time >4 sec
Temperature >40.0°C (104°F)
Hypoxia
Corrected white blood cell count >30,000/mm^3 or <5,000/mm^3
Platelet count <100,000/mm^3
History of pneumococcal sepsis*
Severe pain
Dehydration: poor skin turgor, dry mucous membranes, history of poor fluid intake, or decreased output of urine
Presence of acute chest syndrome (new infiltrate on chest radiograph)
Hemoglobin level <5.0 g/dL
No prophylactic antibiotics
Not immunized

*Or other serious infection requiring hospital admission.
Adapted from Williams JA, Flynn PM, Harris S et al. A randomized study of outpatient treatment with ceftriaxone for selected febrile children with sickle cell disease. *N Engl J Med* 1993;329:472–476.

Outpatient management of fever without a source should be considered in children with the lowest risk of bacteremia and after appropriate cultures are obtained and IV ceftriaxone or another cephalosporin is given. Observation after antibiotic administration is important because children treated with ceftriaxone can develop severe, rapid, and life-threatening immune hemolysis. In the event that *Salmonella* spp. or *Staphylococcus aureus* bacteremia occurs, strong consideration should be given to an evaluation for osteomyelitis with an MRI, given the increased risk of osteomyelitis in children with sickle cell anemia compared to the general population. Screening laboratory and radiologic studies are strongly recommended to identify those at risk for transient red cell aplasia, acute splenic sequestration, and acute chest syndrome (ACS), because many children with these diagnoses present to acute care settings with isolated fever. Screening children and caregivers for psychosocial factors that could impede their return to the hospital in the case of a positive blood culture is essential.

Aplastic Crisis

Human parvovirus B19 infection poses a unique threat for patients with sickle cell disease because this infection results in temporary **red cell aplasia**, limiting the production of reticulocytes and causing profound anemia (see Fig. 507.3 in Chapter 507). Any child with sickle cell disease, fever, and *reticulocytopenia* should be presumed to have parvovirus B19 infection until proven otherwise. However, reticulocytopenia is not a requirement for the diagnosis of a recent parvovirus B19 infection because reticulocytosis and increased nucleated RBCs may be seen in the recovery phase. Diagnostic testing for the presence of human parvovirus B19 with polymerase chain reaction (PCR) testing is superior to using immunoglobulin M (IgM) and IgG titers. The acute exacerbation of anemia is treated conservatively using red cell transfusion when the patient becomes hemodynamically symptomatic or has a concurrent illness, such as ACS or acute splenic sequestration. Children with suspected aplastic crisis should be closely monitored because acute infection with parvovirus B19 is associated with pain, splenic sequestration, ACS, glomerulonephritis, arthropathy, and stroke. Patients with parvovirus-associated aplastic crisis are contagious, and infection precautions should be taken to avoid nosocomial spread of the infection and to avoid exposure of pregnant caregivers who may be at risk for adverse fetal outcomes with acute infection.

Splenic Sequestration

Acute splenic sequestration is a life-threatening complication occurring primarily in infants and young children with sickle cell anemia.

The incidence of splenic sequestration has declined from an estimated 30% to 12.6% with early identification by newborn screening and improved parental education. Sequestration can occur as early as 5 weeks of age but most often occurs in children between ages 6 months and 2 years. Patients with the SC and Sβ$^+$-thalassemia types of sickle cell disease can have acute splenic sequestration events throughout adolescence and adulthood.

Splenic sequestration is associated with rapid spleen enlargement causing left-sided abdominal pain and Hb decline from the patient's baseline. Sequestration may lead to signs of hypovolemia as a result of the trapping of blood in the spleen and profound anemia, with total Hb falling below 3 g/dL. A decrease in WBC and platelet count may also be present. Sequestration may be spontaneous or triggered by fever, bacteremia, or viral infections.

Treatment includes early intervention and maintenance of hemodynamic stability using isotonic fluid or transfusions. Careful blood transfusions with RBCs are recommended to treat both the sequestration and the resultant anemia. Blood transfusion aborts the RBC trapping in the spleen and allows release of the patient's blood cells that have become sequestered, often raising Hb above baseline values. A reasonable approach is to provide only 5 mL/kg of RBCs and/or a posttransfusion Hb target of 8 g/dL, keeping in mind that the goal of transfusion is to prevent hypovolemia. Blood transfusion that results in Hb levels >10 g/dL may put the patient at risk for **hyperviscosity syndrome** because RBCs may be released from the spleen after transfusion.

Repeated episodes of splenic sequestration are common, occurring in 65% of patients. Most recurrent episodes develop within 6 months of the previous episode. Prophylactic splenectomy performed after an acute episode has resolved is the only effective strategy for preventing future life-threatening episodes. Although blood transfusion therapy has been used with the goal of preventing subsequent episodes, evidence strongly suggests that this strategy does not reduce the risk of recurrent splenic sequestration compared to no transfusion therapy. However, a short course of regular RBC transfusions can be used until splenectomy is arranged. Children should be appropriately immunized with meningococcal and pneumococcal vaccines before surgery. Penicillin prophylaxis should be prescribed after splenectomy.

Hepatic and Gallbladder Involvement

See Chapters 408 and 414.

Sickle Cell Pain

Dactylitis, referred to as **hand-foot syndrome**, is often the first manifestation of pain in infants and young children with sickle cell anemia, occurring in 50% of children by their second year of life (Fig. 511.1). Dactylitis often manifests with symmetric or less often unilateral swelling of the hands and/or feet. Unilateral dactylitis can be confused with osteomyelitis, and careful evaluation to distinguish the two is important because treatment differs significantly. Dactylitis requires palliation with pain medications, whereas osteomyelitis requires at least 4-6 weeks of IV antibiotics. Feedback from the parents is needed to determine if pain therapy is successful in relieving pain.

The cardinal clinical feature of sickle cell disease is **acute vasoocclusive pain**. Acute sickle cell pain is characterized as unremitting discomfort that can occur in any part of the body but most often occurs in the chest, abdomen, or extremities. These painful episodes are often abrupt and cause disruption of daily life activities and significant stress for children and their caregivers. A patient with sickle cell anemia has approximately one painful episode per year that requires medical attention, but the frequency is extremely variable. The pattern of symptoms with subsequent episodes may resemble that of previous episodes.

The exact etiology of pain is unknown, but the pathogenesis may be initiated when blood flow is disrupted in the microvasculature by sickled RBCs and other cellular elements, resulting in tissue ischemia. Acute sickle cell pain may be precipitated by physical stress, infection, dehydration, hypoxia, local or systemic acidosis, exposure to cold, and swimming for prolonged periods. However, most pain episodes occur without an identifiable trigger. Successful treatment of these episodes requires education of both caregivers and patients regarding the recognition of symptoms and the optimal management strategy. Given the absence of any reliable objective laboratory or clinical parameter associated with pain, trust between the patient and the treating physician is paramount to successful clinical management. Specific therapy for pain varies greatly but generally includes the use of acetaminophen or a nonsteroidal antiinflammatory drug (NSAID) early in the course of pain, followed by escalation to a combination analgesic regimen using a single-agent short-acting oral opioid, long-acting oral opioid, and continued nonopioid agent.

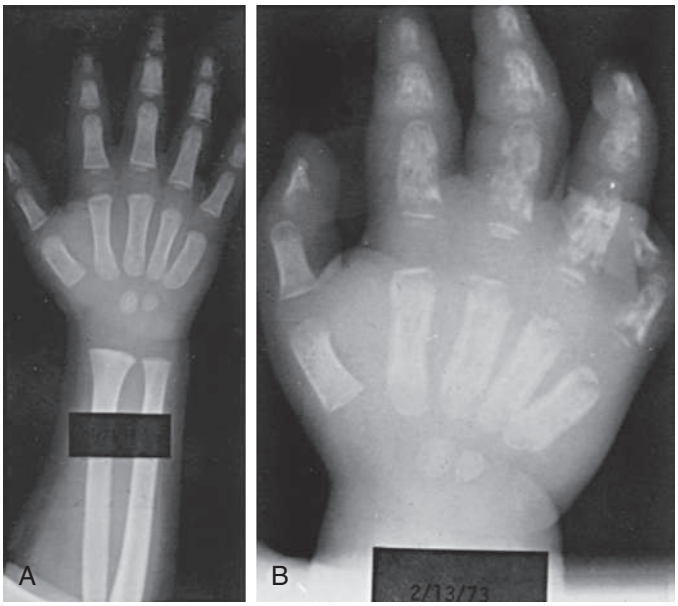

Fig. 511.1 Radiographs of an infant with sickle cell anemia and acute dactylitis. **A**, The bones appear normal at the onset of the episode. **B**, Destructive changes and periosteal reaction are evident 2 weeks later.

The majority of painful episodes in patients with sickle cell disease are managed at home with comfort measures, such as heating pad, relaxation techniques, massage, and oral pain medication. Some patients require treatment in an acute care setting with IV morphine or derivatives of morphine. The primary goal of treatment in these settings is timely administration of analgesics to provide relief of pain. The incremental increase and decrease in the use of the medication to relieve pain roughly parallels the eight phases associated with a chronology of pain and comfort in children (Table 511.3). When pain requires continued parenteral analgesic administration, hospitalization or prolonged stays in day hospitals are required. The average hospital length of stay for children admitted in pain is 4.4 days. The American Society of Hematology and NHLBI clinical guidelines for treating acute and chronic pain in children and adults with sickle cell disease are comprehensive and represent a starting point for treating pain.

The only measure for degree of pain is the patient. Healthcare providers working with children in pain should use a consistent, validated pain scale (e.g., Wong-Baker FACES Scale) for assessing pain. Although pain scales have proved useful for some children, other forms of pain assessment may be required to determine when opioid therapy should be initiated and decreased. Individualized pain plans provide important information for assessment and treatment that address patient preferences.

Several myths have been propagated regarding the treatment of pain in sickle cell disease. The concept that all painful episodes in children should be managed without opioids is without foundation and results in unwarranted suffering on the part of the patient. Blood transfusion therapy during an existing painful episode does not decrease the intensity or duration of the painful episode. Aggressive IV hydration does

Table 511.3 Phases of a Painful Episode in Patients with Sickle Cell Disease

PHASE	DESCRIPTION AND COMFORT MEASURES
DATA FROM CHILDREN	
I	*Baseline* No pain and no comfort measures
II	*Prepain phase* No evidence of pain Child begins to display some prodromal signs and symptoms of VOE (yellow eyes, fatigue) No comfort measures used Caregivers encouraged child to increase fluids to prevent the pain event from occurring
III	*Pain starting point* Child complained of mild "ache-ish" pain in one specific area, which gradually or rapidly increased or "waxed" Mild analgesics (ibuprofen and acetaminophen) given Child maintained normal activities and continued to attend school Caregivers hoped to prevent an increase in pain intensity
IV	*Pain acceleration* Pain continued to escalate; intensity increased from mild to moderate; pain appeared in more areas of the body; child was kept home from school; decreased level of activity; differences in behaviors, appearance, and mood Stronger oral analgesics may be combined with rest, rubbing, heat, distraction, and psychologic comfort
V	*Peak pain experience* Pain continued to escalate Some children were incapacitated and unable to obtain pain relief Pain described as "stabbing," "drilling," "pounding," "banging," "excruciating," "unbearable," or "throbbing" Caregivers sometimes decide to seek help from ED for stronger analgesics and protection from complications such as fever or respiratory distress Caregivers may be exhausted from caring for the child for several days with little or no rest All methods of comfort were used around the clock to reduce the pain and avoid going to the hospital Pain increased despite all efforts Decision is made to take the child to ED
VI	*Pain decrease starting point* Pain begins to resolve after the use of IV fluids and analgesics Analgesics sedate the child and allow the child to sleep for longer periods Pain described as "slowly decreasing" Pain is still sharp and throbbing
VII	*Steady pain decline* Pain decreased slowly or rapidly Child takes more interest in surroundings, roommates, and visitors Child is less irritable Level of activity increased—child may be taken to tub room for warm bath, may watch television, may play games with other children or hospital volunteers Mobility has improved Pain levels reported as "just a little" More animation in behaviors evident
VIII	*Pain resolution* Pain is at a tolerable level Child may be discharged from the hospital on mild oral analgesics; child is at or close to baseline conditions, with behavior, appearance, and mood more normal Caregiver and child attempt to regain, recapture, and catch up with life as it was before the pain event
DATA FROM ADULTS	
I	*Evolving/infarctive phase* 3 days ↓ RBC deformability ↓ Hemoglobin ↑ % of dense RBCs ↑ RDW, ↑ HDW S/S: fear, anorexia, anxiety, ↑ pain

Table 511.3 Phases of a Painful Episode in Patients with Sickle Cell Disease—cont'd

PHASE	DESCRIPTION AND COMFORT MEASURES
II	*Postinfarctive/inflammatory phase* 4-5 days ↓ Hemoglobin ↑ White blood cells (leukocytosis) ↑ Acute-phase reactants C-reactive protein ↑ Reticulocytes, ↑ LDH, ↑ CPK ↑ % dense RBCs ↑ RDW, ↑ HDW S/S: fever, severe steady pain, swelling, tenderness, joint stiffness, joint effusions
III	*Resolving/healing/recovery/postcrisis phase* ↑ RBC deformability Hemoglobin returns to precrisis level Retics return to precrisis levels ↓ % of dense RBCs ↓ RDW, ↓ HDW ↓ ISC Precursors to relapse that happens in phase III: ↑ platelets, ↑ acute-phase reactants (fibrinogen, α_1-acid glycoprotein, osmomucoid), ↑ viscosity, ↑ ESR ↑ Retics expressing the ↑ $\alpha_4\beta_1$-integrin complex ICAM-1

CPK, Creatinine phosphokinase; ED, emergency department; ESR, erythrocyte sedimentation rate; HDW, hemoglobin distribution width; ICAM, intracellular adhesion molecule; ISC, irreversibly sickled cells; IV, intravenous; LDH, lactate dehydrogenase; RBC, red blood cell; RDW, red cell distribution width; S/S, signs and symptoms; VOE, vasoocclusive episode.
Adapted from Jacob E. The pain experience of patients with sickle cell anemia. *Pain Manage Nurs* 2001;2:74–83; with data from Ballas SK, Smith ED. Red blood cell changes during the evolution of the sickle cell painful crisis. *Blood* 1992;79:2154–2163; and Beyer JE, Simmons L, Woods GM, Woods PM. A chronology of pain and comfort in children with sickle cell disease. *Arch Pediatr Adolesc Med* 1999;153:913–920.

not relieve or prevent pain and is appropriate when the patient is dehydrated or unable to drink as a result of the severe pain. *Opioid dependency in children with sickle cell disease is rare and should never be used as a reason to withhold pain medication.* However, patients with multiple painful episodes requiring hospitalization within 1 year or with pain episodes that require hospitalization for >7 days should be evaluated for comorbidities and environmental stressors that are contributing to the frequency or duration of pain. Children with chronic pain should be evaluated for other disease-related complications, including, but not limited to, presence of avascular necrosis, leg ulcers, and vertebral body compression fractures. A careful history is warranted to distinguish chronic pain that often is not relieved by opioids versus recurrent acute prolonged vasoocclusive pain episodes.

Skeletal pain (bone or bone marrow infarction) with or without fever must be differentiated from **osteomyelitis**. Both *Salmonella* spp. and *S. aureus* cause osteomyelitis in children with sickle cell disease, often involving the diaphysis of long bones (in contrast to children without sickle cell anemia, in whom osteomyelitis is in the metaphyseal region of the bone). Differentiating osteomyelitis from a vasoocclusive crisis is often difficult. Clinical signs and symptoms can be consistent with both osteomyelitis and vasoocclusive crises because low-grade fever pain, swelling of the affected area, high WBC counts, and elevated C-reactive protein levels can be present in both. Blood, fluid, and tissue cultures, when positive, are helpful. MRI may be useful for locating an area to obtain fluid for culture. Ultimately, aspiration with or with or without biopsy and culture will be needed to differentiate the two processes (see Chapter 725).

Avascular Necrosis

Avascular necrosis **(AVN)** occurs at a higher rate among children with sickle cell disease than in the general population and is a source of both acute and chronic pain. Most often, the femoral head is affected. AVN of the hip may cause limp and leg-length discrepancy. Other sites affected include the humeral head and mandible. Risk factors for AVN include HbSS disease with α-thalassemia trait, frequent vasoocclusive episodes, and elevated hematocrit (for patients with sickle cell anemia). Optimal treatment of AVN has not been determined, and individual management requires consultation with a sickle cell specialist, orthopedic surgeon, physical therapist, and primary care physician. Initial management should include referral to a pediatric orthopedist and a physical therapist to address strategies to increase strength and decrease weight-bearing daily activities that may exacerbate the pain associated with AVN as well as to determine whether surgical approaches may be beneficial. Opioids are often used but usually can be tapered after the acute pain has subsided. Regular blood transfusion therapy has not been demonstrated as an effective therapy to abate the acute and chronic pain associated with AVN.

Priapism

Priapism, defined as an unwanted painful erection of the penis, affects males of all sickle genotypes but most frequently affects males with sickle cell anemia. The mean age of first episode is 15 years, although priapism has been reported in children as young as 3 years. The actuarial probability of a patient experiencing priapism is approximately 90% by 20 years of age.

Priapism occurs in two patterns: *prolonged*, lasting >4 hours, or *stuttering*, with brief episodes that resolve spontaneously but may occur in clusters and herald a prolonged event. Both types occur from early childhood to adulthood. Most episodes occur between 3 AM and 9 AM. Priapism in sickle cell disease represents a low-flow state caused by venous stasis from RBC sickling in the corpora cavernosa. Recurrent prolonged episodes of priapism are associated with erectile dysfunction (impotence).

The optimal treatment for acute priapism is unknown. Supportive therapy, such as a hot shower, short aerobic exercise, or pain medication, is often used by patients at home. A prolonged episode lasting >4 hours should be treated by aspiration of blood from the corpora cavernosa, followed by irrigation with dilute epinephrine to produce immediate and sustained *detumescence.* Urology consultation is required to initiate this procedure, with appropriate input from a hematologist. Simple blood transfusion with exchange transfusion has been proposed for the acute treatment of priapism, but limited evidence supports this strategy as the initial management. If no benefit is obtained from surgical management, transfusion therapy should be considered. However, detumescence may not occur for up to 24 hours (much longer than with urologic aspiration) after transfusion, and transfusion for priapism has been associated with acute neurologic events. Consultation with a hematologist and urologist will help identify therapies to prevent recurrences.

Neurologic Complications

Neurologic complications associated with sickle cell disease are varied and complex, ranging from acute ischemic stroke with focal neurologic deficit to clinically *silent* abnormalities found on imaging. Before the development of transcranial Doppler ultrasonography to screen for stroke risk among children with sickle cell anemia, approximately 11% experienced an overt stroke before age 20. A functional definition of *overt stroke* is the presence of a focal neurologic deficit lasting for >24 hours and/or abnormal neuroimaging of the brain indicating a cerebral infarct on T2-weighted MRI corresponding to the focal neurologic deficit (Figs. 511.2 and 511.3). A *silent cerebral infarct* lacks focal neurologic findings and is diagnosed by abnormal imaging on T2-weighted MRI. The prevalence of silent infarct among children with HbSS is around 35%. Children with other types of sickle cell disease, such as HbSC or HbSβ$^+$-thalassemia, develop overt or silent cerebral infarcts as well, but at a lower frequency than children with HbSS and HbSβ^0-thalassemia. Other neurologic complications include transient ischemic attacks, headaches that may or may not correlate to degree of anemia, seizures, cerebral venous thrombosis, cerebral vasculopathy, and **posterior reversible encephalopathy syndrome (PRES)**. Chiari I malformations can occur in older children with sickle cell disease. **Fat embolism syndrome** (associated with bone marrow infarction) is a rapidly progressive, potentially fatal complication involving pain, respiratory distress, changes in mental status, and multiorgan system failure. When this syndrome is identified early, exchange transfusion therapy has improved patient survival in small case series. Skull infarction can lead to a subgaleal hematoma or epidural bleed, which may present as altered mental status.

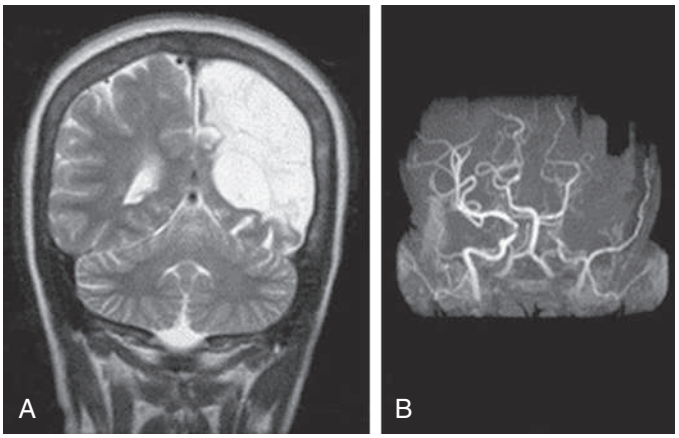

Fig. 511.2 MRI and magnetic resonance angiography (MRA) of the brain. **A**, T2-weighted MRI shows remote infarction of the territories of the left anterior cerebral artery and middle cerebral artery. **B**, MRA shows occlusion of the left internal carotid artery siphon distal to the takeoff of the ophthalmic artery.

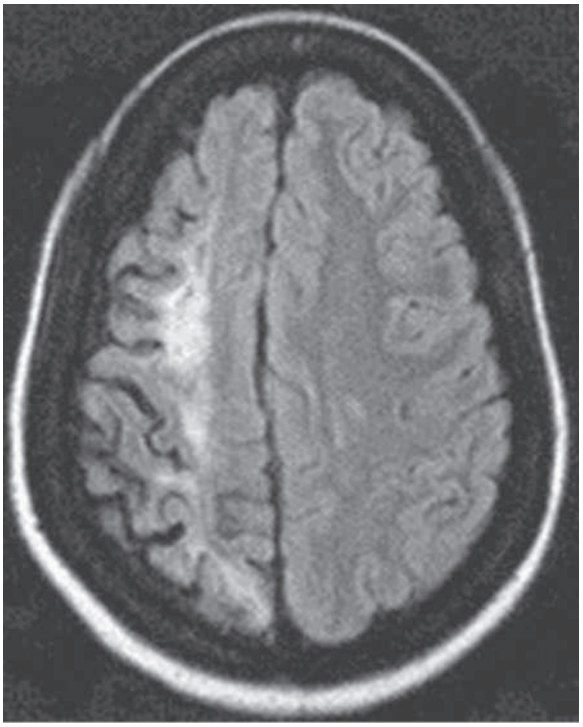

Fig. 511.3 Fast fluid-attenuated inversion recovery sequence MRI of the brain showing a right hemisphere border-zone cerebral infarction in a child with sickle cell anemia. *(From Switzer JA, Hess DC, Nichols F, et al. Pathophysiology and treatment of stroke in sickle-cell disease: present and future. Lancet Neurol 2006;5:501–512.)*

For patients presenting with acute focal neurologic deficit, a prompt pediatric neurologic evaluation and consultation with a pediatric hematologist is recommended. In addition, oxygen administration to keep oxygen saturation (So_2) >96% and blood transfusion within 2 hours of presentation, with a goal of increasing Hb to a maximum of 10 g/dL, is warranted. A timely simple blood transfusion is important because this is the most efficient strategy to dramatically increase oxygen content of the blood, particularly when exchange transfusion is not readily available. However, greatly exceeding this posttransfusion Hb limits oxygen delivery to the brain as a result of hyperviscosity by increasing the Hb significantly over the patient's baseline values. Subsequently, prompt treatment with an exchange transfusion is recommended, either manually or with automated erythrocytapheresis, to reduce the HbS percentage to <30%. Exchange transfusion at the time of acute stroke is associated with a decreased risk of second stroke compared to simple transfusion alone. CT of the head to exclude cerebral hemorrhage should be performed as soon as possible, and, if available, MRI of the brain with diffusion-weighted imaging to distinguish between ischemic infarcts and PRES. MR venography is useful to evaluate the possibility of **cerebral venous thrombosis**, a rare but potential cause of focal neurologic deficit in children with sickle cell disease. MR angiography may identify evidence of cerebral vasculopathy; these images are not critical in the initial time management of a child with sickle cell disease presenting with a focal neurologic deficit but are important for long-term management.

The clinical presentation of PRES or central venous thrombosis can mimic a stroke but would require a different treatment course. For both PRES and cerebral venous thrombosis, the optimal management has not been defined in patients with sickle cell disease, resulting in the need for consultation with both a pediatric neurologist and a pediatric hematologist. The primary approach for **prevention** of recurrent overt stroke is blood transfusion therapy aimed at keeping the maximum HbS concentration <30%. Despite regular blood transfusion therapy, approximately 20% of patients will have a second stroke and 30% of this group will have a third.

Transcranial Doppler Ultrasonography

Primary prevention of overt stroke can be accomplished using screening transcranial Doppler ultrasonography (TCD) assessment of the blood velocity in the terminal portion of the internal carotid and the proximal portion of the middle cerebral artery. Children with sickle cell anemia with an elevated *time-averaged mean maximum* (TAMM) blood flow velocity ≥200 cm/sec (abnormal study) are at increased risk for a cerebrovascular event. A repeat study should be performed within a week to confirm the result. However, a single value ≥220 cm/sec is concerning and does not require repeating before recommending an intervention. A TAMM measurement of <200 but ≥170 cm/sec represents a conditional threshold. A repeat measurement is suggested within a few months because of the high rate of conversion to a TCD velocity >200 cm/sec in this group of patients.

Two distinct methods of measuring TCD velocity are a nonimaging technique and an imaging technique. The *nonimaging* technique was the method used in the stroke prevention trial sponsored by the

National Institutes of Health, whereas most pediatric radiologists in practice use the *imaging* technique. When compared to each other, the imaging technique produces values that are 10–15% below those of the nonimaging technique. The imaging technique uses the *time-averaged mean of the maximum velocity* (TAMX), and this measure is believed to be equivalent to the nonimaging calculation of TAMM. A downward adjustment for the transfusion threshold is appropriate for centers using the imaging method to assess TCD velocity. The magnitude of the transfusion threshold in the imaging technique has not been settled, but a transfusion threshold of a TAMX of 185 cm/sec and a conditional threshold of TAMX of 165 cm/sec seem reasonable. Alternatively, some experts recommend using the same thresholds regardless of technique.

Children with abnormal TCD studies should begin chronic blood transfusion therapy to maintain HbS levels <30% to decrease the risk of first stroke. This strategy results in an over 90% reduction in the rate of overt strokes. Once transfusion therapy is initiated, a subset of patients at low risk for the development of increased TCD values, such as those without MRA-confirmed cerebral vasculopathy, may be able to transition from chronic transfusions to long-term hydroxyurea therapy. Acute stroke risk is decreased when hydroxyurea use and chronic transfusions overlap until a robust therapeutic response to hydroxyurea is achieved.

Pulmonary Complications

Lung disease in children with sickle cell disease is the second most common reason for hospital admission and is associated with significant mortality. **ACS** refers to a life-threatening pulmonary complication of sickle cell disease defined as a new radiodensity on chest radiography. Other clinical definitions include clinical features such as fever, respiratory distress, hypoxia, cough, and chest pain (Fig. 511.4). Even in the absence of respiratory symptoms, very young children with fever should receive a chest radiograph to identify evolving ACS because clinical examination alone is insufficient to identify patients with a new radiographic density. Early detection of ACS may alter clinical management. The radiographic findings in ACS are variable but may include single-lobe involvement, predominantly left lower lobe; multiple lobes, most often both lower lobes; and pleural effusions, either unilateral or bilateral. *ACS may progress rapidly from a simple infiltrate to extensive infiltrates and a pleural effusion.* Therefore continuous pulse oximetry and frequent clinical exams are required, and repeat chest x-ray films may be indicated for progressive hypoxia, dyspnea, tachypnea, and other signs of respiratory distress.

Most patients with ACS do not have a single identifiable cause. Infection is the most well-known etiology, yet only 30% of ACS episodes will have positive sputum or bronchoalveolar culture, and the most common bacterial pathogens are *S. pneumoniae*, *Mycoplasma pneumoniae,* and *Chlamydia* spp. The most frequent event preceding ACS is a painful episode requiring systemic opioid treatment. **Fat emboli** have also been implicated as a cause of ACS, arising from infarcted bone marrow, and can be life-threatening if large amounts are released to the lungs. Fat emboli can be difficult to diagnose but should be considered in any patient with sickle cell disease presenting with rapid onset of respiratory distress and altered mental status changes. Petechial rash may also occur but may be difficult to detect if not carefully sought.

Given that the causes of ACS are varied, recommended management is also multimodal (Table 511.4). The type of opioid, with overuse of morphine being more likely to cause ACS than nalbuphine

Table 511.4 Overall Strategies for the Management of Acute Chest Syndrome

PREVENTION
Incentive spirometry and periodic ambulation in patients admitted for sickle cell pain, surgery, or febrile episodes
Watchful waiting in any hospitalized child or adult with sickle cell disease (pulse oximetry monitoring and frequent respiratory assessments)
Cautious use of intravenous fluids
Intense education and optimum care of patients who have sickle cell anemia and asthma
DIAGNOSTIC TESTING AND LABORATORY MONITORING
Blood cultures, if febrile
Nasopharyngeal samples for viral culture (respiratory syncytial virus, influenza), depending on clinical setting
Complete blood counts every day and appropriate chemistries
Continuous pulse oximetry
Chest radiographs, for persistent or progressive illness
TREATMENT
Blood transfusion (simple or exchange), depending on clinical features; consider maintaining an active type and cross match
Supplemental O_2 for drop in pulse oximetry by 4% over baseline, or values <90%
Empirical antibiotics (third-generation cephalosporin and macrolide)
Continued respiratory therapy (incentive spirometry and chest physiotherapy as necessary)
Bronchodilators and corticosteroids for patients with asthma
Optimum pain control and fluid management

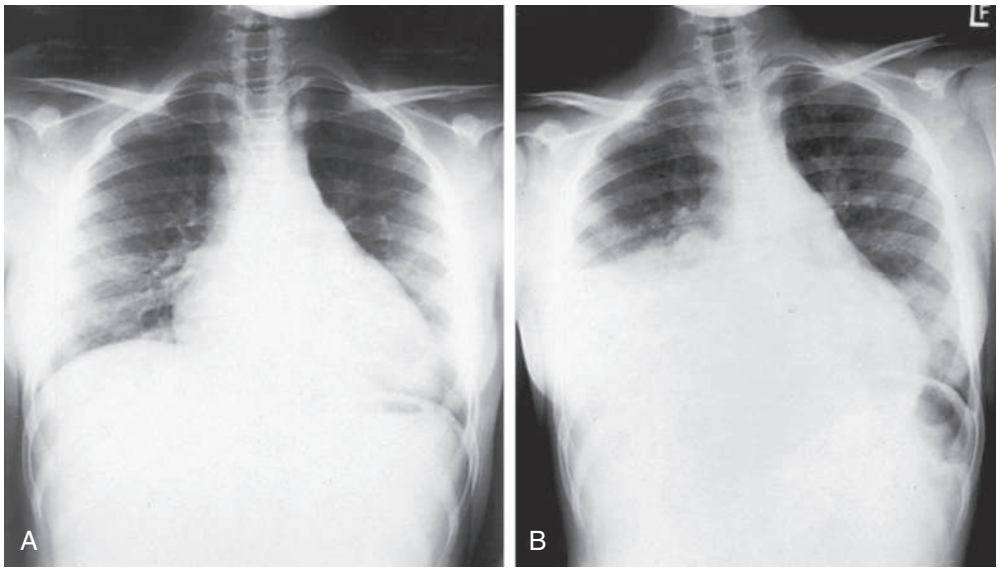

Fig. 511.4 Probable pulmonary infarction in a 15-year-old patient with HbSS. **A,** Frontal radiograph shows consolidation and a small pleural effusion posteriorly in the right lower lobe. **B,** Radiograph obtained <24 hr later shows massive right middle and lower lobe consolidation and effusion. No organisms could be cultured. The diagnosis of "probably pulmonary infarction" was established clinically. *(Courtesy Dr. Thomas L. Stovis, Children's Hospital of Michigan, Detroit. From Kuhn JP, Stovis TL, Haller JO: Caffey's Pediatric Diagnostic Imaging, 10th ed. Philadelphia: Mosby, 2004. p. 1087.)*

hydrochloride, is associated with an increase in the risk of ACS, in part because of sedation and hypoventilation. However, under no circumstance should opioid administration be limited to prevent ACS; rather, other measures must be taken to prevent ACS from developing. In patients with pain, regular use of an **incentive spirometer** at 10-12 breaths every 2 hours can significantly reduce the frequency of subsequent ACS episodes. Because of the clinical overlap between pneumonia and ACS, all episodes should be treated promptly with antimicrobial therapy, including at least a macrolide and a third-generation cephalosporin. A previous diagnosis of asthma or wheezing with ACS should prompt treatment following standard of care for an asthma exacerbation with bronchodilators. The diagnosis of ACS does not negate the recommended management of a patient with asthma exacerbation. Oxygen should be administered for patients who demonstrate hypoxia. Blood transfusion therapy using either simple or exchange (manual or automated) transfusion is the only method to abort a rapidly progressing ACS episode. The decision when to give blood and whether the transfusion should be a simple or exchange transfusion is less clearly defined. Usually, blood transfusions are given when at least one of the following clinical features are present: decreasing So_2, increasing work of breathing, rapidly changing respiratory effort either with or without a worsening chest radiograph, a dropping Hb of 2 g/dL below the patient's baseline, or previous history of severe ACS requiring admission to the intensive care unit.

Pulmonary hypertension has been identified as a major long time risk factor for death in adults with sickle cell anemia. The natural history of pulmonary hypertension in children with sickle cell anemia is unknown. Asymptomatic patients do not require screening for pulmonary hypertension. The initial diagnostic test is an echocardiogram and, depending on the severity of those findings, the echocardiogram should be followed by right-sided heart catheterization. Clinical findings suggestive of pulmonary hypertension include hypoxia or dyspnea at rest or with exertion, comorbid vascular complications (leg ulcers, priapism), elevated N-terminal, pro-B-type natriuretic peptide, or an abnormal 6-minute walk distance.

Renal Disease and Enuresis

Renal disease among patients with sickle cell disease is a major comorbid condition that can lead to premature death. Seven sickle cell disease nephropathies have been identified: gross hematuria, papillary necrosis, nephrotic syndrome, renal infarction, hyposthenuria, pyelonephritis, and renal medullary carcinoma. The presentation of these entities is varied but may include hematuria, proteinuria, renal insufficiency, concentrating defects, or hypertension.

The common presence of **nocturnal enuresis** occurring in children with sickle cell disease is not well defined but is troublesome for affected children and their parents. The overall prevalence of enuresis was 33% in the Cooperative Study of Sickle Cell Disease, with the highest prevalence (42%) among children 6-8 years old. Furthermore, enuresis may still occur in approximately 9% of older adolescents. Patients with sickle cell disease and nocturnal enuresis should have a systematic evaluation for recurrent urinary tract infections, kidney function, and possibly obstructive sleep apnea syndrome. Unfortunately, most children with nocturnal enuresis do not have an etiology, and targeted therapeutic interventions have been of limited success.

Cognitive and Psychologic Complications

Ongoing evaluation of the family unit and identification of the resources available to cope with a chronic illness are critical for optimal management. Children and adolescents with sickle cell disease have decreased quality of life, as measured on standardized assessments, compared to their siblings and children with other chronic diseases. Furthermore, children with sickle cell disease are at great risk for academic failure and have a 20% high school graduation rate, possibly because, among other reasons, approximately one third of children with sickle cell anemia have had a cerebral infarct, either silent or an overt stroke. Early school-age children with sickle cell anemia should have MRI without sedation to screen for silent cerebral ischemia. Children with cerebral infarcts require ongoing cognitive and school performance assessment so that education resources can be focused to optimize educational attainment. Participation in relevant support groups and group activities, such as camps for children with sickle cell disease, may be of direct benefit by improving self-esteem and establishing peer relationships.

Other Complications

In addition to the previous organ dysfunctions, patients with sickle cell disease can have other significant complications. These complications include, but are not limited to, sickle cell retinopathy, delayed onset of puberty, leg ulcers, and complications associated with pregnancy. Optimal treatment for each of these entities has not been determined, and individual management requires consultation with the hematologist and primary care physician. Reproductive health issues are prevalent in children and adolescents with sickle cell disease; therefore hematologists, adolescent medicine teams, and obstetrics/gynecology teams should co-manage patients to provide optimal care.

THERAPEUTIC CONSIDERATIONS

Hydroxyurea

Hydroxyurea is a well-established drug proven effective in reducing the frequency of acute pain episodes. In adults with sickle cell anemia, hydroxyurea decreases the rate of hospitalization for painful episodes by 50% and the rate of ACS and blood transfusion by almost 50%. In addition, adults taking hydroxyurea have shorter hospitalizations and require less analgesic medication during hospitalization.

In children with sickle cell anemia, hydroxyurea is safe and well tolerated. The primary toxicities are limited to myelosuppression that reverses on cessation of the drug. Infants treated with hydroxyurea experience fewer episodes of pain, dactylitis, and ACS; are hospitalized less frequently; and less often require a blood transfusion. Infants treated with hydroxyurea do not experience increased rates of bacteremia or serious infection.

Current recommendations are that all children with sickle cell anemia should be offered hydroxyurea beginning at 9 months of age. Hydroxyurea may be indicated for other sickle cell–related complications, especially in patients who are unable to tolerate other treatments. For patients who either will not or cannot continue blood transfusion therapy to prevent recurrent stroke, hydroxyurea therapy may be a reasonable alternative. The trial assessing the efficacy of hydroxyurea as an alternative to transfusions to prevent a *second* stroke was terminated early after the data safety and monitoring process found an increased stroke rate in the hydroxyurea arm compared to the transfusion arm. Hydroxyurea alone is inferior to transfusion therapy for secondary stroke prevention in patients who do not have contraindications to ongoing transfusions.

The long-term toxicity associated with initiating hydroxyurea in very young children has not yet been established. However, all evidence to date suggests that the benefits far outweigh the risks. For these reasons, very young children starting hydroxyurea require well-informed parents and medical care by pediatric hematologists, or at least co-management by a physician with expertise in immunosuppressive medications. The typical starting dose of hydroxyurea is 15-20 mg/kg once daily, with an incremental dosage increase every 8 weeks of 5 mg/kg, and if no toxicities occur, up to a maximum of 35 mg/kg per day. The infant hydroxyurea study found young children could safely be started at 20 mg/kg/day without increased toxicity. Achievement of the therapeutic effect of hydroxyurea can require several months, and for this reason, initiating hydroxyurea to address short-term symptom relief is not optimal. We prefer to introduce the concept to parents within the first year of life, preferably by 9 months; provide literature that describes both the pros and cons of starting hydroxyurea in children with severe symptoms of sickle cell disease; and educate parents on starting hydroxyurea in asymptomatic children as a preventive therapy for repetitive pain and ACS events. Other effects of hydroxyurea that may vary include an increase in the total Hb level and a decrease in the TCD velocity.

Other medications are available for long-term use in children and adults with sickle cell disease. **Oral L-glutamine** reduces hospitalizations and sickle cell crisis in children age 5 years and older. In addition, **crizanlizumab**, a humanized P-selectin inhibitor administered

monthly IV, is indicated for reducing acute pain in adults and children age 16 years and older. **Voxelotor**, a sickle hemoglobin polymerization inhibitor, is FDA-approved for adults and children 4 years and older. In clinical trials, Voxelotor demonstrated reduced hemolysis in patients with sickle cell disease.

Gene Therapy

The FDA (2023) has approved two different gene therapy approaches to ex vivo gene modification of autologous hematopoietic stem and progenitor cells following myeloablative conditioning. Both therapies are approved for patients ≥12 years of age with recurrent vasoocclusive crises.

Casgevy (exagamglogene autotemcel: exa-cel), involves *gene editing* with CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats / CRISPR associated nuclease 9). Exa-cel specifically targets and inactivates the red cell precursor *BCL11A* gene (a suppressor of fetal hemoglobin expression), which results in increased production of fetal hemoglobin (which does not sickle), resulting in decreased polymerization of sickle hemoglobin.

Lyfgenia (lovotibeglogene autotemcel: lovo-cel) is a *gene addition* approach using a lentiviral vector carrying a modified beta globin gene (beta-A-T87Q globin), thus increasing the levels of hemoglobin A.

Both have been successful in reducing or eliminating vasoocclusive crises after therapy. Lyfgenia has a black box warning for the risk of off target editing /insertional mutagenesis and the possibility of acute myeloid leukemia.

Hematopoietic Stem Cell Transplantation

The only currently approved cure for sickle cell anemia is transplantation with human leukocyte antigen (HLA)–matched hematopoietic stem cells from a sibling donor (allogenic) without sickle cell disease. Because of the limited availability of appropriate sibling donors, research is exploring a wide variety of curative regimens. Clinical trials are underway to explore unrelated and partially matched related donor stem cell transplants.

The most common indications for allogenic transplant are recurrent ACS, stroke, and abnormal TCD. Sibling-matched stem cell transplantation has a lower risk for graft-versus-host disease than unrelated donors. Surveys suggest that younger children may have lower morbidity and mortality. The decision to consider unrelated transplantation should involve appropriate consultation and counseling from physicians with expertise in sickle cell transplantation.

Stem cell transplantation for children with sickle cell disease who have a genetically matched sibling and *few complications* is less commonly performed. The use of hydroxyurea has dramatically decreased the disease burden for the patient and family, with far fewer hospitalizations for pain or ACS episodes and less use of blood transfusions. The field of stem cell transplantation is also progressing such that larger studies involving nonsibling donor and haploidentical donor transplantation. Transplant-related complications caused by conditioning regimens may be decreased by using low-intensity, nonmyeloablative HLA-matched sibling, allogenic stem cell transplantation.

Red Blood Cell Transfusions

RBC transfusions are used frequently both in the treatment of acute complications and to prevent acute or recurrent complications. Typically, short-term transfusions are used to prevent progression of acute complications such as ACS, aplastic crisis, splenic sequestration, and acute stroke, as well as to prevent surgery-related ACS. RBC transfusions are not recommended for uncomplicated acute pain events. Select RBC volumes judiciously to avoid high posttransfusion Hb levels and hyperviscosity. Long-term or chronic transfusion therapy is used to prevent first stroke in patients with abnormal TCD or MRI findings (silent stroke), recurrent stroke, or recurrent ACS. Patients with sickle cell disease are at increased risk of developing *alloantibodies* to less common RBC surface antigens after receiving even a single transfusion. In addition to standard cross matching for major blood group antigens (A, B, O, RhD), more *extended matching* should be performed to identify donor units that are C-, E-, and Kell-antigen matched. Full RBC antigen phenotyping or genotyping for all patients with sickle cell disease should be performed before RBC transfusion whenever possible to have the RBC units least likely to result in alloimmunization available for these patients.

Three methods of **blood transfusion therapy** are used in the management of acute and chronic complications associated with sickle cell disease: automated erythrocytapheresis, manual exchange transfusion (phlebotomy of a set amount of patient's blood followed by rapid administration of donated packed RBCs), and simple transfusion. The decision on which method to use depends on the patient's pretransfusion Hb level, the clinical indication, RBC alloimmunization, and transfusional iron overload. Exchange transfusion, manual or automated, is preferable for patients with new neurologic symptoms. *Automated erythrocytapheresis* is the preferred method for patients requiring chronic blood transfusion therapy because there is a minimum net iron balance after the procedure, followed by manual exchange transfusion. However, this method requires technical expertise, special machines, and good patient venous access. *Manual exchange* is more accessible. However, both methods may expose the patient to more RBC units and possible alloimmunization. *Simple transfusion* therapy may lower donor exposure and be more readily available but may result in higher net iron burden when compared to erythrocytapheresis or exchange transfusion.

Preparation for surgery for children with sickle cell disease requires a coordinated effort from the hematologist, surgeon, anesthesiologist, and primary care provider. Historically, ACS was associated with general anesthesia in patients with sickle cell disease. Blood transfusion before surgery for children with sickle cell disease is recommended to raise Hb level preoperatively to no more than 10 g/dL, to avoid ACS development. Because of better general perioperative care and the use of long-term therapies such as hydroxyurea and chronic transfusions, the decision to transfuse before general anesthesia should be made in conjunction with the medical team who provides sickle cell disease–related care for the patient. When preparing a child with sickle cell disease for surgery with a simple blood transfusion, caution must be used not to elevate Hb level beyond 10 g/dL because of the risk of hyperviscosity syndrome. For children with milder forms of sickle cell disease, such as HbSC or HbSβ-thalassemia, a decision must be made on a case-by-case basis as to whether an exchange transfusion is warranted because a simple transfusion may raise the hemoglobin to an unacceptable level.

Iron Overload

The primary toxic effect of blood transfusion therapy relates to excessive iron stores or iron overload, which can result in organ damage and premature death. Excessive iron stores develop after 100 mL/kg of RBC transfusion, or about 10 transfusions. The assessment of iron overload in children receiving regular blood transfusions is difficult. *The most common and least invasive method of estimating total body iron involves serum ferritin levels.* Ferritin measurements have significant limitations in their ability to estimate iron stores for several reasons, including, but not limited to, elevation during acute inflammation and poor correlation with excessive iron in specific organs, such as the heart and endocrine glands. MRI of the liver has proved to the most effective approach for assessment of iron stores. MRI T2* and MRI R2 and R2* sequences are used to estimate iron levels in the heart and liver. These imaging strategies are more accurate than serum ferritin in estimating heart and liver iron content. The standard for iron assessment previously was liver biopsy, which is an invasive procedure exposing children to the risk of general anesthesia, bleeding, and pain. Liver biopsy alone does not accurately estimate total body iron because iron deposition in the liver is not homogeneous and does not always correlate with iron levels in the heart and other organs. The major advantage of a liver biopsy is that histologic assessment of the parenchyma can be ascertained along with appropriate staging of suspected pathology, particularly cirrhosis.

The primary **treatment** of transfusion-related iron overload requires iron chelation using medical therapy. In the United States, three chelating agents are approved for use in transfusional iron overload. *Deferoxamine* is administered subcutaneously 5 of 7 nights/week over 8 to 12 hours a night. *Deferasirox* is taken by mouth daily, and *deferiprone* is available in tablet forms taken orally twice or three times a day and an oral solution taken three times a day. The FDA approved *deferasirox* for use in patients

age ≥2 years. A pill formulation of deferasirox is available that does not require mixing before oral administration. A sprinkle formulation that is mixed with a soft food such as applesauce also is available for young children who are unable to swallow pills. *Deferiprone* is an older oral chelator that has been widely used outside the United States for many years and was also approved as a first-line agent (see Table 511.8). Because of a 1–2% risk of agranulocytosis, weekly CBC monitoring is recommended, particularly in the first 6 months of treatment and with any febrile illness. Transfusion-related excessive iron stores in children with sickle cell disease should be managed by a physician with expertise in chelation therapy because of the need for close monitoring and due to the risk of significant toxicity from available chelation therapies.

OTHER SICKLE CELL SYNDROMES

The most common sickle cell syndromes besides HbSS are HbSC, HbSβ^0-thalassemia, and HbSβ^+-thalassemia. The other syndromes—HbSD, HbSO^{Arab}, HbSHPFH, HbSE, and other variants—are much less common. Patients with HbSβ^0-thalassemia have a clinical phenotype similar to those with HbSS. In the RBCs of patients with HbSC, crystals of HbC interact with membrane ion transport, dehydrating RBCs and inducing sickling. Children who have HbSC disease can experience the same symptoms and complications as those with severe HbSS disease, but less frequently. Children with HbSC have increased incidence of retinopathy, chronic hypersplenism, and acute splenic sequestration over the life span. The natural history of the other sickle cell syndromes is variable and difficult to predict because of the lack of systematic evaluation.

There is no validated model that can predict the clinical course of an individual with sickle cell disease. A patient with HbSC can have a more severe clinical course than a patient with HbSS. Management of end-organ dysfunction in children with sickle cell syndromes requires the same general principles as managing patients with sickle cell anemia; however, each situation should be managed on a case-by-case basis and requires consultation with a pediatric hematologist.

ANTICIPATORY GUIDANCE

Children with sickle cell disease should receive general health maintenance as recommended for all children, with special attention to disease-specific guidance and infection prevention education. In addition to counseling regarding adherence to penicillin and a vaccination schedule, patients, parents, and caregivers should be instructed to seek immediate medical attention for all febrile illness. In addition, early detection of acute splenic sequestration has been shown to decrease mortality. Therefore parents and caregivers should be educated early and repeatedly about the importance of daily penicillin administration and correct palpation of the spleen.

Prophylactic Penicillin

Children with sickle cell anemia should receive prophylactic oral penicillin VK until at least 5 years of age (125 mg twice daily up to age 3 years, then 250 mg twice daily thereafter). No established guidelines exist for penicillin prophylaxis beyond 5 years of age; some clinicians continue penicillin prophylaxis, and others recommend discontinuation. Penicillin prophylaxis should be continued beyond 5 years in children with a history of pneumococcal infection because of the increased risk of a recurrent infection. An alternative for children who are allergic to penicillin is erythromycin ethylsuccinate.

Immunizations

In addition to penicillin prophylaxis, routine childhood immunizations, as well as the annual administration of influenza vaccine, are highly recommended. Children with sickle cell disease develop functional asplenia and also require immunizations to protect against encapsulated organisms, including additional pneumococcal and meningococcal vaccinations. The U.S. Centers for Disease Control and Prevention (CDC) provides vaccination guidelines at https://www.cdc.gov/vaccines/hcp/acip-recs/index.html.

Spleen Palpation

Splenomegaly is a common complication of sickle cell disease, and splenic sequestration can be life threatening. Parents and primary caregivers should be taught how to palpate the spleen to determine if the spleen is enlarging starting at the first visit, with reinforcement at subsequent visits. Parents should also demonstrate spleen palpation to the provider.

Transcranial Doppler Ultrasound

Primary stroke prevention using TCD has resulted in a decrease in the prevalence of overt stroke among children with sickle cell anemia. Children with HbSS or HbSβ^0-thalassemia should be screened annually with TCD starting at age 2 years. TCD is best performed when the child is quietly awake and in their usual state of health. TCD measurements may be falsely elevated or decreased in the settings of acute anemia, sedation, pain, fever, or immediately after blood transfusions. Screening should occur annually from ages 2-16 years. Abnormal values should be repeated within a week to identify patients at greatest risk of overt stroke. Conditional values should be repeated within at least 3 months, and normal values should be repeated annually. Routine neuroimaging with MRI in asymptomatic patients requires consultation with a pediatric hematologist or neurologist with expertise in sickle cell disease.

Hydroxyurea

Recommendations include offering hydroxyurea therapy to all children with sickle cell anemia starting at 9 months of age regardless of clinical symptoms. Monitoring children receiving hydroxyurea is labor intensive. Hydroxyurea is a chemotherapeutic agent, and patients receiving this agent require the same level of nursing and physician oversight as any child with cancer receiving chemotherapy. The parents must be educated about the consequences of therapy, and when ill, children should be promptly evaluated. Starting doses should be approximately 20 mg/kg/day. CBC with differential and reticulocyte count should be checked within 4 weeks after initiation of therapy or any dose change to monitor for hematologic toxicity, then every 8-12 weeks. Dose escalation should be based on clinical and laboratory parameters. If appropriate, dose increases should be in 5 mg/kg/day increments to a maximum of 35 mg/kg/day.

While receiving hydroxyurea, steady-state absolute neutrophil count should be approximately 2,000/μL or higher and platelet count should be 80,000/μL or higher. However, children may tolerate lower absolute neutrophil counts while receiving hydroxyurea. Holding hydroxyurea and adjusting to lower doses may be required for neutropenia and thrombocytopenia. Hydroxyurea is a pregnancy class D medication, and adolescents should be counseled regarding methods to prevent pregnancy while taking this medication. Close monitoring of the patient requires a commitment by the parents and the patient as well as diligence by a physician to identify toxicity early. Information is scarce regarding the impact of hydroxyurea on fertility, although hydroxyurea has been shown to further reduce sperm count in males with sickle cell disease in several case reports; this effect may be reversible once hydroxyurea is discontinued.

Red Cell Transfusion Therapy

At the initiation of blood transfusion therapy, children with sickle cell disease should have testing to identify the presence of alloantibodies and RBC phenotyping or genotyping, which is performed to identify the best matched blood. RBC units selected should be extended antigen-matched for C, E, and K, when feasible. Goals of transfusion for acute events should be established before initiating therapy, including target posttransfusion Hb level and HbS percentage, or both. For children receiving chronic transfusion therapy, pretransfusion HbS goals should be defined; the most common goal is <30%. Posttransfusion Hb values should be targeted to avoid hyperviscosity. Children, parents, and caregivers should be educated about the symptoms of delayed hemolytic transfusion reactions. Any child with sickle cell disease with a recent history of RBC transfusion who presents with pain, dark urine, increased scleral icterus, or symptoms of worsening anemia should be screened for a delayed hemolytic transfusion reaction after consultation with the blood bank. Children meeting criteria for chronic transfusion therapy should receive annual evaluation for transfusion-transmitted infections, including hepatitis B, hepatitis C, and HIV. After receiving 100 mL/kg RBC transfusions, regular assessments of iron overload should begin, usually including measurements of serum ferritin and MRI assessments for hepatic iron every 1-2 years. Cardiac iron assessments should be

performed in children over 10 years old, especially if there is a history of poor adherence with iron chelation and/or liver iron concentration of 15 mg/g dry weight or higher. For children requiring chelation therapy, audiology and ophthalmology exams should be performed annually and monitoring of liver function and pituitary function performed regularly because of iron deposition.

Pulmonary and Asthma Screening

Pulmonary complications of sickle cell disease are common and life threatening. Asthma is common in children with sickle cell disease, and thus evaluation for asthma symptoms and asthma risk factors should be performed routinely, particularly given the high morbidity and mortality. All children should receive annual screening for signs and symptoms of lower airway disease, such as nighttime cough and exercise-induced cough. In children with symptoms consistent with lower airway disease, consultation with an asthma specialist should be considered. Pulse oximetry readings should be performed during well visits to identify children with abnormally low daytime oxygen saturation. For children with snoring, daytime somnolence, and symptoms associated with obstructive sleep apnea syndrome (OSAS), sleep studies should be performed as necessary.

Retinopathy

Effective therapy is available for retinopathy associated with sickle cell disease. Although all patients are at risk for development of retinopathy, those with sickle cell disease, type SC, are at very high risk. Patients should receive annual screening by an ophthalmologist to identify vascular changes that would benefit from laser therapy. Although changes may occur earlier, children with sickle cell disease should begin annual screenings no later than age 10 years.

Renal Disease

Sickle cell–associated renal disease starts in infancy and may not become clinically evident until adulthood. Chronic kidney disease is common in adults with sickle cell disease, with high morbidity and mortality. Screening protocols for early signs of sickle nephropathy in children have not been adopted due to lack of data. However, when creatinine elevation, microalbuminuria, or macroalbuminuria is detected, a nephrologist should be consulted to determine next steps for further evaluation and possible treatment. The age to begin screening for proteinuria has not been defined, but some experts recommend screening annually after at least 10 years, if not sooner. If proteinuria is detected, urine studies should be repeated with an early-morning urine collection; if the protein remains elevated, the patient should be referred to a pediatric nephrologist. Males with sickle cell disease should also receive counseling regarding the diagnosis and treatment of priapism. Because of the high frequency of enuresis beyond early childhood, approximately 9% between 18 and 20 years of age, parents and caregivers should be educated about the prolonged nature of enuresis in this disease. OSAS is associated with an increased prevalence of enuresis in sickle cell disease. Unfortunately, no evidence-based therapies have been developed to treat enuresis in children and young adults with sickle cell disease. In children with enuresis who have symptoms and clinical features of OSAS, referral to specialists for evaluation is recommended.

Echocardiography

Echocardiography is a screening tool to identify individuals with sickle cell disease who have pulmonary artery hypertension (see the section on "Pulmonary Hypertension"). Studies in adults with sickle cell disease have found that echocardiography is insensitive at identifying individuals truly at risk for pulmonary hypertension, although an elevated tricuspid velocity measurement may still be a risk factor for premature death in adults with sickle cell disease. Routine echocardiograms for pulmonary hypertension screening are not recommended in asymptomatic children; however, they are recommended in patients with leg ulcers, priapism, and connective tissue disease, as well as referral to a pulmonary hypertension specialist for patients with new steady-state cardiorespiratory symptoms, heart failure, or pulmonary embolus.

Additional Screening

Patients with sickle cell disease are at increased risk for behavioral health issues, including anxiety and depression. Screening should be performed at routine and acute visits. AVN of the hips and shoulders is increased in patients with sickle cell disease and may be identified early on routine physical exam. Plain radiographs may not detect early disease; thus, when AVN is suspected and plain films are normal, MRI should be obtained. When AVN is confirmed, patients should be referred promptly to orthopedics and physical therapy.

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511.2 Sickle Cell Trait (Hemoglobin AS)

Kim Smith-Whitley and Janet L. Kwiatkowski

The prevalence of sickle cell trait varies throughout the world; in the United States, the incidence is 7–10% of the Black population. Because all state newborn screening programs include sickle cell disease, for most children, sickle cell trait is first identified on their newborn screen. Communication of sickle cell trait status from infancy to young adulthood for the affected individual, family, and healthcare providers is often inconsistent, and many young adults are unaware of their sickle cell trait status.

By definition, among individuals with sickle cell trait, the HbS level is <50%. The life span of people with sickle cell trait is normal, and serious complications are extremely rare. The CBC is within the normal range (Fig. 511.5B). Hemoglobin analysis is diagnostic, revealing a predominance of HbA, typically >50%, and HbS <50%. Rare complications of sickle cell trait may exist. Sickle cell trait is reported to be associated with exertional rhabdomyolysis in military recruits, and possibly with sudden death during rigorous exercise. However, whether these reports establish sickle cell trait as a risk factor that is nonmodifiable by other genetic factors remains unclear. Other complications reported with sickle cell trait include splenic infarction at high altitude, hematuria, hyposthenuria, deep vein thrombosis, and susceptibility to progressive eye injury after hyphema (Table 511.5). Renal medullary carcinoma has been reported almost exclusively in individuals with sickle cell trait and occurs predominantly in young people.

Children with sickle cell trait do not require limitations on physical activities as long as provisions are made for frequent rest and oral hydration, particularly when participating in physical conditioning or competitive sports. **Sudden death** in persons with sickle cell trait while exercising under extreme conditions is most likely associated with a second genetic factor and/or environmental factors and not the presence of sickle cell trait itself. However, if **exertional rhabdomyolysis** is identified, evaluation by neurology and cardiology should be considered. No causal pathway has been implicated for the presence of sickle cell trait and sudden death. All patients with sickle cell trait who participate in rigorous athletic activities should receive maximum hydration and appropriate rest during exertion, as would be the precautionary steps for all athletes, particularly when participating in hot, humid conditions. The presence of sickle cell trait should never be a reason to exclude a person from athletic participation but rather should serve as an indication that prudent surveillance is necessary to ensure appropriate hydration and prevention of exhaustion from heat or other strenuous exercise. If athletes are to be screened for sickle cell trait, the appropriate procedure is testing using a hemoglobin electrophoresis followed by genetic counseling, along with the knowledge that genetic information may provide opportunities to challenge paternity. Such situations are typically handled by a pediatrician or hematologist accustomed to providing both a balanced approach to genetic counseling and addressing the challenges about paternity.

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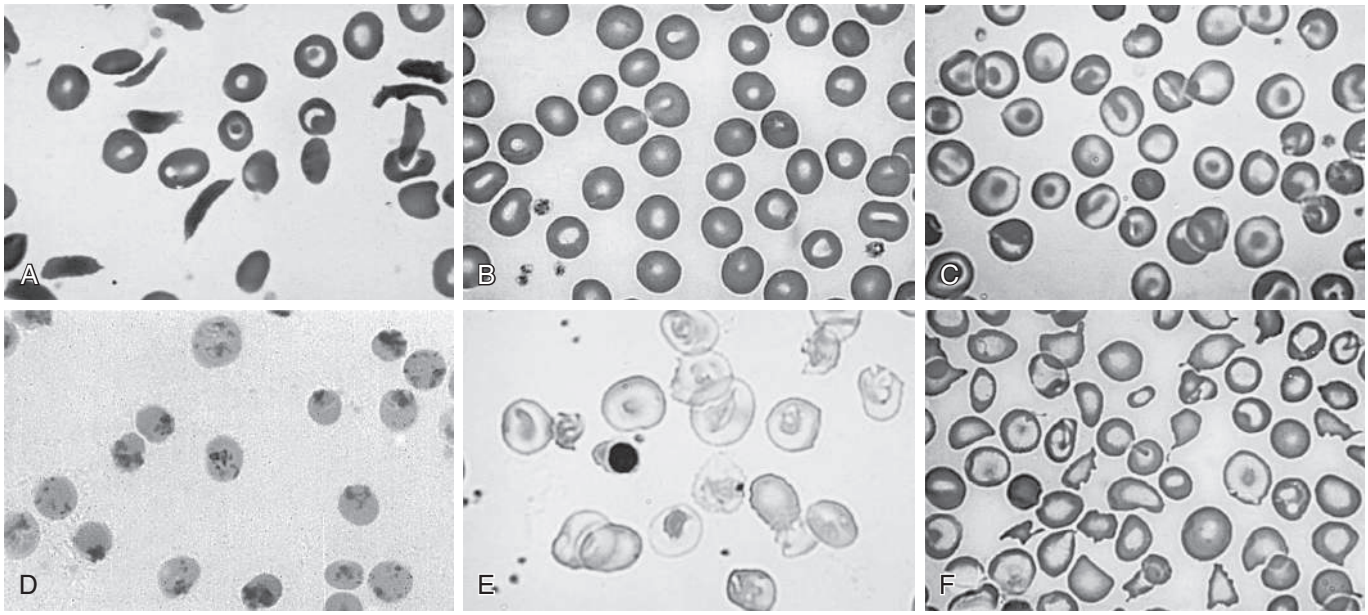

Fig. 511.5 Red blood cell morphology associated with hemoglobin disorders. **A,** Sickle cell anemia (HbSS): target cells and fixed (irreversibly sickled) cells. **B,** Sickle cell trait (HbAS): normal red blood cell (RBC) morphology. **C,** Hemoglobin CC: target cells and occasional spherocytes. **D,** Congenital Heinz body anemia (unstable hemoglobin): RBCs stained with supravital stain (brilliant cresyl blue) reveal intracellular inclusions. **E,** Homozygous β^0-thalassemia: severe hypochromia with deformed RBCs and normoblasts. **F,** Hemoglobin H disease (α-thalassemia): anisopoikilocytosis with target cells. *(Courtesy Dr. John Bolles, The ASH Collection, University of Washington, Seattle.)*

Table 511.5 Complications Reported with Sickle Cell Trait

Renal medullary cancer
Hematuria
Renal papillary necrosis
Hyposthenuria
Splenic infarction at high altitudes
Exertional rhabdomyolysis
Protection against severe complications of falciparum malaria
Microalbuminuria (adults)

From Tsaras G, Owusu-Ansah A, Boateng O, et al. Complications associated with sickle cell trait: a brief narrative review. *Am J Med* 2009;122:507–512.

511.3 Other Hemoglobinopathies

Kim Smith-Whitley and Janet L. Kwiatkowski

HEMOGLOBIN C

The pathogenic variant for HbC is at the same site as in HbS, with substitution of lysine instead of valine for glutamine. In the United States, hemoglobin C trait **(HbAC)** occurs in 1:40 and homozygous hemoglobin C disease **(HbCC)** occurs in 1:5,000 of the Black population. HbAC is asymptomatic. HbCC can result in mild anemia, splenomegaly, and cholelithiasis; rare cases of spontaneous splenic rupture have been reported. Splenic dysfunction does not occur. This condition is usually diagnosed through newborn screening programs. HbC crystallizes, disrupting the red cell membrane, and HbC crystals may be visible on peripheral smear (see Fig. 511.5C).

HEMOGLOBIN E

Hemoglobin E is an abnormal hemoglobin resulting from a qualitative pathogenic variant in the β-globin gene and is the second most common globin pathogenic variant worldwide. Patients may have asymptomatic hemoglobin E trait **(HbAE)** or benign homozygous hemoglobin E disease **(HbEE)**. Compound heterozygous hemoglobin E/β-thalassemia produces clinical phenotypes ranging from moderate to severe anemia, depending on the β-thalassemia pathogenic variant. In California, HbE/β-thalassemia is found almost exclusively in persons of Southeast Asian descent, with a prevalence of 1:2,600 births.

HEMOGLOBIN D

At least 16 variants of hemoglobin exist. HbD-Punjab (Los Angeles) is a rare hemoglobin that is seen in 1–3% of Western Indians and in some Europeans with Asian-Indian ancestry and produces symptoms of sickle cell disease when present in combination with HbS. Heterozygous HbD or hemoglobin D trait **(HbAD)** is clinically silent. Homozygous hemoglobin D disease **(HbDD)** produces a mild to moderate anemia with splenomegaly.

511.4 Unstable Hemoglobin Disorders

Kim Smith-Whitley and Janet L. Kwiatkowski

At least 200 rare unstable hemoglobins have been identified; the most common is **Hb Köln**. Most patients seem to have de novo pathogenic variants rather than inherited hemoglobin disorders. The best-studied unstable hemoglobins are the ones leading to hemoglobin denaturation from pathogenic variants affecting heme binding. The denatured hemoglobin can be visualized during severe hemolysis or after splenectomy as **Heinz bodies**. Unlike the Heinz bodies seen after toxic exposure, in unstable hemoglobins, Heinz bodies are present in reticulocytes and older RBCs (see Fig. 511.5D). Heterozygotes are asymptomatic.

Children with homozygous gene pathogenic variants can present in early childhood with anemia and splenomegaly or with unexplained hemolytic anemia. Hemolysis is increased with febrile illness and with the ingestion of oxidant medications (similar to glucose-6-phosphate dehydrogenase [G6PD] deficiency [see Chapter 512.3]) with some unstable hemoglobins. If the spleen is functional, the blood smear can appear almost normal or have only hypochromasia and basophilic stippling. A diagnosis may be made by demonstrating Heinz bodies, Hb instability, or an abnormal Hb analysis (although some unstable hemoglobins have normal mobility and are not detected on Hb analysis).

Treatment is supportive. Transfusion may be required during hemolytic episodes in severe cases. Oxidative drugs should be avoided, and folate supplementation may be helpful if dietary deficiency is a concern. Splenectomy may be considered in patients requiring recurrent transfusion or demonstrating poor growth, but the complications of splenectomy, including bacterial sepsis, risk of thrombosis, and risk of developing pulmonary hypertension, should be considered before surgery.

511.5 Abnormal Hemoglobins with Increased Oxygen Affinity

Kim Smith-Whitley and Janet L. Kwiatkowski

More than 110 high-affinity hemoglobins have been characterized. These pathogenic variants affect the state of Hb configuration during oxygenation and deoxygenation. Hemoglobin changes structure when in the oxygenated versus the deoxygenated state. The deoxygenated state is termed the **T (tense) state** and is stabilized by 2,3-diphosphoglycerate. When fully oxygenated, hemoglobin assumes the **R (relaxed) state**. The exact molecular interactions between these two states are unknown. High-affinity hemoglobins contain pathogenic variants that either stabilize the R form or destabilize the T form. The interactions between the R and T forms are complex, and the mechanisms of the pathogenic variants are not known. In most cases, the high-affinity hemoglobins can be identified by Hb analysis; approximately 20% must be characterized under controlled conditions where measurements are obtained with the P_{50} lowered to 9-21 mm Hg (normal: 23-29 mm Hg). The decreased P_{50} in these hemoglobins leads to an erythrocytosis with Hb levels of 17-20 g/dL. Levels of erythropoietin and 2,3-DPG are normal. Patients are usually asymptomatic and do not need phlebotomy. If phlebotomy is performed, oxygen delivery could be problematic because of the reduced number of Hb molecules to carry oxygen.

511.6 Abnormal Hemoglobins Causing Cyanosis

Kim Smith-Whitley

Abnormal hemoglobins causing cyanosis, also called **structural methemoglobinemias**, are rare. They are referred to as **M hemoglobins** and represent a group of hemoglobin variants that result from point pathogenic variants in one of the globin chains, α, β, or γ, located in the heme pocket; 13 known variants exist. These unstable hemoglobins lead to hemolytic anemia, most pronounced when the β-globin gene is affected. Clinically, these children are cyanotic from birth, without other signs or symptoms of disease, if the pathogenic variant is in the α-**globin** gene (HbM Boston, HbM Iwate, Hb Auckland). Infants with β-**globin** pathogenic variants become cyanotic later in infancy after the fetal hemoglobin switch (HbM Saskatoon, HbM Chile, HbM Milwaukee 1 and 2). The γ-**chain** pathogenic variants (HbF-M Fort Ripley, HbF-M Osaka, HbF Cincinnati, HbF Circleville, HbF Toms River, HbF Viseu) are all transient, presenting with cyanosis at birth, which resolves during the neonatal period after HbF production discontinues.

The abnormal M hemoglobins exhibit autosomal dominant inheritance and are diagnosed by Hb analysis. HbM variants may not be isolated reliably using Hb analysis (HPLC or IEF); consequently, diagnostic confirmation may require DNA sequencing or mass spectrometry. There is no specific treatment and affected patients do not respond to treatments used for *enzyme-deficient methemoglobinemia*. Beyond cyanosis, individuals are otherwise asymptomatic and do not require additional monitoring. Children with the β-globin form should avoid oxidant drugs. Individuals with all forms have a normal life expectancy and pregnancy course.

Low-affinity hemoglobins have less cyanosis than the M hemoglobins. The amino acid substitutions destabilize the oxyhemoglobin and lead to decreased oxygen saturation. The best characterized are Hb Kansas, Hb Beth Israel, and Hb Denver. Hb analysis (IEF and HPLC techniques) may be normal in affected individuals. When clinically suspected, oxygen affinity studies reveal a right-shifted dissociation curve, and heat testing demonstrates unstable hemoglobin. Children present with mild cyanosis only.

511.7 Hereditary Methemoglobinemia

Kim Smith-Whitley and Janet L. Kwiatkowski

Hereditary methemoglobinemia is a clinical syndrome caused by an increase in the serum concentration of methemoglobin either because of congenital changes in hemoglobin synthesis or of metabolism leading to imbalances in reduction and oxidation of hemoglobin. The iron molecule in hemoglobin is normally in the ferrous state (Fe^{2+}), which is essential for oxygen transport. Under physiologic conditions, there is a slow, constant loss of electrons to released oxygen, and the ferric (Fe^{3+}) form combines with water, producing **methemoglobin (MetHb)**. The newly formed MetHb has a reduced ability to bind oxygen.

Two pathways for MetHb reduction exist. The physiologic and predominant pathway is a reduced form of nicotinamide adenine dinucleotide (NADH)–dependent reaction catalyzed by cytochrome b5 reductase. This mechanism is >100-fold more efficient than the production of MetHb. The alternate pathway uses NAD phosphate generated by G6PD in the hexose monophosphate shunt and requires an extrinsic electron acceptor to be activated (i.e., methylene blue, ascorbic acid, riboflavin). In normal individuals, oxidation of hemoglobin to MetHb occurs at a slow rate, 0.5–3%, which is countered by MetHb reduction to maintain a steady state of 1% MetHb.

MetHb may be increased in the RBC because of exposure to toxic substances or to absence of reductive pathways, such as NADH-cytochrome b5 reductase deficiency. **Toxic methemoglobinemia** is much more common than hereditary methemoglobinemia (Table 511.6). Infants are exceptionally vulnerable to hemoglobin oxidation because their erythrocytes have half the amount of cytochrome b5 reductase seen in adults, hemoglobin F is more susceptible to oxidation than hemoglobin A, and the more alkaline infant gastrointestinal (GI) tract promotes the growth of nitrite-producing gram-negative bacteria. When MetHb levels are >1.5 g/24 hours, cyanosis is visible (15% MetHb); a level of 70% MetHb is lethal. The MetHb level is usually reported as a percentage of normal hemoglobin, and the toxic level is lower at a lower Hb level. Methemoglobinemia has been described in infants who ingested foods and water high in **nitrates**, who were exposed to aniline teething gels or other chemicals, and in some infants with severe gastroenteritis and acidosis. Methemoglobin can color the blood brown (Fig. 511.6). A patient with significant methemoglobinemia is cyanotic and does not respond to 100% oxygen. Arterial oxygen tension will be normal or elevated (if on high FiO_2) despite cyanosis, but blood oxygen saturation determined by multiwavelength co-oximetry will be low. Oxygen saturation calculated from arterial blood gas or pulse oximetry is misleading and inaccurate. Although pulse oximetry is usually lower than normal, it does not reflect the true degree of desaturation.

511.8 Hereditary Methemoglobinemia with Deficiency of NADH Cytochrome b5 Reductase

Kim Smith-Whitley

The first reported inherited disorder causing methemoglobinemia resulted from an enzymatic deficiency of NADH cytochrome b5

Table 511.6 Known Etiologies of Acquired Methemoglobinemia

MEDICATIONS
Benzocaine
Chloroquine
Dapsone
Doxycycline
EMLA (eutectic mixture of local anesthetics) topical anesthetic (lidocaine 2.5% and prilocaine 2.5%)
Flutamide
Lidocaine
Metoclopramide
Nitrates
Nitric oxide
Nitroglycerin
Nitroprusside
Nitrous oxide
Phenazopyridine
Prilocaine
Primaquine
Riluzole
Silver nitrate
Sodium nitrate
Sulfonamides
MEDICAL CONDITIONS
Pediatric gastrointestinal infection, sepsis
Recreational drug overdose with amyl nitrate ("poppers")
Sickle cell disease–related painful episode
MISCELLANEOUS
Aniline dyes
Fume inhalation (automobile exhaust, burning of wood and plastics)
Herbicides
Industrial chemicals: nitrobenzene, nitroethane (found in nail polish, resins, rubber adhesives)
Pesticides
Gasoline octane booster
Nitrate rich vegetables (beets, borage, chard)
Well water

From Ash-Bernal R, Wise R, Wright SM. Acquired methemoglobinemia. *Medicine (Baltimore)* 2004;83:265–273.

reductase, which was classified into two distinct phenotypes. **In type I**, the most common form, the deficiency of NADH cytochrome b5 activity is found only in erythrocytes, with other cell types unaffected. In **type II,** the enzyme deficiency is present in all tissues and results in more significant symptoms beginning in infancy with encephalopathy, intellectual impairment, spasticity, microcephaly, and growth retardation, with death most often by 2 years of age. Both types exhibit an autosomal recessive inheritance pattern.

Cyanosis varies in intensity with season and diet. The time of cyanosis onset also varies, appearing in some patients at birth and others as late as adolescence. Although as much as 50% of the total circulating hemoglobin may be in the form of nonfunctional MetHb, little or no cardiorespiratory distress occurs in these patients, except on exertion.

Daily oral treatment with *ascorbic acid* (200-500 mg/day in divided doses) gradually reduces the MetHb to approximately 10% of the total pigment and alleviates the cyanosis as long as therapy is continued. Chronic high doses of ascorbic acid have been associated with hyperoxaluria and renal stone formation. Ascorbic acid should not be used to treat *acute toxic methemoglobinemia*. When immediately available, poison control should be contacted to verify the most up-to-date therapeutic strategies. As with ascorbic acid, *riboflavin* uses the alternate pathway of MetHb reduction and is most effective when given in high doses. *Methylene blue*, administered via IV (1-2 mg/kg initially), is used to treat toxic methemoglobinemia. An oral dose can be administered (100-300 mg/day) as maintenance therapy.

Fig. 511.6 Normal arterial blood vs methemoglobinemia. Arterial whole blood with 1% methemoglobin *(left)* vs arterial whole blood with 72% methemoglobin *(right)*. Note the characteristic chocolate-brown color of the sample with an elevated methemoglobin level. Both samples were briefly exposed to 100% oxygen and shaken. This quick analysis is a good bedside test for methemoglobinemia. The sample on the *left* turned bright red, whereas the sample on the *right* remained chocolate-brown. *Methods:* Whole blood samples were drawn at the same time from the same person. The measured hemoglobin concentration was 11.7 g/dL. Calculated concentration of methemoglobin: 11.7 g/dL × 0.01 = 0.117 g/dL *(left)* and 11.7 g/dL × 0.72 = 8.42 g/dL *(right)*. An elevated methemoglobin level was made in vitro by adding 0.1 mL of a 0.144 molar solution of sodium nitrate *(right),* and 0.1 mL of normal saline was added as a control *(left)*. Co-oximetry measurements were taken on both samples shortly after the blood was drawn and 20 min after the addition of sodium nitrate solution. Both blood samples were exposed to 100% oxygen before the second measurement. *(Protocol based on personal communication with Dr. Ali Mansouri, December 2002.)*

Methylene blue should not be used in patients with G6PD deficiency. This treatment is ineffective and can cause severe oxidative hemolysis. If methylene blue is given to a patient with G6PD deficiency, symptoms will not improve, and marked hemolysis can develop within 24 hours of administration. Because G6PD deficiency status is rarely known at the time of treatment, a careful history should be elicited. When the history is negative for symptoms of G6PD deficiency, treatment with methylene blue should be initiated judiciously, and the patient should be closely monitored for improvement.

511.9 Syndromes of Hereditary Persistence of Fetal Hemoglobin

Kim Smith-Whitley and Janet L. Kwiatkowski

Hereditary persistence of HbF **(HPFH)** syndromes are a form of thalassemia; pathogenic variants are associated with a decrease in the production of either or both β- and δ-globins. There is an imbalance in the α:non-α synthetic ratio characteristic of thalassemia. More than 20 variants of HPFH have been described. They are deletional, $\delta\beta^0$ (Black, Ghanaian, Italian), nondeletional (Tunisian, Japanese, Australian), linked to the β-globin–gene cluster (British, Italian-Chinese, Black), or unlinked to the β-globin–gene cluster (Atlanta, Czech, Seattle). The $\delta\beta^0$ forms have deletions of the entire δ- and β-globin gene sequences, and the most common form in the United States is the Black **(HPFH 1)** variant. As a result of the δ and β gene deletions, there is production only of γ-globin and formation of HbF. In the homozygous form, no manifestations of thalassemia are present. There is only HbF with very mild anemia and slight microcytosis. When inherited with other variant hemoglobins, HbF is elevated into the 20–30% range; when inherited with HbS, sickle cell disease is ameliorated, with fewer complications.

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511.10 Thalassemia Syndromes

Janet L. Kwiatkowski

Thalassemia refers to a group of genetic disorders of globin-chain production in which there is an imbalance between the α-globin and β-globin chain production. **β-thalassemia** syndromes result from a decrease in β-globin chains, which results in a relative excess of α-globin chains. There are >300 β-thalassemia pathogenic variants that have been characterized. These pathogenic variants can affect any step in the transcription of β-globin genes. **β^0-thalassemia** refers to the absence of production of the β-globin. When patients are homozygous for the β0-thalassemia gene, they cannot make any normal β-globin chains (HbA). **β+-thalassemia** indicates a pathogenic variant that makes decreased amounts of normal β-globin (HbA). Some β-thalassemia pathogenic variants have structural changes such as HbE. Others, such as δβ-thalassemia or HPFH, are variants of β-thalassemia that have decreased production of β-globin gene with increased compensatory production of HbF. β^0-thalassemia syndromes are generally more severe than β^+-thalassemia syndromes, but there is significant variability between the genotype and phenotype. β-thalassemia *major*, commonly called transfusion-dependent thalassemia, refers to severe β-thalassemia that requires early transfusion therapy. β-thalassemia *intermedia* (also known as non–transfusion-dependent thalassemia) is a clinical diagnosis of a patient with a less severe clinical phenotype that usually does not require regular transfusion therapy in childhood. Many of these patients have at least one β^+-thalassemia pathogenic variant. β-thalassemia syndromes usually require a β-thalassemia pathogenic variant in both β-globin genes. Carriers with a single β-globin pathogenic variant are generally asymptomatic, except for microcytosis and mild anemia.

In **α-thalassemia**, there is an absence or reduction in α-globin production usually due to deletions of α-globin genes. Normal individuals have four α-globin genes; the more genes affected, the more severe the disease. **α^0-thalassemia** indicates no α-chains produced from that chromosome (– –/). **α^+-thalassemia** produces a decreased amount of α-globin chain from that chromosome (-alpha/).

The primary pathology in the thalassemia syndromes stems from the *quantity* of globin produced, whereas the primary pathology in sickle cell disease is related to the *quality* of β-globin produced.

EPIDEMIOLOGY

There are >300 different pathogenic variants resulting in absent or decreased globin production. Although most are rare, the 20 most common abnormal alleles constitute 80% of the known thalassemias worldwide; 1.5% of the world's population carries alleles for β-thalassemia, and in Southeast Asia 5–10% of the population carry alleles for α-thalassemia. In the United States, an estimated 2,000 persons have β-thalassemia major.

PATHOPHYSIOLOGY

Two related features contribute to the sequelae of **β-thalassemia syndromes**: inadequate β-globin gene production leading to decreased levels of normal hemoglobin (HbA) and unbalanced α- and β-globin chain production leading to ineffective erythropoiesis. In β-thalassemia α-globin chains are in excess to non–α-globin chains, and α-globin tetramers (α_4) are formed and appear as RBC inclusions. The free α-globin chains and inclusions are very unstable, precipitate in RBC precursors, damage the RBC membrane, and shorten RBC survival, leading to anemia and increased erythroid production (Table 511.7). This results in a marked increase in erythropoiesis, with early erythroid precursor death in the bone marrow. Clinically, this is characterized by a lack of maturation of erythrocytes and an inappropriately low reticulocyte count. This ineffective erythropoiesis and the compensatory massive marrow expansion with erythroid hyperactivity characterize β-thalassemia. Because of the low or absent production of β-globin, the α-chains combine with γ-chains, resulting in HbF ($\alpha_2\gamma_2$) being the dominant hemoglobin. In addition to the natural survival effect, the γ-globin chains may be produced in increased amounts, regulated by genetic polymorphisms. The δ-chain synthesis is not usually affected in β-thalassemia or β-thalassemia trait, and therefore patients have a relative or absolute increase in HbA_2 production ($\alpha_2\delta_2$).

In the **α-thalassemia syndromes**, there is a reduction in α-globin production. Normally, there are four α-globin genes (two from each parent) that control α-globin production. α-thalassemia syndromes vary from complete absence (hydrops fetalis) to only slightly reduced (α-thalassemia silent carrier) α-globin production. In the α-thalassemia syndromes, an excess of β- and γ-globin chains are produced. These excess chains form **hemoglobin Bart's (γ_4)** in fetal life and **HbH** (β_4) after birth. These abnormal tetramers are nonfunctional hemoglobins with very high oxygen affinity. They do not transport oxygen and result in extravascular hemolysis. A fetus with the most severe form of α-thalassemia **(hydrops fetalis)** develops in utero anemia and, without therapeutic intervention, the pregnancy usually results in fetal loss because HbF production requires sufficient amounts of α-globin. In contrast, infants with β-thalassemia major become symptomatic only after birth when HbA predominates and insufficient β-globin production manifests in clinical symptoms.

HOMOZYGOUS β-THALASSEMIA (TRANSFUSION-DEPENDENT BETA THALASSEMIA, BETA THALASSEMIA MAJOR, COOLEY ANEMIA)

Clinical Manifestations

If not treated, children with homozygous β^0-thalassemia usually become symptomatic from progressive anemia, with weakness, poor growth, and cardiac decompensation during the second 6 months of life. Depending on the pathogenic variant and degree of HbF production, regular transfusions are necessary beginning in the second month to second year of life, but rarely later. The decision to transfuse is multifactorial but is not determined solely by the degree of anemia. Persistent hemoglobin level below 7 g/dL in the absence of acute illness is an indication to start transfusions. In addition, the presence of signs of ineffective erythropoiesis, such as growth failure, bone deformities secondary to marrow expansion, and hepatosplenomegaly, are important variables in determining transfusion initiation.

The classic presentation of children with severe disease includes *thalassemic facies* (maxilla hyperplasia, flat nasal bridge, frontal bossing), pathologic bone fractures, marked hepatosplenomegaly, and cachexia and is primarily seen in countries without access to chronic transfusion therapy. Occasionally, patients with moderate anemia develop these features because of severe compensatory, ineffective erythropoiesis.

In nontransfused patients with severe ineffective erythropoiesis, marked **splenomegaly** can develop with hypersplenism and abdominal symptoms. The features of ineffective erythropoiesis include expanded medullary spaces (with massive expansion of the marrow of the face and skull), extramedullary hematopoiesis, and higher metabolic needs (Fig. 511.7). The chronic anemia and increased erythroid drive produce an increase in iron absorption from the GI tract and secondary hemosiderosis-induced organ injury.

Chronic transfusion therapy dramatically improves the quality of life and reduces the complications of severe thalassemia. Transfusion-induced **hemosiderosis** becomes the major clinical complication of transfusion-dependent thalassemia. Each mL of pure packed RBCs contains approximately 1 mg of iron. Physiologically, there is no mechanism to eliminate excess body iron. Iron is initially deposited in the liver and is followed by deposition in the endocrine organs and the heart. This leads to a high rate of hypothyroidism, hypogonadotropic gonadism, growth hormone deficiency, hypoparathyroidism, and diabetes mellitus. Iron deposition in the heart causes heart failure and arrhythmias, and heart disease is the leading cause of death in inadequately chelated patients. Eventually, most patients not receiving adequate iron chelation therapy die from cardiac failure or cardiac arrhythmias secondary to hemosiderosis. Hemosiderosis-induced morbidity can be prevented by adequate iron chelation therapy.

Laboratory Findings

In the United States, some children with β-thalassemia major will be identified on newborn screening because of the detection of only HbF on hemoglobin electrophoresis. However, infants with β^+ pathogenic variants might be missed on newborn screen if small amounts of hemoglobin

Table 511.7 The Thalassemias

THALASSEMIA	GLOBIN GENOTYPE	RED BLOOD CELL FEATURES	CLINICAL FEATURES	HEMOGLOBIN ANALYSIS
α-THALASSEMIA				
1 Gene deletion	−,α/α,α	Normal	Normal	Newborn: Bart's: 1–2%
2 Gene deletion (α-thalassemia trait)	−,α/−,α −, −/α,α	Microcytosis, mild hypochromasia	Normal, mild anemia	Newborn: Bart's: 5–10%
3 Gene deletion hemoglobin H	−,−/−,α	Microcytosis, hypochromic	Mild anemia, transfusions not required	Newborn: Bart's: 20–30%
2 Gene deletion + Constant Spring	$-,-/\alpha,\alpha^{\text{Constant Spring}}$	Microcytosis, hypochromic	Moderate to severe anemia, transfusion, splenectomy.	2–3% Constant Spring, 10–15% HbH
4 Gene deletion	−,−/−,−	Anisocytosis, poikilocytosis	Hydrops fetalis	Newborn: 89–90% Bart's with Gower-1, Gower-2, and Portland
Nondeletional	$\alpha,\alpha/\alpha,\alpha^{\text{variant}}$	Microcytosis, mild anemia	Normal	1–2% variant hemoglobin
β-THALASSEMIA				
β^0 or β^+ heterozygote: trait	β^0/A, β^+/A	Variable microcytosis, mild anemia	Normal	Elevated A_2, variable elevation of F
β^0 or β^+- homozygote or compound heterozygote Thalassemia severe	β^0/β^0, β^+/β^0, $\beta^+\beta^+$, E/β^0, E/β^+	Microcytosis, nucleated RBC	Transfusion dependent	F 98% and A_2 2%, E 30–40% (E/β^0); variably low HbA with β^+
β^0 or β^+ homozygote or compound heterozygote Thalassemia intermedia	β^0/β^0, β^+/β^0, $\beta^+\beta^+$, E/β^0, E/β^+	Hypochromic, microcytosis	Mild to moderate anemia, intermittent transfusions	A_2 2–5%, F 10–30%, HbA variably low levels
Dominant (rare)	B^0/A	Microcytosis, abnormal RBCs	Moderately severe anemia, splenomegaly	Elevated F and A_2
δ-Thalassemia	A/A	Normal	Normal	A_2 absent
$(\delta\beta)^0$-Thalassemia	$(\delta\beta)^0$/A	Hypochromic	Mild anemia	F 5–20%
$(\delta\beta)^+$-Thalassemia Lepore	β^{Lepore}/A	Microcytosis	Mild anemia	Lepore 8–20%
Homozygous Hb Lepore	$\beta^{\text{Lepore}}/\beta^{\text{Lepore}}$	Microcytic, hypochromic	Thalassemia intermedia	F 80%, Lepore 20%
γδβ-Thalassemia	$(\gamma^A\delta\beta)^0$/A	Microcytosis, microcytic, hypochromic	Moderate anemia, splenomegaly, homozygote: thalassemia intermedia	Decreased F and A_2 compared with δβ-thalassemia
γ-Thalassemia	$(\gamma^A\gamma^G)^0$/A	Microcytosis	Insignificant unless homozygote	Decreased F

A are present. An HbFE pattern can be consistent with hemoglobin E β^0-thalassemia, or the more benign hemoglobin EE disease, and needs to be followed up. The lack of standardized neonatal diagnosis of thalassemia disorders requires close follow-up of newborns with unclear thalassemia pathogenic variants and babies from high-risk ethnic groups.

Infants with serious β-thalassemia disorders have a progressive anemia after the newborn period. Microcytosis, hypochromia, and targeting characterize the RBCs. Nucleated RBCs, marked anisopoikilocytosis, and a relative reticulocytopenia are typically seen (see Fig. 511.5E). The Hb level falls progressively often to <6 g/dL unless transfusions are given. The reticulocyte count is commonly <8% and is inappropriately low compared to the degree of anemia caused by ineffective erythropoiesis. The unconjugated serum bilirubin level is usually elevated, but other chemistries may be initially normal. Even if the child does not receive transfusions, iron eventually accumulates with elevated serum ferritin and transferrin saturation. Evidence of bone marrow hyperplasia can be seen on radiographs (see Fig. 511.7).

Early definitive diagnosis is recommended. Newborn screening techniques such as hemoglobin electrophoresis are not definitive. DNA diagnosis of the β-thalassemia pathogenic variants, along with testing for common genetic modifiers of the clinical phenotype, is recommended. Co-inheritance of one or more α-thalassemia deletions is common, and it decreases the severity of the β-thalassemia disease as it improves the α:β chain imbalance. Some patients' pathogenic variants cannot be diagnosed by standard electrophoresis or common DNA probes. Referral of the samples to a tertiary laboratory is indicated, along with parental and family testing. After the definitive diagnosis, families should undergo detailed counseling.

Management and Treatment of Thalassemia

See Figure 511.8.

Transfusion Therapy

β-thalassemia major is a clinical diagnosis that requires the integration of laboratory findings and clinical features. Of patients with homozygous β^0-thalassemia (the most severe pathogenic variants), 15–20% may have a clinical course that is phenotypically consistent with thalassemia intermedia. In contrast, 25% of patients with homozygous β^+-thalassemia, typically a more benign genotype, may have transfusion-dependent thalassemia. Transient clinical events, such as a sudden fall in hemoglobin secondary to an episode of parvovirus requiring transfusion, do not necessarily indicate a transfusion-dependent patient. The long-term observation of the clinical characteristics, such as growth, bony changes, and hemoglobin, are necessary to determine chronic transfusion therapy.

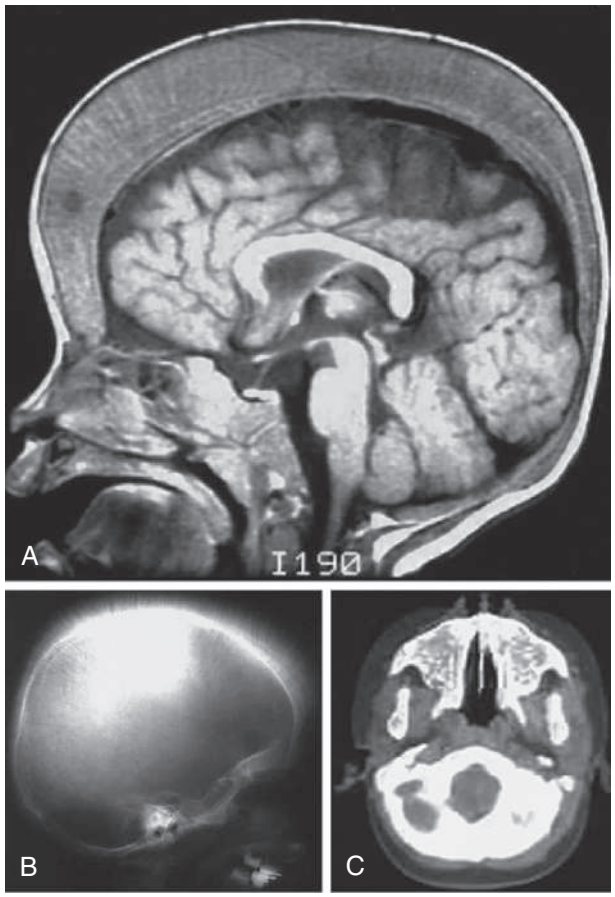

Fig. 511.7 Ineffective erythropoiesis in a 3-yr-old patient who has β-thalassemia major and has not received a transfusion. A, Massive widening of the diploic spaces of the skull as seen on MRI. B, Radiographic appearance of the trabeculae as seen on plain radiograph. C, Obliteration of the maxillary sinuses with hematopoietic tissue as seen on CT scan.

Guidelines for Transfusion Therapy. Patients who require transfusion therapy should have an extended RBC phenotype and/or genotype. Patients should receive RBCs depleted of leukocytes and matched for D, C, c, E, e, and Kell antigens at a minimum. Cytomegalovirus-safe units are indicated in stem cell transplantation candidates. Transfusions should generally be given at intervals of 3-4 weeks, with the goal being to maintain a pretransfusion Hb level of 9.5-10.5 g/dL. Ongoing monitoring for transfusion-associated transmitted infections (hepatitis A, B, and C, HIV), alloimmunization, annual blood transfusion requirements, and transfusion reactions is essential.

Iron Overload Monitoring

Excessive iron stores from transfusion cause many of the complications of β-thalassemia major. Accurate assessment of excessive iron stores is essential to optimal therapy. Serial serum ferritin levels provide a useful screening technique in assessing iron balance trends, but results may not accurately predict quantitative iron stores. Undertreatment or overtreatment of presumed excessive iron stores can occur in managing a patient based on serum ferritin alone. Quantitative measurements of liver iron and cardiac iron by MRI are standard noninvasive methods to assess tissue iron overload; estimation of pancreatic and gonadal iron is being studied. This technology, along with access to multiple chelators, enables targeted chelation therapy for patients with organ-specific hemosiderosis before the onset of overt organ failure. Integration of these imaging technologies with chelation therapy may prevent heart failure, diabetes, and other organ dysfunction.

Quantitative liver iron by approved R2 or R2* MRI is the best indicator of total body iron stores and should be obtained in patients after chronic transfusion therapy has been initiated. The liver iron results will help guide the chelation regimen. Cardiac iron estimation by T2* MRI, is usually obtained starting at 10 years old, but should be obtained earlier in the setting of severe iron overload or if the transfusion and chelation history is not known. There may be a discrepancy between the liver iron and the heart iron because of different rates of tissue loading and unloading and the differential effects of iron chelators on organ-specific iron removal.

Chelation Therapy

Iron-chelation therapy should start as soon as the patient becomes significantly iron-overloaded. In general, this occurs after 1 year of transfusion therapy and correlates with the serum ferritin >1,000 ng/mL and/or a liver iron concentration of >5,000 μg/g dry weight. Iron chelation is not currently labeled for use in children <2 years.

There are three available iron chelators (deferoxamine, deferasirox, and deferiprone); each varies in its route of administration, pharmacokinetics, adverse events, and efficacy (Table 511.8). Combination chelation therapy may be required for high iron burden. The overall goal is to prevent hemosiderosis-induced tissue injury and avoid chelation toxicity. This requires close monitoring of the patients. In general, chelation toxicity increases as iron stores decrease.

Deferoxamine (Desferal) is the most studied iron chelator; it has an excellent safety and efficacy profile. It requires subcutaneous or IV administration because of its poor oral bioavailability and short half-life of <30 minutes, necessitating administration as a continuous infusion over at least 8 hours daily, 5-7 days/week. Deferoxamine is initially started at 25 mg/kg and can be increased to 60 mg/kg in heavily iron-overloaded patients. The major problem with deferoxamine is poor adherence because of the difficult, time-consuming route of administration. Adverse side effects include local skin reactions, ototoxicity, retinal changes, and bone dysplasia with truncal shortening. Maintaining the therapeutic index (deferoxamine dose in mg/kg divided by the serum ferritin) below 0.025 limits these adverse effects.

The *oral* iron chelator **deferasirox** is commercially available in the United States. Of patients treated with deferoxamine, 70% have switched to deferasirox because it is orally available. Deferasirox has a half-life of >16 hours and requires once-daily administration. The drug was initially available as a dispersible tablet that is dissolved in water or juice. Subsequently, a film coated tablet that is swallowed whole and a granule form that is sprinkled on soft food and ingested became available. Dosing is different for the different deferasirox formulations. For the dispersible tablet form, the initial dose typically is 20 mg/kg/day and can be escalated to as high as 40 mg/kg/day based on the iron burden. The dosing for the film-coated tablet and granule forms is 30% lower than the dispersible tablet, with a starting dose of 14 mg/kg/day, which can be escalated to a maximum of 28 mg/kg/day. The most common side effects are GI symptoms, which may be lessened with the film-coated tablet and granule formulations because they do not contain lactose and sodium laureate, which are found in the dispersible tablet and are thought to be responsible for some of the GI symptoms. The most serious side effect of deferasirox is potential kidney damage. Up to 30% of patients have transient increases in creatinine that may require temporary modifications of dosing. This toxicity may occur more commonly in the setting of dehydration. Proteinuria also can develop and, less commonly, a renal Fanconi syndrome may occur. Long-term studies in thousands of patients have not demonstrated progressive renal dysfunction, but isolated cases of renal failure in patients have occurred. In addition, hepatic transaminitis may occur, with an increase to >5 times the upper limit of normal in approximately 8% of patients. All patients require monthly chemistry panels and ongoing monitoring for proteinuria.

Deferiprone, an oral iron chelator, is approved in the United States for use in individuals with transfusional iron overload 3 years and older. Deferiprone has a half-life of approximately 3 hours and requires dosing 3 times daily; a new longer-acting tablet form is available that is

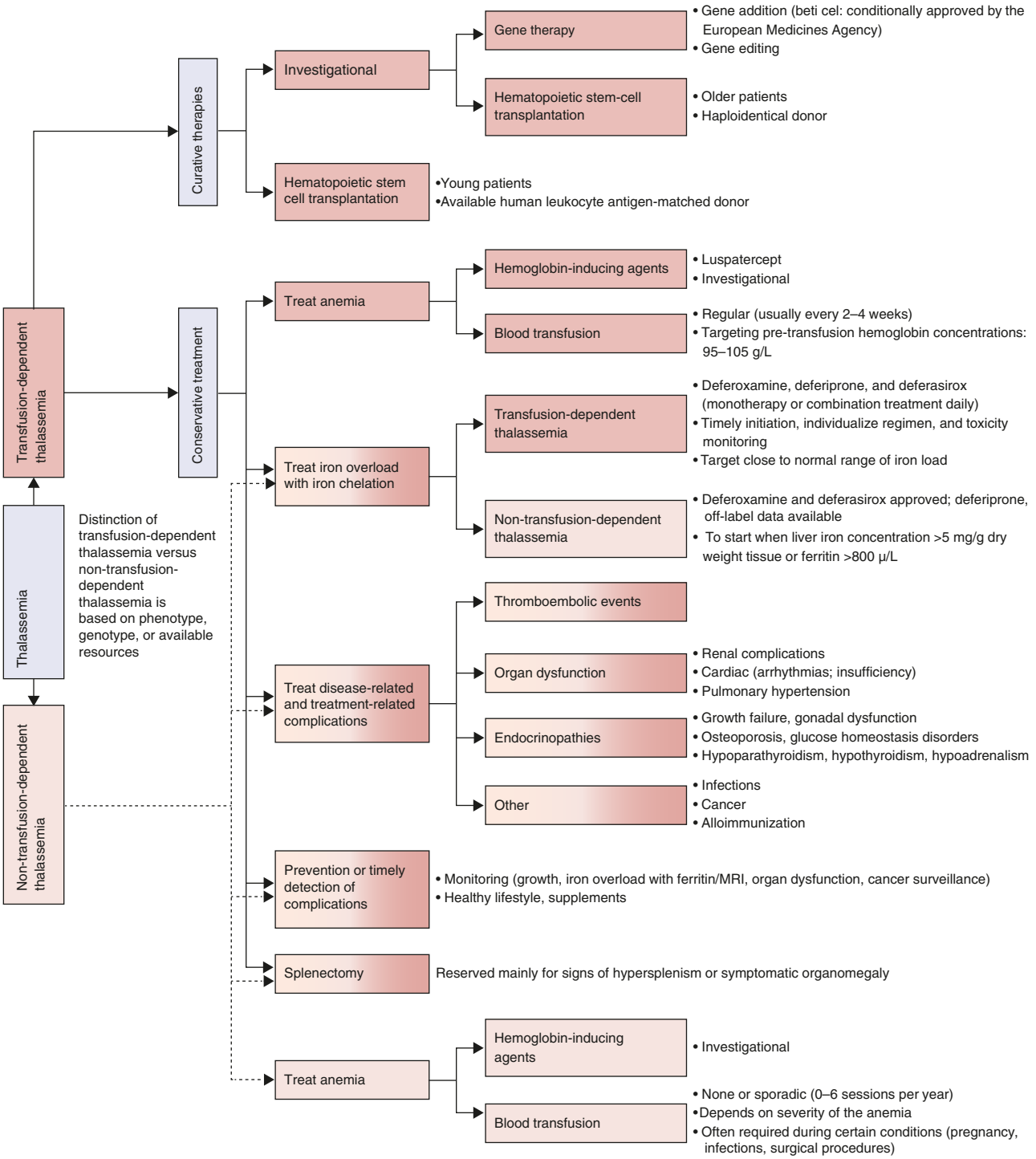

Fig. 511.8 Algorithm for the therapeutic management of thalassemia. *(From Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassemia. Lancet. 2022;399:2310–2322; Fig. 3.)*

dosed twice daily. The starting dose is 75 mg/kg/day and can be escalated to 99 mg/kg/day based on the degree of iron overload. Deferiprone, a small molecule, effectively enters cardiac tissue and may be more effective than other chelators in reducing cardiac hemosiderosis. The most serious side effect of deferiprone is transient agranulocytosis, which occurs in 1-2% of patients and usually in the first year of treatment. It has been associated with rare deaths where patients were not adequately monitored. The use of deferiprone requires frequent CBC monitoring, typically weekly for at least the first 6 months of therapy. Most importantly, the drug should be held and the neutrophil count checked immediately with all febrile illnesses.

As thalassemia patients live longer, the iron chelation goals have changed. Aggressive treatment with combination chelation therapy is often used in heavily iron-overloaded patients to prevent or reverse organ dysfunction. Deferoxamine, in combination with deferiprone, is routinely used in patients with increased cardiac iron. Combination therapy of deferoxamine and deferasirox or with deferasirox and deferiprone may also be efficacious in patients with severe iron overload.

Luspatercept

Luspatercept is a recombinant fusion protein that binds TGFβ family ligands and thus blocks a signaling pathway involved in ineffective

Table 511.8 Properties of Iron Chelators

	DEFEROXAMINE	DEFERASIROX	DEFERIPRONE
Prototype trade name	Desferal	Exjade; Jadenu	Ferriprox
Route of administration	Subcutaneous; intravenous	Oral dispersible tablet; film-coated tablet; granules	Oral solution; oral tablet (3 times per day); oral tablet (twice per day)
Usual dose	Standard, 25–40 mg/kg per day over 8–12 h; low cardiac T2* or heart failure, 50–60 mg/kg per day over 12–24 h	Oral dispersible tablet, 20–40 mg/kg per day once daily; film-coated tablet and granules, 14–28 mg/kg per day once daily	75–100 mg/kg per day divided into 3 doses (oral solution or oral tablet, 3 times per day); 75–100 mg/kg per day divided into 2 doses (oral tablet, twice per day)
Excretion	Urinary (60%), defecation (40%)	Defecation (~90%)	Urinary (~75–90%)
Ability to remove liver iron	High	High	Moderate*
Ability to remove cardiac iron	Moderate†	Moderate‡	High
Adverse events	Local reactions, auditory, ophthalmologic, allergic reactions, bone defects, Yersinia or Klebsiella infections; high doses: pulmonary, neurologic, renal failure	Gastrointestinal, rash, renal impairment (increase in creatinine, proteinuria, proximal renal tubular dysfunction, renal insufficiency), elevated hepatic enzymes, gastrointestinal bleeding or ulcers, hepatic failure, auditory, ophthalmologic	Gastrointestinal, elevated hepatic enzymes, arthropathy, neutropenia or agranulocytosis
EU licensure	Licensed	Patients age 6 years and older with transfusion-dependent thalassemia; patients age 10 years and older with non-transfusion-dependent thalassemia (as a first-line treatment); patients age 2 years and older with transfusion-dependent thalassemia (as a second-line treatment)	Patients with thalassemia major when current chelation therapy is contraindicated or inadequate
USA licensure	Licensed	Patients age 2 years and older with transfusion-dependent thalassemia; patients age 10 years and older with nontransfusion-dependent thalassemia	Patients age 8 years and older with transfusional iron overload and thalassemia syndromes

*Reports of insufficient liver iron removal in some patients at doses of 75 mg/kg per day, but higher dosages, especially for patients with high transfusional iron burden, might be more effective.
†With continuous infusion.
‡Limited data show faster cardiac iron removal with modest hepatic iron loading, but this removal might be less effective in patients with more severe hepatic siderosis.
From Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassemia. Lancet 2022;399:2310–2322; Table 1.

erythropoiesis and results in improved erythroid maturation. The drug is given by subcutaneous injection every 3 weeks at a starting dose of 1 mg/kg/dose, which can be escalated to 1.25 mg/kg/dose if no response after 6 weeks of treatment. In patients with transfusion-dependent β-thalassemia, use of luspatercept was associated with a reduction in RBC requirements in about 70% of treated patients. Luspatercept is approved by the FDA for use in adults with transfusion-dependent β-thalassemia. Adverse effects include bone pain, arthralgia, dizziness, hypertension, and hyperuricemia. The drug also is being studied in nontransfusion dependent thalassemia, where a 1 to 1.5 g/dL rise in hemoglobin has been seen.

Hydroxyurea

Hydroxyurea, a DNA antimetabolite, increases HbF production. It has been most successfully used in sickle cell disease and in some patients with β-thalassemia intermedia. Studies in β-thalassemia major are limited. In many parts of the world, hydroxyurea therapy is used in β-thalassemia intermedia patients. Even though increases in HbF levels are observed, they do not predictively correlate with increase in total Hb in these patients. In general, there appears to be a mean increase in Hb of 1 g/dL (range: 0.1-2.5 g/dL). Hydroxyurea therapy in thalassemia intermedia is associated with a reduced risk of leg ulcers, pulmonary hypertension, and extramedullary hematopoiesis. The initial starting dose for thalassemia intermedia is 10 mg/kg and may be escalated to 20 mg/kg/day. Patients with β-thalassemia are at increased risk of developing cytopenias with hydroxyurea use, which may prevent dose escalation. Close monitoring of the CBC with differential is required.

Hematopoietic Stem Cell Transplantation and Gene Therapy

Hematopoietic stem cell transplantation has cured >3,000 patients who had β-thalassemia major. In low-risk HLA-matched sibling patients, there is at least a 90% survival and an 80% event-free survival. In general, myeloablative conditioning regimens have been used to prevent graft rejection and thalassemia recurrence. Most success has been in children <14 years old without excessive iron stores and hepatomegaly who undergo sibling HLA-matched allogeneic transplantation. All children who have an HLA-matched sibling should be offered the option of bone marrow transplantation. Outcomes of hematopoietic stem cell transplantation with a matched *unrelated donor* using molecular techniques for HLA typing have significantly improved, approaching outcomes with matched sibling donors, but with an increased risk of graft versus host disease. Alternative transplantation regimens for patients without appropriate donors are experimental and have variable success.

Gene therapy using gene addition or gene editing approaches are FDA approved for transfusion-dependent patients with β-thalassemia. Results with gene addition using lentiviral vectors containing a functional β-globin gene have been promising. **Betibeglogene autotemcel** is a rare gene-based, FDA- approved therapy for adults and pediatric patients (≥4 years) with transfusion dependent β-thalassemia. This one-time therapy transduces harvested autologous CD34+ cells with a replicant incompetent vector containing a modified β-globin gene. Approximately 90% of treated patients achieve transfusion independence.

A second approved approach to gene therapy involves gene editing to increase hemoglobin F levels. The first patient treated with transfusion-dependent thalassemia treated with a CRISPR-Cas9 gene editing approach was able to discontinue RBC transfusions while maintaining a normal hemoglobin level, mostly consisting of HbF. This gene approach is indicated for transfusion-dependent β-thalassemia patients ≥12 years of age.

Splenectomy

Splenectomy may be required in thalassemia patients who develop hypersplenism. These patients have a falling steady-state Hb level and/or a rising transfusion requirement. However, splenectomy is less frequently used as a therapeutic option; serious adverse effects of splenectomy are increasingly recognized beyond infection risk. In thalassemia intermedia, splenectomized patients have a marked increased risk of venous thrombosis, pulmonary hypertension, leg ulcers, and silent cerebral infarction compared to nonsplenectomized patients. All patients should be fully immunized against encapsulated bacteria and receive appropriate instructions regarding fever management. Prophylactic penicillin should be administered after splenectomy to prevent sepsis, and families need to be educated on the risk of fever and sepsis.

Preventive Monitoring of Thalassemia Patients

See Table 511.9.

Cardiac Disease

Cardiac disease is the major cause of death in thalassemia. Serial echocardiograms should be monitored to evaluate cardiac function and pulmonary artery pressure. Pulmonary hypertension frequently occurs in nontransfusion dependent thalassemia patients and may be an indication for transfusion therapy. After approximately 8 years of chronic transfusion therapy, cardiac hemosiderosis may occur; consequently, cardiac T2* MRI imaging studies are recommended, typically beginning at 10 years of age. Patients with cardiac hemosiderosis and decreasing cardiac ejection fraction require intensive combination chelation therapy. Periodic electrocardiogram studies also are obtained after age 10 years because of the risk of arrhythmia from cardiac iron overload.

Endocrine Disease

Endocrine function progressively declines with age secondary to hemosiderosis and nutritional deficiencies. Iron deposition in the pituitary and endocrine organs can result in multiple endocrinopathies, including hypothyroidism, growth hormone deficiency, delayed puberty, hypoparathyroidism, diabetes mellitus, osteoporosis, and adrenal insufficiency. Monitoring for endocrine dysfunction starts early, about 5 years of age, or after at least 3 years of chronic transfusions. All children require monitoring of their height, weight, pubertal assessment, and sitting height semiannually. Bone density scans should be obtained starting in the second decade of life given the high rate of osteopenia. Nutritional assessments are required. Most patients need vitamin D, vitamin C, and zinc replacement. Fertility is a growing concern among patients and should be assessed routinely.

Psychosocial Support

Thalassemia imposes major disruption in the family unit and significant obstacles to normal development. Culturally sensitive anticipatory counseling is necessary, and the early use of child life services decreases psychologic trauma of therapy. Early social service consultation to address financial and social issues is mandatory.

OTHER β-THALASSEMIA SYNDROMES

Non–Transfusion-Dependent Thalassemia: β-Thalassemia Intermedia

Non–transfusion-dependent thalassemia (thalassemia intermedia) includes a spectrum of patients who initially are not chronically transfused in infancy but may be sporadically transfused throughout their lifetime. The major determining characteristic of these patients is less α-β–globin chain imbalance than observed in thalassemia major. Sometimes, genetic modifiers alter the primary pathogenic variant severity and improve the globin-chain imbalance. Co-inheritance of α-thalassemia trait or polymorphisms of globin promoters such as BCL11A may lessen disease severity and result in a nontransfusion dependent thalassemia. HbE β-thalassemia is a common cause of both transfusion-dependent and non–transfusion-dependent thalassemia. These secondary

Table 511.9 Risk Factors of Thalassemia Comorbidities

CARDIOVASCULAR	
Left ventricle dysfunction	Anemia; cardiac iron overload
Pulmonary hypertension	Chronic hypoxia; splenectomy hypercoagulability; advanced age; nontransfusion-dependent thalassemia
Arrhythmia	Anemia; cardiac iron overload; thyroid disturbances
Thromboembolic events	Splenectomy; hypercoagulability; iron-induced endothelial damage
HEPATOBILIARY	
Viral hepatitis	Red cell transfusions
Fibrosis or cirrhosis	Liver iron overload
Gallstones	Chronic hemolysis
ENDOCRINOPATHIES	
Growth retardation; delayed puberty; hypogonadism	Pituitary iron deposition (thyroid hormone, hypothalamus-pituitary-gonadal axis, growth hormone-insulin like growth factor disturbances); nutrition; anemia
Glucose intolerance; diabetes	Liver iron overload; pancreatic iron deposition; family predisposition
Thyroid dysfunction	Anemia; thyroid iron deposition; hypopituitarism
Adrenal insufficiency	Anemia; adrenal iron deposition; hypopituitarism
Bone disease	Ineffective erythropoiesis, iron predisposition and chelator toxicity; hypogonadism; hypoparathyroidism
NEOPLASIA	
Hepatocellular carcinoma	Chronic hepatitis B or hepatitis C virus infections; liver iron overload
Thyroid cancer; renal cancer; gastrointestinal cancer; breast cancer; hematologic malignancies	Iron toxicity; chronic anemia; advanced age
OTHER	
Renal dysfunction; tubular dysfunction; nephrolithiasis	Anemia; renal iron deposition; and chelator (especially deferasirox) toxicity
Extramedullary hematopoiesis	Ineffective erythropoiesis
Leg ulcers	Anemia; hypercoagulability
Auditory disturbances	Chelator toxicity
Ophthalmologic disturbances	Chelator toxicity
Infectious complications	Splenic dysfunction; transfusion-related infections; iron overload; use of iron chelation therapy (especially deferoxamine)
Spleen (splenomegaly, hypersplenism)	Ineffective erythropoiesis; extramedullary hematopoiesis

From Kattamis A, Kwiatkowski JL, Aydinok Y. *Thalassemia*. Lancet 2022;399:2310–2322. Table 2.

genetic modifiers play a role in altering the severity of this disorder. Occasionally, patients with a single β-thalassemia pathogenic variant or autosomal dominant β-thalassemia trait have clinical features of thalassemia intermedia, or non–transfusion-dependent thalassemia. Genetic studies of these patients often uncover coinheritance of genetic modifiers that worsens the condition, such as α-gene triplication or an unstable β-globin variant.

Individuals with thalassemia intermedia have significant ineffective erythropoiesis that leads to microcytic anemia with hemoglobin of approximately 7 g/dL (range: 6-10 g/dL). These patients have some of the complications characterized in untransfused thalassemia major patients, but the severity varies depending on the degree of ineffective erythropoiesis. They can develop medullary hyperplasia, hepatosplenomegaly, hematopoietic pseudotumors, pulmonary hypertension, leg ulcers, thrombotic events, and growth failure. Many patients develop hemosiderosis secondary to increased GI absorption of iron requiring chelation. Extramedullary hematopoiesis can occur in the vertebral canal, compressing the spinal cord and causing neurologic symptoms; the latter is a medical emergency requiring immediate local radiation therapy to halt erythropoiesis. Transfusions are indicated in thalassemia intermedia patients with significant clinical morbidity.

Thalassemia trait is often misdiagnosed as iron deficiency in children because the two diagnoses produce similar hematologic abnormalities on CBC. However, *iron deficiency is much more prevalent.* A short course of iron and reevaluation is all that is required to identify children who will need further evaluation. Children who have β-thalassemia trait have a persistently normal RBC distribution width and low mean corpuscular volume (MCV), whereas patients with iron deficiency develop an elevated red cell distribution width (RDW) with treatment. On Hb analysis, patients with β-thalassemia trait have elevated levels of HbA_2 and variably increased HbF. There are "silent" forms of β-thalassemia trait, and if the family history is suggestive, further studies may be indicated.

α-THALASSEMIA SYNDROMES

The same evolutionary pressures that produced β-thalassemia and sickle cell disease produced α-thalassemia. Infants are identified in the newborn period by the increased production of hemoglobin Bart's (γ_4) during fetal life and its presence at birth. The α-thalassemia syndromes occur most frequently in Southeast Asia. Deletions are most common in α-thalassemia. In addition to deletional pathogenic variants, there are nondeletional α-globin gene pathogenic variants, the most common being Constant Spring ($\alpha^{CS}\alpha$); these variants cause a more severe anemia and clinical course than the deletional variants. Normally, there are four α-globin genes. The different phenotypes in α-thalassemia largely result from whether one (α^{+}-thalassemia) or both (α^{0}-thalassemia) α-globin genes are deleted in each of the two loci.

The deletion of one α-globin gene (silent carrier) is not identifiable hematologically. Specifically, no alterations are noted in the MCV and mean corpuscular hemoglobin (MCH). Persons with this deletion are usually diagnosed after the birth of a child with a two-gene deletion or HbH (β_4), but some newborn screening programs report even low concentrations of Hb Bart's. During the newborn period, <3% Hb Bart's is observed. The deletion of one α-globin gene is common in Black persons.

The deletion of two α-globin genes results in α-thalassemia trait. The α-globin alleles can be lost in a *trans* (–α/–α) or *cis* (α,α^{-SEA}) configuration. The *trans* or *cis* pathogenic variants can combine with other pathogenic variants or deletions and lead to HbH or α-thalassemia major. In persons from Africa or of African descent, the most common α-globin deletions are in the *trans* configuration, whereas in persons from or descended from Asia or the Mediterranean region, *cis* deletions are most common.

α-Thalassemia trait (two missing α-globin genes) manifests as a microcytic anemia that can be mistaken for iron-deficiency anemia (see Fig. 511.5F). The Hb analysis is normal, except during the newborn period, when Hb Bart's is typically <8% but >3%. Children with a deletion of two α-globin genes are commonly mistaken to have iron deficiency, given the presence of both low MCV and MCH. The simplest approach to distinguish between iron deficiency and α-thalassemia trait is with a good dietary history. Children with iron-deficiency anemia often have a diet that is low in iron and drink a significant amount of cow's milk. Alternatively, a brief course of iron supplementation along with monitoring of erythrocyte parameters might confirm the diagnosis of iron deficiency. If both parents of a child diagnosed with α-thalassemia trait are carriers in the *cis* conformation, they are at risk for a future hydrops fetalis pregnancy. Thus family screening and genetic counseling are indicated.

The deletion of three α-globin genes leads to the diagnosis of HbH disease. A more severe form of HbH disease may be caused by a nondeletional α-globin pathogenic variant in combination with two gene deletions. HbH Constant Spring ($-\alpha/\alpha,\alpha^{CS}$) is the most common type of nondeletional HbH disease.

In California, where a large population of persons of Asian descent resides, approximately 1:10,000 of all newborns have HbH disease. The simplest manner of diagnosing HbH disease is during the newborn period, when excess in γ-tetramers are present and Hb Bart's is commonly >25%. Obtaining supporting evidence from the parents is helpful. Later in childhood, there is an excess of β-globin chain tetramers that results in HbH. A definitive diagnosis of HbH disease requires DNA analysis. Brilliant cresyl blue can stain HbH, but it is rarely used for diagnosis. Patients with HbH disease have a marked microcytosis, anemia, mild splenomegaly, and, occasionally, scleral icterus or cholelithiasis. Chronic transfusion is not usually required for therapy because the Hb range is 7-11 g/dL, with MCV 51-73 fL, but intermittent transfusions for worsening anemia, particularly in the setting of acute infection, may be needed. Individuals with nondeletional HbH disease are more likely to require transfusions than individuals with deletional HbH disease.

The deletion of all four α-globin gene alleles causes profound anemia during fetal life, resulting in **hydrops fetalis**; the ζ-globin gene must be present for fetal survival. There are no normal hemoglobins present at birth (primarily Hb Bart's, with Hb Gower-1, Gower-2, and Portland). Intrauterine transfusions may rescue the fetus, but congenital abnormalities and neurodevelopmental delay often result. Infants with severe α-thalassemia will have lifelong transfusion dependence, and hematopoietic stem cell transplantation is the only cure.

Treatment of HbH disease requires ongoing monitoring of growth and organ dysfunction. Dietary supplement with folate and multivitamins without iron is indicated. Older patients may develop decreased bone density with calcium and vitamin D deficiency. Vitamin D supplementation is indicated if the level is low, and adequate dietary calcium intake should be encouraged to promote bone health. Iron supplementation should be avoided as patients are at risk of developing iron overload. Intermittent transfusion requirements during intercurrent infection may occur, particularly in nondeletional HbH. Splenectomy is occasionally indicated, and because of the high risk of postsplenectomy thrombosis, aspirin or other anticoagulant therapy after splenectomy should be considered. Hemosiderosis, secondary to GI iron absorption or transfusion exposure, may develop in older patients and require chelation therapy. Because HbH is an unstable hemoglobin sensitive to oxidative injury, oxidative medications should be avoided. At-risk couples for hydrops fetalis should be identified and offered molecular diagnosis on fetal tissue obtained early in pregnancy. Later in pregnancy, intrauterine transfusion can improve fetal survival, but chronic transfusion therapy or bone marrow transplantation for survivors will be required.

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Chapter 512

Enzymatic Defects

512.1 Pyruvate Kinase Deficiency

Allison S. Remiker and Amanda M. Brandow

Congenital nonspherocytic hemolytic anemia occurs in persons homozygous or compound heterozygous for autosomal recessive genes that cause either a marked reduction in red blood cell (RBC) pyruvate kinase (PK) or production of an abnormal enzyme with decreased catalytic activity resulting in impaired conversion of phosphoenolpyruvate to pyruvate. Generation of adenosine triphosphate (ATP) within RBCs at this step is impaired, and low levels of ATP, pyruvate, and the oxidized form of nicotinamide adenine dinucleotide (NAD^+) are found (Fig. 512.1). The concentration of 2,3-diphosphoglycerate is increased; this isomer is beneficial in facilitating oxygen release from hemoglobin but detrimental in inhibiting hexokinase and enzymes of the hexose monophosphate shunt. This decrease of hemoglobin oxygen affinity leads to a rightward shift of the hemoglobin-oxygen dissociation curve and therefore improved oxygen delivery manifesting as tolerance of anemia more than expected for the degree of severity. In addition, an unexplained decrease occurs in the sum of the adenine (ATP, adenosine diphosphate, and adenosine monophosphate) and pyridine (NAD^+ and reduced form of NAD) nucleotides, further impairing glycolysis. Because of decreased ATP, RBCs cannot maintain their potassium and water content; the cells become rigid, and their life span is considerably reduced with precipitous destruction in the liver and/or spleen. This is more pronounced in reticulocytes, which require higher levels of ATP.

ETIOLOGY

There are two mammalian PK genes, but only the *PKLR* gene is expressed in RBCs. More than 300 pathogenic variants are reported in this structural gene, which codes for a 574–amino acid protein that forms a functional tetramer. There is some correlation of the type, location, and amino acid substitution with disease severity. The majority of patients have at least one missense pathogenic variant. Most affected patients are compound heterozygotes for two different PK gene defects. The many possible combinations likely account for the variability in clinical severity. The pathogenic variants 1456 C to T and 1529 G to A are the most common pathogenic variants in the White population.

CLINICAL MANIFESTATIONS AND LABORATORY FINDINGS

The clinical manifestations of **PK deficiency** vary from severe neonatal hemolytic anemia to mild, well-compensated hemolysis first noted in adulthood (Table 512.1). Perinatal complications include premature birth, prenatal anemia or fetal hydrops requiring transfusions, and preterm labor. Severe hyperbilirubinemia, anemia, and, in rare cases, hepatic failure may occur in the neonatal period, and kernicterus has been reported. The hemolysis in older children and adults varies in severity, with hemoglobin values ranging from 2-14 g/dL in various reports and associated pallor, jaundice, scleral icterus, splenomegaly, bone deformities due to extramedullary hematopoiesis, and hyperpigmentation. Reticulocyte counts are often extremely elevated, reflecting the severe ongoing hemolysis. Associated serum chemistries consistent with nonimmune hemolytic anemia are present, including an elevated indirect bilirubin, decreased haptoglobin, increased lactate dehydrogenase (LDH), negative direct antiglobulin test (DAT), and

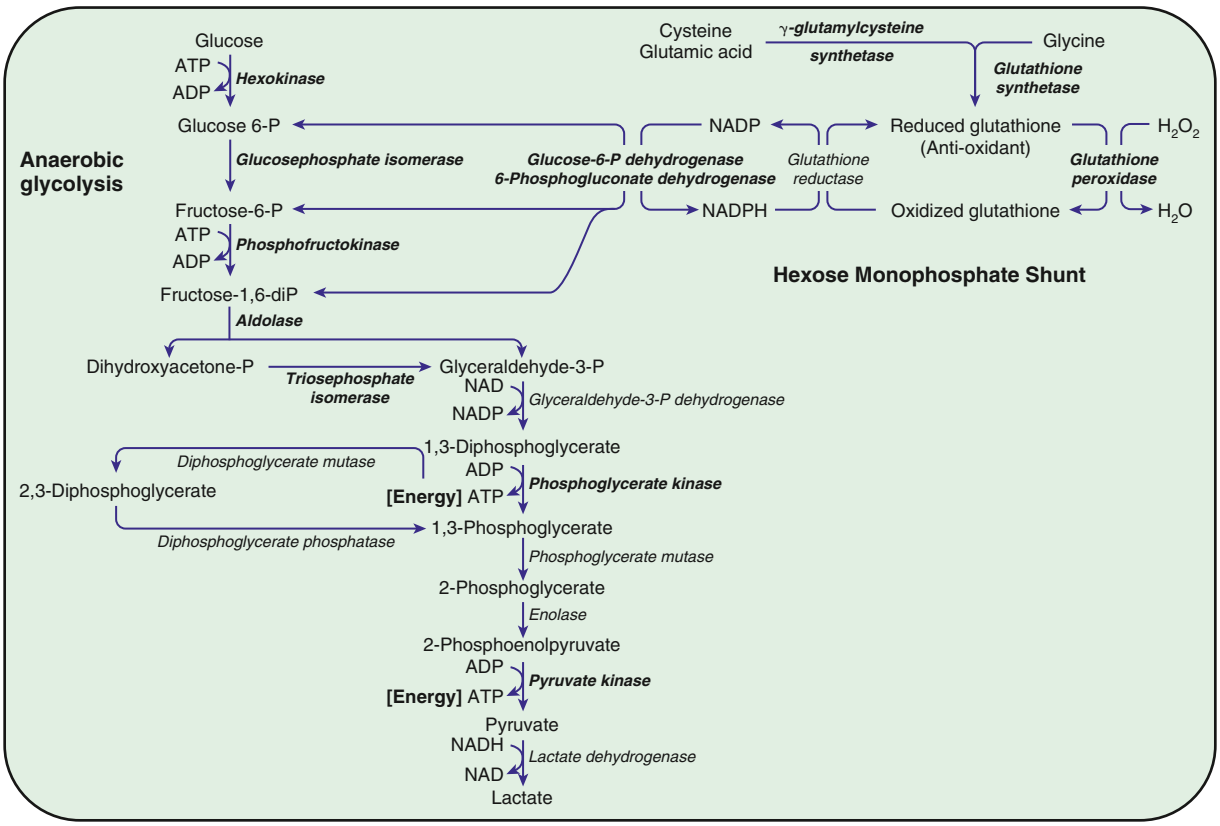

Fig. 512.1 Red blood cell metabolism: glycolysis and hexose monophosphate pathway. The enzyme deficiencies clearly associated with hemolysis are shown in **bold**. ATP, Adenosine triphosphate; ADP, adenosine diphosphate; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of NADP.

Table 512.1 Hexokinase Variants Associated with Hemolytic Anemia

CLINICAL FEATURES			PROPERTIES OF RBC HEXOKINASE			
INHERITANCE	ANEMIA	OTHER	ACTIVITY	KINETIC ABNORMALITIES	STABILITY	MOBILITY
—	+	Congenital malformations	13-24*	0	—	—
Recessive	++		15-20*	+	Normal	Abnormal
Recessive	++		16*	0	—	Abnormal
Recessive	+++	Hydrops fetalis	17			
Recessive	+		20*	0	Normal	Normal
Recessive	++	Low platelet and fibroblast HK activity	20*	0	Low	Normal
Recessive	++	Low platelet HK activity	25*	+	Normal	Abnormal
Recessive	+		25*	0	Low	Normal
Dominant	+	Spherocytes, ovalocytes	30*	0	Low	Normal
Recessive	+	Developmental and cognitive delays	45†	+	Normal	Normal
Recessive	+		50*	0	Normal	Normal
—	+	Congenital malformations	33*	+	—	—
Recessive	+		40-53*	+	Low	Normal
—	+		50*	+	—	—
			53			
Dominant	+		45-91†	+	Normal	Abnormal
Dominant	++	WBC HK activity low	75*	+	Normal	Abnormal
Recessive	±		77†			

*Maximal enzyme activity (V_{max}) compared to reticulocytosis controls.
†Maximal enzyme activity (V_{max}) compared to normal red cells.
HK, Hexokinase; RBC, red blood cell; WBC, white blood cell.
From Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood.* 8th ed. Philadelphia: Elsevier, 2015: 583; Table 17.2.

negative indirect antiglobulin test. A severe form of the disease has a relatively high incidence among the Amish of the Midwestern United States (homozygous splicing pathogenic variant R479H). PK deficiency may possibly provide protection against falciparum malaria. There are long-term effects of chronic hemolysis, including folate deficiency, skin ulcers, gallstones, increased risk of bone fractures, and risk of transient bone marrow aplasia associated with certain viral infections.

Polychromatophilia and **mild macrocytosis** reflect the elevated reticulocyte count. Spherocytes are uncommon, but a few spiculated pyknocytes may be found. Diagnosis relies on demonstration of a marked reduction of RBC PK activity or an increase in the Michaelis-Menten dissociation constant (K_m) for its substrate, phosphoenolpyruvate (high K_m variant). Other RBC enzyme activity is normal or elevated, reflecting the reticulocytosis. No abnormalities of hemoglobin are noted. The white blood cells (WBCs) and platelets have normal PK activity and must be rigorously excluded from the RBC hemolysates used to measure PK activity. Fructose 1,6-disphosphate (FDP) must also be removed because it is an allosteric activator of PK. Heterozygous carriers usually have moderately reduced levels of PK activity. Molecular testing provides additional evidence for the disease. However, due to genotype-phenotype variability with unclear pathogenicity regarding variants, it is recommended to perform initial biochemical testing.

TREATMENT

Intrauterine RBC transfusions are performed in the setting of severe anemia associated with fetal hydrops. Phototherapy and exchange transfusions may be indicated for hyperbilirubinemia in newborns. Transfusions of packed RBCs are necessary for severe anemia or for aplastic crises. If the anemia is consistently severe and frequent transfusions are required, iron chelation may be necessary. Splenectomy should be considered after the child is 5-6 years of age to decrease the need for transfusions and minimize iron overload. Although not curative, splenectomy may be followed by higher Hb levels and by strikingly high (30–60%) reticulocyte counts. Death resulting from overwhelming pneumococcal sepsis has followed splenectomy; thus immunization with vaccines for encapsulated organisms should be given before splenectomy and prophylactic penicillin administered after the procedure. Splenectomy has also been associated with thrombosis and pulmonary hypertension. There is currently no standard curative therapy, although the role of gene therapy and hematopoietic cell transplant are actively being investigated. An oral allosteric PK activator (mitapivat) is being studied in clinical trials involving adults and is under review by the US Food and Drug Administration (FDA). The natural history of the disease is limited and is currently being studied through an international registry.

512.2 Other Glycolytic Enzyme Deficiencies

Allison S. Remiker and Amanda M. Brandow

Chronic nonspherocytic hemolytic anemias of varying severity have been associated with deficiencies of other enzymes in the glycolytic pathway, including hexokinase, glucose phosphate isomerase, and aldolase, which are inherited as autosomal recessive disorders. **Phosphofructokinase deficiency,** which occurs primarily in Ashkenazi Jews in the United States, results in hemolysis associated with a myopathy classified as **glycogen storage disease type VII** (see Chapter 107.1). Clinically, hemolytic anemia is complicated by

muscle weakness, exercise intolerance, cramps, and myoglobinuria. Erythrocyte production is increased to compensate for hemolysis. Enzyme assays for phosphofructokinase yield low values for RBCs and muscle.

Triose phosphate isomerase deficiency is an autosomal recessive disorder affecting many systems. Affected patients have hemolytic anemia, cardiac abnormalities, and lower motor neuron and pyramidal tract impairment, with or without evidence of cerebral impairment. They usually die in early childhood. The gene for triose phosphate isomerase has been cloned and sequenced and is located on chromosome 12.

Phosphoglycerate kinase (PGK) is the first ATP-generating step in glycolysis. At least 23 kindreds with PGK deficiency have been described. PGK is the only glycolytic enzyme inherited on the X chromosome. PGK is important in erythrocytes, the central nervous system (CNS), and skeletal muscle. The phenotype is quite variable and may affect one isolated tissue or a combination of all three. Affected males may present with dysfunction of the CNS with seizures, intellectual disability, and nonspherocytic hemolytic anemia; hemolytic anemia without CNS involvement; or myopathy, myoglobinuria, and progressive weakness. Some patients present with parkinsonism. Female heterozygotes have been shown to have partial symptoms depending on their degree of enzymatic activity, including signs of hemolysis. The gene for PGK is particularly large, spanning 23 kb, and various genetic abnormalities, including nucleotide substitutions, gene deletions, missense, and splicing pathogenic variants, result in PGK deficiency.

DEFICIENCIES OF ENZYMES OF HEXOSE MONOPHOSPHATE PATHWAY

The most important function of the hexose monophosphate pathway is to maintain glutathione in its reduced state (GSH) as protection against the oxidation of RBCs (see Fig. 512.1). Approximately 10% of the glucose taken up by RBCs passes through this pathway to provide the reduced form of NAD phosphate (NADPH) necessary for the conversion of oxidized glutathione to GSH. Maintenance of GSH is essential for the physiologic inactivation of oxidant compounds, such as hydrogen peroxide, that are generated within RBCs. If glutathione, or any compound or enzyme necessary for maintaining it in the reduced state, is decreased, the SH groups of the RBC membrane are oxidized, and the Hb becomes denatured and may precipitate into RBC inclusions called **Heinz bodies**. Once Heinz bodies have formed, an acute hemolytic process results from damage to the RBC membrane by the precipitated Hb, the oxidant agent, and the action of the spleen. The damaged RBCs then are rapidly removed from the circulation.

512.3 Glucose-6-Phosphate Dehydrogenase Deficiency and Related Deficiencies

Allison S. Remiker and Amanda M. Brandow

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most frequent disease involving enzymes of the hexose monophosphate pathway, is responsible for two clinical syndromes: episodic *acute* hemolytic anemia and *chronic* nonspherocytic hemolytic anemia. The most common manifestations are neonatal jaundice and episodic acute hemolytic anemia, which is induced by infections, certain drugs, and, rarely, fava beans. This X-linked deficiency affects >400 million people worldwide, representing an overall 4.9% global prevalence. The global distribution of this disorder parallels that of malaria, representing an example of "balanced polymorphism," in which there is an evolutionary advantage of resistance to falciparum malaria in heterozygous females that outweighs the small negative effect of affected hemizygous males.

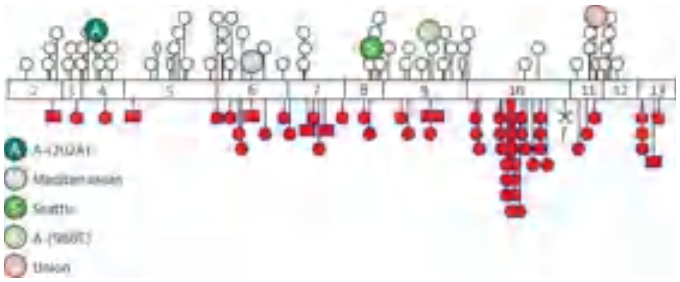

Fig. 512.2 Most common pathogenic variants along coding sequence of *G6PD* gene. Exons are shown as *open numbered boxes*. *Open circles* are pathogenic variants causing classes II and III variants. *Filled circles* represent sporadic pathogenic variants giving rise to severe variants (class I). *Open ellipses* are pathogenic variants causing class IV variants. X is a nonsense pathogenic variant; *f*, a splice site pathogenic variant; *filled squares*, small deletions. 202A and 968C are the two sites of base substitution in G6PD-A. *(From Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371:64–74.)*

The deficiency is caused by inheritance of any of a large number of abnormal alleles of the gene responsible for the synthesis of the G6PD protein. Over 200 pathogenic variants have been described in the gene responsible for the synthesis of the G6PD protein. Many of these pathogenic variants are missense point pathogenic variants leading to amino acid substitutions and destabilization of the G6PD enzyme. Figure 512.2 shows some of the pathogenic variants that cause episodic versus chronic hemolysis. Milder disease is associated with pathogenic variants near the *amino* terminus of the G6PD molecule, and chronic nonspherocytic hemolytic anemia is associated with pathogenic variants clustered near the *carboxyl* terminus near the G6P and NADP-binding sites. The normal enzyme found in most populations is designated G6PD B+. A normal variant, designated G6PD A+, is common in Americans of African descent.

EPISODIC OR INDUCED ACUTE HEMOLYTIC ANEMIA

Etiology

G6PD catalyzes the conversion of glucose 6-phosphate to 6-phosphogluconic acid. This reaction produces NADPH, which maintains GSH (glutathione in its reduced, functional state; see Fig. 512.1). GSH provides protection against **oxidant threats** from certain drugs and infections that would otherwise cause precipitation of hemoglobin (Heinz bodies) or damage the RBC membrane.

Synthesis of RBC G6PD is determined by a gene on the X chromosome. Thus heterozygous females have intermediate enzymatic activity and have two populations of RBCs: one is normal, and the other is deficient in G6PD activity. Because they have fewer susceptible cells, most heterozygous females do not have clinically evident hemolysis after exposure to oxidant drugs. Rarely, the majority of RBCs is G6PD deficient in heterozygous females because the inactivation of the normal X chromosome is random and sometimes exaggerated.

Disease involving this enzyme therefore occurs more frequently in males than in females. Approximately 13% of male Americans of African descent have a variant enzyme **(G6PD A–)** that results in a deficiency of RBC G6PD activity (5–15% of normal). Italians, Greeks, and other Mediterranean, Middle Eastern, African, and Asian ethnic groups also have a high incidence, ranging from 5–40%, of a variant designated **G6PD B– (G6PD Mediterranean)**. In these variants, the G6PD activity of homozygous females or hemizygous males is <5% of normal. Therefore the defect in Americans of African descent is *less severe* than that in Americans of European descent. A third variant enzyme with greatly reduced activity **(G6PD Canton)** occurs in approximately 5% of the Chinese population.

Clinical Manifestations

Most individuals with G6PD deficiency are asymptomatic, with no clinical manifestations of illness unless triggered by infection, drugs, or ingestion of fava beans. Typically, hemolysis ensues in about 24-96 hours after a patient has ingested a substance with **oxidant properties**. In severe cases, hemoglobinuria and jaundice result, and the Hb concentration may fall precipitously. Drugs that elicit hemolysis in these individuals include fluoroquinolones, methylene blue, sulfonylureas, rasburicase, and antimalarials, such as primaquine and dapsone (Table 512.2). There is mixed evidence regarding the safety data of aspirin and sulfa-containing medications, which are generally thought to be safe. Chemical exposures to henna compounds and naphthalene (e.g., mothballs and deodorant) have also been implicated. The degree of hemolysis varies with the inciting agent, amount ingested, and severity of the enzyme deficiency. In some individuals, ingestion of fava beans also produces an acute, severe hemolytic syndrome, known as **favism**. Fava beans contain divicine, isouramil, and convicine, which ultimately lead to production of hydrogen peroxide and other reactive oxygen products. Favism is thought to be more frequently associated with the G6PD Mediterranean and G6PD Canton variants.

In the G6PD A– variant, the stability of the folded protein dimer is impaired, and this defect is accentuated as the RBCs age. Thus hemolysis decreases as older RBCs are destroyed, even if administration of the drug is continued. This recovery results from the *age-labile enzyme*, which is abundant and more stable in younger RBCs (Fig. 512.3). The associated reticulocytosis produces a compensated hemolytic process in which the blood hemoglobin may be only slightly decreased, despite continued exposure to the offending agent.

G6PD deficiency can produce hemolysis in the neonatal period. In G6PD A–, spontaneous hemolysis and hyperbilirubinemia have been observed in preterm infants. In newborns with the G6PD B– and G6PD Canton varieties, hyperbilirubinemia and even kernicterus may occur. Neonates with co-inheritance of G6PD deficiency and a pathogenic variant of the promoter of uridine-diphosphate-glucuronyl transferase (UGT1A1), seen in **Gilbert syndrome**, have more severe neonatal jaundice. When a pregnant woman ingests oxidant drugs, they may be transmitted to her G6PD-deficient fetus, and hemolytic anemia and jaundice may be apparent at birth. Similarly, medications may be transmitted to a neonate through breast milk.

Laboratory Findings

The onset of acute hemolysis usually results in a precipitous fall in hemoglobin and hematocrit. If the episode is severe, the Hb-binding proteins, such as haptoglobin, are saturated, and free hemoglobin may appear in the plasma and subsequently in the urine. Hemolysis is intravascular and extravascular. Unstained or supravital preparations of RBCs reveal precipitated hemoglobin, or Heinz bodies. The RBC inclusions are not visible on the Wright-stained blood film. Cells that contain these inclusions are seen only within the first 3-4 days of illness because they are rapidly cleared from the blood. Also, the blood film may contain RBCs with what appears to be a bite taken from their periphery ("bite cells") and **polychromasia** (evidence of bluish, larger RBCs), representing reticulocytosis (Fig. 512.4).

Diagnosis

The diagnosis depends on direct or indirect demonstration of reduced G6PD activity in RBCs. By direct measurement, enzyme activity in affected persons is ≤10% of normal, and the reduction of enzyme activity is more extreme in Americans of European descent and in Asians than in Americans of African descent. Satisfactory screening tests are based on decolorization of methylene blue, reduction of methemoglobin, or fluorescence of NADPH. Confirmatory testing is available using quantitative NADPH formation measured spectrophotometrically. Immediately after a hemolytic episode, reticulocytes and young RBCs predominate. These young cells have significantly higher enzyme activity than do older cells in the A– variety (African). *Testing may therefore have to be deferred for a few weeks before a diagnostically low*

Table 512.2 Agents Precipitating Hemolysis in Glucose-6-Phosphate Dehydrogenase Deficiency

DEFINITE RISK OF AHA	"POSSIBLE" RISK OF AHA	POSSIBLE ASSOCIATION (LESS LIKELY)
ANTIMALARIAL DRUGS		
Dapsone	Chloroquine	
Pamaquine	Quinidine	
Primaquine	Quinine	
Tafenoquine		
OTHER DRUGS		
Ciprofloxacin	Aspirin*	Chloramphenicol
Glibenclamide	Menadiol sodium phosphate	Dimercaptosuccinic acid
Methylene blue	Sulfadiazine	Glibenclamide
Moxifloxacin	Sulfasalazine	Mepacrine
Nalidixic acid	Sulfonylureas	Vitamin K analogs
Nitrofurantoin	Tolonium chloride	
Norfloxacin		
Ofloxacin		
Phenazopyridine		
Rasburicase and pegloticase		
Sulfamethoxazole/cotrimoxazole		
Henna (cosmetic use)		

*Hemolysis is dose related. Typical 75 to 100 mg per day, widely used for prophylaxis of cardiovascular events, is safe for G6PD-deficient persons.
AHA, acute hemolytic anemia.
From Luzzatto L, Ally M, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. *Blood.* 2020;136:1225–1240; Table 1.

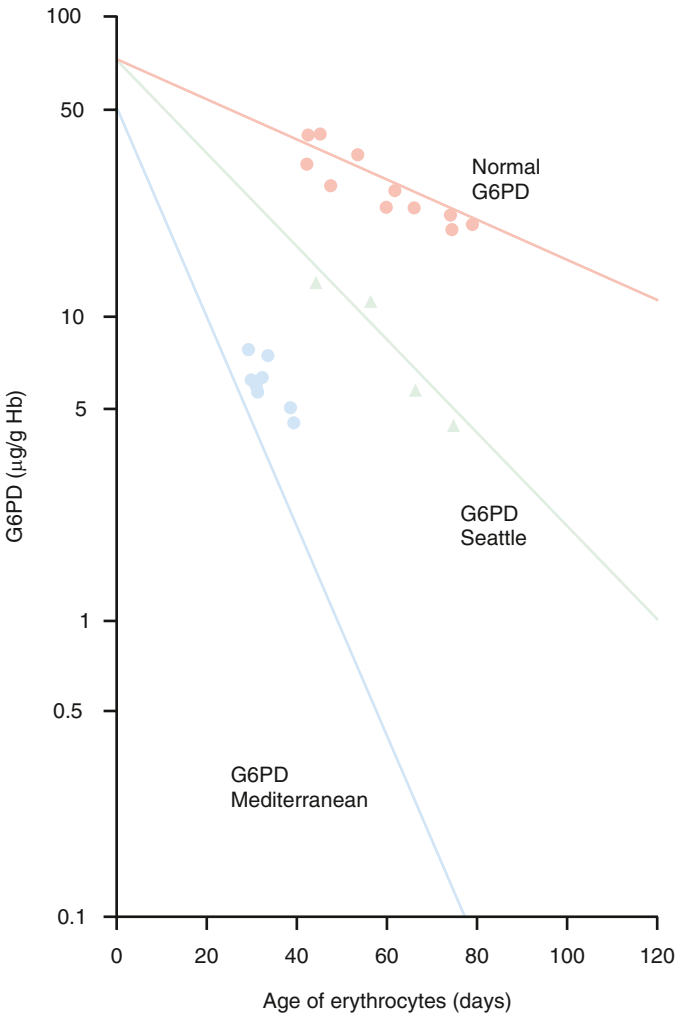

Fig. 512.3 Main mechanism of G6PD deficiency in RBCs is in vivo instability of mutant enzyme. For many G6PD variants, such as the two studied here, this is essentially acceleration of a process that takes place normally with the aging of RBCs in the circulation. Hb, Hemoglobin. *(Adapted from Morelli A, Benatti U, Gaetani GF, et al. Biochemical mechanisms of glucose-6-phosphate dehydrogenase deficiency. Proc Natl Acad Sci USA. 1978;75:1979–1983.)*

level of enzyme can be shown. The diagnosis can be suspected when G6PD activity is within the low-normal range in the presence of a high reticulocyte count. G6PD variants also can be detected by electrophoretic and molecular analysis. G6PD deficiency should be considered in any neonatal patients with hyperbilirubinemia and borderline low G6PD activity.

Prevention and Treatment

Prevention of hemolysis constitutes the most important therapeutic measure. When possible, males belonging to ethnic groups with a significant incidence of G6PD deficiency (e.g., Greeks, southern Italians, Sephardic Jews, Filipinos, southern Chinese, Black Americans, Thais) should be tested for the defect before known oxidant drugs are given.

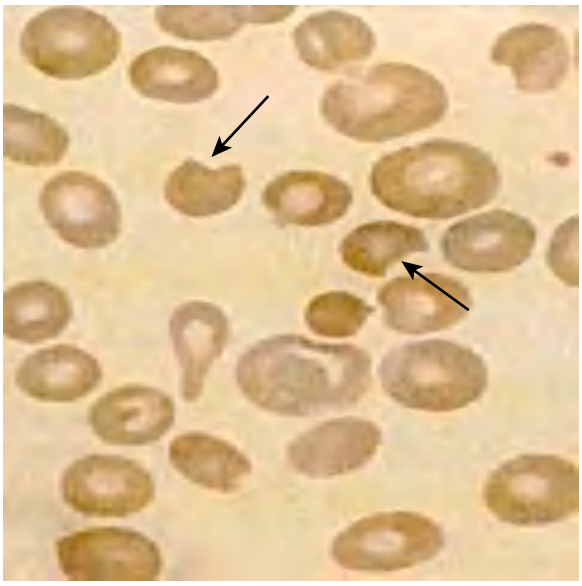

Fig. 512.4 Morphologic erythrocyte changes (anisopoikilocytosis, bite cells) during acute hemolysis in a G6PD-deficient patient. *Arrows* show bite cells. *Anisopoikilocytosis* is abnormality in the shape or size of erythrocytes. *(From Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371:64–74.)*

The usual doses of aspirin and trimethoprim-sulfamethoxazole do not cause clinically relevant hemolysis in the A– variety. Aspirin administered in doses used for acute rheumatic fever (50-100 mg/kg/24 hr) may produce a severe hemolytic episode. Infants with severe neonatal jaundice who belong to these ethnic groups also require testing for G6PD deficiency because of their heightened risk for this defect. If severe hemolysis has occurred, supportive therapy may require blood transfusions, although recovery is the rule when the oxidant agent is discontinued.

CHRONIC HEMOLYTIC ANEMIAS ASSOCIATED WITH DEFICIENCY OF G6PD OR RELATED FACTORS

Chronic **nonspherocytic hemolytic anemia** has been associated with profound deficiency of G6PD caused by enzyme variants, particularly those defective in quantity, activity, or stability. The gene defects leading to chronic hemolysis are located primarily in the region of the NADP-binding site near the carboxyl terminus of the protein (see Fig. 512.2). These include the Loma Linda, Tomah, Iowa, Beverly Hills, Nashville, Riverside, Santiago de Cuba, and Andalus variants. Persons with G6PD B– enzyme deficiency occasionally have chronic hemolysis, and the hemolytic process may worsen after ingestion of oxidant drugs. Splenectomy is of little value in these types of chronic hemolysis.

Other enzyme defects may impair the regeneration of GSH as an oxidant "sump" (see Fig. 512.1). Mild, chronic nonspherocytic anemia has been reported in association with decreased RBC GSH, resulting from γ-glutamylcysteine or GSH synthetase deficiencies. Deficiency of 6-phosphogluconate dehydrogenase has been associated primarily with drug-induced hemolysis, and hemolysis with hyperbilirubinemia has been related to a deficiency of GSH peroxidase in newborn infants.

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Chapter 513

Hemolytic Anemias Resulting from Extracellular Factors—Immune Hemolytic Anemias

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IMMUNE HEMOLYTIC ANEMIAS

A number of extrinsic agents and disorders may lead to premature destruction of red blood cells (RBCs) at a rate where hemolysis exceeds hematopoietic replacement. Among the most clearly defined are antibodies associated with immune hemolytic anemias. The hallmark of this group of diseases is the positive result of the direct antiglobulin **(Coombs)** test (DAT), which detects a coating of immunoglobulin or components of complement on the RBC surface. The most important immune hemolytic disorder in pediatric practice is hemolytic disease of the newborn (erythroblastosis fetalis), caused by transplacental transfer of maternal antibody active against the RBCs of the fetus, that is, isoimmune hemolytic anemia (see Chapter 140).

Various other immune hemolytic anemias are **autoimmune** (Table 513.1). Often, no underlying cause can be found; this is the *primary* or idiopathic type. If the autoimmune hemolysis is associated with an underlying disease, including various infections (Epstein-Barr virus [EBV], rarely HIV, cytomegalovirus, and mycoplasma), autoimmune or inflammatory diseases (autoimmune lymphoproliferative disease [ALPS], systemic lupus erythematosus [SLE], rheumatoid arthritis, type 1 diabetes mellitus, ulcerative colitis), primary immunodeficiency diseases (common variable immunodeficiency [CVID], Wiskott-Aldrich syndrome), neoplasms (lymphoproliferative, solid tumors), or posttransplant (solid organ or allogeneic stem cell), it is considered *secondary* (Table 513.2). As many as 20% of episodes of autoimmune hemolysis are drug-induced (e.g.,

Table 513.1 Diseases Characterized by Immune-Mediated Red Blood Cell Destruction

AUTOIMMUNE HEMOLYTIC ANEMIA CAUSED BY WARM-REACTIVE AUTOANTIBODIES
Primary (idiopathic)
Secondary
Lymphoproliferative disorders
Connective tissue disorders (especially systemic lupus erythematosus)
Nonlymphoid neoplasms (e.g., ovarian tumors)
Chronic inflammatory diseases (e.g., ulcerative colitis)
Immunodeficiency disorders
AUTOIMMUNE HEMOLYTIC ANEMIA CAUSED BY COLD-REACTIVE AUTOANTIBODIES (CRYOPATHIC HEMOLYTIC SYNDROMES)
Primary (idiopathic) cold agglutinin disease
Secondary cold agglutinin disease
Lymphoproliferative disorders
Infections (*Mycoplasma pneumoniae*, Epstein-Barr virus)
Paroxysmal cold hemoglobinuria
Primary (idiopathic)
Viral syndromes (most common)
Congenital or tertiary syphilis
DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA (SEE TABLE 513.2)
Hapten/drug adsorption (e.g., penicillin)
Ternary (immune) complex (e.g., quinine, quinidine)
True autoantibody induction (e.g., methyldopa)

Adapted from Packman CH. Autoimmune hemolytic anemias. In Rakel R (ed). *Conn's Current Therapy*. Philadelphia: Saunders, 1995: 305.

Table 513.2 Secondary Etiologies Causing Autoimmune Cytopenias in Children

DISEASES ASSOCIATED WITH AUTOIMMUNE CYTOPENIAS IN CHILDREN
Lymphoproliferative Disorders
Autoimmune lymphoproliferative syndrome (ALPS)*
Rosai-Dorfman disease
Castleman disease
Ras-associated leukoproliferative disorder
Immune Deficiencies
Common variable immune deficiency (CVID)*
Selective IgA deficiency
Chromosome 22q11.2 deletion (DiGeorge or velocardiofacial syndrome)
Severe combined immunodeficiency syndromes
IPEX syndrome
Rheumatologic Conditions
Systemic lupus erythematosus (SLE)*
Antiphospholipid antibody syndrome*
Juvenile idiopathic arthritis
Sjögren syndrome
Sarcoid
Malignancies
Non-Hodgkin lymphoma
Acute lymphoblastic leukemia
Myelodysplastic syndrome
Hodgkin lymphoma
Chronic Infections
EBV
HIV**
Helicobacter pylori
CMV
HCV
Other
Celiac disease
Inflammatory bowel disease
RECOMMENDED EVALUATION FOR CHILDREN WITH CHRONIC SINGLE LINEAGE AUTOIMMUNE CYTOPENIAS OR MULTI-LINEAGE AUTOIMMUNE CYTOPENIAS*
Flow cytometry for double negative T cells (ALPS)
ANA (SLE)
Anti-phospholipid antibodies
Quantitative immunoglobulins (CVID)
Specific antibody titers (CVID)
T cells subsets (CD3/CD4, CD3/CD8)
Ferritin
HIV**
FOXP3 testing (IPEX)
Exome or specific gene panels

*Consider screening for these conditions in children with chronic single lineage autoimmune cytopenias or multi-lineage autoimmune cytopenias.

**Consider also screening for HIV in adolescents with chronic single lineage or multi-lineage autoimmune cytopenias. Other diseases should be considered if the history or physical are suggestive of the underlying condition. It is extremely rare for the other conditions to present with autoimmune cytopenias and no other signs or symptoms suggestive of the underlying disease. Thus, the utility of routine screening is low.

EBV, Epstein-Barr virus; CMV, cytomegalovirus; HCV, hepatitis C virus; ANA, antinuclear antibody; IPEX, immune dysregulation, polyendocrinopathy, enteropathy X-linked.

Modified from Teachey DT, Lambert MP. Diagnosis and management of autoimmune cytopenias in childhood. *Pediatr Clin North Am*. 2013;60:1489–1511; Table 2.

methyldopa, L-dopa, checkpoint inhibitors). Other drugs (penicillins, cephalosporins) cause hemolysis by means of "drug-dependent antibodies"—antibodies directed toward the drug and in some cases toward an RBC membrane antigen as well.

Etiology

Autoimmune hemolytic anemia (AIHA) is caused by the production of anti-RBC autoantibodies generated by tissue and self-reactive B lymphocytes circulating in the periphery supported by T helper lymphocytes. The complement system induces sequential activation of the membrane attack complex (MAC) leading to erythrocyte lysis typically in the liver and circulation. RBCs are opsonized by the autoantibodies or complement then phagocytosed primarily in the spleen and lymph tissue. Antibody-dependent cell-mediated cytotoxicity (ADCC) induced by cytotoxic $CD8^+$ T lymphocytes and natural killer (NK) cells are an additional important mechanism contributing to AIHA also predominantly in lymphoid organs and the spleen.

The underlying cause of autoimmunity is loss of central self-tolerance in the early stages of lymphocyte differentiation, as well as loss of peripheral self-tolerance driven by Tregs and $CD8^+$ suppressor T lymphocytes. Loss of tolerance allows for autoreactive cells to avoid negative selection, and/or suppression of autoantibodies fails to occur appropriately in the periphery. It is theorized patients who develop AIHA have an underlying predisposition to losing such self-tolerance, which is stimulated by environmental factors.

The autoantibody may be produced as an inappropriate immune response to an RBC antigen or to another antigenic epitope similar to an RBC antigen, known as *molecular mimicry.* Alternatively, an infectious agent may alter the RBC membrane so that it becomes "foreign" or antigenic to the host.

The autoantibodies that form in AIHA have antibody class, antigenic reactivity, and thermal characteristics associated with binding and/or destroying erythrocytes. The temperature predilection is associated with corresponding nomenclature, including warm (wAIHA), cold (cold agglutinin disease [CAD]), paroxysmal cold hemoglobinuria (PCH), and rare mixed forms.

AUTOIMMUNE HEMOLYTIC ANEMIAS ASSOCIATED WITH "WARM" ANTIBODIES

Etiology

Warm autoimmune hemolytic anemia (wAIHA) is the most common form in children found in as many as 90% of cases. Autoantibodies are "warm-reactive" meaning they preferentially bind to erythrocytes at 37°C. Extravascular hemolysis occurs in the spleen. Typically, the antibodies are immunoglobulin (Ig) G, and react to epitopes (antigens) that are "public" or common to all human RBCs, such as Rh proteins. When IgG is present in exceptional quantity, it can fix complement, which results in intravascular hemolysis.

Clinical Manifestations

Patients with wAIHA present with symptoms consistent with anemia and nonspecific signs of hemolysis. Onset may be acute, with pallor, jaundice, icterus, hemoglobinuria, abdominal pain, fever, shortness of breath, and dizziness, or more gradual with primarily fatigue and pallor. Cardiovascular compromise is rare. The spleen is usually enlarged and is the primary site of destruction of IgG-coated RBCs.

Laboratory Findings

In many cases, anemia is profound with hemoglobin (Hb) levels <6 g/dL. Considerable spherocytosis and polychromasia (reflecting the reticulocyte response) are present. More than 50% of the circulating RBCs may be reticulocytes, and nucleated RBCs usually are present. In some cases, a *low reticulocyte count* may be found, particularly early in the episode due to similar epitopes on RBC precursors in the marrow, marrow suppression if there is concurrent infection, and/or a "shocked" marrow after a severe hemolytic event. Leukocytosis is common. The platelet count is usually normal, but concomitant immune thrombocytopenic purpura sometimes occurs (**Evans syndrome**). Patients with Evans syndrome often have or eventually develop a chronic disease, including SLE, an immunodeficiency syndrome (common variable immunodeficiency), or autoimmune lymphoproliferative syndrome.

Results of the **DAT (Coombs test)** are strongly positive, and free antibody can sometimes be demonstrated in the serum **(indirect Coombs test)**. These antibodies are active at 35–40°C (95–104°F) ("warm" antibodies) and most often belong to the IgG class. They do not require complement for activity. Antibodies from the serum and those eluted from the RBCs react with the RBCs of many persons in addition to those of the patient. They often have been regarded as nonspecific panagglutinins, but careful studies have revealed specificity for RBC antigens of the Rh system in 70% of patients (50% of adult patients). Complement, particularly fragments of C3b, may be detected on the RBCs in conjunction with IgG. The Coombs test result is *rarely* negative because of the sensitivity of the Coombs reaction. A minimum of 260-400 molecules of IgG per cell is necessary on the RBC membrane to produce a positive reaction. Special tests are required to detect the antibody in cases of "Coombs-negative" autoimmune hemolytic anemia. In warm antibody hemolytic anemia, the direct Coombs test may detect IgG alone, both IgG and complement fragments, or solely complement fragments if the level of RBC-bound IgG is below the detection limit of the anti-IgG Coombs reagent.

Treatment

Transfusions may provide only transient benefit but may be lifesaving in cases of severe anemia until the effects of other treatments are observed. All tested units for transfusion are serologically incompatible. It is important to identify the patient's ABO blood group to avoid a hemolytic transfusion reaction mediated by anti-A or anti-B. The blood bank should also test for the presence of an underlying alloantibody, which could cause rapid hemolysis of transfused RBCs. Patients who have been neither previously transfused nor pregnant are unlikely to harbor an alloantibody. Early consultation between the clinician and the blood bank physician is essential. Failure to transfuse a profoundly anemic infant or child may lead to serious morbidity and even death.

Patients with mild disease and compensated hemolysis may not require treatment. *If the hemolysis is severe and results in significant anemia or symptoms, treatment with glucocorticoids is initiated.* Glucocorticoids decrease the rate of hemolysis by blocking macrophage function through downregulating Fcγ receptor expression, decreasing the production of the autoantibody, and perhaps enhancing the elution of antibody from the RBCs. Initial dosing is dependent on the degree of anemia and symptoms. In patients who present with Hb <6 to 7 g/dL or severe symptoms, initial treatment with intravenous (IV) methylprednisolone 0.5-1 mg/kg every 6 to 8 hours in the first 24-72 hours is typically indicated. With rapid deterioration or severity of anemia, high-dose methylprednisolone 30 mg/kg with maximum 1 g daily for 3 days may be required. When hemolytic anemia remains severe despite glucocorticoid therapy, or if very large doses are necessary to maintain a reasonable Hb level, intravenous immunoglobulin (IVIG) may be tried. Long-term use of IVIG has not been shown to be effective in the majority of children. In children who present with mild to moderate anemia with Hg ≥7 to 8 g/dL and an appropriate reticulocyte count, oral prednisone 2 mg/kg/day may be started. Response is typically seen within 2-4 weeks after initiation of steroids and considered adequate with Hb concentration >9 to 10 g/dL. Hb, reticulocyte count, lactate dehydrogenase, and DAT should be monitored for response. Treatment should be continued until the rate of hemolysis decreases, with the dose then gradually reduced, typically over at least 4 months. The Coombs test result may remain positive even after the Hb level returns to normal. It is usually safe to discontinue prednisone once the direct Coombs test result becomes negative.

The majority of patients (approximately 80%) have an initial response to glucocorticoids within the first month of treatment; however, it is common to require prolonged or multimodal therapies over time. If a patient is unable to taper prednisone effectively within 1-2 months after diagnosis, addition of a second-line therapy should be considered.

Relapse occurs in 15–40% of patients with wAIHA, typically within the first 6-12 months after initial response to treatment. If this occurs, resumption of the lowest dose of prednisone that was effective in treating the patient is recommended. Because of the adverse effects of long-term steroid use, alternative agents could be considered if relapse continues to occur.

There are limited data in second-line therapies for wAIHA in children. **Rituximab**, a monoclonal antibody that targets B lymphocytes, the source of antibody production, *is useful in chronic cases refractory to conventional therapy.* Plasmapheresis has been used in refractory cases but generally is not helpful because most hemolysis is in the extravascular space. Splenectomy may be beneficial but is complicated by a heightened risk of infection with encapsulated organisms, particularly in patients <6 years old. Prophylaxis is indicated with appropriate vaccines (pneumococcal, meningococcal, and *Haemophilus influenzae* type b) before splenectomy and with oral penicillin after splenectomy. Thrombosis and pulmonary hypertension are also increasingly recognized problems after splenectomy. In refractory disease, several immunomodulating agents have been used in small case series or reports including azathioprine, 6-mercaptopurine, cyclosporine, mycophenolate mofetil, tacrolimus, sirolimus, and monoclonal antibodies (e.g., obinutuzumab, daratumumab, alemtuzumab, bortezomib).

Prognosis

Acute idiopathic autoimmune hemolytic disease in childhood varies in severity but is self-limited; death from untreatable anemia is rare. Approximately 30% of patients have chronic hemolysis, often associated with an underlying disease, such as SLE, immunodeficiency, or lymphoma. The presence of antiphospholipid antibodies in adult patients with immune hemolysis predisposes to thrombosis. Mortality in chronic cases depends on the primary disorder.

AUTOIMMUNE HEMOLYTIC ANEMIAS ASSOCIATED WITH "COLD" ANTIBODIES OR COLD AGGLUTININ DISEASE

"Cold" antibodies agglutinate RBCs at temperatures <37°C (98.6°F). They are primarily of the IgM class and require complement for hemolytic activity. The highest temperature at which RBC agglutination occurs is called the thermal amplitude. A higher thermal amplitude antibody—that is, one that can bind to RBCs at temperatures achievable in the body—results in hemolysis with exposure to a cold environment. High antibody titers are associated with high thermal amplitude.

Cold antibodies usually have specificity for the oligosaccharide antigens of the I/i system. They may occur in primary or idiopathic cold agglutinin disease, secondary to infections such as those from *Mycoplasma pneumoniae* and EBV, or secondary to lymphoproliferative disorders. After *M. pneumoniae* infection, the anti-I levels may increase considerably, and occasionally, enormous increases may occur to titers ≥1/30,000. The antibody has specificity for the I antigen and thus reacts poorly with human cord RBCs, which possess the i antigen but exhibit low levels of I. Patients with infectious mononucleosis occasionally have CAD, and the antibodies in these patients often have anti-i specificity. This antibody causes less hemolysis in adults than in children because adults have fewer i molecules on their RBCs. Spontaneous RBC agglutination is observed in the cold and in vitro, and RBC aggregates are seen on the blood film (rouleaux formation). Mean corpuscular volume (MCV) may be spuriously elevated because of RBC agglutination and reticulocytosis. The severity of the hemolysis is related to the thermal amplitude of the antibody, which itself partly depends on the IgM antibody titer.

When very high titers of cold antibodies are present and active near body temperature, severe intravascular hemolysis with hemoglobinemia and hemoglobinuria may occur and may be heightened on a patient's exposure to cold (external temperature or ingested foods). Each IgM molecule has the potential to activate a C1 molecule, so that large amounts of complement are found on the RBCs in CAD. These sensitized RBCs may undergo intravascular complement-mediated lysis or may be destroyed in the liver and spleen. Only complement, not IgM, is detected on RBCs because the IgM is removed during the washing steps of the DAT.

CAD is less common in children than in adults and more frequently results in an acute, self-limited episode of hemolysis. RBC transfusion is indicated based on symptoms and severity of anemia. If transfusion is required, blood should be warmed before administration. Glucocorticoids are much less effective in CAD than in hemolytic disease with warm antibodies. Patients should avoid exposure to cold and should be treated for any underlying disease. In the uncommon patient with severe hemolytic disease, immunosuppression and plasmapheresis can be used. Successful treatment of CAD has been reported with rituximab, which effectively depletes B lymphocytes. Splenectomy is not useful in CAD.

Paroxysmal Cold Hemoglobinuria

Paroxysmal cold hemoglobinuria is mediated by the Donath-Landsteiner (D-L) hemolysin, which is an IgG cold-reactive autoantibody with anti-P specificity. In vitro, the D-L antibody binds to RBCs in the cold, and the RBCs are lysed by complement as the temperature is increased to 37°C. A similar sequence is thought to occur in vivo as RBCs move from the cooler extremities to warmer parts of the circulation. Most reported cases are self-limited; many patients experience only one paroxysm of hemolysis. Congenital or acquired syphilis was once the most common underlying cause of paroxysmal cold hemoglobinuria, but currently, most cases are associated with nonspecific viral infections. Treatment includes transfusion for severe anemia and avoidance of cold ambient temperatures.

Drug-Induced Hemolytic Anemia

Drugs (penicillin or sometimes cephalosporins) that cause hemolysis through the "hapten" mechanism (immune but not autoimmune) bind tightly to the RBC membrane. Antibodies to the drug, either newly or previously formed, bind to the drug molecules on RBCs, mediating their destruction in the spleen. In other cases, certain drugs, such as quinine and quinidine, do not bind to RBCs, but rather form part of a "ternary complex," consisting of the drug, an RBC membrane antigen, and an antibody that recognizes both (see Table 513.3). Methyldopa and sometimes cephalosporins may, by unknown mechanisms, incite true autoantibodies to RBC membrane antigens, so that the presence of the drug is not required to cause hemolysis. Cephalosporins are the most common cause of drug-induced immune hemolytic anemia.

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Table 513.3 Selected Drugs That Cause Immune-Mediated Hemolysis

MECHANISM	DRUG ADSORPTION (HAPTEN)	TERNARY (IMMUNE) COMPLEX	AUTOANTIBODY INDUCTION
Direct antiglobulin test	Positive (anti-IgG)	Positive (anti-C3)	Positive (anti-IgG)
Site of hemolysis	Extravascular	Intravascular	Extravascular
Medications	Penicillins Cephalosporins 6-Mercaptopurine Tetracycline Oxaliplatin Hydrocortisone	Cephalosporins Quinidine Amphotericin B Hydrocortisone Rifampin (Rifadin) Metformin Quinine Probenecid Chlorpromazine Oxaliplatin	α-Methyldopa Cephalosporins Oxaliplatin L-Dopa Procainamide Ibuprofen Diclofenac (Voltaren) Interferon alfa

Adapted from Packman CH. Hemolytic anemia resulting from immune injury. In Kaushansky K, Lichtman MA, Beutler E, et al, (eds): *Williams Hematology*. 8th ed. New York: McGraw-Hill, 2010.

Chapter 514

Hemolytic Anemias Secondary to Other Extracellular Factors

Allison S. Remiker and Amanda M. Brandow

FRAGMENTATION HEMOLYSIS

See Table 506.2 in Chapter 506.

Red blood cell (RBC) destruction may occur in hemolytic anemias because of **mechanical injury** as the cells traverse a damaged vascular bed. Damage may be microvascular when RBCs are sheared by fibrin in the capillaries during intravascular coagulation or when renovascular disease accompanies the **hemolytic-uremic syndrome (HUS)** (see Chapter 560.5) or **thrombotic thrombocytopenic purpura (TTP)** (see Chapter 533.5). Larger vessels may be involved in **Kasabach-Merritt syndrome** (giant hemangioma and thrombocytopenia; see Chapter 554) or when a replacement heart valve is poorly epithelialized. The blood film shows many *schistocytes*, or fragmented cells, as well as polychromatophilia, reflecting the reticulocytosis (see Fig. 507.4F in Chapter 507 on Castleman disease). Secondary iron deficiency may complicate the intravascular hemolysis because of urinary hemoglobin and hemosiderin iron loss (see Fig. 506.2 in Chapter 506). **Treatment should be directed toward the underlying condition**, and the prognosis depends on the effectiveness of this treatment. The benefit of transfusion may be transient because the transfused cells are destroyed as quickly as those produced by the patient.

It is critical to determine the precise etiology of the fragmentation hemolysis because the treatment depends on the underlying problem (Table 514.1). **Acquired TTP** results from an antibody to an enzyme (ADAMTS13) that regulates the size of von Willebrand multimers. The lack of this enzyme results in a marked increase in multimer size and a resultant *thrombotic microangiopathy*. **Congenital TTP** can result in the inability to produce adequate amounts of the enzyme ADAMTS13 and results in the same pathophysiology. The treatment for acquired TTP involves plasma exchange (PEX) to remove the antibody and replace the ADAMTS13, in addition to immunosuppressive therapy with glucocorticoids. Treatment should be initiated as soon as possible when there is a high index of suspicion, even before confirmation of the diagnosis because of high morbidity and mortality. Once diagnosis has been confirmed, rituximab is suggested. Use of caplacizumab, an anti-von Willebrand factor monoclonal antibody, is used in adult patients with severe disease, although it has not been approved by the US Food and Drug Administration (FDA) in children. The treatment for congenital TTP involves scheduled plasma infusions to replace the ADAMTS13. Recombinant ADAMTS13 is under investigation for use in acquired and congenital TTP. In contrast, HUS results from *Shiga* toxin produced by *Escherichia coli* 0157 and may not be helped by plasmapheresis, and treatment is largely based on supportive measures. **Atypical HUS** involves activation of the alternative complement pathway and can be treated with *eculizumab* (anti-C5), an inhibitor of the complement pathway. Plasmapheresis may reduce the RBC fragmentation and improve the platelet count but has little effect on the tissue (kidney) vasculopathy, and thus is not usually recommended. **Pneumococcal-induced HUS** results from neuraminidase produced by the bacteria, which damages the membranes of the RBCs and the kidney, exposing the **T-antigen**. Plasma contains natural antibody to the T-antigen, producing hemolysis, renal damage, and a thrombotic microangiopathy. Thus, patients with T-antigen activation from suspected pneumococcal-induced HUS should not be given plasma infusions because this will significantly exacerbate the RBC hemolysis and can lead to life-threatening anemia.

THERMAL INJURY

Extensive burns may directly damage the RBCs and cause hemolysis that results in the formation of *spherocytes*. Blood loss and marrow suppression may contribute to anemia and require blood transfusion. Erythropoietin (EPO) has been used as treatment for diminished RBC production. There may be a role of early use of propranolol, which has been shown to restore erythrocyte progenitors.

Table 514.1 Thrombotic Microangiopathies

DISEASE*	PATHOPHYSIOLOGY	LAB FINDINGS	MANAGEMENT
Thrombotic thrombocytopenic purpura (TTP)	*Acquired:* Ab to ADAMTS13 *Congenital:* Inadequate ADAMTS13 production	Ab to ADAMTS13 ADAMTS13 <10%	*Acquired:* Plasma exchange (PEX) Glucocorticoids +/- Rituximab *Congenital:* Scheduled plasma infusions, recombinant ADAMTS13
Hemolytic-uremic syndrome (HUS)	*Escherichia coli* 0157, *Shiga* toxin	*E. coli* 0157, *Shiga* toxin	Supportive ? Value of plasmapheresis
Atypical HUS	Complement-mediated alternative pathway	ADAMTS13 >10% Decreased factors H and I (inhibitors of complement)†	Eculizumab (Ab to C5) Plasmapheresis not indicated
Pneumococcal-induced HUS	Neuraminidase-induced RBC, platelet, and kidney damage Exposure of T-antigen on RBC and kidney	Pneumococcal infection ADAMTS13 >10%	Plasmapheresis with albumin for neuraminidase and endogenous T Ab removal Avoid plasma infusions, which will exacerbate RBC hemolysis
Disseminated intravascular coagulation (DIC)	Sepsis, shock, endotoxin	Decreased fibrinogen, increased fibrin split products, decreased clotting factors and platelets	Treat underlying condition; replace factors and platelets if bleeding

*All show fragmentation hemolytic anemia, thrombocytopenia, and potential renal and other organ damage. An elevated lactate dehydrogenase and reduced haptoglobin usually are present secondary to hemolysis.
†May be related to inherited defect in factor H or I.
Ab, Antibody; RBC, red blood cell.

RENAL DISEASE

The anemia of uremia is multifactorial in origin. EPO production may be decreased, and the marrow suppressed by toxic metabolites. Furthermore, the RBC life span often is shortened because of retention of metabolites and organic acidemia. The use of EPO in chronic renal disease can decrease the need for blood transfusion. Abnormalities in the hepcidin-ferroportin pathway provide an additional pathway contributing to anemia associated with chronic kidney disease. Investigative use of medications targeting this pathway are being evaluated in patients. Hemodialysis is also implicated in causing fragmentation of erythrocytes.

LIVER DISEASE

A change in the ratio of cholesterol to phospholipids in the plasma may result in changes in the composition of the RBC membrane and shortening of the RBC life span. Some patients with liver disease have many target RBCs on the blood film, whereas others have a preponderance of *spiculated cells.* These morphologic changes reflect the alterations in the plasma lipid composition.

TOXINS AND VENOMS

Bacterial sepsis caused by *Haemophilus influenzae,* staphylococci, or streptococci may be complicated by accompanying hemolysis. Particularly severe hemolytic anemia has been observed in clostridial infections and results from a hemolytic clostridial toxin. Large numbers of spherocytes may be seen on the blood film. **Spherocytic hemolysis** also may be noted after bites by various snakes, including cobras, vipers, and rattlesnakes, which have phospholipases in their venom. Large numbers of bites by insects, such as bees, wasps, and yellow jackets, also may cause spherocytic hemolysis by a similar mechanism (see Chapter 768).

WILSON DISEASE

See Chapter 405.2.

An acute and self-limited episode of hemolytic anemia may precede by years the onset of hepatic or neurologic symptoms in Wilson disease. This event appears to result from the toxic effects of free copper on the RBC membrane. The blood film often (but not always) shows large numbers of spherocytes, and the Coombs test result is negative. Because early diagnosis of Wilson disease permits prophylactic treatment with penicillamine and prevention of hepatic and neurologic disease, correct assessment of this rare type of hemolysis is important.

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Section 4

Polycythemia (Erythrocytosis)

Chapter 515

Polycythemia

Allison S. Remiker and Amanda M. Brandow

Polycythemia exists when the hemoglobin concentration and/or hematocrit and total red blood cell (RBC) volume exceed the upper limits of normal in peripheral blood. In postpubertal individuals, an RBC mass >25% above the mean normal value (based on body surface area) or a hemoglobin level >16.5 g/dL (in males) or >16.0 g/dL (in females) and/or hematocrit >49% (in males) or >48% (in females) indicate **absolute erythrocytosis**. A decrease in plasma volume, such as occurs in acute dehydration and burns, may result in a high hemoglobin value. These situations are more accurately designated as **hemoconcentration** or **relative polycythemia** because the RBC mass is not increased, and normalization of the plasma volume restores hemoglobin to normal levels. Once the diagnosis of true polycythemia is made, sequential studies should be done to determine the underlying etiology (Fig. 515.1).

CLONAL POLYCYTHEMIA (POLYCYTHEMIA VERA)

Pathogenesis

Polycythemia vera is an acquired clonal myeloproliferative neoplastic disorder, which is rare in children and adolescents. The median age of diagnosis is >60 years. Although primarily manifesting as erythrocytosis, thrombocytosis and leukocytosis can also be seen. When isolated severe thrombocytosis exists in the absence of erythrocytosis, the myeloproliferative disorder is called **essential thrombocythemia**. A gain-of-function pathogenic variant involving exon 12 or 14 (V617F) of *JAK2,* a cytoplasmic tyrosine kinase, is found in >90% of adult patients with polycythemia vera, but in <30% of children with this condition. The erythropoietin receptor is normal, and serum erythropoietin levels are low. Patients without *JAK* pathogenic variants may have variants in the calreticulin or thrombopoietin receptor genes. Risk factors for development of polycythemia vera include a family history of polycythemia vera and presence of an autoimmune disorder.

Clinical Manifestations

Patients with polycythemia vera usually have splenomegaly. Erythrocytosis may cause hypertension, headache, early satiety, or neurologic symptoms and increases the risk of thrombosis. Granulocytosis may cause diarrhea or pruritus from histamine release. Thrombocytosis (with or without platelet dysfunction) may cause thrombosis or hemorrhage. Bleeding or acquired von Willebrand disease with extreme thrombocytosis, and complications of pregnancy may occur. Long-term effects include progression to myelofibrosis, myelodysplasia, and/or acute leukemia. These events are rare in children. Table 515.1 lists the diagnostic criteria for polycythemia vera.

Treatment

Phlebotomy and low-dose aspirin are the initial treatments of choice to alleviate symptoms of hyperviscosity and decrease the risk of thrombosis. Iron supplementation should be given to prevent viscosity problems from iron-deficient microcytosis or thrombocytosis. If these treatments are unsuccessful, the patient has progressive splenomegaly, thrombocytosis, or leukocytosis, or has high-risk features (i.e., age >60 or history of thrombosis), antiproliferative treatments (hydroxyurea, pegylated interferon, or busulfan) may be helpful. The use of ruxolitinib (a JAK2 inhibitor) is approved in hydroxyurea-resistant or hydroxyurea-intolerant adult patients, but the long-term effectiveness remains under investigation. There are limited data in the treatment of children, given the rarity of the condition. Often only phlebotomy is required. Transformation of the disease into myelofibrosis or acute leukemia is rare in children.

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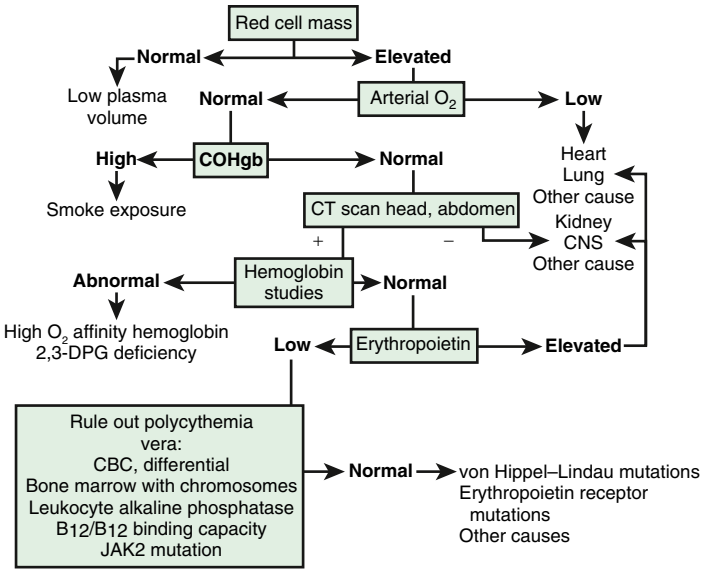


Fig. 515.1 Algorithm showing sequential studies in the evaluation of polycythemia. CBC, Complete blood count; CNS, central nervous system; COHgb, carboxyhemoglobin; 2,3-DPG, 2,3-diphosphoglycerate.

Table 515.1 World Health Organization (WHO) Diagnostic Criteria for Polycythemia Vera

MAJOR CRITERIA
1. Hb >16.5 g/dL (males) or Hb >16.0 g/dL (females)
   or
   Hct >49% (males) or >48% (females)
   or
   Elevated red cell mass >25% above mean normal predicted value
2. Bone marrow (BM) biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic mature megakaryocytes (difference in sizes)
3. Presence of *JAK2* or JAK2 exon 12 mutation

MINOR CRITERIA
1. Subnormal serum erythropoietin level

DIAGNOSIS
All three major criteria
or
First two major criteria and the minor criterion*

*Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in males (hematocrit, 55.5%) or 16.5 g/dL in females (hematocrit >49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Hb, Hemoglobin; Hct, hematocrit.

Modified from Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127: 2391–405.

Table 516.1 Differential Diagnosis of Polycythemia

CLONAL (PRIMARY)
Polycythemia vera

NONCLONAL
Congenital
High–oxygen affinity hemoglobinopathy (e.g., hemoglobin Chesapeake, Malmo, San Diego)
Erythropoietin receptor mutations (primary familial and congenital polycythemia [PFCP])
Methemoglobin reductase deficiency
Hemoglobin M disease
2,3-Diphosphoglycerate deficiency

Acquired
Hormonal
- Adrenal disease: virilizing hyperplasia, Cushing syndrome
- Athletic performance enhancing substances (e.g., anabolic steroids, androgens, recombinant erythropoiesis stimulating agents)
- Malignant tumors: adrenal, cerebellar, hepatic, other
- Renal disease: cysts, hydronephrosis, renal artery stenosis

Hypoxia
- Altitude
- Cardiac disease
- Lung disease
- Sleep apnea
- Central hypoventilation
- Chronic carbon monoxide exposure including smoking

Neonatal: delayed cord clamping (placental-fetal transfusion)
- Normal intrauterine environment
- Placental insufficiency (preeclampsia, maternal chronic hypertension, placental abruption)
- Twin-twin or maternal-fetal hemorrhage
- Perinatal asphyxia
- Infants of diabetic mothers
- Intrauterine growth restriction
- Trisomy 13, 18, or 21
- Adrenal hyperplasia
- Maternal-congenital hyperthyroidism

Spurious
Plasma volume decrease

Chapter 516

Nonclonal Polycythemia

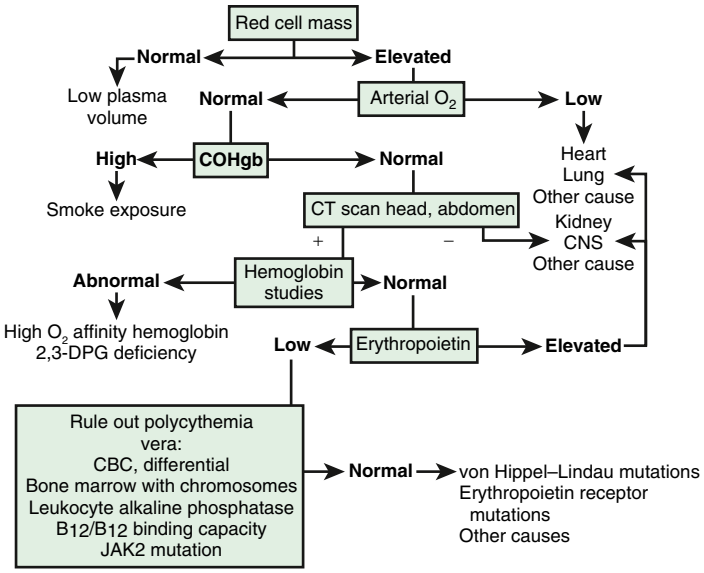
Allison S. Remiker and Amanda M. Brandow

PATHOGENESIS

Nonclonal polycythemia is diagnosed when polycythemia is caused by a physiologic process that is not derived from a single cell (Table 516.1). Nonclonal polycythemia can be congenital or acquired (secondary).

Congenital Polycythemia

Lifelong or familial polycythemia should trigger a search for a congenital problem. These inherited conditions may be transmitted as dominant or recessive disorders instigating erythrocytosis



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2. Bone marrow (BM) biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic mature megakaryocytes (difference in sizes)
3. Presence of *JAK2* or JAK2 exon 12 mutation

MINOR CRITERIA
1. Subnormal serum erythropoietin level

DIAGNOSIS
All three major criteria
or
First two major criteria and the minor criterion*

*Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in males (hematocrit, 55.5%) or 16.5 g/dL in females (hematocrit >49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

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Hormonal
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- Athletic performance enhancing substances (e.g., anabolic steroids, androgens, recombinant erythropoiesis stimulating agents)
- Malignant tumors: adrenal, cerebellar, hepatic, other
- Renal disease: cysts, hydronephrosis, renal artery stenosis

Hypoxia
- Altitude
- Cardiac disease
- Lung disease
- Sleep apnea
- Central hypoventilation
- Chronic carbon monoxide exposure including smoking

Neonatal: delayed cord clamping (placental-fetal transfusion)
- Normal intrauterine environment
- Placental insufficiency (preeclampsia, maternal chronic hypertension, placental abruption)
- Twin-twin or maternal-fetal hemorrhage
- Perinatal asphyxia
- Infants of diabetic mothers
- Intrauterine growth restriction
- Trisomy 13, 18, or 21
- Adrenal hyperplasia
- Maternal-congenital hyperthyroidism

Spurious
Plasma volume decrease

Chapter 516

Nonclonal Polycythemia

Allison S. Remiker and Amanda M. Brandow

PATHOGENESIS

Nonclonal polycythemia is diagnosed when polycythemia is caused by a physiologic process that is not derived from a single cell (Table 516.1). Nonclonal polycythemia can be congenital or acquired (secondary).

Congenital Polycythemia

Lifelong or familial polycythemia should trigger a search for a congenital problem. These inherited conditions may be transmitted as dominant or recessive disorders instigating erythrocytosis

by augmenting hypoxia sensing or abnormalities in oxygen sensing. Autosomal dominant causes include hemoglobins that have increased oxygen affinity (P_{50} [partial pressure of oxygen in the blood at which the hemoglobin is 50% saturated] <20 mm Hg), erythropoietin receptor pathogenic variants resulting in an enhanced effect of erythropoietin, or variants in the von Hippel–Lindau *(VHL), EGLN1*, or *EPAS1* genes that result in altered intracellular oxygen sensing. Another rare cause is autosomal recessive 2,3-diphosphoglyceric acid deficiency, which leads to a left shift of the oxygen dissociation curve, increased oxygen affinity, and consequent polycythemia.

Subtle decreases in oxygen delivery to tissues may cause polycythemia. Congenital **methemoglobinemia** resulting from an autosomal recessive deficiency of cytochrome b5 reductase may cause cyanosis and polycythemia (see Chapter 511.7). Most affected individuals are asymptomatic. Neurologic abnormalities may be present in patients whose enzyme deficits are not limited to hematopoietic cells. Hemoglobin M disease (autosomal dominant) causes methemoglobinemia and can lead to polycythemia. Cyanosis may occur in the presence of as little as 1.5 g/dL of methemoglobin but is uncommon in other hemoglobin variants unless hyperviscosity results in localized hypoxemia.

In rare cases, there is an undefined inherited lesion causing primary familial and congenital polycythemia (PFCP), which is described as an elevation of erythrocyte mass and hemoglobin, hypersensitivity to EPO, and low serum EPO in the setting of a normal hemoglobin-oxygen dissociation curve.

Acquired (Secondary) Polycythemia

Polycythemia may be present in clinical situations associated with chronic arterial oxygen desaturation. Cardiovascular defects involving right-to-left shunts and pulmonary diseases interfering with proper oxygenation are the most common causes of hypoxic polycythemia. Clinical findings usually include cyanosis, hyperemia of the sclerae and mucous membranes, and clubbing of the fingers. As the hematocrit rises to >65%, clinical manifestations of hyperviscosity, such as headache and hypertension, may require phlebotomy. Living at high altitudes also causes hypoxic polycythemia; the hemoglobin level increases approximately 4% for each rise of 1,000 m (~3,300 ft) in altitude. Smoking has been associated with polycythemia secondary to tissue hypoxia, elevation of carbon monoxide, and volume contraction. Partial obstruction of a renal artery rarely results in polycythemia. Polycythemia has also been associated with benign and malignant tumors that secrete paraneoplastic erythropoietin. Exogenous or endogenous excess of anabolic steroids also may cause polycythemia. A common spurious cause is a decrease in plasma volume, as occurs in moderate to severe dehydration.

DIAGNOSIS

See Chapter 515; Figure 515.1 outlines sequential studies to evaluate polycythemia.

TREATMENT

For mild disease, observation is sufficient. When the hematocrit is >65–70% (hemoglobin >23 g/dL), blood viscosity greatly increases. Periodic phlebotomy may prevent or decrease symptoms such as headache, dizziness, or exertional dyspnea. Apheresed blood should be replaced with plasma or saline to prevent hypovolemia in patients accustomed to a chronically elevated total blood volume. Increased demand for red blood cell production may cause iron deficiency. Iron-deficient microcytic red cells are more rigid, further increasing the risk of intracranial and other thromboses in patients with polycythemia. Periodic assessment of iron status should be performed, and iron deficiency should be treated.

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Section 5

The Pancytopenias

Chapter 517

Inherited Bone Marrow Failure Syndromes with Pancytopenia

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Pancytopenia refers to a reduction below normal values of all three peripheral blood lineages: leukocytes, platelets, and erythrocytes. Identifying the etiology of pancytopenia usually requires microscopic examination of the peripheral blood smear, as well as bone marrow biopsy and aspirate specimens to assess overall cellularity and morphology. The three general categories of pancytopenia are related to bone marrow pathologies and can frequently be differentiated based on bone marrow findings.

Pancytopenia with hypocellular bone marrow on biopsy is seen with *inherited bone marrow failure syndromes* (IBMFSs) with pancytopenia, *acquired* aplastic anemia of varied etiologies, and the *hypoplastic* variant of *myelodysplastic syndrome* (MDS). **Pancytopenia with cellular bone marrow** is seen with primary bone marrow disease (e.g., acute leukemia, myelodysplasia) and secondary to autoimmune disorders (e.g., autoimmune lymphoproliferative syndrome, systemic lupus erythematosus), vitamin B_{12} or folate deficiency, storage diseases (e.g., Gaucher, Niemann-Pick), overwhelming infection, sarcoidosis, and hypersplenism. **Pancytopenia with bone marrow infiltration** can be seen in metastatic solid tumors, myelofibrosis, hemophagocytic lymphohistiocytosis, and osteopetrosis. It is important to note that exceptions exist with regard to this classification; IBMFSs can manifest as normocellular or hypercellular bone marrow at early stages of presentation or in cases where MDS develops.

Inherited pancytopenias with hypocellular bone marrow are IBMFSs that feature decreased bone marrow production of the three major hematopoietic lineages occurring on an inherited basis and resulting in anemia, neutropenia, and thrombocytopenia. Patients may have single-lineage or bi-lineage cytopenia at presentation and gradually develop pancytopenia over time. All disorders for which a genetic basis has been deciphered have thus far been shown to be monogenic. Transmission of pathogenic variant genes is mendelian and in an autosomal dominant, autosomal recessive, or X-linked manner (Table 517.1). Modifying genes and acquired factors may also be operative. Inherited pancytopenias account for approximately 30% of cases of pediatric bone marrow failure. Clinical features may help differentiate the IBMFS disorders (Table 517.2), but whole exome sequencing (WES) or specific bone marrow failure syndrome gene panels help to confirm the diagnosis.

FANCONI ANEMIA

Etiology and Epidemiology

Fanconi anemia (FA) is a rare multisystem hereditary disorder resulting in the development of bone marrow failure in those affected and a predisposition to malignancy, including myelodysplasia (MDS), acute myeloid leukemia (AML), and epithelial cancers. Individuals with FA

Table 517.1 Inherited Bone Marrow Failure Syndromes with Multilineage Cytopenia and Familial MDS/AML: Inheritance, Variant Genes, and Affected Pathways

DISORDER	INHERITANCE	GENE	AFFECTED PATHWAYS
Fanconi anemia	AR	*FANCA, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG/XRCC9, FANCI, FANCJ/BRIP1, FANCL/PHF9, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRCA1, FANCT/UBE2T, FANCU/XRCC2, FANCV/REV7, FANCW/RFWD3*	DNA repair, homologous recombination, ribosome biogenesis *(FANCI)*
	XLR	*FANCB*	DNA repair, homologous recombination
Mixed Fanconi anemia/xeroderma pigmentosa/Cockayne syndrome	AR	*ERCC1/XPF*	DNA repair
Shwachman-Diamond syndrome	AR	*SBDS, DNAJC21, EFL1*	Ribosome biogenesis
	AD	*SRP54*	Co-translational protein modification
Dyskeratosis congenita	XLR	*DKC1*	Telomere maintenance, ribosome biogenesis
	AD	*TINF2, TERC, TERT, RTEL1, ACD(TPP1), PARN*	Telomere maintenance, RNA processing
	AR	*TERT, RTEL1, ACD(TPP1), WRAP53(TCAB1), CTC1, POT1, RPA1, PARN, NOP10, NHP2*	Telomere maintenance, RNA processing, ribosome biogenesis
Congenital amegakaryocytic thrombocytopenia	AR	*MPL*	Hematopoietic cytokine receptor
SRP72-associated hereditary aplastic anemia/MDS	AD	*SRP72*	Co-translational protein modification
ERCC6L2-associated hereditary aplastic anemia/MDS	AR	*ERCC6L2*	Transcription
THPO-associated hereditary aplastic anemia/MDS	AR/AD	*THPO*	Hematopoietic cytokine
Reticular dysgenesis	AR	*AK2*	Nucleotide metabolism
Cartilage-hair hypoplasia	AR	*RMRP, POP1, NEPRO*	rRNA and mitochondrial RNA processing
Pearson's syndrome	Maternal	*mDNA*	Mitochondrial DNA genes
Familial thrombocytopenia with predisposition to AML	AD	*RUNX1/CBFA2*	Hematopoietic cytokines
	AD	*ETV6*	Transcription repression
GATA2-associated disorders (MonoMac syndrome, Emberger syndrome, familial MDS syndrome)	AD	*GATA2*	Transcription
Bone marrow failure and diabetes		*DUT*	Nucleotide metabolism
Familial MDS/AML (others)		*CEBPA*	Transcription
Familial MDS/AML (others)		*DDX41*	RNA helicase
Seckel syndrome	AR	*CEP152, CENPJ, CEP63, NIN, PLK4, CDK5RAP2; ATR, RBBP8, ATRIP, DNA2*	Centriole/Centrosome duplication and function; DNA damage sensing and repair, and checkpoint signaling activation
Schimke immunoosseous dysplasia	AR	*SMARCAL1*	Chromatin remodeling
Dubowitz syndrome	AR	*NSUN2; LIG4*	Cytosine methylation in various RNA types; DNA non-homologous end joining repair
	AD	-14q32, -17q24, -19q13	
Rothmund-Thomson syndrome	AR	*RECQL4*	Chromosome segregation
Nijmegen breakage syndrome	AR	*NBN*	DNA repair

AD, Autosomal dominant; AML, acute myeloid leukemia AR, autosomal recessive; MDS, myelodysplastic syndrome; UK, unknown; XLR, X-linked recessive.

Table 517.2 Clinical Manifestations and Laboratory Findings in Inherited Bone Marrow Failure Syndrome

SYNDROME	NONHEMATOLOGICAL CLINICAL MANIFESTATIONS	LABORATORY FINDINGS
Fanconi anemia	Short stature, low birth weight, microcephaly, microphthalmia, hearing loss, triangular face, micrognathia, cardiac anomalies, tracheoesophageal fistula, esophageal atresia, kidney anomalies, hypoplastic thenar eminence, clinodactyly, café-au-lait spots	Pancytopenia, macrocytosis, elevated Hb F, increased chromosome breakage in clastogenic assay
Dyskeratosis congenita	Mucocutaneous triad (skin pigmentation, nail dysplasia, oral leukoplakia), short stature, low birth weight, failure to thrive, pulmonary fibrosis, stenosis of the esophagus, liver fibrosis	Pancytopenia, macrocytosis, elevated Hb F, very short telomeres
Diamond-Blackfan anemia	Low birth weight, short stature, developmental delay, anomalies in craniofacial skeleton, eyes, heart, visceral organs, and limbs	Anemia, elevated red blood cell adenosine deaminase, macrocytosis, elevated Hb F
Shwachman-Diamond syndrome	Exocrine pancreatic insufficiency, failure to thrive, malabsorption, short stature, neurodevelopment and skeletal abnormalities	Neutropenia, low serum isoamylase, low serum trypsinogen
Severe congenital neutropenia	Recurrent infection	Neutropenia
Congenital amegakaryocytic thrombocytopenia	Nonsyndromic (occasionally, growth retardation, cardiac anomalies, psychomotor developmental delay)	Thrombocytopenia, reduced megakaryocytes
GATA2 deficiency	Lymphedema, immunodeficiency, atypical mycobacterial infections	Neutropenia, anemia, thrombocytopenia
SAMD9/SAMD9L disorders	MIRAGE (SAMD9): MDS, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy	Transient or permanent cytopenia
	Ataxia–pancytopenia syndrome (SAMD9L): cerebellar atrophy and white matter hyperintensities, gait disturbance, nystagmus	
MECOM-associated syndromes	Radioulnar synostosis, clinodactyly, hearing loss, cardiac/renal malformation	Thrombocytopenia
Pearson syndrome	Pancreatic insufficiency, failure to thrive, microcephaly, ptosis, Kearns-Sayre syndrome	Neutropenia, anemia, pancytopenia, lactic acidosis, hyperglycemia

Hb F, Hemoglobin F; MDS, myelodysplastic syndrome.
Modified from Park M. Overview of inherited bone marrow failure syndromes. *Blood Res.* 2022;57(S1):49–54. Table 1.

often have congenital malformations and high sensitivity to alkylating agents and radiation. The estimated frequency of FA is 1 in 200,000 in most populations but is higher in Ashkenazi Jews (1:30,000) and Afrikaners (1:22,000). Carrier frequency is approximately 1:200-300 in most populations. Pathogenic variants in 22 genes, designated *FANC* genes, have been reported to cause FA or FA-like disease. All pathogenic variants except for one are inherited in an autosomal recessive manner. One uncommon form of FA is X-linked recessive. FA occurs in all ethnic groups. At presentation, patients may have typical physical anomalies and abnormal hematologic findings (majority of patients), normal physical features but abnormal hematologic findings (about one third of patients), or physical anomalies and normal hematologic findings (unknown percentage of patients). There can be sibling discordance in clinical and hematologic manifestations, even in affected monozygotic twins.

Pathology

All FA genes code for proteins that play roles in various cellular pathways and most prominently in DNA cross linking and repair. Patients with FA have faulty DNA repair and increased chromosomal fragility caused by DNA interstrand cross-linking agents such as diepoxybutane (DEB) and mitomycin C (MMC). Cell fusion of FA cells with normal cells or with cells from some unrelated patients with FA produces a corrective effect on chromosomal fragility, a process called *complementation.* The classic FA phenotype that clearly defines the FA-associated genes *(FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BACH1/BRIP1, FANCL, FANCM, FANCN/PALB2, FANCP/SLX4, FANCQ/ERCC4, UBE2T, REV7, RFWD3)* includes the triad of **bone marrow failure**, **congenital anomalies**, and **elevated chromosome fragility**. These genes can be mutated in patients who have one or all of the components of the triad. Genes that were found to be associated with one or two but not all three of the components are **FA-like genes** *(FANCO/RAD51C, RAD51, FANCS/BRCA1, FANCR/EXCC2). FANCA* accounts for approximately 64% of FA cases, *FANCC* for 14%, and *FANG* for 9%. *FANCB*, *FANCD1/BRCA2*, *FANCD2*, *FANCE,* and *FANCF* are collectively mutated in almost 13% of FA patients. The remaining genes are pathogenic variants in rare cases.

The proteins encoded by wild-type *FANC* genes are involved in the DNA damage recognition and repair biochemical pathway. Therefore aberrant proteins lead to genomic instability and chromosome fragility. FANC proteins are involved in other cellular activities, such as reactive oxygen species detoxification, energetic metabolism, and cytokine signaling. Thus FANC pathogenic variants likely affect several cellular and biochemical roles of the respective proteins, which eventually leads to the FA phenotype. The observed disease complexity and heterogeneity is likely caused by the involvement of multiple cellular and biochemical pathways both in unrelated individuals and in family members with the same genetic pathogenic variant.

Clinical Manifestations

The most common congenital anomalies in FA are skeletal and include absence of radii and/or thumbs that are hypoplastic, supernumerary, bifid, or absent. Anomalies of the feet, congenital hip dislocation, and leg abnormalities can also be seen (Fig. 517.1 and Table 517.3). Skin hyperpigmentation of the trunk, neck, and intertriginous areas;

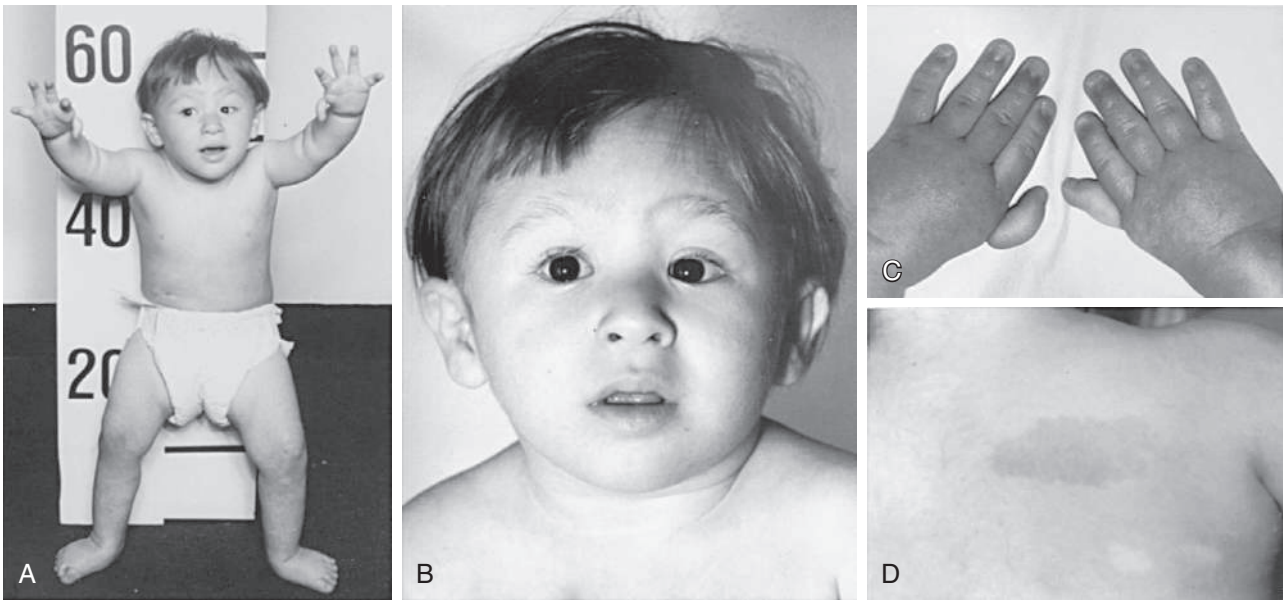

Fig. 517.1 A 3-year-old boy with Fanconi anemia who exhibits several classic phenotypic features. **A,** Front view. **B,** Face. **C,** Hands. **D,** Back right shoulder. The features to be noted include short stature, dislocated hips, microcephaly, a broad nasal base, epicanthal folds, micrognathia, thumbs attached by a thread, and café-au-lait spots with hypopigmented areas beneath. *(From Nathan DC, Orkin SH, Ginsburg D, et al, eds: Nathan and Oski's Hematology of Infancy and Childhood, 6th ed. Philadelphia: Saunders, 2003. p. 285.)*

Table 517.3 Specific Types of Anomalies in Fanconi Anemia

SKIN (40%)
Generalized hyperpigmentation on the trunk, neck, and intertriginous areas; café-au-lait spots; hypopigmented areas

BODY (40%)
Short stature, delicate features, small size, underweight

UPPER LIMBS (35%)
Thumbs (35%): absent or hypoplastic; supernumerary, bifid, or duplicated; rudimentary; short, low set, attached by a thread; triphalangeal, tubular, stiff, hyperextensible
Radii (7%): absent or hypoplastic (only with abnormal thumbs); absent or weak pulse
Hands (5%): clinodactyly; hypoplastic thenar eminence; six fingers; absent first metacarpal; enlarged, abnormal fingers; short fingers; transverse crease
Ulnae (1%): dysplastic or absent

LOWER LIMBS (5%)
Feet: toe syndactyly, abnormal toes, flat feet, short toes, clubfeet, six toes, supernumerary toe
Legs: congenital hip dislocation, Perthes disease, coxa vara, abnormal femur, thigh osteoma, abnormal legs

GONADS
Males (25%): hypogenitalia, undescended testes, hypospadias, abnormal genitalia, absent testis, atrophic testes, azoospermia, phimosis, abnormal urethra, micropenis, delayed development
Females (2%): hypogenitalia; bicornuate uterus; abnormal genitalia; aplasia of uterus and vagina; atresia of uterus, vagina, and ovary

OTHER SKELETAL ANOMALIES
Head (20%) and face (2%): microcephaly, hydrocephalus, micrognathia, peculiar face, birdlike face, flat head, frontal bossing, scaphocephaly, sloped forehead, choanal atresia, dental abnormalities
Neck (1%): Sprengel deformity; short, low hairline; webbed
Spine (2%): spina bifida (thoracic, lumbar, cervical, occult sacral), scoliosis, abnormal ribs, sacral agenesis, sacrococcygeal sinus, Klippel-Feil syndrome, vertebral anomalies, extra vertebrae

EYES (20%)
Small eyes, strabismus, epicanthal folds, short or almond-shaped palpebral fissures, hypertelorism, ptosis, slanting, cataracts, astigmatism, blindness, epiphora, nystagmus, proptosis, small iris

EARS (10%)
Deafness (usually conductive); abnormal shape; atresia; dysplasia; low set, large, or small; infections; abnormal middle ear; absent eardrum; dimples; rotated; canal stenosis

KIDNEY (20%)
Ectopic or pelvic; abnormal, horseshoe, hypoplastic, or dysplastic; absent; hydronephrosis or hydroureter; infections; duplicated; rotated; reflux; hyperplasia; no function; abnormal artery

GASTROINTESTINAL SYSTEM (5%)
High-arched palate, atresia (esophagus, duodenum, jejunum), imperforate anus, tracheoesophageal fistula, Meckel diverticulum, umbilical hernia, hypoplastic uvula, abnormal biliary ducts, megacolon, abdominal diastasis, Budd-Chiari syndrome

UROGENITAL
Males (25%): micropenis, penile-scrotal fusion, undescended or atrophic or absent testes, hypospadias, chordae, phimosis, azoospermia
Females (2%): bicornuate uterus, aplasia or hypoplasia of vagina and uterus, atresia of vagina, hypoplastic uterus, hypoplastic or absent ovary, hypoplastic fused labia

CARDIOPULMONARY SYSTEM (6%)
Patent ductus arteriosus, ventricular septal defect, abnormal heart, peripheral pulmonic stenosis, aortic stenosis, coarctation, absent lung lobes, vascular malformation, aortic atheromas, atrial septal defect, tetralogy of Fallot, pseudotruncus, hypoplastic aorta, abnormal pulmonary drainage, double aortic arch, cardiac myopathy

CENTRAL NERVOUS SYSTEM (3%)
Hyperreflexia, Bell palsy, central nervous system arterial malformation, moyamoya syndrome, Arnold-Chiari malformation, stenosis of internal carotid artery, small pituitary gland, absent corpus callosum
Slow development (10%)

Abnormalities are listed in the approximate order of frequency within each category.
Adapted from Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev.* 2010;24:101–122.

café-au-lait spots; and vitiligo, alone or in combination, occur with similar frequency. Short stature is common and in some patients is aggravated by subnormal growth hormone (GH) secretion or hypothyroidism. Male patients with FA may have an underdeveloped penis; undescended, atrophic, or absent testes; and hypospadias or phimosis, and all are infertile. Females can have malformations of the vagina, uterus, and ovary, and all have reduced fertility. Many patients have characteristic facial dysmorphisms, including microcephaly, small eyes, epicanthal folds, and abnormal shape, size, or positioning of the ears (see Fig. 517.1). Kidneys may be ectopic, pelvic, horseshoe-shaped, hypoplastic, dysplastic, or absent. Cardiovascular (CV) and gastrointestinal (GI) malformations also occur. Approximately 10% of patients with FA are cognitively delayed. Neonates with FA usually have intrauterine growth restriction (IUGR) and low birthweight and may show malformations consistent with VACTERL/VACTERL-H association (vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula with esophageal atresia, renal and limb structural abnormalities with hydrocephalus).

Bone marrow failure usually appears within the first decade of life. Thrombocytopenia, red blood cell (RBC) macrocytosis, and increased fetal hemoglobin (Hb F), as a result of bone marrow stress, often appear first. At these stages, bone marrow aspirate and biopsy often show a hypocellular specimen. Subsequently, patients develop neutropenia and then anemia. Severe aplasia develops in most cases, usually over a few years.

Cancer Risk and Other Complications

In addition to the low blood counts and physical anomalies, patients with FA have a high risk of developing cancer. The most frequent solid tumors are *squamous cell carcinomas* (SCCs) of the head and neck (600-fold higher risk than the general population) and carcinoma of the upper esophagus (2,000-fold higher risk), the vulva (3,000-fold higher risk), and/or anus, cervix, and lower esophagus. Onset of solid-tumor malignancy is much sooner than that seen in the general population, with median age of onset of SCC in the FA population occurring at 33 years vs 60–70 years in the general population. Human papillomavirus (HPV) is suspected in the pathogenesis of SCC. Benign and malignant liver tumors can occur (adenomas, hepatomas) and are usually associated with androgen therapy for aplastic anemia. Androgens are also implicated in the etiology of peliosis hepatis (blood-filled hepatic sinusoids), which is reversible when androgen therapy is discontinued. Clonal bone marrow cytogenetic abnormalities are common in FA and on follow-up can either be stable, intermittently detected, or progressive. The cumulative incidence of clonal and malignant myeloid transformation by age 18 years, which includes clonal cytogenetic marrow abnormalities, MDS, and AML, is approximately 75%. One study indicated that by age 40 years, the cumulative incidence of leukemia is 33%.

Diagnosis

FA should be considered in all children and young adults with unexplained cytopenias. Abnormal hematologic findings and characteristic physical anomalies suggest the diagnosis, which can be confirmed with a lymphocyte chromosomal breakage study done with and without the addition of cross-linking agents such as DEB and MMC. Increased chromosome fragility is indicated by spontaneously occurring chromatid breaks, rearrangements, gaps, endoreduplications, and chromatid exchanges in blood lymphocytes cultured with phytohemagglutinin, as well as in cultured skin fibroblasts, underscoring the constitutional nature of FA. With the addition of DEB or MMC, fragility is strikingly enhanced in lymphocyte cultures of patients with FA compared with those of controls. Abnormal chromosome breakage analysis and genetic testing for prenatal diagnosis can be performed on amniotic fluid cells or on tissue from a chorionic villus biopsy. No other inherited pancytopenia is associated with a prominent in vitro hypersensitivity to DEB or MMC by the chromosomal breakage study. However, 10–15% of patients with suspected FA have *somatic mosaicism* and may not show the characteristically high degree of chromosomal fragility in their lymphocytes, reflecting the presence of mixed populations of somatic cells, some with two abnormal alleles and some with only one. The latter population of lymphocytes derives from a portion of hematopoietic stem cells (HSCs) that underwent spontaneous somatic gene correction on one allele. Testing of skin fibroblasts should be performed if the suspicion of FA is high despite negative testing on peripheral blood lymphocytes.

Next-generation sequencing (NGS) gene panels and WES are the tests of choice for FA. NGS is an efficient and accurate method for diagnosing FA but can occasionally be limited by difficulties in interpreting previously unreported variants or in unraveling pathogenic variants due to reversion variants or technical matters. When no definite causative point pathogenic variants are found, high-resolution copy number variation analysis techniques are employed, followed by a genome-wide search for pathogenic variants in novel associated genes. Pathogenic variants in one of the 22 FA genes are found in over 95% of FA cases.

Extensive screening for potential medical problems is necessary after the diagnosis of FA is established. Imaging using radiation should be minimized as much as possible because of the carcinogenic risk inherent to this genetic instability disease; MRI should replace CT whenever possible. In addition to detailed review of the past medical history and thorough physical examination, the screen should include ultrasonographic examination of the abdomen and echocardiography to rule out internal congenital anomalies. Other imaging may be done as necessary and based on the initial screen. Subspecialty consultations for anomalies and disabilities that have been identified can be arranged during this interval. If growth velocity is below expectations, endocrine evaluation is needed to assess for GH and thyroid deficiency. Blood work should include evaluation of renal, liver, thyroid, metabolic, and immune systems.

Treatment

A hematologist, preferably one who specializes in IBMFSs, with a multidisciplinary team should manage patients with FA. At diagnosis, a detailed assessment of the patient's blood counts, bone marrow function, growth, development, and other organ function should be carried out.

If hematologic abnormalities are mild to moderate and stable and there is no transfusion requirement, patients can be observed closely with peripheral blood counts every 3 months and bone marrow aspiration surveillance every year for clonal cytogenetic abnormalities, MDS, and AML. Bone marrow biopsy might also be intermittently done during bone marrow testing to evaluate changes in percentage of cellularity and fibrosis. More frequent monitoring can be applied when deemed necessary, as when a decline in blood counts occurs. Glucose levels should be performed annually or biannually, depending on the degree of hyperglycemia found on initial testing. Screening for hypothyroidism should be performed yearly. Patients should be assessed for solid tumors at least annually, with a careful physical examination that includes comprehensive inspection of the skin, oral cavity, and other organs for unusual masses. After a certain age (e.g., 10 years) or after HSC transplant (HSCT), fluoroscopic examination of the orolaryngeal cavity and occult fecal blood testing are also recommended. Beginning at menarche, female patients should be screened annually for gynecologic cancer. Administration of HPV quadrivalent vaccine to decrease the risk of SCC is advised.

Chromosome fragility (and/or targeted genetic testing) should be offered to siblings and parents of affected patients for identification of other affected individuals. It is noteworthy that heterozygosity for several FA genes (e.g., *FANCN*, *FANCD1*, *FANCS*) is associated with cancer development. Human leukocyte antigen (HLA) typing of patients, biological parents, and full siblings for future HSCT should also occur early.

HSCT is the only curative therapy for the hematologic abnormalities observed in FA patients. Outcomes have improved because of modified reduced-intensity regimens that include fludarabine, low dose cyclophosphamide, and antithymocyte globulin with or without low-dose busulfan. Most regimens do not use radiation. These regimens have decreased the toxicities experienced by FA patients, who have high sensitivity to DNA-damaging agents such as alkylating drugs and irradiation. Those who undergo transplant using an HLA-identical sibling

donor without irradiation in the preparative regimen have an overall 3-5-year survival rate of >90%.

Traditionally, HSCT of FA patients with matched unrelated donors (MUD) or mismatched related donors have been a challenge because of the high degree of HSCT-related toxicity and death. However, major progress has been made in this regard. Several reports of radiation-free HSCT with in vivo or ex-vivo T-cell depleted, peripheral blood $CD34^+$ selected MUD graft and conditioning with fludarabine, rabbit antithymocyte globulin (ATG), low-dose cyclophosphamide, and low-dose busulfan demonstrated 80% or higher probabilities of overall and disease-free survival 1–5 years post HSCT. Improvement in high-resolution HLA typing contributes to better selection of unrelated-donor selection and a better outcome. Transplantation using haploidentical donors is on the rise and is showing promising outcomes, similar to those seen with unrelated donors.

Those transplanted before they receive multiple transfusions or develop clonal and malignant myeloid transformation (MDS or AML) do better. Survival rates are higher for patients who undergo transplant at <10 years of age. Molecular technology has led to preimplantation genetic diagnosis on parent-derived blastomeres, allowing for the unaffected ones to be implanted and resulting in the creation of an HLA-matched sibling donor without FA.

Androgens produce a response in approximately 70% of patients, heralded by reticulocytosis and a rise in hemoglobin within 1-2 months. White blood cell (WBC) counts may increase next, followed by platelet counts. After the initial response is seen, counts may continue to improve over many months until a maximum response is achieved. If a low dose is initially employed, the androgen dose can be increased every 3-4 weeks as long as no major side effects are seen and until the desired response is achieved. If a high dose is initially employed, androgen dosage can be slowly reduced to the minimum dose that maintains the required blood counts. Oral danazol and oxymetholone are currently the two most commonly used androgenic drugs. Patients typically stop responding to androgens after several months or years, as their bone marrow failure progresses or as they develop MDS or AML. Thus androgen therapy is not curative but is used rather as a bridge while waiting for a suitable donor for HSCT or while weighing the risks and benefits of transplant. Side effects of androgens include masculinization, increased linear growth, increased mood swings or aggressiveness, elevated hepatic enzymes, cholestasis, peliosis hepatis, and liver tumors. Screenings for these should be performed regularly.

The potential for recombinant growth factor (cytokine) therapy for FA has not been defined. Granulocyte colony-stimulating factor (G-CSF) can usually induce an increase in the absolute neutrophil count; however, there may be a heightened risk of expansion of bone marrow cells with clonal cytogenetic abnormalities such as monosomy 7. In one study, combination therapy consisting of G-CSF given subcutaneously daily or every 2 days along with erythropoietin given subcutaneously or intravenously 3 times per week resulted in improved neutrophil counts in most patients and a sustained rise in hemoglobin and platelet levels in approximately one third of patients. Most patients lost the response because of progression of bone marrow disease.

Prognosis

Improvements in supportive care, careful surveillance of known complications, prompt intervention, and improved transplant techniques have resulted in patients with FA surviving into their 30s. Unfortunately, there is an increased risk of solid tumors after HSCT. For example, head and neck cancer risk is increased 4.4-fold and is accelerated by approximately 15 years compared with nontransplanted patients. The cumulative incidence of malignancy 20 years after transplant is 35–40%. Some of the increased risk might be attributed to the use of DNA-damaging agents or the occurrence of graft-versus-host disease (GVHD).

SHWACHMAN-DIAMOND SYNDROME

Etiology and Epidemiology

Shwachman-Diamond syndrome (SDS) is an inherited disorder caused by pathogenic variants in one of four genes. It occurs in all racial and ethnic groups. SDS is a multisystem disorder. However, the nonhematologic manifestations of SDS are substantially different and usually include exocrine pancreatic insufficiency and skeletal abnormalities such as metaphyseal dysplasia (Table 517.4). SDS is a **ribosomopathy**, and the underlying defect is in ribosome assembly.

Table 517.4 Major Clinical Features of Shwachman-Diamond Syndrome

CLINICAL FEATURE	TOTAL/AVERAGE
Number of patients	225
Neutropenia	90%
Severe (≤500/μL)	46%
Anemia	46%
Thrombocytopenia	42%
Pancytopenia	21%
Exocrine pancreatic insufficiency	98%
Liver (elevated transaminases)	61%
Skeletal abnormalities	70%
Metaphyseal dysostosis	53%
Rib cage abnormalities	35%
Short stature (<3rd percentile)	66%

Data from Ginzberg H, Shin J, Ellis L, et al. Shwachman syndrome: phenotypic manifestations of sibling sets and isolated cases in a large patient cohort are similar. *J Pediatr*. 1999;135:81–88; Cipolli M, D'Orazio C, Delmarco A, et al. Shwachman's syndrome: pathomorphosis and long-term outcome. *J Pediatr Gastroenterol Nutr*. 1999;29:265–272; and Kuijpers TW, Alders M, Tool AT, et al. Hematologic abnormalities in Shwachman-Diamond syndrome: lack of genotype-phenotype relationship. *Blood*. 2005;106:356–361.

Pathology

Four genes have been linked to SDS. *SBDS* is the first gene that was described to have biallelic variants in SDS in 2003. *SBDS* maps to chromosome 7q11 and accounts for 80–90% of SDS cases. *SBDS* plays a role in the late stage of the pre-60S ribosome subunit maturation, binding to the *EFL1* GTPase and facilitating the release of eIF6 to enable 80S monosome formation. *DNAJC21* is the second reported SDS gene. The function of the human *DNAJC21* is required for the release and recycling of the Arx1/Alb1 heterodimer from the pre-60S biogenesis factors. The third SDS gene discovered is *EFL1*. *DNAJC21* and *EFL1*-associated SDS are autosomal recessive. Monoallelic pathogenic variants in *SRP54*, a protein involved in co-translational protein modification, mainly cause severe congenital neutropenia phenotype. Nevertheless, a small proportion of the patients have partial or classical SDS phenotype. The underlying genetic defects of SDS indicate that the last step in ribosome biogenesis is associated with pancytopenia (most often neutropenia) and a hypoplastic bone marrow. Defects in ribosomal proteins that are involved in earlier stages of ribosome subunit maturation and are structural components of the ribosome are associated with predominantly anemia and pure red cell aplasia.

Exocrine pancreatic insufficiency is caused by failure of exocrine pancreatic acinar development in SDS, and fatty replacement of pancreatic tissue is prominent. **Bone marrow failure** is characterized by dysfunctional HSCs, accelerated apoptosis of bone marrow progenitors, and a defective bone marrow microenvironment that does not support and maintain normal hematopoiesis.

Clinical Manifestations

Most patients with SDS have symptoms of fat malabsorption from birth that are caused by pancreatic insufficiency, but steatorrhea is not always obvious. Approximately 50% of patients appear to exhibit an improvement in pancreatic enzyme secretion as they age. The clinical picture can be dominated by complications from neutropenia, anemia,

or thrombocytopenia. Bacterial and fungal infections secondary to neutropenia, neutrophil dysfunction, and immunodeficiency can occur. A major concern is the development of MDS and acute leukemia, most often AML.

Short stature is a consistent feature of SDS. Most patients show normal growth velocity yet remain consistently below the 3rd percentile for height and weight. The occasional SDS adult achieves the 25th percentile for height. Although skeletal abnormalities are variable, classic findings are metaphyseal dysplasia, osteopenia, delayed appearance of secondary ossification centers, short or flared ribs, and thoracic dystrophy.

Some patients have hepatomegaly and elevations of liver enzymes. Most patients have dental abnormalities and poor oral health. Many have neurocognitive problems and poor social skills.

Laboratory Findings

Exocrine pancreatic insufficiency in SDS is associated with reduced age-adjusted serum trypsinogen and pancreatic isoamylase levels. Because serum pancreatic isoamylase is physiologically low in the first 3 years of life, and because reduced serum trypsinogen is typically seen in young infants and often improves with age, testing both enzymes is helpful. Fecal elastase is often reduced in SDS patients. Fat-soluble vitamin (A, D, E, and K) absorption is impaired, and thus measurement of vitamin A, D, and E levels, as well as prothrombin time (or international normalized ratio [INR]), is helpful to assess the consequences of fat malabsorption. Ultrasound or CT scan can visualize fatty replacement of pancreatic tissue. Fat malabsorption can be proven by assay on a 72-hour stool collection.

Neutropenia is observed in about 70% of patients with SDS at presentation and is seen in close to 100% of patients on follow-up. It is chronic but can be persistent or intermittent and mild, moderate, or severe. It has been identified in some neonates during an episode of sepsis. Neutrophils may have a defect in mobility, migration, and chemotaxis because of alterations in neutrophil cytoskeletal or microtubular function. Anemia, thrombocytopenia, and pancytopenia are seen in 40–66%, 40–60%, and 21–44% of cases, respectively. Pancytopenia can be severe as a result of full-blown aplastic anemia. Bone marrow aspirate and biopsy specimens show varying degrees of bone marrow hypoplasia and fat infiltration. However, at a young age or when patients develop MDS or leukemia, the bone marrow can be normocellular or even hypercellular.

Patients may also have B-cell defects with one or more of the following: low immunoglobulin G (IgG) or IgG subclasses, low percentage of circulating B lymphocytes, decreased in vitro B-cell proliferation, and lack of specific antibody production. Patients may have a low percentage of circulating T cells, subsets, or natural killer cells and decreased in vitro T-cell proliferation.

Diagnosis

The clinical diagnosis of SDS relies on having evidence of two of the following: bone marrow dysfunction, exocrine pancreatic insufficiency, and metaphyseal dysplasia. However, atypical presentations have been identified, such as MDS without previous documentation of low blood counts. Up to 20% of patients lack clear evidence of exocrine pancreatic defects at diagnosis. Furthermore, 20–60% lack metaphyseal dysplasia at diagnosis because this bony anomaly often develops with age. Therefore it is recommended that all patients with either hypoplastic/aplastic bone marrow or exocrine pancreatic insufficiency or metaphyseal dysplasia of unknown etiology be considered for SDS genetic testing. Genetic analysis for *SBDS*, *DNAJC21*, and *EFL1* are definitive in all or almost all cases of SDS. A patient with neutropenia and an *SRP54* pathogenic variant who does not have exocrine pancreatic insufficiency or metaphyseal dysplasia should be considered as having severe congenital neutropenia.

Pearson syndrome, consisting of refractory sideroblastic anemia, cytoplasmic vacuolization of bone marrow precursors, metabolic acidosis, exocrine pancreatic insufficiency, and a diagnostic mitochondrial DNA pathogenic variant, is similar to SDS, but the clinical course, morphologic features of the bone marrow, and gene pathogenic variant are different. Severe anemia requiring transfusion, rather than neutropenia, is present in Pearson syndrome from birth to 1 year of age. SDS shares some manifestations with **Fanconi anemia**, such as bone marrow dysfunction and growth failure, but patients with SDS are readily distinguished because of pancreatic insufficiency with fat malabsorption, fatty changes within the pancreatic body visualized by imaging, characteristic skeletal abnormalities not seen in FA, and a normal chromosomal breakage study with DEB and MMC. Distinguishing SDS from **dyskeratosis congenita (DC)** may not be possible based solely on clinical findings and pancreatic enzyme levels; telomere length measurement may facilitate a correct diagnosis. *In difficult cases of IBMFSs that cannot be easily classified, comprehensive genetic testing using an NGS panel of all known IBMFS genes or unbiased testing using WES/genome sequencing is likely to assist in establishing a diagnosis.*

Predisposition to Cancer

Patients with SDS are predisposed to MDS and leukemic transformation. Approximately 25% of patients develop clonal marrow cytogenetic abnormalities, MDS, or leukemia by age 18 years. About one third of patients have been reported to develop leukemia by age 30 years. Isochromosome 7q [i(7q)] is particularly common and is rarely seen in other conditions. i(7q) might be related to the presence of pathogenic variant *SBDS* on 7q11 and likely confers a compensatory effect by increasing *SBDS* transcribing alleles (although they remain variant). Other clonal chromosome abnormalities include monosomy 7, i(7q) combined with monosomy 7, deletions or translocations involving part of 7q, and deletions of 20q [Del(20q)]. The i(7q) and del(20q) are associated with relatively low risk and very slow progression to MDS or leukemic transformation.

Treatment

Fat malabsorption responds to oral pancreatic enzyme replacement and supplemental fat-soluble vitamins, administered according to guidelines similar to those for cystic fibrosis. A long-term plan should be initiated to periodically monitor changes in peripheral blood counts that require corrective action and to look for early evidence of malignant myeloid transformation. The latter also requires periodic bone marrow aspirations for smears and cytogenetic testing, as well as bone marrow biopsy. A common recommendation is to perform bone marrow testing every 1-3 years and complete blood counts every 3 months.

Daily *subcutaneous G-CSF* for profound neutropenia is effective in inducing a sustained increase in neutrophils. Some patients require transfusion support for management of severe anemia or thrombocytopenia. Experience with erythropoietin is limited. Androgens have been used in few published cases (with and without steroids); some patients showed response, and some did not.

The only curative option for severe bone marrow failure and advanced MDS or leukemia in SDS is allogeneic HSCT. Traditional myeloablative HSCT results in treatment-related mortality in 35–50% of patients. Reduced-intensity conditioning regimens that incorporate *fludarabine* appear to be safer and have been shown to be effective in SDS. Graft failure poses a challenge, possibly due to a stromal cell defect that is not corrected by HSCT. Results of treatment for advanced MDS and AML are generally limited, and outcome is typically poor.

Prognosis

The accurate life expectancy of SDS patients is unknown; analysis of published cases revealed a median survival of 35 years. Because the number of undiagnosed patients with mild or asymptomatic disease is unknown, the overall prognosis may be better than previously thought.

Although all patients have some degree of hematologic cytopenia at diagnosis, the changes in most patients are mild to moderate and do not require therapeutic intervention. Severe neutropenia responds well to G-CSF, but there is a concern that G-CSF may promote the growth of evolving MDS or leukemia clones because of the agent's powerful

growth stimulus on bone marrow cells. HSCT for severe bone marrow failure has produced a 50–70% survival rate, but safer protocols need to be introduced. Malignant bone marrow transformation remains ominous.

Approximately 50% of patients experience spontaneous conversion from exocrine pancreatic insufficiency to pancreatic sufficiency as a result of improvement in pancreatic enzyme secretion.

DYSKERATOSIS CONGENITA

Etiology and Epidemiology

DC is an inherited multisystem telomere disorder. A diagnostic triad of mucocutaneous features was proposed when the disorder was first described and included **dysplastic nails**, lacy **reticular pigmentation** of the upper chest and/or neck, and **oral leukoplakia** (Fig. 517.2). *However, this triad is not present in all individuals.* If it occurs, skin and nail findings usually become apparent in the first 10 years of life, whereas oral leukoplakia may be noticed later. Manifestations tend to progress as patients age. Hematological manifestations are actually the most common features in this disease. Varying degrees of bone marrow failure are seen in approximately 90% of patients. Severe aplastic anemia occurs in approximately 50% of cases, with the age of onset varying according to the genetic group. In some genetic groups, the disease usually starts in the first decade of life (e.g., *DKC1*, *TINF2, PARN*), whereas in others, it typically starts after the first decade (e.g., *TERT, TERC*). In addition to progressive bone marrow failure, patients with DC are also at high risk for pulmonary and hepatic fibrosis, other congenital anomalies, and a predisposition to solid tumors and MDS or AML. DC is rare, with an incidence in childhood of approximately 4 cases per 1 million population per year.

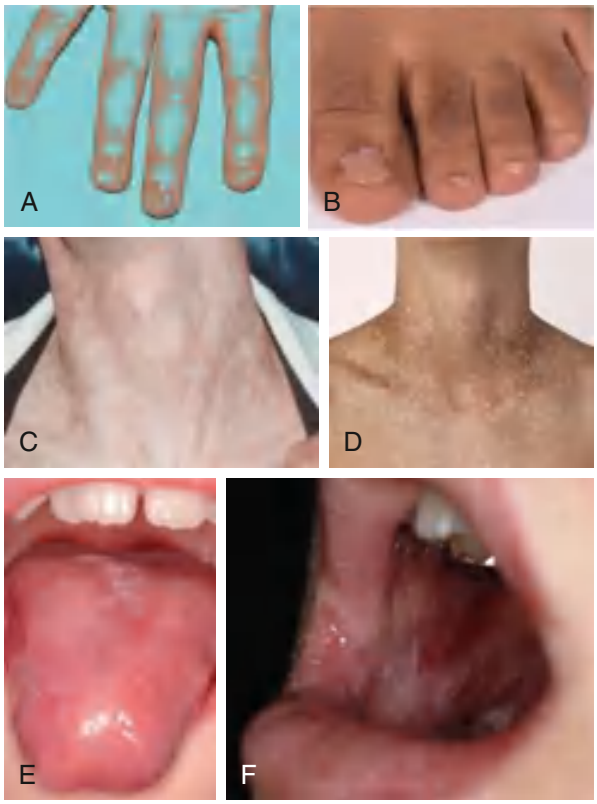

Fig. 517.2 Features of the diagnostic triad in dyskeratosis congenita. A and B, Dystrophic nails on hands and feet. C and D, Lacy reticular pigmentation on neck and upper thorax. E and F, Oral leukoplakia on tongue and buccal mucosa. *(A-C and E from Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24:101–122, Fig. 8; D and F from Savage SA, Alter BP. Dyskeratosis congenital. Hematol Oncol Clin North Am. 2009;23:215–231)*

Pathology

DC is genetically heterogeneous, and patients have pathogenic variants in genes that encode components of the telomerase complex *(TERT, TERC)*, telomere-capping complex *(CTC1, STN1)*, T-loop unwinding and telomere replication *(RTEL1, RPA1)*, telomerase trafficking *(WRAP53*/TCAB1*)*, TERC-associated factors that stimulate telomerase activity *(DKC1, NOP10, NHP2, NAF1)*, TERC-maturation factors *(PARN)*, and telomere-shelterin complex *(TINF2, POT1, ACD)*.

All components are critical for telomere maintenance. The **X-linked recessive** form of DC maps to Xq28, and many pathogenic variants have been identified in the *DKC1* gene, which codes for the nuclear protein dyskerin. The **autosomal dominant** form of disease is caused by pathogenic variants in *TINF2, TERC, TERT, RTEL1, ACD, NAF1,* and *RPA1*. **Autosomal recessive** DC is linked to pathogenic variants in *NOP10, NHP2, TCAB1/WRAP53, CTC1,* and *STN1,* as well as *TERT, TERC, RTEL1, PARN*, and *ACD*. Because of impaired telomere maintenance in all three inherited forms of DC, extremely short telomeres (<1st percentile for age) are demonstrated in the peripheral blood cells of all patients. Finding extremely short telomeres in lymphocytes performed by automated multicolor flow fluorescence in situ hybridization (FISH) has 97% sensitivity and 91% specificity for DC. Approximately 70% of individuals who meet clinical diagnostic criteria of DC have a pathogenic variant in one of the known DC-related genes. Pathogenic variants in *DKC1* are most common (20–25% of individuals), followed by *TINF2* (12–20% of individuals), *TERC* (5–10% of individuals), *RTEL1*, *TERT,* and *CTC1*. The remainder of the genetic pathogenic variants have been described in only a few families. Bone marrow failure is likely caused by progressive attrition and depletion of HSCs because of premature senescence, apoptosis, or chromosome instability, which manifests as pancytopenia.

Clinical Manifestations

The clinical criteria for classic DC include the presence of at least two of the four major features—abnormal skin pigmentation, nail dystrophy, leukoplakia, and bone marrow failure—and two or more of the other somatic features known to occur in DC. However, making a diagnosis continues to be challenging because individuals develop clinical features of DC at variable rates and ages, even within the same family. Further, some of the patients have only one complication (e.g., isolated bone marrow failure or isolated pulmonary fibrosis). In about one quarter of individuals with DC, pathogenic variants in the known DC-related genes cannot be identified. The spectrum ranges from individuals who develop bone marrow failure first, then years later develop other classic findings such as nail abnormalities, to others who have severe nail problems and abnormalities of skin pigmentation at presentation but normal bone marrow function. In classic disease, skin pigmentation and nail changes typically appear first, usually in the first decade of life. Bone marrow failure usually develops within the first two decades, with 80% of patients developing bone marrow failure by age 30 years and almost 90% of patients having bone marrow failure at some point in their life.

Lacy reticulated **skin pigmentation** affecting the face, neck, chest, and arms is a common finding (89%). The degree of pigmentation increases with age and can involve the entire skin surface. There may also be a telangiectatic erythematous component. Nail dystrophy of both hands and feet is the next most common finding (88%). It usually starts with longitudinal ridging, splitting, or pterygium formation and may progress to complete nail loss. **Leukoplakia** usually involves the oral mucosa (78%), especially the tongue, but may also be seen in the conjunctiva and the anal, urethral, or genital mucosa. Excessive tearing **(epiphora)** secondary to nasolacrimal duct obstruction is common and observed in about 30% of individuals. Approximately 25% of individuals have learning difficulties and/or developmental delay. Hyperhidrosis of the palms and soles, hair loss and graying, dental caries or loss, esophageal stricture, pulmonary disease with reduced diffusion capacity and/or a restrictive defect due to pulmonary fibrosis and abnormalities in pulmonary vasculature, and short stature are each seen in approximately 15–20% of individuals.

Ocular abnormalities include conjunctivitis, blepharitis, loss of eyelashes, strabismus, cataracts, and optic atrophy. **Skeletal** abnormalities include osteoporosis, avascular necrosis of the hips or shoulders, abnormal bone trabeculation, scoliosis, and mandibular hypoplasia. **Genitourinary** abnormalities include hypoplastic testes, hypospadias, phimosis, urethral stenosis, and horseshoe kidney. **GI** findings, such as vascular lesions causing bleeding, hepatomegaly, peptic ulceration, and fibrosis, are seen in 10% of cases.

Laboratory Findings

The initial hematologic change in DC is usually thrombocytopenia, anemia, or both, followed by pancytopenia and aplastic anemia. The red cells are often macrocytic, and the Hb F is elevated. Initial bone marrow specimens may be normocellular or hypercellular, but with time, a symmetric depletion of all hematopoietic lineages ensues. Some patients have immunologic abnormalities, including reduced or elevated immunoglobulin values, decreased B- and/or T-lymphocyte counts, and reduction or absence of lymphocyte proliferative responses to phytohemagglutinin. This is particularly common and severe in the *DKC1*-associated disease. Primary skin fibroblasts in culture have abnormal morphologic features and doubling rate and show numerous unbalanced chromosome rearrangements, such as dicentrics, tricentrics, and translocations, in the absence of DEB or MMC. These findings provide evidence of a defect that predisposes patient cells to chromosomal rearrangements and possibly to DNA damage.

The hallmark of DC is very short telomeres, below the first percentile for age. However, some patients do not have short telomeres. Further, adult patients tend to have telomere length at the low range of normal rather than below the first percentile.

Diagnosis

The diagnosis of DC can often be made based on medical and family history and physical examination. The following abnormalities are seen in patients with DC but not in those with FA: nail dystrophy, early-onset leukoplakia, tooth abnormalities, hyperhidrosis of the palms and soles, and hair loss. There are several relatively more severe forms of DC. **Hoyeraal-Hreidarsson syndrome** is a multisystem disorder that presents in early childhood, which requires the features of DC along with **cerebellar hypoplasia** to establish the diagnosis. Patients have the classic diagnostic DC triad, in addition to developmental delay, IUGR, and bone marrow failure. Hoyeraal-Hreidarsson syndrome is genetically heterogeneous and caused by X-linked recessive pathogenic variants in *DKC1*. Some patients may also have severe immunodeficiency. **Revesz syndrome** has many of the features of DC and presents in early childhood. Bilateral exudative retinopathy is required to establish a diagnosis. Patients may also have intracranial calcifications, IUGR, developmental delay, and bone marrow failure. Pathogenic variants of *TINF2* are involved in Revesz syndrome, making it mostly an autosomal dominant condition, but a few patients have been described without an identified pathogenic variant. Individuals with these severe forms of DC have even shorter telomere lengths than those with classic DC. **Coats plus syndrome** is caused by compound heterozygous pathogenic variants in the *CTC1* gene or the *STN1* gene and has overlapping features with DC, including sparse and graying hair, dystrophic nails, and anemia. Telomeres can be very short, but patients display variable telomere length. Coats plus syndrome is characterized by retinal telangiectasia and exudates, intracranial calcification, leukodystrophy, brain cysts, osteopenia, GI bleeding, and portal hypertension caused by the development of vasculature ectasias in the stomach, small intestine, and liver.

Laboratory testing and imaging are an important part in establishing a diagnosis but also evaluate the spectrum of patient organ involvement. These tests include, but are not limited to, the laboratory tests described in the section "Laboratory Findings," as well as ultrasound of the abdomen, pulmonary function tests, liver enzymes, and nutritional elements (such as ferritin, folic acid, and vitamin B12). MRI of the head is useful when the patient has developmental delay and ataxia. Baseline bone marrow testing is critical. Annual evaluation of the bone marrow and complete blood counts every 3 months are common practice.

Because of genomic instability, imaging involving radiation should be limited to those that may affect management.

Cancer Risk and Other Complications

Patients with DC are predisposed to MDS and AML, as well as to solid tumors. Cancer usually develops in the third and fourth decades of life. The cumulative incidence of solid cancers and leukemia by the age of 65–70 years were estimated as 20% and 5–10%, respectively. The cumulative incidence of MDS was predicted to be 20% by the age of 50 years. The actuarial risk of clonal and malignant myeloid disease is 25% by age 18 years.

Forty percent of the cancers in such patients are SCCs of the head and neck (tongue, mouth, pharynx). SCCs of the skin and GI tract (esophagus, stomach, colon), as well as anorectal adenocarcinoma, are common. Patients may develop multiple separate primaries in different sites involving the tongue and nasopharynx.

Other life-threatening complications include pulmonary fibrosis, liver fibrosis, and severe GI bleeding.

Treatment

Androgens can induce improvement of bone marrow function in approximately 70% of patients, and in some, this treatment can result in normal trilineage blood counts for a number of years. Patients with DC become refractory to androgens as aplastic anemia progresses due to HSC depletion. They also tend to be more sensitive to the side effects of androgens than FA patients, making it important to start with lower doses and to monitor for side effects frequently. When the response is maximal, the androgen dose can be slowly reduced to the minimum dose required to maintain desired and safe blood cell counts but cannot be stopped.

Cytokine therapy with granulocyte-macrophage colony stimulating factor (GM-CSF) or with G-CSF alone or combined with erythropoietin appears to offer potential benefit, at least in the short term. Use of cytokines needs to be balanced with a potential growth-promoting effect of these medications on as-yet undetected MDS or AML cells.

Allogeneic HSCT is the only curative option for severe bone marrow failure, MDS, and AML. Long-term survival, even with sibling HLA-matched HSC donors, has traditionally been poor at about 50%. Morbidity and mortality result from transplant-related complications such as graft failure, GVHD, sepsis, or venoocclusive disease or from emergence of DC-related complications, such as pulmonary fibrosis and GI bleeding related to vascular anomalies. The high morbidity and mortality rate after HSCT is likely caused by ongoing tissue sensitivity and aging because of the telomere maintenance defect.

Prognosis

Considerable heterogeneity exists in DC, and some data about genotype-phenotype correlations are available. Patients with certain genetic groups (e.g., monoallelic *TERC, TERT*) may develop severe aplastic anemia or fibrosis of the liver and lungs, but these complications may appear later on in life and may not be accompanied by multisystem involvement. Patients with other genetic groups (e.g., monoallelic pathogenic variants in *DKC1* or *TINF2 and biallelic pathogenic variants in PARN, ACD, RTEL1, TERC or TERT*) appear to have more physical anomalies and a higher incidence and earlier onset of aplastic anemia and cancer. The mean age of death for patients with DC who are diagnosed in childhood is approximately 30 years. However, patients who are diagnosed during adulthood may have a mild disease or be completely asymptomatic. The main causes of death are bone marrow failure, complications of HSCT, cancer, fatal pulmonary problems, and GI bleeding.

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA

Etiology and Epidemiology

Congenital amegakaryocytic thrombocytopenia **(CAMT)** is less common than FA, SDS, and DC. It is transmitted in an autosomal recessive manner. CAMT typically manifests in infancy as isolated thrombocytopenia caused by reduction or absence of bone marrow megakaryocytes

with initial preservation of granulopoietic and erythroid lineages. Pancytopenia due to aplastic anemia often ensues in the first few years of life. Development of MDS and AML was reported in patients with CAMT, as well as persistent aplastic anemia.

The defect in CAMT is directly related to pathogenic variants in *MPL*, the gene for the receptor of thrombopoietin (THPO). *THPO* is a growth factor that promotes HSC survival and stimulates megakaryocyte proliferation and maturation. Heterozygotes of the pathogenic variant gene have normal hematology, whereas affected individuals have pathogenic variants in both alleles. Genotype-phenotype correlations predict disease course and prognosis. **Nonsense pathogenic variants** cause a complete loss of function of the THPO receptor, resulting in persistently low platelet counts in early infancy due to absence of megakaryocytes and a fast progression to pancytopenia and aplastic anemia **(CAMT type I)**. Impaired stem cell survival with *MPL* nonsense pathogenic variants explains the evolution of CAMT into aplastic anemia because THPO also has an antiapoptotic and cell survival effect on HSCs. **Missense pathogenic variants** of *MPL* are associated with a milder disease course, a later presentation, a partial and transient increase in platelets during the first year of life after presentation, and a delayed onset, if any, of pancytopenia, indicating residual receptor function **(CAMT type II)**. Biologically active plasma THPO is consistently elevated in all patients with CAMT. A small proportion of patients with the clinical picture of CAMT have no pathogenic variants in *MPL*, but more recently have been found to have homozygous pathogenic variants in *THPO*.

Clinical Manifestations

Intracranial hemorrhage is a major risk; about 25% of the patients develop this complication either in utero (13%), at birth (4%), or within the first 4 weeks of life (7%). Patients with CAMT commonly have a petechial rash, bruising, or bleeding. Onset of symptoms may depend on the severity of pathogenic variants and ranges from birth to the first year of life. Most patients with CAMT have normal physical and imaging features. About 10–20% of published phenotypic CAMT cases involved physical anomalies. The most common anomalies are neurologic. Findings related to cerebellar and cerebral atrophy are frequent, and developmental delay is a prominent feature. Congenital heart disease is rare but includes atrial and ventricular septal defects, patent ductus arteriosus, tetralogy of Fallot, and coarctation of the aorta. Some of these occur in combinations. Other anomalies include abnormal hips or feet, kidney malformations, eye anomalies, and cleft or high-arched palate. Some patients have microcephaly and abnormal facies.

Laboratory Findings

Thrombocytopenia is the major laboratory finding in CAMT. At birth, most patients present with thrombocytopenia but not all. Typically, at first, thrombocytopenia appears with normal hemoglobin levels and WBC counts. Peripheral blood platelets are reduced or totally absent. As in other IBMFSs, RBCs may be macrocytic. Hemoglobin F may be elevated, and there may be increased expression of i antigen. Initial bone marrow aspirates and biopsy specimens show normal cellularity with marked reduction or absence of megakaryocytes. Most patients develop pancytopenia between 6 months to 2 years of age. In patients in whom aplastic anemia develops, bone marrow cellularity is decreased, with fatty replacement; erythropoietic and granulopoietic lineages are also symmetrically reduced.

Diagnosis

If thrombocytopenia persists beyond the neonatal period or is associated with adequate platelet transfusion response and no obvious precipitating cause such as infections or immunologic reactions, a bone marrow aspirate and biopsy are indicated. Deficient megakaryocytes in such cases suggest the diagnosis, and genetic analysis will confirm it. If CAMT occurs at birth or shortly after, it must be distinguished from other causes of inherited and acquired neonatal thrombocytopenia. Thrombocytopenia with absent radii **(TAR syndrome)** is distinguished from CAMT because radii are absent in TAR. The distinction from DC may be evident by a lack of mucocutaneous, neurologic, and immunologic findings that are characteristic of the early-onset forms of DC. Telomere lengths below the first percentile matched for age to healthy controls is characteristic of DC and not CAMT. CAMT blood lymphocytes do not show increased chromosomal breakage when exposed to DEB, distinguishing the disease from FA.

Cancer Predisposition

CAMT can evolve into MDS and AML, but the true risk cannot be defined because most patients undergo early transplantation. Typical course in patients with transformation includes thrombocytopenia, aplastic anemia, followed by MDS (e.g., with monosomy 7 or trisomy 8) and leukemia.

Therapy and Prognosis

The mortality rate from thrombocytopenic bleeding, complications of aplastic anemia, or leukemic transformation in patients with *MPL* **nonsense** pathogenic variants is close to 100% if bone marrow function is not improved. Patients with **missense** pathogenic variants have a milder course but may still have serious complications. HSCT is the only curative option. The majority of patients with CAMT who undergo HSCT are cured, especially if the procedure is performed with HLA-matched sibling donors. Before transplantation, platelet transfusion should be used carefully. Platelet count should not always be the sole indication for treatment, but symptoms such as clinical bleeding are an appropriate trigger. Single-donor, leuko-reduced platelets are preferred to minimize sensitization. In a patient who is a candidate for HSCT, all blood products should be irradiated and cytomegalovirus safe. The role of thrombomimetic agents such as eltrombopag or romiplostim might be suitable for some patients (CAMT type II) and need to be studied further. However, the promotion of fibrosis by these agents and the risk of MDS and leukemia in CAMT render HSCT the preferred treatment for patients with severe cytopenia. Romiplostim has shown good and sustained response in those with pathogenic variants in *THPO*, and there have been no reports of acute leukemia or MDS in this subgroup of CMPL patients, but the numbers of patients with pathogenic variants in *THPO* are small.

GATA2-RELATED DISORDERS

The *GATA2* gene codes for a hematopoietic transcription factor that is crucial for the proliferation and maintenance of early hematopoietic cells. Heterozygous germline pathogenic variants, inherited in an autosomal dominant fashion and that are spontaneous in the majority of cases, result in various phenotypes that range from mild cytopenia to severe immunodeficiency and myeloid neoplasia. Approximately two thirds of pathogenic variants in *GATA2* are null, and one third are missense substitutions. Germline pathogenic variants in *GATA2* have been recognized as a major MDS and AML predisposition syndrome. Approximately 150 unique *GATA2* germline pathogenic variants have been identified in about 550 patients.

Clinical manifestations include monocytopenia and *Mycobacterium avium* complex (MonoMAC syndrome) infections, MDS with lymphedema (Emberger syndrome), familial MDS/AML, primary MDS, and chronic neutropenia and dendritic cell, monocyte, and B and NK lymphoid deficiency (DCML deficiency). Hematologic presentation is variable. Some patients present with cytopenia, a hypocellular marrow and monocytopenia, whereas others have severe immunodeficiency, and others still present with MDS/AML and no preexisting relevant medical history or cytopenia. Immune deficiency results in HPV-related infections such as warts or generalized verrucosis, disseminated nontuberculous mycobacterial, and systemic bacterial and fungal infections. Recurrent respiratory tract infections can result in lung disease, such as pulmonary alveolar proteinosis. Few patients have autoimmune dysregulation manifesting as autoimmune cytopenia, arthritis, lupus, and others. Constitutional abnormalities, such as lymphedema, hydrocele, and congenital deafness, as well as abnormalities in pulmonary, CV, urogenital, and neurological systems have been observed in approximately 50% of patients (Fig. 517.3).

In children and adolescents with primary MDS, pathogenic variants in *GATA2* are a predominant germline predisposition accounting for 7% of all MDS cases, 15% of patients with advanced MDS, and 37% of patients

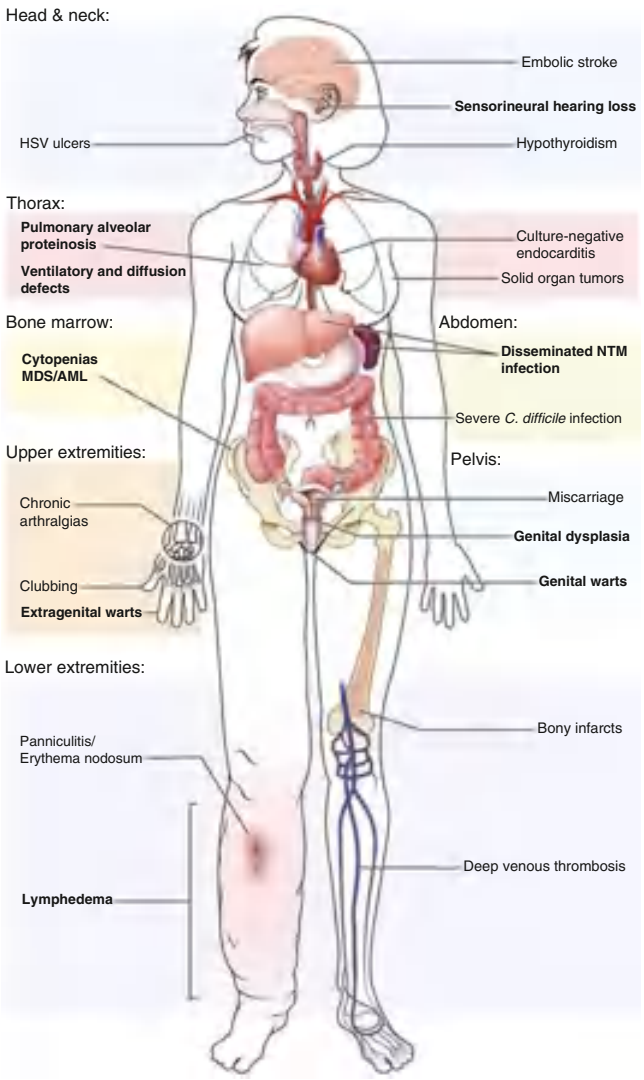

Fig. 517.3 Clinical features of GATA2 deficiency by organ system. Common clinical problems are indicated in bold. AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; NTM, non-tuberculous mycobacterium. *(From National Institute of Allergy and Infectious Diseases [NIAID] Health Information Fact Sheet.* https://www.niaid.nih.gov/sites/default/files/GATA2-Factsheet.pdf.*)*

with MDS and monosomy 7. Among children with MDS, the prevalence of *GATA2* pathogenic variants increases with age; 66% of adolescents with MDS and monosomy 7 carry germline *GATA2* pathogenic variants. Median age of diagnosis with *GATA2*-related MDS is approximately 10 years.

Patients should be followed by a hematologist with expertise in bone marrow failure/MDS and by an immunologist. Blood counts should be done every 3 months, and bone marrow aspirate and biopsy with cytogenetics should be carried out annually. HSCT is the only curative option for MDS and carries an overall survival ranging from 60–85% depending on donor type and MDS disease severity.

SAMD9/9L-RELATED DISORDER

Sterile alpha motif domain-containing protein 9 *(SAMD9)* and the paralogue gene *SAMD9*-like *(SAMD9L)* are located side-by-side on chromosome 7q21. They are interferon and tumor necrosis factor (TNF)-α responsive proteins that play a role in antiviral response, tumor suppression, inflammation, development, and endosomal fusion. Gain of function pathogenic variants, mostly missense, in these genes have variable penetrance. Thirty-eight pathogenic variants have been identified in approximately 110 patients.

Multiple organs can be affected in those with germline *SAMD9/9L* pathogenic variants, including the hematologic, immunologic, endocrine, and neurologic systems (see Table 517.2). The term **MIRAGE syndrome** was given to a constellation of abnormalities that include MDS, infection, restriction of growth (starting in utero), adrenal hypoplasia/insufficiency (with early onset), genital phenotypes (such as 46XY females and testicular dysgenesis), and enteropathy (often with reflux). Thrombocytopenia and anemia are often present at birth. In some patients, the anemia and thrombocytopenia resolve during infancy. Patients with *SAMD9L* pathogenic variants might show disease-specific neurological findings with very variable age of onset. Severe cerebellar ataxia is observed in some, whereas others have cerebellar atrophy, dysmetria, nystagmus, white matter abnormalities, and loss of Purkinje cells. The majority of patients in both syndromes have hematologic manifestations which range from single lineage cytopenia to pancytopenia with hypocellular marrow, MDS with monosomy 7, or deletion 7q. Some patients have nonsyndromic *SAMD9/9L* related MDS.

SAMD9/9L pathogenic variants are seen in 8–17% of pediatric MDS. The median age of presentation with SAMD9/9L related MDS is approximately 10 years. Ninety percent of children present in the early stage of MDS (refractory cytopenia of childhood), and another 10% present with advanced MDS. Transient monosomy 7 syndrome, the disappearance of monosomy 7 clones from the bone marrow, has been described in a few young children. It was hypothesized that pathogenic variants in the growth inhibitory genes, *SAMD9*/9L, confer gain of function that further suppress cell growth. Hence, monosomy 7, which depletes one allele of *SAMD9*/9L, reduces the growth inhibitory effect of the pathogenic variants. Somatic revertant mosaicism with expansion of benign, corrected hematopoiesis, has also been reported with normalization of blood counts and marrow cellularity for up to 20 years from diagnosis.

Monitoring and treatment depend on the degree of blood count abnormalities, the presence of MDS, and the presence of syndromic disease. Children who received HSCT for *SAMD9*/9L–related MDS showed an overall survival of 85% in one study.

OTHER INHERITED APLASTIC ANEMIAS

A substantial number of genes that are associated with bone marrow failure with pancytopenia have been identified with the emergence of whole genome screening methods (see Table 517.1). The specific gene-associated disorders may vary by phenotype but frequently include physical malformations, familial distribution, early age of disease onset, pancytopenia, and a risk of MDS and AML. Significant overlap exists between inherited pancytopenia syndromes and familial MDS and AML syndromes.

SRP72-Related Disorder

This autosomal dominant disorder is characterized by familial aplastic anemia and MDS. Some patients also have deafness. *SRP72* encodes for a signal recognition particle 72 protein. SRP72 is part of a ribonucleoprotein complex that mediates targeting of secretory proteins to the endoplasmic reticulum.

ERCC6L2-Related Disorder

This autosomal recessive disorder features multilineage cytopenia, MDS, and physical malformations. Neurological and cranial abnormalities are common in *ERCC6L2*-related disorder and include developmental delay, microcephaly, ataxia, dysmetria, generalized brain volume loss, retinal dystrophy, and low-set ears. ERCC6L2 regulates transcription by RNA Pol II through an interaction with DNA-PK; thereby, it promotes resolution of R loops and minimizes transcription-associated genome instability. The inherited BMF syndrome caused by biallelic pathogenic variants in ERCC6L2 is thus a primary transcription deficiency and may not cause a DNA repair defect.

Dubowitz Syndrome

Dubowitz syndrome is characterized by a peculiar facies, infantile eczema, small stature, and mild microcephaly. The face is small, with a shallow supraorbital ridge, a nasal bridge at the same level as the forehead, short palpebral fissures, variable ptosis, and micrognathia. There

is a predisposition to cancer in these patients. Approximately 10% of patients have hematopoietic disorders, including moderate pancytopenia, hypoplastic anemia, bone marrow hypoplasia, and full-blown aplastic anemia. Patients also have an increased risk of lymphoma and neuroblastoma. In about one third of patients, a genetic cause can be identified and includes biallelic pathogenic variants in *NSUN2* (an RNA methyltransferase), biallelic pathogenic variants in *LIG4* (a nuclear DNA ligase), or one copy deletion in several chromosome sites including 14q32, 17q24, -14q32, -17q24, and -19q13.

Seckel Syndrome

Seckel syndrome, sometimes called "bird-headed dwarfism," is a developmental disorder characterized by marked growth failure and mental deficiency, microcephaly, a hypoplastic face with a prominent nose, and low-set and/or malformed ears. Approximately 25% of patients have aplastic anemia or malignancies. The syndrome is genetically heterogeneous and is associated with biallelic pathogenic variants in *ATR*, *RBBP8*, *CENPJ*, *CEP152*, *CEP63*, *NIN,* or *ATRIP.* A deletion at the14q21-q22 locus has also been described.

Reticular Dysgenesis

Reticular dysgenesis is a severe form of immunodeficiency. It is mainly characterized by severe lymphopenia and neutropenia. Patients typically present with severe infections at birth or shortly thereafter. On physical examination, lymph nodes and tonsils are absent, and a thymic shadow on radiographs cannot be seen. Laboratory tests show absence of cellular and humoral immunity. Anemia, thrombocytopenia, and aplastic anemia may be evident. Bone marrow specimens show markedly reduced myeloid and lymphoid elements. Clonogenic assays of hematopoietic progenitors consistently show reduced to absent colony growth, suggesting that the disorder has its origins at the HSC level. The disease is inherited in an autosomal recessive fashion and is caused by biallelic pathogenic variants in mitochondrial *AK2*. The only curative therapy is HSCT.

Schimke Immunoosseous Dysplasia

Schimke immunoosseous dysplasia is an autosomal recessive disorder caused by pathogenic variants in the chromatin remodeling gene *SMARCAL1*. Patients have spondyloepiphyseal dysplasia with exaggerated lumbar lordosis and a protruding abdomen. There are pigmentary skin changes and abnormally discolored and configured teeth. T-cell immunodeficiency, progressive renal insufficiency, nephrotic syndrome, and early atherosclerosis are common causes of morbidity and mortality. Approximately 50% of patients have hypothyroidism; 50% have cerebral ischemia; 10% have bone marrow failure with neutropenia, thrombocytopenia, and anemia; and about 5% are predisposed to non-Hodgkin lymphoma. Eighty percent of patients have lymphopenia and altered cellular immunity. Bone marrow transplantation has been successfully applied to patients with bone marrow failure or immunodeficiency.

Cartilage Hair Hypoplasia

Cartilage-hair hypoplasia features metaphyseal dysostosis, short-limbed dwarfism, and fine sparse hair. Skeletal abnormalities may also include scoliosis, lordosis, chest deformity and varus lower limbs. GI abnormalities such as aganglionic megacolon have been reported. Most reported cases are of Finnish or Amish descent. In over 80% of patients, biallelic causal pathogenic variants in the noncoding RNA gene *RMRP* (RNA Component of Mitochondrial RNA Processing Endoribonuclease) can be found. Recently, biallelic pathogenic variants in *POP1* and in *NEPRO* have also been found to be associated with the disease. POP1 is a component of the RNAse-MRP endoribonuclease complex. NEPRO is a nucleolar and neural progenitor protein that interacts with multiple subunits of the RNase MRP complex. The complex plays a role in rRNA and in mitochondrial RNA processing by cleavage at a priming site during replication.

The hematological abnormalities include macrocytic anemia. In most cases the anemia is mild and self-limited, but a proportion of patients have severe and persistent anemia that requires regular RBC transfusions. Lymphopenia occurs in 65% of patients. Severe immunodeficiency can occur, often with severe anemia. Neutropenia has been reported in 25% of patients. HSCT has been used successfully to reconstitute the hemato/immunological system. Lymphomas and basal cell carcinoma occur at an increased frequency among these patients.

Pearson Syndrome

Pearson syndrome is a mitochondrial metabolic disorder with various bone marrow impairments. Insufficiency of the exocrine pancreas, caused by acinar cell atrophy and fibrosis, develops in 30% of cases. The syndrome is caused by a maternally inherited deletion of mitochondrial DNA (mtDNA) that encodes enzymes that are critical to oxidative phosphorylation. Severe macrocytic anemia requiring transfusions is commonly present within the first year of life, sometimes at birth. Bone marrow aspirate typically shows ringed sideroblasts and prominent vacuolization of erythroid and myeloid precursors. However, cases with pure red cell aplasia mimicking Diamond Blackfan anemia have been reported. Pancytopenia may occur alone or in association with hepatic failure, renal tubulopathy, and lactic acidosis. In such cases, platelet transfusions may also be required. Erythropoietin has been used for the anemia of renal failure. G-CSF is indicated to prevent infections in patients with severe neutropenia. HSCT is traditionally not recommended for the hematopoietic complications of Pearson syndrome due to their tendency to improve spontaneously in most cases; nonetheless, HSCT has been used in rare cases with Pearson syndrome, and engraftment was achieved. Interestingly, HSCT was associated not only with improved hematopoiesis but also with resolution of lactacidemia and acidosis. Patients may need pancreatic enzyme replacement if they develop malabsorption due to exocrine pancreatic insufficiency.

Immune-Related Pancytopenias

Primary immunodeficiency, autoimmune, lymphoproliferative, and hemophagocytic disorders can be associated with pancytopenia. Autoantibodies, hypersplenism, and bone marrow involvement are potential mechanisms. Recognizable disorders include common variable immunodeficiency syndrome (Chapter 165), hemophagocytic lymphohistiocytosis (Chapter 556.2), autoimmune lymphoproliferative syndrome (Chapter 174.7), and other lymphoproliferative disorders.

UNCLASSIFIED INHERITED BONE MARROW FAILURE SYNDROMES

Unclassified IBMFSs are heterogeneous disorders that in many cases were shown to present as new syndromes. In other cases, they may be atypical presentations of already characterized diseases. Unbiased approaches, such as study of comprehensive genetic panels of all known IBMFS genes regardless of the specific hematologic (e.g., isolated neutropenia or pancytopenia) or nonhematologic manifestations, or WES/genome sequencing, have proved this to be true. These disorders do not fit into classic genetic bone marrow failure diseases because all features of any one disease may not be evident at presentation. All are characterized by various cytopenias caused by underproductive bone marrow with or without physical manifestations. Compared with patients with classical disorders who present at a median age of 1 month, patients with unclassified disorders present later, at a median age of 9 months. Patients with unclassified IBMFSs can manifest single or multilineage cytopenia, aplastic anemia, MDS, or malignancy with variable expression of malformations. Table 517.5 lists criteria for the diagnosis, which include evidence of chronic bone marrow failure, in addition to factors that indicate a high likelihood of inherited disease (e.g., family history, congenital anomalies, young age at presentation).

When patients present later and without physical malformations, an acquired etiology cannot be ruled out. Detailed genetic testing for known IBMFS genes followed by approaches to discover novel genes and novel syndromes by WES/genome sequencing may identify an inherited etiology and define the disease.

Determining the actual genetic cause helps group patients according to diseases and guides counseling and proper medical care. Implementing a treatment plan is urgent in many cases. In such patients, the management should be according to the type of complications that the patient has at presentation and the lessons that can be learned from published experience on unclassified cases in the literature.

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Table 517.5 The Canadian Inherited Marrow Failure Registry Criteria for Unclassified Inherited Bone Marrow Failure Syndromes

FULFILLS CRITERIA 1 AND 2:
1. Does not fulfill criteria for any categorized inherited bone marrow failure syndrome*
2. Fulfills both of the following:
FULFILLS AT LEAST TWO OF THE FOLLOWING:
a. Chronic cytopenia(s) detected on at least two occasions over at least 3 months†
b. Reduced marrow progenitors or reduced clonogenic potential of hematopoietic progenitor cells or evidence of ineffective hematopoiesis‡
c. High fetal hemoglobin for age‡
d. Red blood cell macrocytosis (not caused by hemolysis or a nutritional deficiency)
FULFILLS AT LEAST ONE OF THE FOLLOWING:
a. Family history of bone marrow failure
b. Presentation at age <1 year
c. Anomalies involving multiple systems to suggest an inherited syndrome

*The criteria for most common syndromes are described in Tsangaris E, Klaasen R, Fernandez CV, et al. Genetic analysis of inherited bone marrow failure syndromes from one prospective, comprehensive, and population-based cohort and identification of novel mutations. *J Med Genet*. 2011;48:618–628.

†Cytopenia is defined according to the affected cell: neutropenia, neutrophil count of $<1.5 \times 10^9$/L; thrombocytopenia, platelet count of $<150 \times 10^9$/L; anemia, hemoglobin concentration of >2 standard deviations below mean, adjusted for age.

‡Hemoglobinopathies with ineffective erythropoiesis and high fetal hemoglobin should be excluded by clinical or laboratory testing.

Modified from Teo JT, Klaassen R, Fernandez CV, et al. Clinical and genetic analysis of unclassifiable inherited bone marrow failure syndromes. *Pediatrics*. 2008;22:e139–e148.

Table 518.1 Etiology of Acquired Aplastic Anemia

Radiation, Drug, and Chemicals
Predictable: chemotherapy, benzene
Idiosyncratic: chloramphenicol, antiepileptics, gold, 3,4-methylenedioxymethamphetamine, nonsteroidal antiinflammatory drugs (NSAIDs), antibiotics
See also Table 518.2
Viruses
Cytomegalovirus
Epstein-Barr
Hepatitis B
Hepatitis C
Hepatitis non-A, non-B, non-C (seronegative hepatitis)
HIV
COVID-19
Immune Diseases
Eosinophilic fasciitis
Hypoimmunoglobulinemia
Thymoma
Common variable immunodeficiency syndrome *(NFKB1)*
Pregnancy
Paroxysmal Nocturnal Hemoglobinuria
Marrow Replacement
Leukemia
Myelodysplasia
Myelofibrosis
Autoimmune
Nutritional
Vitamin B_{12}
Folate
Copper
Other
Cryptic dyskeratosis congenita (no physical stigmata)
Telomerase reverse transcriptase haploinsufficiency
Atypical presentation of genetic marrow failure syndromes
Leishmaniasis

Chapter 518

Acquired Pancytopenias

John H. Fargo and Jeffrey D. Hord

ETIOLOGY AND EPIDEMIOLOGY

Therapeutic and recreational drugs, environmental toxins, infectious agents, radiation, and immune disorders can result in pancytopenia by direct destruction of hematopoietic progenitors, disruption of the marrow microenvironment, or immune-mediated suppression of marrow elements (Tables 518.1 and 518.2). A careful history of exposure to known risk factors should be obtained for every child presenting with pancytopenia. Even in the absence of the classic associated physical findings, the possibility of a genetic predisposition to bone marrow failure must also be considered (see Chapter 517). Many cases of acquired marrow failure in childhood are idiopathic, in that no causative agent is identified. Many are probably immune mediated through cytotoxic T lymphocytes and cytokine destruction of marrow stem and progenitor cells. Patients with an initial diagnosis of acquired aplastic anemia may have developed somatic pathogenic variants in genes associated with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Clonal hematopoiesis resulting from these acquired somatic genetic variants may over time lead to the development of MDS or AML. The overall incidence of **acquired aplastic anemia** is relatively low, with an approximate incidence in both children and adults in the United States and Europe of 2–6 cases per 1 million population per year. The incidence is higher in Asia, with as many as 14 cases per 1 million per year in Japan.

Severe bone marrow suppression can develop after exposure to many different therapeutic drugs and environmental chemicals, including certain chemotherapeutic agents, insecticides, antibiotics, anticonvulsants, nonsteroidal antiinflammatory drugs (NSAIDs), and recreational drugs. Some of the most notable agents are benzene, chloramphenicol, gold, and 3,4-methylenedioxymethamphetamine (MDMA, or "ecstasy").

A number of viruses can either directly or indirectly result in bone marrow failure. Parvovirus B19 is classically associated with isolated red blood cell (RBC) aplasia, but in patients with sickle cell disease or immunodeficiency, it can result in transient pancytopenia (see Chapter 298). Prolonged pancytopenia can occur after infection with many of the hepatitis viruses, herpesviruses, Epstein-Barr virus (see Chapter 301), cytomegalovirus (see Chapter 302), and HIV (see Chapter 322).

Immune-mediated acquired pancytopenia is also found to be prominent among acute, sporadic cases of aplastic anemia. Immunologic conditions, such as seronegative hepatitis, eosinophilic fasciitis, and thymoma, have been implicated in aplastic anemia.

Patients with evidence of bone marrow failure should also be evaluated for inherited forms of marrow failure, **paroxysmal nocturnal hemoglobinuria** (PNH; see Chapter 510), and collagen vascular diseases. Pancytopenia without peripheral blasts may be caused by bone marrow replacement by malignant cells including leukemia and solid tumor cells, such as neuroblastoma.

Table 518.2 Drugs and Toxins Associated with Aplastic Anemia

DOSE DEPENDENT
Antineoplastic Agents
Antimetabolites: fluorouracil, mercaptopurine, 6-thioguanine, methotrexate, cytosine arabinoside, gemcitabine, fludarabine, cladribine, pentostatin, hydroxyurea
Alkylating and cross-linking agents: busulfan, cyclophosphamide, chlorambucil, nitrogen mustard, melphalan, cisplatin, carboplatin, ifosfamide, nitrosoureas (BCNU and CCNU), mitomycin C
Cytotoxic antibiotics: daunorubicin, doxorubicin, mitoxantrone
Plant alkaloids: vinblastine, paclitaxel
Topoisomerase inhibitors: etoposide
Antimicrobial Agents
Chloramphenicol, dapsone, fluorocytosine
Antiinflammatory and Antirheumatic Agent
Colchicine
Insecticides
Chlordane, chlorophenothane (DDT), lindane, parathion
Other Chemicals
Benzene
Benzene-containing chemicals: kerosene, chlorophenols, carbon tetrachloride

DOSE INDEPENDENT*
Idiosyncratic, likely immune mediated
Antimicrobial Agents
Chloramphenicol, dapsone, sulfonamides, tetracycline, methicillin, amphotericin, quinacrine, chloroquine, pyrimethamine
Anticonvulsants
Hydantoins, carbamazepine, phenacemide, primidone, ethosuximide
Antiinflammatory Agents
Phenylbutazone, indomethacin, ibuprofen, oxyphenbutazone, sulindac, naproxen
Antiarrhythmic Drugs
Quinidine, tocainide, procainamide
Metals
Gold, arsenic, mercury, bismuth
Antihistamines
Cimetidine, ranitidine, chlorpheniramine, pyrilamine, tripelennamine
Diuretics
Acetazolamide, furosemide, chlorothiazide, methazolamide
Hypoglycemic Agents
Chlorpropamide, tolbutamide
Antithyroid Drugs
Propylthiouracil, potassium perchlorate, methylthiouracil, methimazole, carbimazole
Antihypertensive Agents
Methyldopa, enalapril, captopril
Sedatives
Chlordiazepoxide, chlorpromazine, meprobamate, prochlorperazine

*Most agents listed in this group should be considered to be possibly associated with aplastic anemia.
From Bagby GC. Aplastic anemia and related bone marrow failure states. In: Goldman L, Schafer AI, eds. *Goldman-Cecil Medicine*, 26th ed. Philadelphia: Elsevier, 2020: Table 156.2.

PATHOLOGY AND PATHOGENESIS

The hallmark of aplastic anemia is peripheral pancytopenia, coupled with hypoplastic or aplastic bone marrow. The severity of the clinical course is related to the degree of myelosuppression. **Severe aplastic anemia** is defined as a condition in which ≥2 cell components have become seriously compromised (absolute neutrophil count <500/mm^3, platelet count <20,000/mm^3, reticulocyte count <1% after correction for hematocrit) in a patient whose bone marrow biopsy material has <30% cellularity. Approximately 65% of patients who first present with **moderate aplastic anemia** (absolute neutrophil count 500–1,500/mm^3, platelet count 20,000–100,000/mm^3, reticulocyte count <1%) eventually progress to meet the criteria for severe disease if they are simply observed. Bone marrow failure may be a consequence of a direct cytotoxic effect on hematopoietic stem cells (HSCs) from a drug or chemical or may result from either cell-mediated or antibody-dependent cytotoxicity. There is strong evidence that many cases of idiopathic aplastic anemia are caused by an immune-mediated process, with increased circulating cytotoxic T lymphocytes producing cytokines (interferon-γ) that suppress hematopoiesis. Abnormal telomere length and telomerase activity in granulocytic precursors of patients with aplastic anemia suggest that early apoptosis of hematopoietic progenitors may play a role in the pathogenesis of this disease.

Cytogenetic abnormalities associated with aplastic anemia include uniparental disomy of 6p, monosomy 7/deletion of 7q, and trisomy 8, 6, or 15. Genes associated with aplastic anemia include telomere complex genes *(TERT, TERC)* and *BCOR/BCORL, PIGA, DNMT3A,* and *ASXL1*.

CLINICAL MANIFESTATIONS, LABORATORY FINDINGS, AND DIFFERENTIAL DIAGNOSIS

Pancytopenia results in increased risks of cardiac failure, fatigue, infection, and bleeding. Acquired pancytopenia is typically characterized by anemia, leukopenia, and thrombocytopenia in the setting of elevated serum cytokine values. Other treatable disorders, such as cancer, collagen vascular disorders, PNH, and infections, that may respond to specific therapies (IV immunoglobulin for parvovirus), should be considered in the differential diagnosis. Careful examination of the peripheral blood smear for RBC, leukocyte, and platelet morphologic features is important. A reticulocyte count should be performed to assess erythropoietic activity. In children, the possibility of *congenital* pancytopenia must always be considered, and chromosomal breakage analysis should be performed to evaluate for **Fanconi anemia** (see Chapter 517) and telomere length to evaluate for telomeropathies. The presence of fetal hemoglobin suggests congenital pancytopenia but is not diagnostic. To assess for possible PNH, flow cytometric analysis of erythrocytes and granulocytes for CD55 and CD59 is the most sensitive test. Bone marrow examination should include both aspiration and biopsy, and the marrow should be carefully evaluated for morphologic features, cellularity, and cytogenetic abnormalities.

TREATMENT

The treatment of children with acquired pancytopenia requires comprehensive supportive care coupled with an attempt to treat the underlying etiology for marrow failure. For patients with a human leukocyte antigen (HLA)–matched family member donor, **allogeneic hematopoietic stem cell transplantation (HSCT)** offers a 90% chance of long-term survival. Preparative regimens vary but typically consist of cyclophosphamide, fludarabine, and horse antithymocyte globulin (ATG). Preliminary data also suggest that children with severe aplastic anemia can be successfully transplanted using *alemtuzumab* (humanized monoclonal antibody against CD52 on lymphocytes)–based conditioning. The risks associated with bone marrow transplantation include the immediate complications of transplantation, graft failure, and graft-versus-host disease. Late adverse effects associated with transplantation may include secondary cancers, cataracts, short stature, hypothyroidism, and gonadal dysfunction (see Chapters 179 and 180). Only approximately 20% of patients have an HLA-matched family member donor, so matched-related HSCT is not an option for the majority of patients.

For patients without a sibling donor, the major form of therapy is **immunosuppression** with horse ATG and cyclosporine, with a response rate of 60–70%. The median time to response is 6 months. As many as 30–60% of patient responders experience relapse after discontinuation of immunosuppression, and some patients must continue cyclosporine for several years to maintain a hematologic

response. Among those who relapse after immunosuppression, approximately 50% show response to a second course of ATG and cyclosporine. To accelerate neutrophil recovery, a hematopoietic colony-stimulating factor (e.g., granulocyte CSF, granulocyte-macrophage CSF) is sometimes added to ATG and cyclosporine for treatment of patients with very severe neutropenia (absolute neutrophil count <200/mm^3), but there is no clear evidence that this treatment influences response rate or survival. Higher baseline reticulocyte count correlates with a higher probability of response to immunosuppression and survival. There is an inverse correlation between telomere length and the probability of relapse after immunosuppression.

For patients who show no response to immunosuppression or who experience relapse after immunosuppression, **matched-unrelated HSCT and T-cell–depleted haploidentical family member–donor HSCT** are treatment options, with a response rate approaching 90%. Cord blood transplants have been performed in this refractory group of pediatric patients, with survival approximating 90%. Ongoing studies using **eltrombopag** (an oral thrombopoietin mimetic agent) have shown promise in patients ≥15 years of age (mostly adults) with refractory disease. The use of eltrombopag resulted in a hematologic response with improvements in platelet and neutrophil counts and hemoglobin levels in over half the patients. In patients who responded, bone marrow biopsies demonstrated trilineage normalization of hematopoiesis, with some showing a durable response. **High-dose cyclophosphamide** has been used successfully in the treatment of patients who are not good candidates for HSCT and have not had an adequate response to immunosuppression. This therapy leads to prolonged severe pancytopenia, increasing the risk of life-threatening infection, especially fungal infections. Other therapies that have been used in the past with inconsistent results include androgens, corticosteroids, and plasmapheresis. **Alemtuzumab** as monotherapy in relapsed disease showed improved response rates and 3-year survival compared to additional courses of ATG and cyclosporine.

COMPLICATIONS

The major complications of severe pancytopenia are predominantly related to the risk of life-threatening bleeding from prolonged thrombocytopenia or to infection secondary to protracted neutropenia. Patients with protracted neutropenia as a result of bone marrow failure are at risk not only for serious bacterial infections but also for invasive mycoses. Patients who have been transfused with RBCs regularly over a long period are at increased risk of developing alloantibodies to RBC antigens and may require iron chelation therapy for transfusional iron overload. The general principles of supportive care that have evolved from the use of chemotherapy-related myelosuppression to treat patients with cancer should be fully extended to the care of patients with acquired pancytopenia.

PROGNOSIS

Spontaneous recovery from pancytopenia rarely occurs. If left untreated, severe pancytopenia has an overall mortality rate of approximately 50% within 6 months of diagnosis and of >75% overall, with infection and hemorrhage being the major causes of morbidity and mortality. The majority of children with acquired severe aplastic anemia show response to allogeneic marrow transplantation or immunosuppression, leaving them with normal or near-normal blood cell counts.

PANCYTOPENIA CAUSED BY MARROW REPLACEMENT

Processes that either infiltrate or replace the bone marrow can manifest as acquired pancytopenia. Infiltration can be caused by malignancy (classically, neuroblastoma or leukemia) or result from myelofibrosis, MDS, or osteopetrosis. Although uncommon, evidence of hypoplastic anemia can precede the onset of acute leukemia, generally by a few months. This relationship is important to appreciate in evaluating and monitoring children who present with what appears to be acquired aplastic anemia. Morphologic examination of the peripheral blood and bone marrow and marrow cytogenetic studies are critically important in making the diagnoses of leukemia, myelofibrosis, and MDS.

Myelodysplasia is very rare in children, but when it occurs, its clinical course is more aggressive than the same category of MDS in adults. Pediatric MDS can be subdivided into **refractory cytopenia of childhood** (peripheral blasts <2% and marrow blasts <5%) and **MDS with excess blasts** (peripheral blasts 2–19% and/or marrow blasts 5–19%). Disease in children with >20% blasts is usually defined as AML.

Myelodysplastic syndromes are a heterogeneous group of bone marrow failure disorders that have in common ineffective hematopoiesis that leads to pancytopenia over time. In one group, there are somatic variants (in >25 genes) leading to MDS. In another group, usually in younger patients (<55 years), there is autoimmune suppression of hematopoiesis by clonal expansion of T lymphocytes, particularly in those patients who look similar to patients with idiopathic aplastic anemia. In all patients, other causes of MDS (medications and vitamin B_{12}, folate, or copper deficiencies) must be ruled out.

A number of inherited conditions are associated with an increased risk for development of MDS, including Down syndrome, severe congenital neutropenia, Noonan syndrome, Fanconi anemia, trisomy 8 mosaicism, neurofibromatosis, Shwachman-Diamond syndrome, and some familial MDS syndromes caused by pathogenic variants in *ANKRD26, CEBPA, DDX41, ETV6, GATA2, RUNX1,* and *SRP72* genes. Significant clonal abnormalities are found within the marrow of approximately 50% of patients with MDS, with monosomy 7 being most common but prognostically neutral. Those with a structurally complex karyotype have a very poor outcome (see Chapter 517).

The transition time from pediatric MDS to acute leukemia is relatively short, 14–26 months, so aggressive treatment such as HSCT must be considered shortly after diagnosis. With allogeneic HSCT, the survival rate is approximately 60%. One exception to such an aggressive therapeutic approach is MDS and AML in children with Down syndrome because these diseases in this specific population are very responsive to conventional chemotherapy, with long-term survival rates >80%.

The decision on how to treat a child with MDS who lacks a suitable HSC donor should be made with the specific clonal abnormality found within the child's marrow taken into consideration. Lenalidomide produces the best responses among patients who have the chromosomal abnormality 5q−. Immunosuppressive therapy with ATG and cyclosporine is most effective in patients with trisomy 8, especially in the presence of a PNH clone. Imatinib mesylate targets mutations in the tyrosine kinase receptor family of genes found in patients with t(5;12) and del(4q12). The DNA hypomethylating agents azacitidine and decitabine have also been used in treating MDS without a known molecular target and have some effect.

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Section 6

Blood Component Transfusions

Chapter 519

Red Blood Cell Transfusions and Erythropoietin Therapy

Patricia E. Zerra and Cassandra D. Josephson

Red blood cells (RBCs) are transfused to increase the oxygen-carrying capacity of the blood, with the goal to increase or maintain satisfactory tissue oxygenation. This goal may not be achieved simply by increasing the hemoglobin (Hb) concentration or hematocrit (Hct) by an RBC transfusion because tissue oxygenation depends on several additional factors, including oxygen off-loading from RBCs, microvascular blood flow, and diffusion of oxygen into tissue cells. Although some attempts have been made to accurately relate posttransfusion Hb or Hct values to changes in posttransfusion tissue oxygenation (e.g., improvements in the ratio of cerebral vs mesenteric oxygenation patterns assessed by serial near-infrared spectroscopy measurements), decisions to transfuse RBCs per physiologic indications, rather than degree of anemia, remain investigational. This information can be applied to approaches for transfusion in both preterm infants/neonates and children/adolescents. However, neonates, especially extremely low birthweight infants (≤1,000 g), are not "small" children (i.e., RBC physiology and pathophysiology of anemia of prematurity are unique); thus RBC transfusions for neonates and children are considered separately.

RBC TRANSFUSION IN CHILDREN AND ADOLESCENTS

Guidelines for RBC transfusions in children and adolescents are based on maintaining a specified Hb or Hct level considered to be optimal (per the best evidence available) for the clinical condition present at the time of transfusion. The guidelines are similar to those for adults (Table 519.1). Transfusions may be given more stringently to children because normal Hb levels are lower in healthy children than in adults and, as is often the case, most children do not have the underlying multiorgan, cardiorespiratory, and vascular diseases that develop with aging in adults to suggest a need for RBC transfusions. As a result, children may compensate better for RBC loss than elderly adults, thus requiring less RBC transfusion support. In general, there is increasing enthusiasm for applying patient blood management strategies, encompassing conservative transfusion practices (i.e., accepting lower pretransfusion Hct values to "trigger" an RBC transfusion) to all patient ages with evidence-based support.

In the **perioperative period,** it is unnecessary for most children to maintain Hb of ≥8 g/dL, a level frequently desired for adults. Instead, Hb of ≥7 g/dL is an acceptable level, although the optimal value for individual patients is based on clinical and laboratory circumstances, as influenced by the following factors. The desired preoperative Hb level should consider the estimated blood loss for the surgical procedure planned and the rate of bleeding. There should be a compelling reason to prescribe any postoperative RBC transfusion, such as continued bleeding with hemodynamic instability because most children (without continued bleeding) can restore their RBC mass with iron therapy (in a relatively short time).

Table 519.1 Guidelines for Pediatric Red Blood Cell Transfusions*†

CHILDREN AND ADOLESCENTS
1. Maintain stable status with acute loss of >25% of circulating blood volume.
2. Maintain hemoglobin >7.0 g/dL† in the perioperative period.
3. Maintain hemoglobin >12.0 g/dL with *severe* cardiopulmonary disease.
4. Maintain hemoglobin >12.0 g/dL during extracorporeal membrane oxygenation.
5. Maintain hemoglobin >7.0 g/dL with *symptomatic* chronic anemia.
6. Maintain hemoglobin >7.0 g/dL with *marrow failure*.
INFANTS ≤4 MO OLD
1. Maintain hemoglobin >12.0 g/dL with *severe* pulmonary disease.
2. Maintain hemoglobin >12.0 g/dL during extracorporeal membrane oxygenation.
3. Maintain hemoglobin >10.0 g/dL with *moderate* pulmonary disease.
4. Maintain hemoglobin >12.0 g/dL with *severe* cardiac disease.
5. Maintain hemoglobin >10.0 g/dL preoperatively and during *major* surgery.
6. Maintain hemoglobin >7.0 g/dL postoperatively.
7. Maintain hemoglobin >7.0 g/dL with *symptomatic* anemia.

*Words in *italics* must be defined for local transfusion guidelines.
†Pretransfusion blood hemoglobin (Hb) level (convert to hematocrit values if preferred by multiplying Hb values by 3) "triggering" an RBC transfusion. Hb values to maintain vary among published reports, and the guideline values to maintain should be determined locally to fit the practices judged to be optimal by local physicians.

The most important measures in the treatment of **acute hemorrhage** are to control the hemorrhage and, if blood loss is modest, to restore the circulating blood volume and tissue perfusion with crystalloid or, less often, colloid solutions. If the estimated blood loss is >25% of the circulating blood volume (>15 mL/kg of an estimated 60 mL/kg total estimated blood volume) *and* the patient's condition is unstable despite initial intravenous (IV) fluids, RBC transfusions should be given without undue hesitation, along with plasma transfusions at a 1:1 ratio of RBC/plasma volumes. Some experts recommend transfusing platelets early if bleeding is sustained or "massive" (i.e., approximating one blood volume or 60 mL/kg, which may occur very quickly in infants and small pediatric patients). Details of combined RBC and plasma transfusions, the volume ratio transfused, and considerations for adding platelet transfusions to treat bleeding patients are controversial. Accordingly, each hospital should develop and follow a "massive transfusion" protocol to ensure consistent practices.

In critically ill children with severe cardiac or pulmonary disease requiring assisted ventilation, it is common practice to maintain the Hb level close to the normal range, although the efficacy of this practice has not been well documented. A similar approach is used for children with acute cardiac, pulmonary, or cardiopulmonary disorders managed with extracorporeal membrane oxygenation (ECMO).

The pretransfusion Hb level or Hct that should "trigger" an RBC transfusion remains controversial (i.e., restrictive or a low pretransfusion level vs liberal or a high pretransfusion level) despite a substantial amount of published information, including randomized clinical trials. The current trend in critical care settings is to transfuse RBCs conservatively, following restrictive guidelines, and to permit modest anemia because there appears to be no disadvantage to conservative/restrictive transfusion practices, and some patients with Hb levels maintained close to the normal range by RBC transfusions (i.e., liberal guidelines) have poorer outcomes. Studies in critically ill adults demonstrated better outcomes when Hb level was maintained at 7–9 g/dL vs 10–12 g/dL. Anemic adults with *significant cardiac disease* did better with Hb level maintained at 13 g/dL rather than 10 g/dL. A similar study in children admitted to intensive care units found no inferiority when RBC

transfusions were given by restrictive guidelines *(transfusion threshold of 7 g/dL)*. It must be remembered that the children studied were in stable clinical status and needed few transfusions. Therefore, results of the trial cannot be automatically generalized to all patients admitted to ICUs because unstable critically ill children (who were not included in the study) may need more liberal RBC transfusion approaches.

With **chronic anemia,** the decision to transfuse RBCs should not be based solely on blood Hb levels because children compensate well and may be asymptomatic despite low Hb levels. Patients with iron-deficiency anemia are often treated successfully with oral iron alone, even at Hb levels <5 g/dL. Factors other than Hb concentration to be considered in the decision to transfuse RBCs include (1) the patient's symptoms, signs, and compensatory capacities; (2) the presence of underlying cardiorespiratory, vascular, and central nervous system disease; (3) the cause and anticipated course of the anemia; and (4) alternative therapies, such as recombinant human erythropoietin (EPO) therapy, which is known to reduce the need for RBC transfusions and to improve the overall condition of children with chronic renal insufficiency (see Chapter 572.2). In anemias that are likely to be permanent, it is also important to balance the detrimental effects of the degree of long-standing anemia on growth and development against the potential toxicity associated with repeated transfusions (i.e., iron overload and risks of transfusion-transmitted diseases) given to maintain the Hb concentration at a specified level. RBC transfusions for disorders such as sickle cell anemia and thalassemia are discussed in Chapters 511.1 and 511.10.

RBC TRANSFUSION IN PRETERM INFANTS AND NEONATES

For neonates, almost all aspects of RBC transfusions remain controversial—the accepted indications for RBC transfusions, restrictive vs liberal pretransfusion Hb/Hct levels, optimal RBC product to be transfused, and fresh vs stored RBC units—and clinical practices vary greatly. Generally, RBCs are given to maintain an Hb value believed to be the most desirable for each neonate's clinical status. Restrictive guidelines (i.e., lower pretransfusion Hb/Hct levels) have been compared to more liberal transfusion practices, but both short-term and long-term results and outcomes have been inconsistent and controversial, particularly as to neurodevelopmental status. Two multicenter randomized controlled trials showed no difference in survival or neurodevelopmental outcome in premature infants receiving RBC transfusion based on liberal versus restrictive Hb thresholds. Importantly, a lower Hb threshold was *not inferior* to a higher threshold for RBC transfusion. However, these studies had limitations, and accordingly, conventional guidelines are recommended to avoid problems caused by undertransfusion or overtransfusion (see Table 519.1).

During the first few weeks of life, all neonates experience a decline in circulating RBC mass caused by physiologic factors and, in sick premature infants, by phlebotomy blood losses. In healthy term infants, the nadir Hb value rarely falls to <11 g/dL at age 10-12 weeks. This benign drop in Hb does not require transfusions. In contrast, the decline occurs earlier and is more pronounced in premature infants, in whom the mean Hb concentration falls to approximately 7 g/dL in infants weighing <1 kg at birth, resulting in the **anemia of prematurity**, for which there often is need for RBC transfusions, particularly when the anemia is worsened by blood draws for laboratory testing.

A key reason that the nadir Hb values of premature infants are lower than those of term infants is the premature infant's relatively diminished plasma EPO level in response to anemia (see Chapters 139 and 496). Another factor is the rapid disappearance of EPO from infant plasma (i.e., accelerated metabolism). Low plasma EPO levels provide a rationale for the possible use of recombinant EPO in the treatment of anemia of prematurity; treatment with EPO and iron effectively stimulate neonatal erythropoiesis. Recombinant EPO has not been widely accepted as a treatment for anemia of prematurity (see Chapter 139).

Many low birthweight preterm infants need RBC transfusions (see Table 519.1). Although the practice to maintain a very high Hb level >13 g/dL (or Hct >40%) was once widely recommended, currently more restrictive guidelines have been suggested. However, one prospective, observational, multisite, birth cohort study of very low birthweight infants (≤1500 g) found a sixfold increased risk of necrotizing enterocolitis (NEC) when an infant's hemoglobin was ≤8 g/dL. Importantly, RBC transfusion in a given week did not significantly increase the rate of NEC in this population. Consistent with the rationale for oxygen delivery in neonates with severe respiratory disease, it also seems appropriate to keep the Hb value relatively high in neonates with *severe cardiac disease* leading to either cyanosis or congestive heart failure, but convincing and consistent data are lacking.

The optimal Hb level for neonates facing major surgery has not been established. However, it seems reasonable to begin surgery in neonates with the Hb level no lower than 10 g/dL (hematocrit >30%) and to maintain that value during major surgery because even modest blood loss will have a relatively large effect on the small blood volume of the neonate. Neonates with underlying pulmonary problems have limited ability to compensate for anemia due to the inability to increase ventilation and the inferior off-loading of oxygen because of the diminished interaction between fetal Hb and 2,3-diphosphoglycerate. Postoperatively, a lower pretransfusion Hb value should be followed to "trigger" a transfusion.

Stable neonates do not require RBC transfusion, regardless of their blood Hb levels, unless they exhibit clinical symptoms attributable to anemia. Proponents of RBC transfusions for symptomatic anemia in preterm neonates believe that the low RBC mass contributes to tachypnea, dyspnea, tachycardia, apnea and bradycardia, feeding difficulties, and lethargy, which can be alleviated by transfusion of RBCs. However, anemia is only one of several possible causes of these problems, and RBC transfusions should only be given when clinical benefit seems likely.

RBC PRODUCT AND DOSE

The RBC product of choice to transfuse neonates, infants, children, and adolescents is prestorage *leukocyte-reduced* RBCs suspended in an anticoagulant/preservative storage solution at Hct of approximately 60–70% for storage up to 35–42 days. For those infants with birthweight <1,500 g, *irradiation* is recommended to prevent transfusion-associated graft-versus-host disease. Additionally, both leukoreduction and cytomegalovirus-seronegative RBC units have been recommended by some (estimated risk of *transfusion-transmission cytomegalovirus* [TT-CMV] infection: 0–0.3% per unit) as first-line therapy to prevent TT-CMV. However, given the low risks of TT-CMV with improvements in modern leukoreduction techniques, others have recommended use of leukoreduced blood without need for CMV antibody testing as an acceptably safe and low-risk alternative. RBC transfusion should not be delayed if CMV-negative blood is unavailable, and CMV-untested leukoreduced blood should be used because the risk of TT-CMV is relatively low, with possibly more harm to the patient if transfusion is withheld or delayed. The usual dose is 10–15 mL/kg, but transfusion volumes vary greatly depending on clinical circumstances (continued vs arrested bleeding, hemolysis). For neonates, some prefer a centrifuged RBC concentrate (Hct 70–90%). Unless transfusions are being given to treat rapid bleeding, RBCs are infused slowly (over 2–4 hours) at a dose of approximately 10–15 mL/kg. In this small-volume setting, because of the small quantity of extracellular fluid transfused and the slow rate of infusion, the type of RBC anticoagulant/preservative solution does not pose any risk for premature infants when the dose does not exceed 20 mL/kg. However, the additive solutions (e.g., AS-1, AS-3, AS-5) have not been studied by comparative randomized clinical trials in the setting of >20 mL/kg dosing or massive transfusion settings such as cardiopulmonary bypass, ECMO, or massive transfusion for trauma. Although a few anecdotal reports suggest RBCs in additive solutions are safe for large-volume transfusions while awaiting more definitive information, some hospitals that manage these complex neonates and children maintain separate inventories of different RBC products earmarked either for neonates and infants (e.g., citrate-phosphate-dextrose or citrate-phosphate-dextrose-adenine) or for older children (e.g., additive solutions).

STORAGE AGE OF RBC UNITS

The historical practice of transfusing fresh RBCs (<7 days of storage) for the small-volume (15 mL/kg) transfusions commonly given was supplanted several years ago in most centers by reserving a single unit of RBCs for an infant, from which multiple aliquots were obtained for transfusions as needed throughout the 42 days of storage. Concerns about high concentrations of extracellular potassium, loss of 2,3-diphosphoglycerate, altered RBC shape and deformability, and nitric oxide quenching were found not to pose clinically significant problems. Preterm neonates allocated to "fresh RBC" (<7 days' storage) transfusions vs "stored RBC" (up to 42 days' storage) transfusions have no advantage for fresh RBC transfusions in altering the composite clinical outcome of mortality, plus NEC, retinopathy of prematurity, bronchopulmonary dysplasia, and intraventricular hemorrhage, or of the individual disorders.

For children weighing >30 kg who are to undergo elective surgery for whom RBC transfusions are likely to be needed, autologous RBC transfusions offer an alternative to donor allogeneic RBCs. **Preoperative autologous** blood collections from the patient occur up to 6 weeks before the surgery and require careful considerations for the volume to be drawn, vascular access, and use of EPO and iron to help restore the donated RBCs. **Acute normovolemic hemodilution** occurs in the preoperative period, in which blood is withdrawn from the patient and replaced with saline, a task often difficult in centers without experience in the process. **Salvaged autologous blood** is collected from blood loss during the operation but is impractical unless the volume of blood salvaged is fairly large to permit washing and transfusion of a significant number of RBCs. Because of all these difficulties, plus the relative safety of the usual allogeneic blood supply, autologous RBC transfusions are not typically used in the pediatric setting.

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Chapter 520

Platelet Transfusions

Patricia E. Zerra and Cassandra D. Josephson

CHILDREN AND ADOLESCENTS

Guidelines for platelet (PLT) support of children and adolescents with quantitative and qualitative PLT disorders are similar to those for adults, in whom the risk of life-threatening bleeding occurring after injury or spontaneously can be related to the severity of thrombocytopenia, although somewhat imprecisely (Table 520.1).

For children and adolescents with overt bleeding, therapeutic PLT transfusions should be given when the blood PLT count falls below 50×10^9/L and repeated as needed to maintain the PLT count $>50 \times 10^9$/L during bleeding and for 48 hours after bleeding ceases to allow the clot to "stabilize." Similarly, for a major invasive procedure (e.g., surgical), the PLT count should be maintained $>50 \times 10^9$/L until any bleeding that occurs ceases and the patient is stable. For minor invasive procedures (e.g., lumbar puncture, intravascular catheter placement), practices vary. It is reasonable to maintain the PLT count $>25 \times 10^9$/L, although these procedures often are performed in children with cancer or recent transplants, and it is important to be mindful of possible clotting abnormalities and anemia that may affect hemostasis beyond the effects of thrombocytopenia. Historical studies of patients with thrombocytopenia resulting from bone marrow failure suggest that the risk of spontaneous bleeding increases when blood PLT levels fall to $<20 \times 10^9$/L, particularly when hemorrhagic risk factors (infection, organ failure, clotting abnormalities, minor skin/mucosal bleeding, mucosal lesions, severe graft-versus-host disease [GVHD], anemia) are present. In this high-risk setting, prophylactic PLT transfusions are given to maintain a PLT count $>20 \times 10^9$/L. This threshold has been challenged by several studies of adult patients, who in many instances were carefully selected to be in relatively good clinical condition *without* hemorrhagic risk factors. Consequently, a lower PLT transfusion trigger of 10×10^9/L is recommended for stable (i.e., low-risk) patients.

Table 520.1 Guidelines for Pediatric Platelet (PLT) Transfusion*

CHILDREN AND ADOLESCENTS
1. Maintain PLT count $>50 \times 10^9$/L with bleeding.
2. Maintain PLT count $>50 \times 10^9$/L with *major invasive* procedure; $>25 \times 10^9$/L with minor.
3. Maintain PLT count $>20 \times 10^9$/L and *marrow failure* WITH hemorrhagic risk factors.
4. Maintain PLT count $>10 \times 10^9$/L and *marrow failure* WITHOUT hemorrhagic risk factors.
5. Maintain PLT count at any level with PLT dysfunction PLUS bleeding or invasive procedure.
INFANTS ≤4 MO OLD
1. Maintain PLT count $>100 \times 10^9$/L with bleeding or during extracorporeal membrane oxygenation.
2. Maintain PLT count $>50 \times 10^9$/L and an invasive procedure.
3. Maintain PLT count $>20 \times 10^9$/L and *clinically stable.*
4. Maintain PLT count $>50 \times 10^9$/L and *clinically unstable and/or bleeding or not when on indomethacin, nitric oxide, antibiotics, etc., affecting PLT function.*
5. Maintain PLT count at any level with PLT dysfunction PLUS bleeding invasive procedure.

*Words in *italics* must be defined for local transfusion guidelines.

In practice, severe thrombocytopenia that is prolonged beyond 1 week usually becomes complicated by the development of risk factors, including fever, antimicrobial therapy, GVHD, active bleeding, need for an invasive procedure, disseminated intravascular coagulation, and liver or kidney dysfunction with clotting abnormalities. In these situations, prophylactic PLT transfusions are given to maintain relatively high PLT counts (e.g., at least $>30 \times 10^9$/L). Despite the desire by some physicians to elevate the blood PLT count to 80×10^9/L or 100×10^9/L, there are no definitive data to justify a true benefit of PLT transfusions given at a PLT count $>50 \times 10^9$/L, unless bleeding is ongoing despite a PLT count between 50 and 100×10^9/L *and* thrombocytopenia seems to be the only cause for the bleeding.

Qualitative PLT disorders may be *inherited* or *acquired,* as in advanced hepatic or renal insufficiency or when blood flows through an extracorporeal circuit, such as during extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass. In patients with inherited disorders, PLT transfusions are justified only if the risk of significant bleeding is quite high or if bleeding is overt because inherited PLT dysfunction often is lifelong and repeated transfusions may lead to alloimmunization and refractoriness (i.e., poor response to PLT transfusions). Accordingly, prophylactic PLT transfusions are rarely justified unless an invasive procedure is planned, and therapeutic PLT transfusions must be given judiciously.

When managing patients with PLT dysfunction, it is important to remember that an abnormal test result with a modern PLT function device or, historically, a bleeding time more than twice the upper limit of normal provides diagnostic evidence of PLT dysfunction. However, an abnormal bleeding time or any other abnormal laboratory test is poorly predictive of hemorrhagic risk or the need to transfuse PLTs. Alternative therapies, particularly desmopressin acetate, should be considered to avoid PLT transfusions. Antiplatelet medications (nonsteroidal antiinflammatory drugs) should also be avoided.

INFANTS AND NEONATES

In neonates, thrombopoiesis and the risks of bleeding are substantially different from that in older children; the approach to thrombocytopenia and PLT transfusions likewise differs (see Table 520.1). **Thrombopoietin** (TPO) levels are higher in healthy neonates than in older individuals. Relative to adult PLT progenitors, *megakaryocyte* progenitors of neonates are more sensitive to TPO, have higher proliferative potential, and give rise to larger megakaryocyte colonies. Fetal/neonatal megakaryocytes are smaller in size and have lower ploidy than do their adult counterparts; this

information is important because small megakaryocytes of low ploidy produce fewer PLTs than do larger megakaryocytes of higher ploidy. Presumably, this allows the expanding marrow of the growing fetus and neonate to be supplied with sufficient numbers of megakaryocytes, yet not allowing blood PLT counts to become excessively high during proliferation because of the lower numbers of PLTs produced by each megakaryocyte.

An important contrasting point is that older children and adults respond to situations of increased demand for PLTs by first increasing megakaryocyte size and ploidy, which is followed in 3–5 days by increased megakaryocyte number. In thrombocytopenic neonates, megakaryocyte numbers but not size increase. Moreover, although cytoplasmic maturation is achieved per TPO stimulation, increases in ploidy are relatively diminished and actually appear to be inhibited by TPO, resulting in large numbers of small megakaryocytes that are cytoplasmically mature but with low ploidy and, consequently, lower PLT production.

PLT counts ≥150 × 10^9/L are present after 17 weeks' gestational age, and it is accepted that neonates have PLT counts in the same range as older children and adults (150,000–450,000/μL). However, other data suggest a lower limit of 120,000/μL for extremely small preterm infants. Approximately 1% of term infants demonstrate PLT counts <150 × 10^9/L, but bleeding in such infants is rare. In contrast, 18–35% of preterm neonates treated in intensive care units (ICUs) exhibit PLT counts <150 × 10^9/L at some time during admission, with approximately 4% overall receiving PLT transfusions. Notably, when only extremely low birthweight preterm infants (<1 kg) were considered in one report, 70% had PLT counts <150 × 10^9/L, and 5–9% of infants received platelet transfusions.

Debate continues in the United States as to the appropriate prophylactic platelet transfusion threshold for neonates, with a wide range in practice patterns. Multiple pathogenetic mechanisms underlie thrombocytopenia in these sick neonates, including predominantly accelerated PLT destruction plus diminished PLT production, as evidenced by decreased numbers of megakaryocyte progenitors and relatively low upregulation of TPO levels during thrombocytopenia, when compared with thrombocytopenic children and adults.

PLT counts <100 × 10^9/L pose significant clinical risks for premature neonates. Bleeding time may be prolonged at PLT counts <100 × 10^9/L in infants with birthweight <1,500 g, and PLT dysfunction is suggested by bleeding times (a test no longer performed) that are disproportionately long for the degree of thrombocytopenia. The risk of hemorrhage may be increased in thrombocytopenic infants. However, in a randomized trial, transfusing PLTs prophylactically whenever the PLT count fell to <150 × 10^9/L (i.e., at the lower limit of normal range) to maintain the average PLT count at >200 × 10^9/L, compared to not transfusing PLTs until the PLT count fell to <50 × 10^9/L to maintain the average PLT count at approximately 100 × 10^9/L, *did not result* in a lower incidence of intracranial hemorrhage (28% vs 26%, respectively). A U.S. multicenter observational study of very low birthweight infants in six neonatal ICUs found wide variation in PLT thresholds for transfusion, ranging from 10,000 to 139,000/μL in the first week of life and <10,000/μL to >50,000/μL after the first week. The most common thresholds were 80,000–89,000/μL in the first 7 days of life and 40,000–49,000/μL after the first 7 days. Furthermore, after controlling for severity of thrombocytopenia, the authors found PLT transfusions were not associated with a lower risk of intraventricular hemorrhage. Thus, there is no documented benefit for prophylactic PLT transfusions to maintain PLT counts within the normal range or to correct moderate thrombocytopenia (PLT count >50 × 10^9/L). As an exception, infants with inherited PLT dysfunction disorders *and* bleeding, as well as infants at high risk of bleeding because of acquired PLT dysfunction, such as during ECMO, typically receive transfusions to keep their PLT counts >100 × 10^9/L.

One randomized clinical trial reported a significantly higher rate of new major hemorrhage or death within 28 days of randomization in very low birthweight neonates given prophylactic PLT transfusions at a pretransfusion PLT count of 50,000/μL (26%) vs a pretransfusion PLT count of 25,000/μL (19%), irrespective of their baseline risk of bleeding. Results are too preliminary to permit changes in practice but support other published findings indicating no need to maintain normal PLT counts and add to the belief that a PLT count of 50,000/μL is too high to serve as the pretransfusion PLT count for transfusions in stable low birthweight neonates.

Table 520.1 lists pediatric PLT transfusion guidelines that are acceptable to many neonatologists. One particularly contentious issue is how to manage critically ill neonates receiving agents known to adversely affect PLT function (e.g., indomethacin, nitric oxide, antibiotics). Some reports suggest increased risk of bleeding for these neonates, but the efficacy of PLT transfusions has not been convincingly proven, particularly when given prophylactically. For optimal PLT transfusion practices, each hospital should modify their guidelines to comply with local practices, with audits and reviews done to avoid violations of the recommended practices.

PLATELET PRODUCTS AND DOSING

In the United States, two types/sources of PLT units are available, although any one blood bank or hospital may stock only one of these types. Whole blood–derived PLT units **(PLT concentrates)** and PLT units collected by apheresis **(apheresis PLTs)** differ in their PLT content and plasma volume. Although a PLT concentrate contains approximately 5.5-10 × 10^{10} PLTs in approximately 50 mL, and 1 apheresis PLT unit contains at least 3 × 10^{11} PLTs in 300–600 mL, the PLT content may vary considerably among different blood suppliers. Accordingly, it is prudent for hospital blood banks to confirm the composition of the PLT units they issue for transfusion, at the very least by contacting their blood supplier. It is often easier to use PLT concentrates for infants and small children because apheresis PLTs usually need to be prepared as *aliquots* to provide the correct dose (10–15 mL/kg). However, many blood centers exclusively provide only one type of PLT component. Platelet products generally have a 5-day expiration time, although 7-day storage has been approved for apheresis platelet units within certain stipulations to reduce the risk of bacterial contamination and are stored at room temperature with constant agitation.

The posttransfusion goal of most PLT transfusions is to raise the PLT count well above 50 × 10^9/L, hopefully to ≥100 × 10^9/L. These increases can be achieved consistently in children weighing up to 30 kg by infusion of 5–10 mL/kg of standard (unmodified) PLT concentrates, obtained either from PLT concentrates or apheresis PLTs. For larger children, the appropriate dose is 4-8 pooled PLT concentrates or 1 apheresis unit. Because PLT concentration/quantity varies in different PLT products made available for transfusion, each hospital should monitor posttransfusion PLT counts to determine the dose that works best locally. PLT concentrates may be transfused as rapidly as the patient's overall condition permits, certainly within 2 hours, but not longer than 4 hours. Neonates/infants requiring repeated PLT transfusions should receive leukocyte-reduced blood products to diminish HLA alloimmunization and PLT refractoriness and to reduce the risk of transfusion-transmission cytomegalovirus infection (TT-CMV). For those infants weighing <1,500 g at birth, *irradiation* is recommended to prevent transfusion associated GVHD. Additionally, some recommend both leukoreduction and CMV-seronegative RBC units (estimated risk of TT-CMV infection: 0–0.3% per unit) as first-line therapy to prevent TT-CMV. However, given the low risks of TT-CMV with improvements in modern leukoreduction techniques, others have recommended use of leukoreduced blood from CMV-untested donors as an acceptably safe and low-risk alternative. PLT transfusion should not be delayed if CMV-negative units are unavailable; CMV-untested leukoreduced units should be used because the risk of TT-CMV is relatively low and more harm may come to the patient if transfusion is withheld or delayed.

Routinely reducing the volume of PLT concentrates for infants and small children by additional centrifugation steps is both unnecessary and unwise. Transfusion of 10–15 mL/kg of an unmodified PLT concentrate is adequate because it adds approximately 10 × 10^9 PLTs to 70 mL of blood (estimated intravascular blood volume of 1 kg neonate), a dose/volume calculated to increase the PLT count by 100 × 10^9/L. This calculated increment is consistent with actual posttransfusion increment reported in patients. Moreover, 10–15 mL/kg is not an excessive transfusion volume, provided that the intake of other IV fluids, medications, and nutrients is monitored and adjusted.

It is important to select PLT units for transfusion with the donor ABO group identical to that of the neonate/infant and to avoid repeated transfusion of group O PLTs to group A or B recipients, because passive transfusion anti-A or anti-B in group O plasma can occasionally lead to intravascular hemolysis.

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Chapter 521

Neutrophil (Granulocyte) Transfusions

Patricia E. Zerra and Cassandra D. Josephson

Table 521.1 lists guidelines for granulocyte transfusion (GTX). GTX has been used sparingly in older infants and children. The current ability to collect markedly higher numbers of neutrophils from donors stimulated with combined recombinant granulocyte colony-stimulating factor (G-CSF) plus dexamethasone has led to renewed interest for patients with neutropenic infections, particularly when severe neutropenia is prolonged (e.g., in the setting of placental/cord blood hematopoietic progenitor cell transplantation). As a result, higher neutrophil yields are collected with this approach, making the addition of GTX to antibiotics a therapeutic consideration. This is especially true at institutions where neutropenic patients continue to die of progressive bacterial and fungal infections or to suffer substantial morbidity despite optimal antiinfection measures, including antibiotics and recombinant myeloid growth factors.

GRANULOCYTE TRANSFUSIONS FOR CHILDREN

The use of GTX added to antibiotics for children with **severe neutropenia** (neutrophil count $<0.5 \times 10^9$/L) because of bone marrow failure is similar to that for adults. Unfortunately, two randomized clinical trials comparing antibiotics plus GTX from donors stimulated with G-CSF plus dexamethasone vs antibiotics without GTX to treat neutropenic infections in children have not provided definitive guidelines. However, in practice, neutropenic patients with bacterial infections usually show response to antibiotics alone, provided bone marrow function recovers within the first 7–10 days of infection onset, so that severe neutropenia is relatively brief. Children with newly diagnosed acute lymphoblastic leukemia show rapid response to induction chemotherapy and are rarely candidates for GTX. In contrast, infected children with more sustained bone marrow failure and consequent severe neutropenia (e.g., acute myeloblastic leukemia, malignant neoplasms resistant to treatment, severe aplastic anemia, placental/cord blood hematopoietic progenitor cell transplant recipients) may benefit when GTX is added to antibiotics.

Currently, the efficacy of GTX obtained from G-CSF plus dexamethasone–stimulated donors for bacterial sepsis unresponsive to antibiotics in patients with severe neutropenia (neutrophil count $<0.5 \times 10^9$/L) *is not well supported* by trials in children. Furthermore, GTX efficacy for yeast and fungal infections remains unproven, despite transfusing GTX with relatively large numbers of neutrophils.

Children with **qualitative neutrophil defects (neutrophil dysfunction)** usually have adequate or even increased numbers of blood neutrophils but develop serious infections because their neutrophils kill pathogenic microorganisms inefficiently. Neutrophil dysfunction syndromes are rare; accordingly, no definitive studies have established GTX efficacy. However, several patients with progressive life-threatening infections have shown striking improvement with the addition of GTX, often given for long periods, to antimicrobial therapy. These disorders are chronic and thus associated with an increased risk of alloimmunization to leukocyte antigens, specifically to Kell system antigens on the red blood cells in some patients with chronic granulomatous disease, and GTX is recommended only when serious infections are clearly unresponsive to antimicrobial drugs.

Table 521.1 Guidelines for Pediatric Granulocyte Transfusions*

CHILDREN AND ADOLESCENTS
1. Severe neutropenia (blood neutrophil count $<0.5 \times 10^9$/L) and infection (bacterial, yeast, or fungal) *unresponsive or progressive* despite appropriate antimicrobial therapy.
2. Qualitative neutrophil defect, neutropenia not required, and infection (bacterial or fungal) *unresponsive or progressive* to appropriate antimicrobial therapy.
INFANTS ≤4 MO OLD†
Blood neutrophil count $<3.0 \times 10^9$/L in first week of life or $<1.0 \times 10^9$/L thereafter and *fulminant* bacterial infection.

*Words in *italics* must be defined for local transfusion guidelines.
†No longer commonly used.

GRANULOCYTE TRANSFUSION FOR NEONATES

Neonates are unusually susceptible to severe bacterial infections, and a number of defects of neonatal defenses contribute to this susceptibility, including actual or "relative" neutropenia. Neonates with fulminant sepsis who exhibit *relative neutropenia* (blood neutrophil count $<3.0 \times 10^9$/L during the first week of life and $<1.0 \times 10^9$/L thereafter) and a severely diminished neutrophil marrow storage pool (with <10% of nucleated marrow cells being postmitotic neutrophils) are at risk of dying if treated only with antibiotics. Despite this risk, GTX has not provided the solution. GTX is rarely used in neonates because the results of clinical trials are mixed and not uniformly convincing, and it is difficult to obtain neutrophil apheresis concentrates in a timely fashion.

Current data are insufficient to conclude that recombinant myeloid growth factors have a role in treating septic neonates, despite demonstration that both G-CSF and granulocyte-macrophage CSF enhance myelopoiesis and raise neutrophil counts in infants. In contrast to the uncertain role of G-CSF and GM-CSF to treat infections in many clinical settings, it is important to remember that G-CSF is efficacious for the long-term treatment of several types of severe **congenital neutropenias**.

GRANULOCYTE PRODUCT

If the decision to provide a GTX has been made, an adequate dose of neutrophils/granulocytes collected by leukapheresis must be transfused as shortly after collection as possible. To facilitate this goal, experienced donors with recently performed negative testing for HIV and hepatitis (usually within the past 30 days) are selected. Granulocyte donors should be documented to be cytomegalovirus antibody negative (seronegative). These donors should also be ABO/Rh crossmatch compatible with the recipient because there is a large volume of red blood cells in the granulocyte product.

Granulocyte Product Dosing

Neonates and **infants** weighing <10 kg should receive $1\text{-}2 \times 10^9$/kg neutrophils per each GTX. **Larger infants** and **small children** should receive a minimal total dose of 1×10^{10} neutrophils per each GTX. The preferred dose for **adolescents** is $5\text{-}8 \times 10^{10}$ neutrophils per each GTX, a dose requiring donors to be stimulated with G-CSF plus dexamethasone. GTX should be given daily until either the infection resolves or the blood neutrophil count is sustained above 1.5×10^9/L for a few days. Because neutrophils transfused through the GTX often passively increase the blood neutrophil count, it may be necessary to skip 1-2 days of GTXs to accurately assess whether endogenous myelopoiesis and neutrophil production have recovered.

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Chapter 522

Plasma Transfusions

Patricia E. Zerra and Cassandra D. Josephson

Guidelines for plasma transfusion in pediatric patients are similar to those for adults but with the understanding that plasma levels of coagulant and anticoagulant proteins can be developmentally low in preterm infants (Table 522.1). Therefore transfusions of plasma and plasma-derived commercial concentrates should be determined by actual bleeding or a significant risk of bleeding, not simply by prolonged clotting time results. Plasma is transfused to replace clinically significant congenital or acquired deficiencies of plasma proteins for which more highly purified protein concentrates, treated to reduce infectious disease risks, or recombinant products are not available. Plasma and plasma derivatives are required to provide clotting proteins when bleeding is actually occurring or in settings when prevention of bleeding is deemed critical.

PLASMA PRODUCTS AND PATIENT TESTING

Two interchangeable plasma products are available for transfusion, plasma frozen within 8 hours of collection **(fresh-frozen plasma [FFP])** and plasma frozen within 24 hours of collection **(F24)**. Although levels of factors V and VIII are modestly reduced in F24 (generally, not more than 25% lower), they are equally efficacious for all indications for which plasma is transfused in infants and children (see Table 522.1). Recommendations for the volume of plasma to be transfused vary with the specific protein being replaced and the severity of the deficiency, but a starting dose of 15 mL/kg is usually sufficient to elevate plasma levels satisfactorily.

Transfusion of plasma is efficacious for the treatment of deficiencies of clotting factors II, V, X, and XI. Deficiencies of factor XIII and fibrinogen are treated either with cryoprecipitate or specific commercial concentrates; for patients being given large doses of plasma (e.g., in massive transfusion settings or to treat bleeding in liver failure), however, additional sources of fibrinogen may not be necessary because plasma contains large amounts of fibrinogen. It is always useful to include a measurement of plasma fibrinogen (a separate test) when performing clotting assays, including prothrombin time (PT)/international normalized ratio (INR) and activated partial thromboplastin time (aPTT).

PLASMA TRANSFUSION IN CHILDREN

Transfusion of plasma is not recommended for the treatment of patients with severe hemophilia A or B, von Willebrand disease, or factor VII deficiency because safer plasma-derived and recombinant factor products for VII, VIII, IX, and von Willebrand factor are available. Moreover, mild to moderate hemophilia A and certain types of von Willebrand disease can be treated with intranasal or intravenous desmopressin (see Chapter 526). An important use of plasma is for rapid reversal of the effects of warfarin in patients who are actively bleeding or who require emergency surgery (in whom functional deficiencies of vitamin K–dependent factors II, VII, IX, and X cannot be rapidly reversed by vitamin K administration). Plasma-derived and virally inactivated prothrombin "complex" concentrates can also be used for this purpose.

Results of screening coagulation tests (PT/INR, aPTT, thrombin time, and plasma fibrinogen level) should not be assumed to reflect the integrity of the coagulation system or be regarded as indications for plasma transfusions. This is particularly true for neonates. To justify plasma transfusions, coagulation test results must be related to the patient's clinical condition in regard to bleeding and the risk of bleeding. Transfusion of plasma in patients with chronic liver disease and prolonged clotting times is not recommended unless bleeding is present, or an invasive procedure is planned because prophylactic correction of the clotting factor deficiencies is brief and of questionable benefit.

Plasma also contains several anticoagulant proteins (antithrombin III, protein C, and protein S) whose deficiencies have been associated with thrombosis. In select situations, plasma as replacement therapy along with anticoagulant treatment may be appropriate in patients with these disorders; when available, purified concentrates are preferred. Other indications for plasma include replacement fluid during plasma exchange in patients with **thrombotic thrombocytopenic purpura** (i.e., thrombotic microangiopathies) or other disorders for which plasma is likely to be beneficial. This includes plasma exchange in a patient with overt bleeding caused by the underlying disorder (e.g., Goodpasture syndrome, vasculitis) or disorders with significant severe coagulopathy that would substantially worsen with replacement by albumin solutions only. *Plasma is not indicated for correction of hypovolemia or as immunoglobulin replacement therapy because safer alternatives exist (albumin or crystalloid solutions and IV immunoglobulin, respectively).*

PLASMA TRANSFUSION IN NEONATES

In neonates, clotting times are "physiologically" prolonged because of developmental deficiency of clotting proteins; plasma should be transfused only after reference to normal values is adjusted for the birthweight and age of the infant (*not* to normal ranges for older children and adults). The indications for plasma in neonates include (1) reconstitution of red blood cell (RBC) concentrates to simulate whole blood for use in massive transfusions (exchange transfusion, cardiac bypass surgery, and extracorporeal membrane oxygenation), (2) hemorrhage secondary to vitamin K deficiency, (3) disseminated intravascular coagulation *with* bleeding, and (4) bleeding in congenital coagulation factor deficiency when more specific treatment is either unavailable or inappropriate. The use of prophylactic plasma transfusion to prevent intraventricular hemorrhage in premature infants is not recommended. Plasma should not be used as a suspending agent to adjust the hematocrit values of RBC concentrates before small-volume RBC transfusions to neonates because it offers no apparent medical benefit over the use of sterile solutions such as crystalloid and albumin. Similarly, the use of plasma in partial exchange transfusion for the treatment of neonatal hyperviscosity syndrome is unnecessary because safer crystalloid or colloid solutions are available.

In the treatment of bleeding infants, *cryoprecipitate* is often considered because of its small infusion volume. However, cryoprecipitate contains significant quantities of only fibrinogen, von Willebrand factor, and factors VIII and XIII. Thus it is not effective for treating the usual clinical situation in bleeding infants with multiple clotting factor deficiencies. However, cryoprecipitate is an excellent source of fibrinogen (much more concentrated than frozen plasma), and with a dose of 1–2 units/kg, the patient's fibrinogen level can be quickly raised by 60–100 mg/dL.

In preliminary studies, infusions of very small volumes of recombinant activated factor VII have been lifesaving in patients with hemorrhage caused by several mechanisms. Because the efficacy and toxicity of factor VIIa have not been fully defined in these "off-label" uses (not approved by the U.S. Food and Drug Administration), it must be considered "experimental" therapy at this time.

Table 522.1 Guidelines for Children and Infants for Plasma Transfusions*

1. *Severe* clotting factor deficiency AND bleeding.
2. *Severe* clotting factor deficiency AND an invasive procedure.
3. *Emergency reversal* of warfarin effects.
4. Dilutional coagulopathy and bleeding (e.g., massive transfusion).
5. Anticoagulant protein (antithrombin III, proteins C and S) replacement.
6. Plasma exchange replacement fluid for thrombotic thrombocytopenic purpura or for disorders with overt bleeding or in which there is risk of bleeding because of clotting protein abnormalities (e.g., liver failure).

*Words in *italics* must be defined for local transfusion guidelines.

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Chapter 523

Risks of Blood Transfusions

Patricia E. Zerra and Cassandra D. Josephson

The greatest risk of a blood transfusion is mistakenly receiving a transfusion intended for another patient. *Misidentification* is usually a result of mistakes made in labeling the patient's blood sample sent to the blood bank or not accurately matching the unit with the patient at the bedside when the blood is transfused. This risk is particularly high for infants, especially if ABO type-specific or type-compatible blood is transfused, because (1) identification bands may not be attached directly to their bodies, (2) difficulties in drawing blood samples for pretransfusion compatibility testing may lead to deviations from usual policies, and (3) infants cannot speak to identify themselves. Thus particular care must be taken to ensure accurate patient and blood sample identification.

INFECTIOUS RISKS OF TRANSFUSION

Although the infectious disease risks of allogeneic blood transfusions are extremely low, transfusions must be given judiciously because "emerging infections," such as Ebola or Zika virus, when they first arise, pose a potential threat until they are studied definitively and, accordingly, are of constant concern, and testing is not done for every microorganism possibly transmitted by blood transfusions (Table 523.1 and Fig. 523.1). Taking nucleic acid amplification testing (NAT) and all other donor-screening activities (antibody and epidemiology screening) into account, a current estimate of the risk of transfusion-associated **HIV** infection is approximately 1 per every 2 million donor exposures. Similarly, with NAT, the risk of hepatitis C virus **(HCV)** infection is 1 per every 1.5-2 million donor exposures (see Table 523.1). NAT identifies circulating microbial nucleic acids that appear in the window before antibodies develop, and NAT is used routinely to detect HIV, HCV, West Nile virus, hepatitis B virus, *Trypanosoma cruzi, Babesia microti,* and Zika virus.

Transfusion-associated cytomegalovirus **(CMV)** has been nearly eliminated by transfusion of leukocyte-reduced cellular blood products or by selection of blood collected from donors who are seronegative for antibody to CMV. Although it is logical to hypothesize that first collecting blood components from CMV-seronegative donors and then removing the white blood cells (WBCs) might further improve safety, little data are available to document the superior efficacy of this combined approach. However, in a recent prospective birth cohort study of premature infants with birthweight ≤1,500 g, a combined approach of leukoreduction and CMV-seronegative cellular blood components yielded 0% transfusion transmission of CMV (15.3% cumulative incidence at 12 weeks of maternal breast milk transmission from CMV-seropositive mothers) in >300 transfused infants studied. Similar uncontrolled reports of hematopoietic stem cell transplant patients found 0% transmission of CMV from leukoreduced blood products from donors of unknown CMV antibody status. Therefore considerable care must be taken not to place children at risk of delayed or missed transfusions while awaiting/searching for blood from CMV-seronegative donors, then to leukoreduce (i.e., risks must not be taken for practices with no established benefits).

Further data on these two mitigation strategies revealed that large quantities of CMV viral material are present "free" in the plasma of healthy-appearing donors during the early phase of primary infection (while CMV antibodies are either absent ["window" phase] or are newly emerging and at low, inconsistently detected levels in plasma), rather than being leukocyte associated, as occurs with CMV as substantial quantities of IgG antibodies appear. As a result of this biology of CMV primary infection, plasma "free" virus will not be removed by leukoreduction during early infection, and CMV-seronegative donors who may be asymptomatic or deny symptoms of infection during blood donor screening will be misclassified as being CMV safe. They are not necessarily as safe because antibody is below the limits of detection, while plasma "free" CMV is plentiful during early infection. Because almost all plasma "free" CMV disappears and becomes almost exclusively cell associated, once donors are CMV seropositive with antibody present for several months, some propose that the best method to reduce CMV risk may be leukoreduction of blood from donors known to be CMV seropositive for at least 1 year. However, data to prove the efficacy of this proposal are lacking, and in practice, several studies have shown that the most efficacious method currently available to prevent transfusion-transmitted CMV is to perform leukoreduction in the

Table 523.1 Estimated Risks in Transfusion per Unit Transfused in the United States

ADVERSE EFFECT (INFECTIOUS)	ESTIMATED RISK
Human immunodeficiency virus (HIV)-1 and HIV-2	1:2 million
Hepatitis B	1:2 million
Hepatitis C	1:2 million
Human T-cell lymphotropic virus (HTLV) I and II	1:3 million
Bacterial contamination (usually platelets)	1:100,000
Malaria	<1:3 million
Hepatitis A	Unknown
Parvovirus	Unknown
Dengue fever	Unknown
Cytomegalovirus	<1:3 million
Babesiosis	Unknown
West Nile virus	<1:3 million
Trypanosoma cruzi	<1:3 million
Leishmania spp.	Unknown
Variant Creutzfeldt-Jakob prion disease	Unknown
Zika virus	<1:3 million
Syphilis	Unknown
ADVERSE EFFECT (NONINFECTIOUS)	**ESTIMATED RISK**
Febrile nonhemolytic reaction	1:800
Mild-moderate allergic reaction	1:1,000
Severe allergic reactions	1:40,000
Transfusion-associated circulatory overload (TACO)	1:8,500
Hypotensive	1:11,000
Acute hemolytic transfusion reaction	1:95,000
Transfusion-associated dyspnea	1:15,000
Transfusion-related acute lung injury (TRALI)	1:70,000
Delayed hemolytic transfusion reaction	1:20,000
Transfusion-associated graft-versus-host disease	Uncommon
Immunomodulation	Unknown
Posttransfusion purpura	1:30,000
Life-threatening reactions, requiring major medical intervention	1:20,000

Adapted from Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. *Blood* 2019;133:1854–1864; and Savinkina AA, Haass KA, Sapiano MRP, et al. Transfusion-associated adverse events and implementation of blood safety measures – findings from the 2017 National Blood Collection and Utilization Survey. *Transfusion* 2020;60:S10–S16.

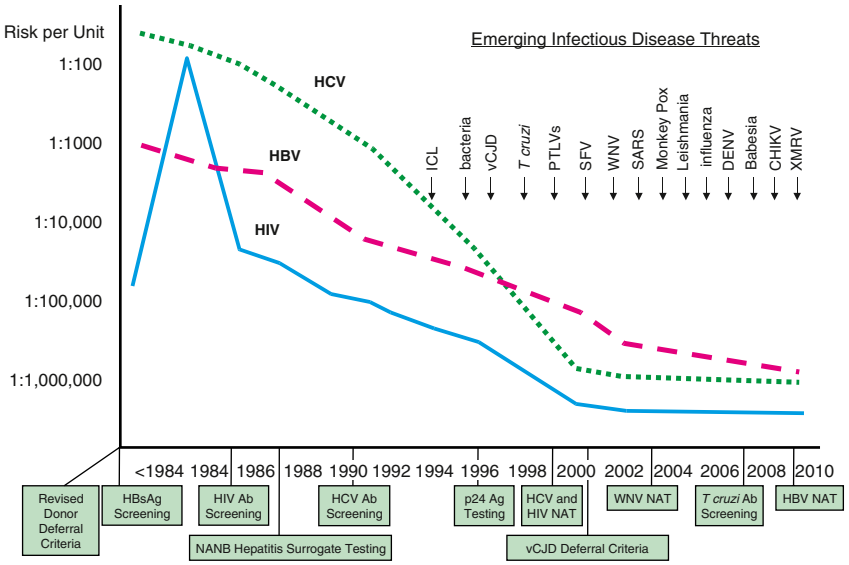

Fig. 523.1 Risks of major transfusion-transmitted viruses related to interventions and accelerating rate of emerging infectious diseases of concern to blood safety. Evolution of the risks of transmission by blood transfusion for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Major interventions to reduce risks are shown below the timeline on the *x* axis. Emerging infectious disease threats in the past 20 years are shown above in the *top right quadrant* of the figure. Ab, Antibody; Ag, antigen; CHIKV, chikungunya virus; DENV, dengue virus; HBsAg, hepatitis B surface antigen; ICL, idiopathic CD4 T-lymphocytopenia; NANB, non-A, non-B hepatitis; NAT, nucleic acid amplification testing; PTLVs, primate T-lymphotropic viruses; SARS, severe acute respiratory syndrome; SFV, simian foamy virus; vCJD, variant Creutzfeldt-Jakob disease; WNV, West Nile virus; XMRV, xenotropic murine leukemia virus–related virus. *(From Busch MP. Transfusion-transmitted viral infections: Building bridges to transfusion medicine to reduce risks and understand epidemiology and pathogenesis. 2005 Emily Cooley Award Lecture. Transfusion. 2006;46:1624–1640.)*

blood center/bank without regard for the CMV antibody status of the donor/unit (i.e., leukoreduction alone performed by the blood center/bank, not at the bedside, is sufficient in most cases).

Additional infectious risks include other types of **hepatitis** (A, B, E) and **retroviruses** (human T-cell lymphotropic virus types I and II, HIV-2), syphilis, parvovirus B19, Epstein-Barr virus, human herpesvirus 8, West Nile virus, yellow fever vaccine virus, malaria, babesiosis, *Anaplasma phagocytophilum*, Chagas disease, and Zika virus. Variant Creutzfeldt-Jacob disease has also been transmitted by blood transfusions in humans. All are reported very infrequently, but nonetheless provide the rationale to transfuse only when true benefits are likely.

NONINFECTIOUS RISKS OF TRANSFUSION

Transfusion-associated risks of a noninfectious nature that may occur include hemolytic and nonhemolytic transfusion reactions, circulatory fluid overload, **graft-versus-host disease** (GVHD), electrolyte and acid-base imbalances, iron overload if repeated red blood cell (RBC) transfusions are needed long term, increased susceptibility to oxidant damage, exposure to plasticizers, hemolysis with T-antigen activation of RBCs, posttransfusion purpura, **transfusion-related acute lung injury** (TRALI), posttransfusion immunosuppression and immunomodulation, and alloimmunization (Fig. 523.2; see Table 523.1). The risk of TRALI may be reduced by avoiding transfusion of plasma or platelets from female donors, who were possibly alloimmunized to leukocyte antigens during pregnancy or by selecting donors (e.g., males) who are likely to be negative for human leukocyte antigen (HLA) antibodies.

Immunologic adverse effects, including immunosuppression, immunomodulation, and alloimmunization, may be reduced by leukoreduction. Transfusion reactions and alloimmunization to RBC and leukocyte antigens seem to be uncommon in infants, perhaps because of developmental immaturity of the immune system or deficient cytokine production. When they do occur, adverse effects are seen primarily in massive transfusion settings, such as exchange transfusions and trauma or surgery, in which relatively large quantities of blood components are needed but are rare when small-volume transfusions are usually given.

Premature infants are known to have immune dysfunction, but their relative risk of posttransfusion GVHD is controversial. The postnatal age of the infant, the number of immunocompetent lymphocytes in the transfusion product, the degree of HLA compatibility between donor and recipient, and other, poorly described phenomena determine which infants are truly at risk for GVHD. Regardless, many centers caring for preterm infants transfuse exclusively irradiated cellular products. As an alternative, pathogen-reduction technology has been documented to prevent GVHD and can substitute for irradiation. Directed donations with blood drawn from blood relatives must always be irradiated because of the risk of engraftment with transfused HLA-homozygous, haploidentical lymphocytes. Cellular blood products given as intrauterine or exchange transfusions should be irradiated, as should transfusions for patients with severe congenital immunodeficiency disorders (severe combined immunodeficiency syndrome and DiGeorge syndrome requiring heart surgery) and transfusions for recipients of hematopoietic progenitor cell transplants. Other groups who are potentially at risk but for whom no conclusive data are available include patients given T-cell antibody therapy (antithymocyte globulin or OKT3), those with organ allografts, those receiving immunosuppressive drug regimens, and those infected with HIV.

As an alternative, pathogen-reduction technology has been documented to prevent T-cell proliferation, and thus TA-GVHD may be used as a substitute for irradiation. Current practice uses **irradiation** from a cesium, cobalt, or linear acceleration source at doses ranging from 1,500 to 2,500 centigray (cGy); a maximum dose of 2,500 cGy is required to hit the center of the irradiation field and a minimum of 1,500 cGy delivered to any other portion of the cannister. All cellular blood components should be irradiated, except those frozen without a cryoprotectant agent and to be rendered as "acellular" products, such as plasma and cryoprecipitate, which do not require irradiation. Leukocyte reduction cannot be substituted for irradiation to prevent GVHD. However, as mentioned, pathogen-reduction technologies have been demonstrated to be efficacious.

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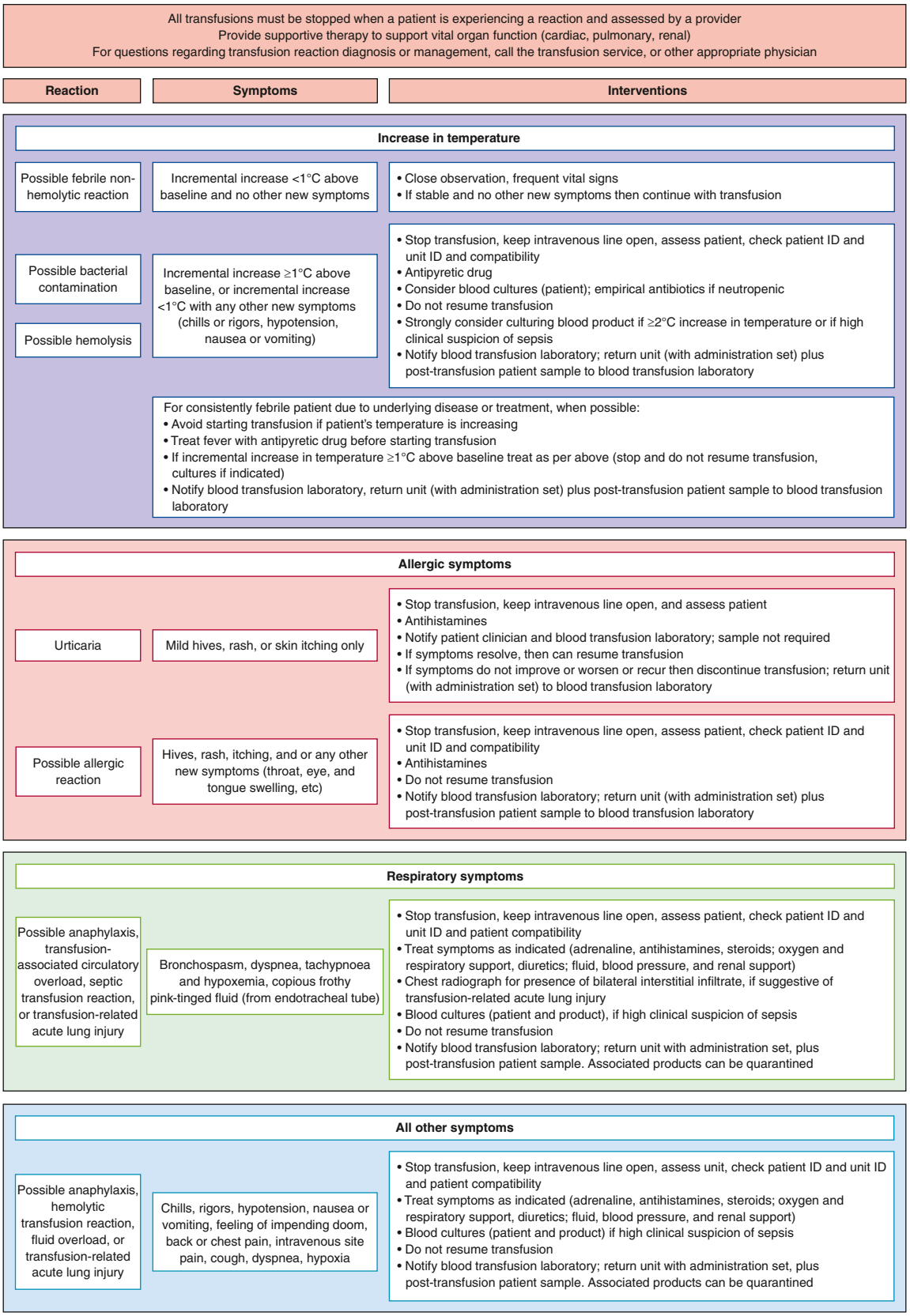

Fig. 523.2 Transfusion reaction decision tree. Algorithm to guide assessment and actions to take when a transfusion reaction is initially identified. Actions should proceed from left to right. *(From Delaney M, Wendel S, Bercovitz RS. Transfusion reactions: prevention, diagnosis, and treatment. Lancet 2016;388:2825–2836. Fig 2.)*

Section 7

Hemorrhagic and Thrombotic Diseases

Chapter 524

Hemostasis

Brian R. Branchford, Benjamin J. Samelson-Jones, Leslie J. Raffini, and Veronica H. Flood

Hemostasis is the biological process that limits hemorrhage after blood vessel injury. An initial platelet plug (primary hemostasis) is bolstered by the product of the coagulation cascade: a factor XIII-cross-linked fibrin clot (secondary hemostasis). Extraneous fibrin is lysed by plasmin (fibrinolysis), and normal blood flow is restored through the previously damaged vessel. If hemostasis is impaired, varying degrees of bleeding may occur depending on the hemostatic defect and the site of injury. Excess coagulation activity can lead to thrombotic complications. The hemostatic response therefore needs to be rapid and locally regulated, such that trauma neither results in hemorrhage nor triggers a systemic thrombotic reaction. When a platelet adheres to a site of vascular injury, the activated platelet surface provides a phospholipid reaction surface where clotting factors bind and create a "thrombin burst." Active clotting is controlled by negative feedback loops that inhibit the clotting process when procoagulant products come in contact with intact endothelium. The main components of the hemostatic process are the **vessel wall, platelets, coagulation factors, anticoagulant proteins,** and **fibrinolytic system.** Many components of the hemostasis system are multifunctional. Fibrinogen serves as the ligand between platelets during platelet aggregation and as the substrate for thrombin to produce fibrin. Platelets provide the reaction surface on which clotting reactions occur, form the plug at the site of vessel injury, release procoagulant compounds from their granules, and contract to constrict and limit clot size.

THE HEMOSTATIC PROCESS

The intact vascular endothelium is the primary barrier against hemorrhage and thrombosis. The endothelial cells that line the vessel wall normally inhibit coagulation and provide a smooth surface that permits rapid blood flow.

After vascular injury, vasoconstriction occurs, and flowing blood comes in contact with the subendothelial matrix. **Von Willebrand factor (VWF)** tethers platelets to the site of injury by binding subendothelial matrix components such as collagen and platelets, via the glycoprotein Ib complex on platelet membranes. Platelet binding of VWF initiates complex cellular signaling, activating the platelets and triggering secretion of storage granules containing adenosine diphosphate (ADP), serotonin, and stored plasma and platelet membrane proteins. On activation, the platelet receptor for fibrinogen, $\alpha IIb\beta_3$, is switched on ("inside out" signaling) to bind fibrinogen and triggers the aggregation and recruitment of other platelets to form the platelet plug. Multiple physiologic agonists can trigger platelet activation and aggregation, including ADP, collagen, thrombin, and arachidonic acid. Aggregation involves the interaction of specific receptors on the platelet surface with plasma hemostatic proteins, primarily fibrinogen.

One of the subendothelial matrix proteins that is exposed after vascular injury is *tissue factor*. Exposed tissue factor binds to factor VII and activates the *clotting cascade* (Fig. 524.1). The activated clotting factor then initiates the activation of the next sequential clotting factor in a systematic manner. During the process of platelet activation, internalized platelet phospholipids (primarily phosphatidylserine) become externalized supporting membrane binding of the principal enzyme complexes of coagulation: the extrinsic and intrinsic Xase complexes and the prothrombinase complex. These complexes are localized to the platelet surface and bring together the active proteolytic enzyme, an activated cofactor, and the zymogen substrate that will form the next active enzyme in the cascade. This sequence of the coagulation cascade serves as a biochemical amplifier where a small amount of activated proteolytic enzyme can rapidly produce a large amount of downstream components, in a temporally and spatially controlled manner. In vivo, small amounts of activated factor VII (VIIa) are present, so the system is always poised to act. Near the bottom of the cascade, the multipotent enzyme *thrombin* is formed. Thrombin converts fibrinogen into fibrin, activates factors V, VIII, and XI, and aggregates platelets. Activation of factor XI by thrombin amplifies further thrombin generation and contributes to inhibition of fibrinolysis. Thrombin also activates factor XIII. The stable fibrin-platelet plug is ultimately formed through clot retraction and cross linking of the fibrin clot by factor XIIIa.

Virtually all procoagulant proteins are balanced by anticoagulant proteins that regulate or inhibit procoagulant function. Four clinically important, naturally occurring anticoagulants regulate the extension of the clotting process: antithrombin **(AT)**, protein C, protein S, and tissue factor pathway inhibitor **(TFPI)**. AT is a serine protease inhibitor (SERPIN) that regulates primarily factor Xa and thrombin and to a lesser extent, factors IXa, XIa, and XIIa. When thrombin encounters intact endothelium, thrombin binds to *thrombomodulin*, its endothelial receptor. The thrombin-thrombomodulin complex then converts **protein C** into activated protein C. In the presence of the cofactor **protein S**, activated protein C proteolyzes and inactivates factor Va and factor VIIIa. Inactivated factor Va is also a functional anticoagulant that inhibits clotting. TFPI limits activation of factor X by factor VIIa and tissue factor and shifts the activation site of tissue factor and factor VIIa to that of factor IX (Fig. 524.2).

Once a stable fibrin-platelet plug is formed, the fibrinolytic system limits its extension and proteolyzes the clot **(fibrinolysis)** to reestablish vascular integrity. Plasmin, generated from plasminogen by either urokinase-like or tissue-type plasminogen activator, degrades the fibrin clot. In the process of dissolving the fibrin clot, fibrin degradation products are produced. The fibrinolytic pathway is regulated by plasminogen activator inhibitors and α_2-antiplasmin, as well as by the thrombin-activatable fibrinolysis inhibitor (TAFI).

PATHOLOGY

Congenital deficiency of an individual procoagulant protein leads to a bleeding disorder, whereas deficiency of an anticoagulant (clotting factor inhibitor) predisposes the patient to thrombosis. In acquired hemostatic disorders, there are frequently multiple problems with homeostasis that perturb and dysregulate hemostasis. A primary illness (sepsis) and its secondary effects (shock and acidosis) activate coagulation and fibrinolysis and impair the host's ability to restore normal hemostatic function. When sepsis triggers **disseminated intravascular coagulation,** platelets, procoagulant clotting factors, and anticoagulant proteins are consumed, leaving the hemostatic system unbalanced and prone to bleeding or clotting (see Chapter 532). Similarly, newborn infants and patients with severe liver disease have synthetic deficiencies of both procoagulant and anticoagulant proteins. Such dysregulation causes the patient to be predisposed to both hemorrhage and thrombosis with mild or moderate triggers that result in major alterations in the hemostatic process.

In the laboratory evaluation of hemostasis, parameters are manipulated to allow assessment of isolated aspects of hemostasis and limit the multifunctionality of some of its components. The coagulation process is studied in plasma anticoagulated with citrate to bind calcium, with added phospholipid to mimic the reaction surface normally provided by the platelet membrane and with a stimulus to trigger clotting. Calcium is added to restart the clotting process. This process does not necessarily reflect the in vivo pathways of clot formation.

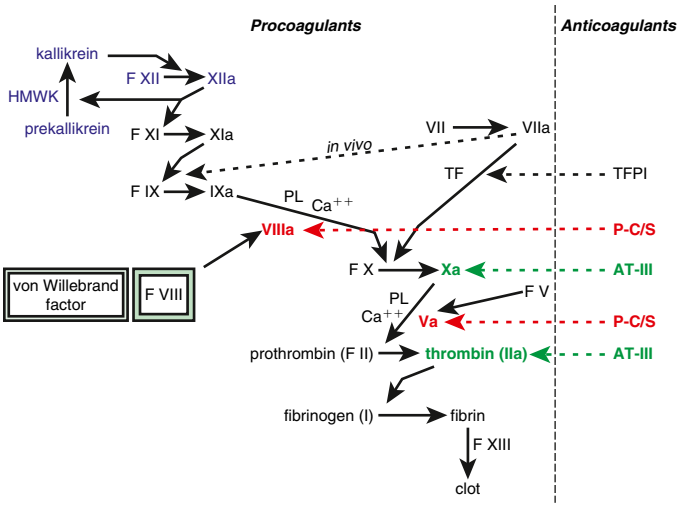

Fig. 524.1 The clotting cascade, with sequential activation and amplification of clot formation. Many of the factors (F) are activated by the clotting factors shown above them in the cascade. The activated factors are designated by the addition of an *a*. On the *right side*, the major anticoagulants and the sites that they regulate are shown. Tissue factor pathway inhibitor (TFPI) regulates tissue factor (TF); factor VIIa, protein C, and protein S (P-C/S) regulate factors VIII and V; and antithrombin III (AT-III) regulates factor Xa and thrombin (factor IIa). The *dotted line* shows that, in vivo, TF and factor VIIa activate both factors IX and X, but that, in vitro, only the activation of factor X is measured. Unactivated factor VIII, when bound to its carrier protein, von Willebrand factor (VWF), is protected from protein C inactivation. When thrombin, or factor Xa activates factor VIII, it becomes unbound from VWF, at which point it can participate with factor IXa in the activation of factor X in the presence of phospholipid (PL) and Ca^{++} (the "tenase" complex). Factor XIIIa cross-links the fibrin clot and thereby makes it more stable. Prekallikrein, high-molecular-weight kininogen (HMWK), and factor XII are shown in *purple* because they do not have a physiologic role in coagulation, although they contribute to the clotting time in partial thromboplastin time (PTT).

524.1 Clinical and Laboratory Evaluation of Hemostasis

Brian R. Branchford, Benjamin J. Samelson-Jones, Leslie J. Raffini, and Veronica H. Flood

CLINICAL HISTORY

For most hemostatic disorders, the clinical history provides the most useful information. To evaluate for a bleeding disorder, the history should determine the sites of bleeding, the severity and duration of hemorrhage, and the age at onset. Was the bleeding spontaneous, or did it occur after trauma? Was there a previous personal or family history of similar problems? Did the symptoms correlate with the degree of injury or trauma? Does bruising occur spontaneously? Are there lumps with bruises for which there is minimal trauma? If the patient had previous surgery or significant dental procedures, was there any increased bleeding? If a child or adolescent has had surgery that affects the mucosal surfaces, such as a tonsillectomy or major dental extractions, the absence of bleeding usually rules out a hereditary bleeding disorder. Delayed or slow healing of superficial injuries may suggest a hereditary bleeding disorder. In postpubertal females, it is important to take a careful menstrual history. Because some common bleeding disorders, such as **von Willebrand disease (VWD)**, have a fairly high prevalence, mothers and family members may have the same mild bleeding disorder and may not be cognizant that the child's menstrual history is abnormal. Females with mild VWD who have a moderate history of bruising frequently have a reduction in symptoms during pregnancy or after administration of oral contraceptives. Some medications, such as aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs), inhibit platelet function and increase bleeding symptoms in patients with a low platelet count or abnormal hemostasis. Standardized bleeding scores have been developed but have not been widely adopted in pediatrics.

Outside the neonatal period, thrombotic disorders are relatively rare until adulthood. In the neonate, physiologic deficiencies of procoagulants and anticoagulants place the hemostatic mechanism at greater risk for imbalance, and clinical events can lead to either hemorrhage or thrombosis. If a child or teenager presents with deep vein thrombosis

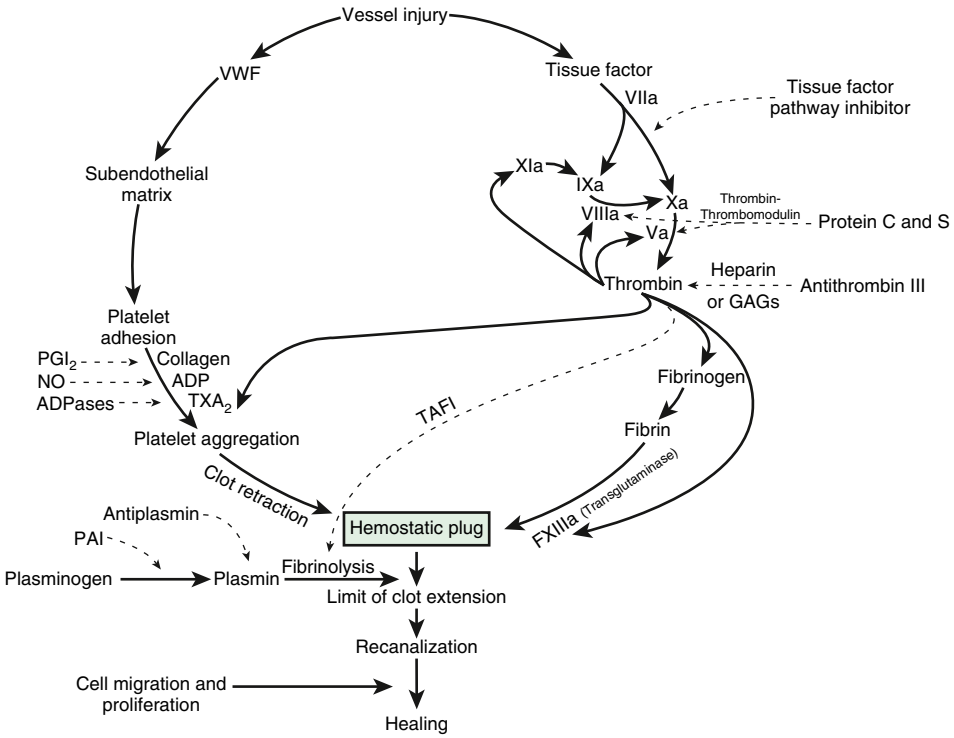

Fig. 524.2 The hemostatic mechanism. ADP, Adenosine diphosphate; GAGs, glycosaminoglycans; NO, nitric oxide; PGI_2, prostacyclin (prostaglandin I_2); PAI, plasminogen activator inhibitor; TAFI, thrombin-activated fibrinolytic inhibitor; TXA_2, thromboxane A_2; VWF, von Willebrand factor.

(DVT) or pulmonary embolism (PE), a detailed family history must be obtained to evaluate for DVT, PE, myocardial infarction (MI), or cerebrovascular accident (stroke) in other family members. The presence of **thrombosis**, especially in the absence of a provoking agent in the child or teenager, should induce the clinician to take a careful family history and consider an evaluation for a hereditary or acquired predisposition to thrombosis.

PHYSICAL EXAMINATION

The physical examination should focus on whether bleeding symptoms are associated primarily with the mucous membranes or skin (*mucocutaneous* bleeding) or with the muscles and joints (*deep* bleeding). The examination should determine the presence of petechiae, ecchymoses, hematomas, hemarthroses, or mucous membrane bleeding. Patients with defects in platelet–blood vessel wall interaction (VWD or platelet function defects) usually have mucocutaneous bleeding, which may include epistaxis, menorrhagia, petechiae, ecchymoses, occasional hematomas, and less frequently, hematuria and gastrointestinal bleeding. Individuals with a clotting deficiency of factor VIII or IX (hemophilia A or B) have symptoms of deep bleeding into muscles and joints, with much more extensive ecchymoses and hematoma formation. Patients with mild VWD or other mild bleeding disorders may have no abnormal findings on physical examination. Individuals with disorders of the collagen matrix and vessel wall may have loose joints and lax skin associated with easy bruising **(Ehlers-Danlos syndrome)**.

Patients undergoing evaluation for thrombotic disorders should be asked about swollen, warm, tender extremities (venous thrombosis), unexplained dyspnea or persistent "pneumonia," especially in the absence of fever (PE), and skin changes suggestive of chronic thrombosis (dilated collateral veins). Arterial thrombi usually cause an acute, dramatic impairment of organ function, such as stroke, MI, or a painful, white, cold extremity (Table 524.1).

LABORATORY TESTS

In patients who have a positive bleeding history or who are actively hemorrhaging, a platelet count, prothrombin time (PT), and partial thromboplastin time (PTT) should be performed as screening tests (Fig. 524.3). In individuals with abnormal screening tests, further evaluation should be based on those results (Table 524.2). In a patient with an abnormal bleeding history and a positive family history, normal screening tests should not preclude further laboratory evaluation, which may include a thrombin time, VWF testing, and platelet function studies. Historically, bleeding time and platelet function analysis **(PFA-100)** have been used as screening tests, but neither has proved to be useful in diagnosis of mild bleeding disorders.

There are no useful routine screening tests for *hereditary* thrombotic disorders. If the family history is positive or clinical thrombosis is unexplained, specific thrombophilia assays should be performed. Thrombosis is rare in children, and when it is present, the possibility of a hereditary predisposition should be considered (see Chapter 527).

Platelet Count

Platelet count is essential in the evaluation of the child with a positive bleeding history because thrombocytopenia is the most common acquired cause of a bleeding diathesis in children. Patients with a platelet count of $>50 \times 10^9$/L rarely have significant clinical bleeding. **Thrombocytosis** in children is usually reactive and is not associated with bleeding complications. Persistent, severe thrombocytosis in the absence of an underlying illness may require evaluation for the very rare pediatric presentation of essential thrombocythemia or polycythemia vera.

Prothrombin Time and Activated Partial Thromboplastin Time

Because clotting (coagulation) factors were named in the order of discovery, they do not necessarily reflect the sequential order of activation (Table 524.3; see also Table 524.2). Only two factors have commonly used names: fibrinogen (factor I) and prothrombin (factor II). The dual mechanisms of activating clotting have been termed the **intrinsic** (surface activation) and **extrinsic** (tissue factor–mediated) pathways. PT measures the activation of clotting by tissue factor (thromboplastin) in the presence of calcium. The tissue factor–factor VIIa complex activates factor X. Whether factor X is activated by the intrinsic or the extrinsic pathway, factor Xa on the platelet phospholipid surface complexes with factor Va and calcium (the "prothrombinase" complex) to activate prothrombin to thrombin (also referred to as *factor IIa*). Once thrombin is generated, fibrinogen is converted to a fibrin clot, the endpoint of the reaction (see Fig. 524.2). PT is not prolonged with deficiencies of factors VIII, IX, XI, and XII. In most laboratories, the normal PT value is 10-13 seconds. PT has been standardized using the international normalized ratio **(INR)** so that values can be compared from one laboratory or instrument to another. This ratio is used to determine similar degrees of anticoagulation with vitamin K antagonists, such as warfarin.

Partial Thromboplastin Time

The intrinsic pathway involves the initial activation of factor XII, which is accelerated by two other plasma proteins, prekallikrein and high-molecular-weight kininogen. In the clinical laboratory, factor XII is activated using a surface (silica or glass) or a contact activator, such as ellagic acid. Factor XIIa, in turn, activates factor XI to factor XIa, which then catalyzes factor IX to factor IXa. On the platelet phospholipid surface, factor IXa complexes with factor VIIIa and calcium to activate factor X ("tenase" complex).

This process is accelerated by interaction with phospholipid and calcium at the steps involving factors V and VIII. An isolated deficiency of

Table 524.1 Signs and Symptoms of Thrombosis

LOCATION OF CLOT	PRESENTING SYMPTOMS	RADIOGRAPHIC DIAGNOSIS
Venous thrombus in limbs	Swelling, pain, and/or redness	Venous ultrasonography with Doppler
Arterial thrombus in limbs	Cool limbs, diminished or absent pulse	Arterial ultrasonography with Doppler
Cerebral venous sinus thrombosis	Headache, nausea and/or vomiting, lethargy, change in mental status	CT venography or MR venography of head/brain
Pulmonary embolism	Chest pain, shortness of breath, pleuritis	CT angiography
Thrombus in the heart	Chest pain, shortness of breath, pleuritis	Echocardiogram
Portal vein thrombosis	Abdominal pain, nausea and/or vomiting, anorexia	Right upper quadrant ultrasonography with Doppler
Renal vein thrombosis	Hematuria, abdominal mass, abdominal pain, thrombocytopenia	Renal ultrasonography with Doppler or CT abdomen/pelvis with IV contrast

CT, Computed tomography; IV, intravenous; MR, magnetic resonance.
Modified from Han H, Hensch L, Hui SKR, Teruya J. Evaluation and management of coagulopathies and thrombophilias in pediatric patients. *Clin Lab Med.* 2021;41:83–100. Table 5.

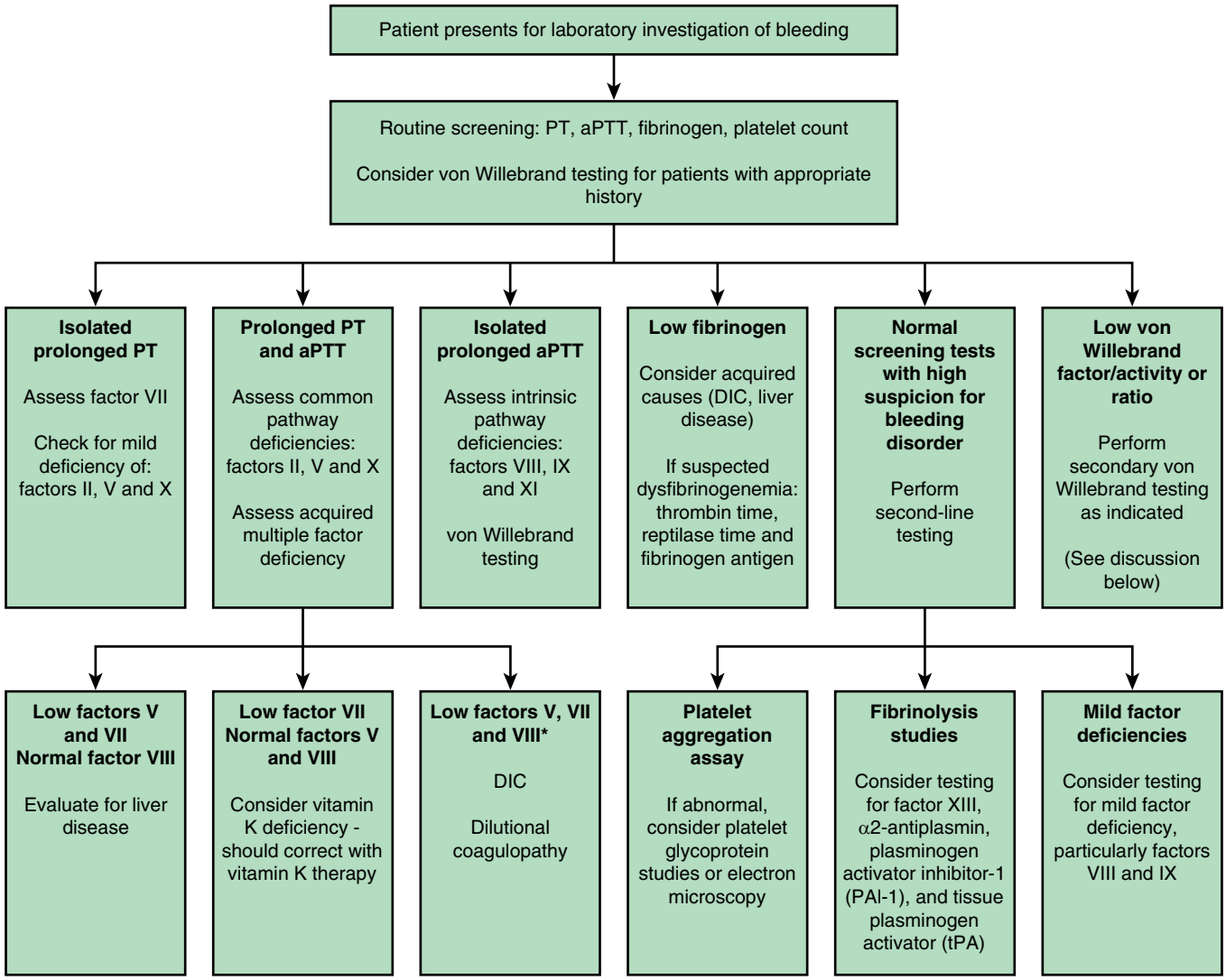

Fig. 524.3 Testing algorithm based on the screening tests (PT, aPTT, fibrinogen, and platelet count). DIC, disseminated intravascular coagulation. *Note: Factor VIII can be normal or increased in these settings secondary to the acute phase response. *(From Han H, Hensch L, Hui SKR, Teruya J. Evaluation and management of coagulopathies and thrombophilias in pediatric patients. Clin Lab Med. 2021;41:83–100. Fig. 1.)*

a single clotting factor may result in isolated prolongation of PT, PTT, or both, depending on the location of the factor in the clotting cascade. This approach is useful in determining hereditary clotting factor deficiencies; however, in acquired hemostatic disorders encountered in clinical practice, more than one clotting factor is frequently deficient, so the relative prolongation of PT and PTT must be assessed.

Measurement of PTT as performed in the clinical laboratory is actually "activated" PTT; most refer to it as **PTT**. This test measures the initiation of clotting at the level of factor XII through sequential steps to the final clot endpoint. It does not measure factor VII, factor XIII, or anticoagulants. PTT uses a contact activator (silica, kaolin, or ellagic acid) in the presence of calcium and phospholipid. Because of differences in reagents and laboratory instruments, the normal range for PTT varies among hospital laboratories. Normal ranges for PTT have much more interlaboratory variability than those for PT.

Thus the mechanisms studied by PT and PTT allow the evaluation of clotting factor deficiencies, even though these pathways may not be the same as those occurring physiologically. The PTT can be prolonged by deficiencies of factor XII, prekallikrein, and high-molecular-weight kininogen, yet these deficiencies do not result in bleeding,

Thrombin Time

Thrombin time (TT) measures the final step in the clotting cascade, in which fibrinogen is converted to fibrin. The normal TT varies between laboratories but is usually 11-15 seconds. Prolongation of TT occurs with reduced fibrinogen levels (*hypofibrinogenemia* or *afibrinogenemia*), with dysfunctional fibrinogen *(dysfibrinogenemia)*, or in the presence of substances that interfere with fibrin polymerization, such as heparin and fibrin split products. If heparin contamination is a potential cause of prolonged TT, a reptilase time can be ordered.

Reptilase Time

Reptilase time uses snake venom to clot fibrinogen. Unlike thrombin time, reptilase time is not sensitive to heparin and is prolonged only by reduced or dysfunctional fibrinogen and fibrin split products. Therefore, if TT is prolonged but reptilase time is normal, the prolonged TT is caused by heparin and does not indicate the presence of fibrin split products or reduced concentration or function of fibrinogen.

Mixing Studies

If there is unexplained prolongation of PT or PTT, a mixing study is usually performed. Normal plasma is added to the patient's plasma, and the PT or PTT is repeated. Correction of PT or PTT by 1:1 mixing with normal plasma suggests deficiency of a clotting factor because a 50% level of individual clotting proteins is sufficient to produce normal PT or PTT. If the clotting time is not corrected or only partially corrected, an **inhibitor** is usually present. An inhibitor of clotting may be either a chemical similar to heparin that delays coagulation or an

Table 524.2 Causes of Bleeding Associated with Prolonged Prothrombin Time and Activated Partial Thromboplastin Time

ISOLATED PROLONGED PT	ISOLATED PROLONGED aPTT	PROLONGED PT AND aPTT	CAUSES OF BLEEDING WITHOUT PT/aPTT PROLONGATION
FVII deficiency	Hemophilia A (FVIII deficiency)	Disseminated intravascular coagulation	FXIII deficiency
Mild common pathway factor deficiency (II, V, and X), fibrinogen deficiency	Hemophilia B (FIX deficiency)	Dilutional coagulopathy	von Willebrand disease
Early vitamin K deficiency	Hemophilia C (FXI deficiency)	Liver synthetic dysfunction	Platelet dysfunction
Early liver synthetic dysfunction	von Willebrand disease	Marked vitamin K deficiency	Mild deficiency of FVIII, FIX, and FXI
Direct Xa inhibitor	Acquired FVIII, FIX, or FXI inhibitors	Common pathway factor deficiency (FII, FV, and FX), severe fibrinogen deficiency	α_2-antiplasmin deficiency and other causes of hyperfibrinolysis
—	Heparin	Warfarin or direct thrombin inhibitor, DOAC (not always)	Collagen vascular diseases (i.e., Ehlers-Danlos syndrome)
—	—	Increased tissue factor pathway inhibitor, FV Amsterdam or East Texas bleeding disorder	DOAC

DOAC, Direct oral anticoagulant.
From Han H, Hensch L, Hui SKR, Teruya J. Evaluation and management of coagulopathies and thrombophilias in pediatric patients. *Clin Lab Med*. 2021;41:83–100. Table 1.

Table 524.3 Coagulation (Clotting) Factors

CLOTTING FACTOR	SYNONYM	DISORDER
I	Fibrinogen	Congenital deficiency (afibrinogenemia) or dysfunction (dysfibrinogenemia)
II	Prothrombin	Congenital deficiency or dysfunction
V	Labile factor, proaccelerin	Congenital deficiency (parahemophilia)
VII	Stable factor or proconvertin	Congenital deficiency
VIII	Antihemophilic factor	Congenital deficiency is hemophilia A (classic hemophilia)
IX	Christmas factor	Congenital deficiency is hemophilia B (sometimes referred to as Christmas disease)
X	Stuart-Prower factor	Congenital deficiency
XI	Plasma thromboplastin antecedent	Congenital deficiency (sometimes referred to as hemophilia C)
XII	Hageman factor	Congenital deficiency is not associated with clinical symptoms
XIII	Fibrin-stabilizing factor	Congenital deficiency

antibody directed against a specific clotting factor or the phospholipid used in clotting tests. In the inpatient setting the most common cause of a prolonged PTT is **heparin contamination** of the sample. The presence of heparin in the sample can be ruled in or out by adding *heparinase* to the sample and repeating the PTT. If the mixing study is not corrected or if its result becomes more prolonged and the patient has clinical bleeding, an inhibitor of a specific clotting factor (antibody directed against the factor), most often factor VIII, factor IX, or factor XI, may be present. If the patient has no bleeding symptoms and both PTT and the mixing study are prolonged, a **lupus-like anticoagulant** (see Chapter 531) is often present. Patients with these findings usually have a long PTT but do not bleed.

Platelet Aggregation

When a qualitative platelet function defect is suspected, platelet aggregation testing is usually ordered. Platelet-rich plasma from the patient is activated with a series of agonists (ADP, epinephrine, collagen, thrombin or thrombin-receptor peptide, and ristocetin). Some platelet aggregometers measure specific adenosine triphosphate release from the platelets, as measured by the generation of luminescence via lumi-aggregometry, and are more sensitive in detecting abnormalities of the platelet release reaction from storage granules. Repeat testing or testing of other symptomatic family members can help to determine the hereditary nature of the defect. Many medications, especially aspirin, other NSAIDs, and valproic acid, alter platelet function testing. Figure 524.1 provides an approach to the differential diagnosis of many common bleeding disorders based on screening tests.

Factor XIII

The PT and PTT reflect only fibrin formation and do not measure cross-linking by factor XIII. Therefore specific testing is required to identify factor XIII deficiency. Clot solubility testing can be performed but only identifies the most severely affected individuals. Quantitative measurement of factor XIII levels can also be performed.

Testing for Thrombotic Predisposition

Hereditary predisposition to thrombosis is associated with a reduction of anticoagulant function (protein C, protein S, AT III); the presence of

Table 524.4 Reference Values for Coagulation Tests in Healthy Children

PARAMETER	15 D-4 WK	1-5 MO	6-11 MO	1-5 YR	6-10 YR	11-17 YR	ADULT RANGES
SCREENING TESTS							
PT (sec)	11.2 (9.5-12.6)	11.0 (9.7-12.8)	11.0 (9.8-13.0)	11.3 (9.9-13.4)	11.7 (10.0-14.6)	11.8 (10.0-14.1)	10.9 (9.2-12.2)
PTT (sec)	35.4 (27.6-45.6)	33.5 (24.8-40.7)	32.4 (25.1-40.7)	31.6 (24.0-39.2)	31.6 (26.9-38.7)	31.0 (24.6-38.4)	31.7 (27.8-36.3)
COAGULATION PROTEINS							
Fibrinogen (g/L)	2.54 (1.43-4.02)	2.26 (1.50-3.76)	2.33 (1.57-3.60)	2.73 (1.88-4.13)	2.78 (1.89-4.75)	2.66 (1.77-4.20)	2.75 (2.17-3.42)
FII (IU/mL)	56 (45-74)	75 (47-111)	92 (74-117)	99 (49-130)	90 (68-132)	94 (48-119)	101 (75-132)
FV (IU/mL)	100 (69-124)	100 (60-147)	102 (59-160)	111 (73-188)	101 (82-141)	97 (62-125)	99 (61-142)
FVII (IU/mL)	76 (55-108)	88 (43-141)	88 (52-128)	82 (48-124)	77 (55-135)	82 (55-133)	95 (59-151)
FVIII (IU/mL)	96 (65-153)	85 (50-187)	75 (53-132)	95 (59-167)	87 (61-154)	93 (43-155)	97 (56-146)
FIX (IU/mL)	44 (30-77)	53 (29-105)	77 (51-107)	84 (53-129)	80 (55-156)	97 (60-138)	112 (70-131)
FX (IU/mL)	85 (66-92)	89 (68-122)	100 (76-134)	99 (60-153)	99 (71-162)	93 (64-131)	106 (73-150)
FXI IU/mL)	56 (33-75)	64 (28-126)	86 (61-126)	92 (58-154)	83 (32-154)	84 (55-139)	105 (49-139)
FXII (U/mL)	69 (25-81)	76 (38-137)	109 (48-156)	107 (50-175)	84 (49-154)	92 (49-154)	108 (47-157)
FXIII (IU/mL)	86 (78-100)	83 (55-133)	92 (51-137)	97 (49-137)	97 (54-142)	106 (64-133)	100 (68-138)
Von Willebrand antigen (IU/mL)	163 (46-220)	102 (36-217)	79 (48-199)	89 (41-186)	80 (45-141)	92 (56-123)	91 (43-144)
ANTICOAGULANT PROTEINS							
Antithrombin (IU/mL)	41 (33-63)	80 (29-120)	96 (63-122)	67 (61-128)	97 (64-136)	97 (64-136)	112 (83-126)
Protein C (IU/mL)	38 (30-115)	82 (28-128)	85 (44-151)	86 (61-144)	91 (39-170)	95 (66-127)	119 (69-149)
Protein S (IU/mL)	90 (29-115)	82 (33-154)	88 (52-1138)	97 (60-149)	105 (67-162)	99 (53-147)	102 (58-138)
Plasminogen (U/mL)	53 (41-83)	69 (38-110)	81 (49-126)	92 (60-178)	92 (52-158)	92 (58-131)	94 (63-135)
D-dimer (ng/mL)	530 (445-1200)	515 (90-878)	270 (133-844)	280 (88-780)	275 (60-567)	245 (69-580)	277 (109-560)

PT, Prothrombin time; PTT, partial thromboplastin time.
Data expressed as median (95% confidence interval).
Data adapted from Toulon P, Berruyer M, Brionne-François M, et al. Age dependency for coagulation parameters in paediatric populations. Results of a multicentre study aimed at defining the age-specific reference ranges. *Thromb Haemost.* 2016;116:9–16.

a factor V molecule that is resistant to inactivation by protein C (factor V Leiden); elevated levels of procoagulants (a pathogenic variant of the prothrombin gene); or a deficiency of fibrinolysis (plasminogen deficiency); and the rare metabolic disease homocystinuria (see Chapter 527).

Tests of the Fibrinolytic System

Euglobulin clot lysis time is a screening test used in some laboratories to assess fibrinolysis. More specific tests are available in most laboratories to determine the levels of plasminogen, plasminogen activator, and inhibitors of fibrinolysis. An increase in fibrinolysis may be associated with hemorrhagic symptoms, and a delay in fibrinolysis is associated with thrombosis.

DEVELOPMENTAL HEMOSTASIS

The normal newborn infant has reduced plasma concentrations of several procoagulants and anticoagulants compared to adults (see Table 524.4), and this is even more pronounced in preterm infants. During gestation, there is progressive maturation and increase of the clotting factors synthesized by the liver. The extremely premature infant has prolonged PT and PTT values, as well as a marked reduction in anticoagulant protein levels (protein C, protein S, and AT). Levels of fibrinogen, factors V and VIII, VWF, and platelets are near-normal throughout the later stages of gestation (see Chapter 142). Because protein C and protein S are physiologically reduced, factors V and VIII, which are present at normal levels at birth, are not balanced with their regulatory proteins. In contrast, the physiologic deficiency of vitamin K–dependent procoagulant proteins (factors II, VII, IX, and X) is partially balanced by the physiologic reduction of AT. The net effect is that newborns (especially premature infants) are at increased risk for complications of bleeding, clotting, or both. Establishing absolute cutoff values for normal ranges of coagulation proteins in newborn and preterm infants is challenging because of the age-dependent variation and heterogeneity among laboratory instruments and reagents. Repeat testing (or testing parents) may be necessary to confirm a diagnosis of an inherited deficiency.

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Chapter 525

Hereditary Clotting Factor Deficiencies (Bleeding Disorders)

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Inherited deficiencies in the coagulation factors responsible for forming cross-linked fibrin clots (as detailed in Chapter 524) can result in bleeding disorders. **Hemophilia A** (factor VIII deficiency) and **hemophilia B** (factor IX deficiency) are the most common and serious congenital coagulation factor deficiencies. The clinical findings in hemophilia A and hemophilia B are virtually identical. Factor XI deficiency is a rare autosomal bleeding disorder discussed in Chapter 525.2. Reduced levels of the *contact factors* (factor XII, high molecular weight kininogen, and prekallikrein) are associated with significant prolongation of *activated partial thromboplastin time* (aPTT; also referred to as **PTT**) but are not associated with clinical bleeding, as discussed in Chapter 525.3.

525.1 Factor VIII or Factor IX Deficiency (Hemophilia A or B)

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) are the most common severe inherited bleeding disorders.

PATHOPHYSIOLOGY

The activated forms of factors VIII and IX participate in the enzyme complex responsible for the sustained proteolytic activation of factor X during coagulation. Together with phospholipids and calcium, they form the intrinsic tenase (or factor X activating) complex. Hemophilia A and hemophilia B have similar clinical manifestations because, molecularly, both disorders result in a deficiency of factor X activation, which, in turn, results in insufficient thrombin generation (see Fig. 524.1 in Chapter 524 for a depiction of the in vitro clotting process as it occurs in the test tube). In hemostatically normal individuals, hemorrhage after an injury is constrained by the initial formation of the platelet plug, which is stabilized by the generation of a cross-linked fibrin clot. In people with hemophilia A or B, clot formation is delayed and ineffectual. Inadequate thrombin production leads to failure to form a tightly cross-linked fibrin to support the platelet plug. When untreated bleeding occurs in a closed space, such as a joint, bleeding may stop as the result of **tamponade**. With open wounds, in which tamponade cannot occur, even minor injuries may result in significant blood loss, with a highly friable clot forming slowly; rebleeding occurs during physiologic thrombolysis or with minimal new traumas.

CLINICAL MANIFESTATIONS

The severity of the bleeding phenotype in hemophilia A and B is strongly associated with the amount of residual factor VIII or factor IX, respectively. **Severe hemophilia** is characterized by having <1% normal factor activity, and bleeding is frequent and often spontaneous. Patients with **moderate hemophilia** have factor levels of 1–5% normal and bleed excessively with mild trauma. Individuals with **mild hemophilia** have factor levels 5–40% normal and usually only bleed abnormally after major trauma or surgery; people with mild hemophilia may go many years before the condition is diagnosed.

Because neither factor VIII nor factor IX crosses the placenta, bleeding symptoms may be present at birth. Between 1% and 4% of neonates with hemophilia have intracranial hemorrhages, mostly from birth trauma. However, about 35% and 70% of male infants with severe and nonsevere hemophilia, respectively, *do not bleed* with circumcision. Thus, in the absence of a positive family history (50% of cases), hemophilia may go undiagnosed in the newborn period. Obvious symptoms, such as easy bruising, intramuscular hematomas, and hemarthroses, begin when the child starts to cruise. Bleeding from minor traumatic lacerations of the mouth (torn frenulum) may persist for hours or days and may cause the parents to seek medical evaluation. Even in patients with severe hemophilia, only 90% have evidence of increased bleeding by a year of age.

Although bleeding may occur in any area of the body, the hallmark of hemophilia bleeding is **hemarthrosis**, which can occur spontaneously or after minor trauma. The earliest joint hemorrhages appear most often in the ankles. In the older child and adolescent, hemarthroses of the knees and elbows are also common. Whereas the child's early joint hemorrhages are recognized only after major swelling and fluid accumulation in the joint space, older children are frequently able to recognize bleeding before the physician does: patients report a warm, tingling sensation in the affected joint as the first sign of a joint hemorrhage. Repeated bleeding episodes into the same joint may result in a "target" joint, which becomes more susceptible to recurrent bleeding because of the pathologic changes (see "Chronic Complications").

Although most **muscular hemorrhages** are clinically evident because of localized pain or swelling, bleeding into the iliopsoas muscle requires specific mention. A patient may lose large volumes of blood into the iliopsoas muscle, verging on hypovolemic shock, with only a vague area of referred pain in the groin. The hip is held in a flexed, internally rotated position because of irritation of the iliopsoas. The diagnosis is made clinically from the inability to extend the hip but should be confirmed with ultrasonography, CT, or MRI (Fig. 525.1).

Life-threatening bleeding in the patient with hemophilia is caused by bleeding into vital structures (central nervous system [CNS], upper airway) or by exsanguination (external trauma, gastrointestinal [GI] or iliopsoas hemorrhage). Prompt treatment with clotting factor concentrate for these life-threatening hemorrhages is imperative. If head trauma is of sufficient concern to suggest radiologic evaluation, factor replacement should *precede* radiologic evaluation. Life-threatening hemorrhages require replacement therapy to achieve a level equal to that of normal plasma (100 IU/dL or 100%; see section below on "Treatment").

LABORATORY FINDINGS AND DIAGNOSIS

A reduced level of factor VIII or factor IX will often result in a *prolonged PTT*. In severe hemophilia, the PTT value is usually 2-3 times the upper limit of normal. However, depending on the PTT reagents, people with mild hemophilia can have a PTT in the normal range. Results of the other screening tests of the hemostatic mechanism (platelet count, bleeding time, prothrombin time [PT]) are normal. The specific activity assays for factors VIII and IX confirm the diagnosis of hemophilia. One IU of each factor activity is defined as that amount in 1 mL of normal plasma, referenced against a standard established by the World Health Organization (WHO). Thus 100 mL of normal plasma has 100 IU/dL (100% normal activity) of each factor. The term *% activity* refers to the percentage found in normal plasma (100% activity). Factor concentrates are also referenced against an international WHO standard, so treatment doses are usually referred to in IU.

In the newborn period, factor VIII activity values may be artificially elevated because of the acute-phase response elicited by the birth process. This artificial elevation may cause an infant with mild hemophilia A to have a normal or near-normal measured level. Patients with severe hemophilia A do not have detectable levels of factor VIII. In contrast, factor IX levels are physiologically low in all newborns and only reach adult values at about 6 months of age. If severe hemophilia is present in the family, an undetectable level of factor IX is diagnostic of severe hemophilia B. In some patients with mild factor IX deficiency, the presence of low factor IX activity can be confirmed only after several months of life.

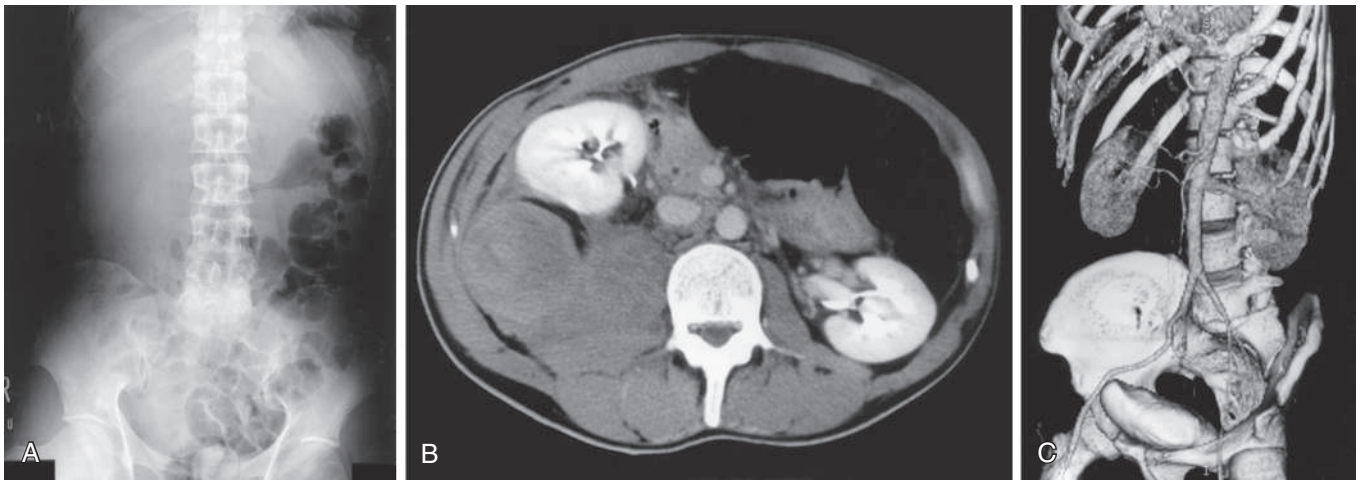

Fig. 525.1 Massive hematoma into the iliopsoas muscle in a patient with hemophilia B. A male with severe deficiency of factor IX (hemophilia B) was admitted for right lower abdominal pain of progressively increasing severity and tenderness. He had had a common cold with severe cough and loss of appetite for approximately 1 week. **A,** Abdominal radiograph shows presence of the psoas sign on the right side and left-shifted colon gas. **B,** CT scan shows massive hematoma in the right iliopsoas muscle, resulting in anterior translocation of the right kidney. **C,** Reconstructed 3-dimensional image shows more clearly the kidney translocation and the extended, but intact, large vessels. These are useful findings for the diagnostic procedures because progressive right lower abdominal pain may closely simulate acute appendicitis. The hemorrhage was successfully managed by replacement of factor IX for 1 week without any recurrence. The patient did not have any inhibitors to factor IX. *(From Miyazaki K, Higashihara M. Massive hemorrhage into the iliopsoas muscle. Intern Med. 2005;44:158–159.)*

The mixing of normal plasma with hemophilia plasma usually corrects PTT value, unless the patient has an inhibitor, as discussed later. An inhibitor titer can be quantified by the Bethesda assay, where 1 Bethesda Unit (BU) neutralizes 1 IU/mL of factor.

DIFFERENTIAL DIAGNOSIS

In young infants with severe bleeding manifestations, the differential diagnosis includes severe thrombocytopenia; severe platelet function disorders, such as Bernard-Soulier syndrome and Glanzmann thrombasthenia; type 3 (severe) von Willebrand disease; and vitamin K deficiency.

GENETICS

The genes for factor VIII and IX (*F8* and *F9*, respectively) are carried near the terminus of the long arm of the X chromosome, making hemophilia an X-linked disorder. It occurs in approximately 1/5,000 males, with 80% having factor VIII deficiency and 20% having factor IX deficiency. Hemophilia appears in all ethnic groups. A third of cases are estimated to be the result of a spontaneous pathogenic variant. Approximately 50% of cases of severe hemophilia A have a specific *F8* change, in which there is an internal inversion within the factor VIII gene (intron 22 inversion). Meanwhile, 98% of hemophilia patients have an identified genetic cause of their hemophilia, which can facilitate diagnosis, prenatal testing, and identification of female carriers.

Some **female carriers** (heterozygotes) have sufficiently low factor level to have abnormal bleeding. Factor levels should be determined in all known or potential carriers to assess the need for treatment in the event of surgery or clinical bleeding. Female carriers with factor levels <40% normal are considered to have hemophilia and should be treated as such. Notably, about 0.2% of people with severe and moderate hemophilia are female, mostly because of skewed lyonization or Turner syndrome. Female carriers with factor levels >40% normal can also have abnormal bleeding and are termed *symptomatic hemophilia carriers*. Historically, female carriers were thought to be asymptomatic, but newer data prove these individuals can also have significant bleeding.

TREATMENT

Disease-specific therapy started early in life is the hallmark of excellent hemophilia care (Table 525.1). Treatment can be divided into on-demand therapy (hemostatic interventions to stop bleeding) and prophylaxis therapy to prevent bleeding. *Prophylaxis is the standard of care for children with severe hemophilia* in countries with sufficient medical resources. Prophylaxis decreases catastrophic bleeding, prevents hemarthrosis, and preserves joint health. The types of hemostatic agents available for hemophilia A and hemophilia B are rapidly expanding, with several new classes of medications receiving regulatory approval (Table 525.2).

On-Demand Treatment

To treat acute bleeding, nothing works better than replacing the deficient coagulation factor. When mild to moderate bleeding occurs, values of factor VIII or factor IX must be raised to hemostatic levels, approximately 50% of normal activity. For life-threatening or major hemorrhages, the dose should aim to achieve levels of 100% activity. Both plasma-derived and recombinant factor VIII and factor IX concentrates are available. Replacement therapy requires intravenous administration, which patients and families often learn to do themselves. The half-lives of standard factor VIII and factor IX are 8-12 hours and 18-24 hours, respectively, although there is wide variability between patients with younger children often having shorter half-lives. There are also bioengineered factor VIII and factor IX products designed to have longer half-lives (termed *extended half-life products*), which reduce the frequency of intravenous administrations necessary to treat bleeds and provide prophylaxis.

Table 525.1 summarizes the treatment recommendations of some common types of hemorrhage in a patient with hemophilia. The calculation of the factor replacement dose is as follows, with the difference between factor VIII and factor IX being because of their different volumes of distribution:

$$\text{Factor VIII dose (IU)} = [\%\ \text{desired raise}] \times [\text{weight (kg)}] \times 0.5$$

$$\text{Factor IX dose (IU)} = [\%\ \text{desired raise}] \times [\text{weight (kg)}] \times 1.3$$

Therefore emergency dosing for factor VIII deficiency is approximately 50 units per kg and emergency dosing for factor IX deficiency is approximately 100-130 units/kg.

It is important to note that patients can have variable recoveries, and different factor products also have slightly different recoveries. Many patients will carry dosing cards detailing their recommended treatments. In general, doses are rounded up to vial size so as not to waste product.

For some patients with *mild* factor VIII deficiency, **desmopressin acetate** can be used to stimulate the cellular release of endogenously

Table 525.1 Treatment of Hemophilia *Without* Inhibitors

TYPE OF HEMORRHAGE	HEMOPHILIA A	HEMOPHILIA B
Hemarthrosis*	50-60 IU/kg factor VIII concentrate† on day 1, then 20 IU/kg the following day. Consider every other day until joint function is normal or back to baseline. Consider prophylaxis.	80-100 IU/kg factor IX concentrate‡ on day 1; then 40 IU/kg the following day. Consider every other day until joint function is normal or back to baseline. Consider prophylaxis.
Muscle or significant subcutaneous hematoma	50 IU/kg factor VIII concentrate; 20 IU/kg every other day treatment may be needed until resolved.	80 IU/kg factor IX concentrate‡; 40 IU/kg every 2-3 days may be needed until resolved.
Mouth, deciduous tooth, or tooth extraction	20 IU/kg factor VIII concentrate†; antifibrinolytic therapy§; remove loose deciduous tooth.	40 IU/kg factor IX concentrate‡; antifibrinolytic therapy§; remove loose deciduous tooth.
Epistaxis	Apply pressure for 15-20 min; pack with petrolatum gauze; give antifibrinolytic therapy§; 20 IU/kg factor VIII concentrate† if this treatment fails.‖	Apply pressure for 15-20 min; pack with petrolatum gauze; antifibrinolytic therapy§; 30 IU/kg factor IX concentrate‡ if this treatment fails.#
Major surgery, life-threatening hemorrhage	50-75 IU/kg factor VIII concentrate; then initiate 25 IU/kg q8-12h to maintain trough level >50 IU/dL for 5-7 days; then 50 IU/kg q24h to maintain trough >25 IU/dL for 7 days; monitor factor VIII levels.	80-120 IU/kg factor IX concentrate‡, then 50-60 IU/kg q12-24h to maintain factor IX at >40 IU/dL for 5-7 days, and then at >30 IU/dL for 7 days; monitor factor IX levels.
Iliopsoas hemorrhage	50 IU/kg factor VIII concentrate; then 25 IU/kg q12h until asymptomatic; then 20 IU/kg every other day, for a total of 10-14 days.**	100 IU/kg factor IX concentrate‡; then 50-60 IU/kg q12-24h to maintain factor IX at >40 IU/dL until patient is asymptomatic; then 40-50 IU every other day, for a total of 10-14 days.**
Hematuria	Bed rest; 1.5× maintenance fluids; if not controlled in 1-2 days, 20 IU/kg factor VIII concentrate; if not controlled, give prednisone (unless patient is HIV-infected).	Bed rest; 1.5× maintenance fluids; if not controlled in 1-2 days, 40 IU/kg factor IX concentrate‡; if not controlled, give prednisone (unless patient is HIV-infected).

*For hip hemarthrosis, orthopedic evaluation for possible aspiration is advisable to prevent avascular necrosis of the femoral head.
†For mild or moderate hemophilia, desmopressin 0.3 μg/kg can be used instead of factor VIII concentrate if the patient is known to respond with a hemostatic level of factor VIII; if repeated doses are given, monitor factor VIII levels for tachyphylaxis.
‡Stated doses apply for recombinant factor IX concentrate; different dosing may apply for long-acting recombinant factor IX concentrates or plasma-derived factor IX.
§Do not give antifibrinolytic therapy until 4-6 hr after a dose of prothrombin complex concentrate.
#Nonprescription coagulation-promoting products may be helpful.
**Repeat radiologic assessment should be performed before discontinuation of therapy.
HIV, Human immunodeficiency virus; IU, international units; q12-24h, every 12 to 24 hours.
Adapted from Di Paola J, Montgomery RR, Gill JC, Flood VH. Hemophilia and von Willebrand disease. In: Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015: pp. 1028–1054.

Table 525.2 Strengths and Weaknesses of the Old and New Therapeutic Options for Hemophilia

	STRENGTHS	WEAKNESSES
Standard half-life clotting factor concentrates	Effective for both bleeding control and prevention; well established safety and effectiveness profile for decades; measurable FVIII and FIX concentrations as surrogate marker of effectiveness; can result in normal concentrations of FVIII and FIX	Frequent intravenous injections; inhibitor development
Extended half-life clotting factor concentrates	Effective for both bleeding control and prevention; a reduced number of injections; higher trough concentrations; measurable FVIII and FIX concentrations as surrogate markers of effectiveness; can result in normal concentrations of FVIII and FIX	Intravenous route; inhibitor development
Nonreplacement therapies (emicizumab, fitusiran, anti-tissue factor pathway inhibitor antibodies, SerpinPC)	Subcutaneous route; infrequent injections; standard doses for all patients	Need for adjunctive hemostatic treatment; steady state of coagulation activity not within the normal range; thrombotic risk
Gene therapy	Single intravenous injection; restoration of endogenous FVIII and FIX production	Preexisting immunity against adeno-associated viral vectors; immune response against vectors and transfected cells; unknown durability of transduction; need for immunosuppressive therapy; unknown long-term safety

FVIII, factor VIII; FIX, factor IX.
From Mancuso ME, Mahlangu JN, Pipe SW. The changing treatment landscape in haemophilia: from standard half-life clotting factor concentrates to gene editing. *Lancet.* 2021;397:630–640. Table 5.

produced factor VIII to increase its plasma levels. In patients with moderate or severe factor VIII deficiency, the stored levels of factor VIII are inadequate, and desmopressin treatment is ineffective. Desmopressin can be administered intravenously or intranasally, with the latter facilitating home treatment. The intranasal formulation is a higher concentration of desmopressin than that used to treat enuresis or hypopituitarism; the dose is 150 μg (1 spray) for children weighing <50 kg and 300 μg (2 sprays) for children and young adults weighing >50 kg. Most centers administer a trial of desmopressin to determine if the factor VIII levels increase sufficiently for hemostasis. Because desmopressin triggers the release of cellularly stored factor VIII, usually it can only be given twice in a 1-2 day period. The most serious complication is hyponatremia, which can be mitigated by fluid restrictions for the 24 hours after administration. Desmopressin is not effective in the treatment of factor IX deficiency.

Mucosal bleeding often benefits from antifibrinolytic agents such as aminocaproic acid or tranexamic acid that can be given orally or intravenously.

PROPHYLAXIS

Many patients are given lifelong prophylaxis to prevent spontaneous joint bleeding. The World Federation of Hemophilia recommends prophylaxis for children with severe hemophilia and for adults with joint disease. Usually, such programs are initiated at the time of the first or second joint hemorrhage. Young children often require the insertion of a central catheter to ensure venous access for factor replacements. Such regimens are expensive but highly effective in preventing or greatly limiting the degree of joint pathology; complications include central line infection and thrombosis.

The goal of prophylaxis is generally to maintain a measurable plasma factor level (1–2%), when assayed just before the next infusion (trough level); this converts patients from severe to moderate hemophilia. For standard half-life products, this typically requires intravenous administration 2-4 times per week; this frequency is reduced with prophylaxis with extended half-life products.

Another class of hemophilia drugs that promotes hemostasis without factor VIII or factor IX is termed **nonfactor therapies**. Emicizumab is approved for prophylaxis for hemophilia A. Emicizumab is a bispecific, humanized monoclonal antibody that can bridge activated factor IX and factor X to restore factor X activation. It is administered subcutaneously and is given only 1-4 times per month after four weekly loading doses. Because emicizumab mimics some of the biochemistry of activated factor VIII, it dramatically shortens PTT times. It is not used to treat acute bleeds.

Efanesoctocog alfa, a modified extended half-life single recombinant factor VIII protein that decouples recombinant factor VIII from endogenous von Willebrand factor, has demonstrated efficacy in patients with severe hemophilia A. **Fitusiran**, a small interfering RNA molecule that reduces antithrombin synthesis, has been used successfully for prophylaxis in patients with hemophilia A or B with or without inhibitors. **Gene therapy** (valoctocogene roxaparvovec for hemophilia A; etranacogene dezaparvovec for hemophilia B) has demonstrated efficacy in reducing bleeding in adult patients with severe hemophilia.

SUPPORTIVE CARE

Effective measures to avoid trauma include anticipatory guidance, including the use of car seats, seatbelts, and bike helmets and the avoidance of high-risk behaviors. Older males should be counseled to avoid violent contact sports, but this issue is admittedly a challenge; however, active lifestyles are also encouraged to limit obesity. Early psychosocial intervention helps the family achieve a balance between overprotection and permissiveness. Patients with hemophilia should avoid aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) that affect platelet function.

Inhibitor Formation

The formation of neutralizing antibodies, termed *inhibitors*, is the major complication of factor VIII or factor IX replacement therapy. They develop in 20–30% of severe hemophilia A patients and 3–10% of severe hemophilia B patients that receive factor therapy, usually within 20 exposures to factor. Inhibitors may be identified during routine laboratory surveillance or become clinically apparent when bleeding episodes fail to respond to appropriate replacement therapy. There are both patient and product risk factors for inhibitor development, with the underlying hemophilia-causing gene pathogenic variant being the most important with the majority of people with large-gene deletions developing inhibitors. Prophylaxis with plasma-derived factor VIII products decreases the risk of inhibitor development about 1.7-fold compared to recombinant factor VIII products. Intensive early factor exposure during surgery or major trauma increases the risk of inhibitor development, likely through initiating immunologic danger signaling. Patients with inhibitors always require referral to a center that cares for many such patients and has a comprehensive hemophilia program.

Clinically, inhibitor titers are demarcated as low-titer (≤5 BU) and high-titer (>5 BU). Low-titer inhibitors can often be overcome by large amounts of factor, while high-titers necessitate the use of bypassing agents, which can circumvent the inhibitor to promote hemostasis. The two bypassing agents available that can treat acute bleeds are activated prothrombin complex concentrates and recombinant activated factor VII, both of which are administered intravenously. Prophylaxis regimens for bypassing agents have been described, but most hemophilia A patients with inhibitors currently use emicizumab prophylaxis because of its efficacy and ease of administration.

Inhibitor eradication has been prioritized, and it is still recommended since the advent of emicizumab. Low-titer or transient inhibitors often disappear with continued regular factor infusions. Higher titer inhibitors often require immune tolerance induction regimens, in which high doses of factor are administered to attempt to develop immune tolerance. This procedure is successful in about two-thirds of severe hemophilia A patients. Immune tolerance induction with factor IX products is complicated by allergic reactions and the development of nephrotic syndrome in some patients. Rituximab, corticosteroids, and other immunosuppressives have been used in patients with high-titer inhibitors in whom immune tolerance programs have failed, although the use of immunosuppressive therapy has declined with the use of emicizumab prophylaxis for factor VIII deficiency inhibitor patients.

CHRONIC COMPLICATIONS

Long-term complications of hemophilia A and B include chronic arthropathy and the risk of transfusion-transmitted infectious diseases, although both complications have been substantially mitigated with modern hemophilia care, including early initiation of prophylaxis and the use of recombinant protein products or plasma-derived products with advanced donor screening and highly active viricidal procedures.

Chronic arthropathy is the major long-term disability associated with hemophilia. The natural history of severe hemophilia without prophylaxis is one of cyclic recurrent hemorrhages into specific joints, including hemorrhages into the same (target) joint. In young children, the joint distends easily, and a large volume of blood may fill the joint until tamponade ensues or therapy intervenes. After joint hemorrhage, proteolytic enzymes are released by white blood cells into the joint space, and heme iron induces macrophage proliferation, leading to inflammation of the synovium. The synovium thickens and develops frondlike projections into the joint that are susceptible to being pinched and may induce further hemorrhage. The cartilaginous surface becomes eroded and ultimately may even expose raw bone, leaving the joint susceptible to articular fusion. In the older patient with advanced arthropathy, bleeding into the target joint, with its thickened synovium, causes severe pain, because the joint has little space to accommodate blood. Once a target joint develops, the patient is usually given short- or long-term prophylaxis to prevent progression of the arthropathy and reduce inflammation.

In the past, plasma-derived products tragically transmitted hepatitis B virus, hepatitis C virus, and HIV to large numbers of people with hemophilia. Modern plasma-derived products pose a very low risk of spreading identified bloodborne pathogens, but the risk of transmitting unidentified pathogens or emerging agents such as prions remains largely undefined.

COMPREHENSIVE CARE

Patients with hemophilia are best managed through comprehensive hemophilia care centers. Such centers are dedicated to patient and family education and the prevention and treatment of the complications of

hemophilia, including chronic joint disease and inhibitor development. Such centers involve a team that includes physicians, nurses, orthopedists, physical therapists, and psychosocial workers, all focused on hemophilia care. Education remains crucial in hemophilia care because patients who are receiving prophylaxis may be less experienced in recognizing bleeding episodes than affected children from previous eras.

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525.2 Factor XI Deficiency

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Factor XI deficiency (previously known as hemophilia C) is an autosomal disorder associated with mild to moderate bleeding symptoms. Bleeding is usually after trauma and surgery, with bleeding most commonly occurring at sites of high fibrinolytic activity, such as the oropharynx and urogenital tract. This focality is likely secondary to the role of factor XI in augmenting thrombin generation for the subsequent activation of *thrombin-activatable fibrinolysis inhibitor* (TAFI). Heavy menstrual bleeding is also common in females with factor XI deficiency. Factor XI deficiency can occur in any ethnic group but is frequently encountered in Ashkenazi Jews with a heterozygous frequency of 1 in 11 individuals. Factor XI deficiency usually (but not always) results in a prolonged PTT; specific factor XI activity assays are diagnostic.

The bleeding phenotype is poorly predicted by the measured factor XI activity. Some patients with severe deficiency (<20% normal activity) may have minimal or no symptoms, whereas other individuals with higher levels experience excessive surgical bleeding. Because spontaneous bleeding is very rare, treatment is focused on perioperative management. Although factor XI concentrates are available in other countries, in the United States, factor XI has to be replaced with fresh-frozen plasma (FFP). For minor surgeries or bleeds, antifibrinolytics such as aminocaproic acid or tranexamic acid are frequently used. For major surgeries, 15-25 mL/kg of FFP in combination with antifibrinolytics is recommended. The factor XI half-life is 50 hours.

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525.3 Deficiencies of the Contact Factors (Nonbleeding Disorders)

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Deficiency of the "contact factors"—factor XII, prekallikrein, and high molecular weight kininogen—causes a prolonged PTT but no bleeding symptoms. Because these contact factors function at the step of initiation of the intrinsic clotting system by the reagent used to determine PTT, the PTT is markedly prolonged when these factors are absent. Thus there is the paradoxical situation in which PTT is extremely prolonged with no evidence of clinical bleeding. It is important that individuals with these findings be well informed about the meaning of their clotting factor deficiency because they do not need treatment, even for major surgery.

525.4 Factor VII Deficiency

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Factor VII deficiency is a rare autosomal bleeding disorder that ranges in its presentation from mild to severe bleeding. There is a poor correlation between the clinical severity and the measured factor VII level, although major bleeding is more frequent in patients with <1% activity. Epistaxis and menorrhagia are the most common symptoms, although hemarthroses and intracranial hemorrhages also occur, with the latter reported in 1% of symptomatic cases.

Patients with this deficiency have greatly prolonged PT but normal PTT. Specific factor VII assays are diagnostic. Some recombinant factor VIIa products are approved for the treatment of factor VII deficiency at a dose of 15-30 mcg/kg, which is lower than the dose approved for hemophilia with inhibitors. The plasma half-life of factor VII is 2-6 hours and usually at least three doses at this frequency are required for major bleeding or surgery. Prophylaxis regimens with 2-3 administrations per week have been described in patients with severe bleeding.

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525.5 Factor X Deficiency

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Factor X deficiency is a rare autosomal recessive disorder with variable severity. Mild deficiency results in mucocutaneous and posttraumatic bleeding, whereas severe deficiency (<1–10% normal activity) is associated with joint, GI, and intracranial hemorrhages. Intracranial and umbilical bleeding can be the presenting symptom for neonates. Heterozygous carriers usually have activity >50% normal and are asymptomatic.

A reduced factor X level is associated with prolongation of both PT and PTT; factor X specific assays are diagnostic. Replacement treatment is with FFP or prothrombin complex concentrate (PCC) or plasma-derived factor X concentrates. The plasma half-life of factor X is approximately 40-60 hours, and prophylaxis regimens with PCC or factor X concentrates administered 1-2 times per week are described for patients with severe bleeding.

Although it is rarely a problem in pediatric patients, about 10% of cases of **light-chain amyloidosis** are associated with factor X deficiency, resulting from the adsorption of factor X on the amyloid protein. In the setting of amyloidosis, replacement therapy often is not successful because of the rapid clearance of factor X.

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525.6 Prothrombin (Factor II) Deficiency

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Prothrombin (factor II) deficiency is caused either by a markedly reduced prothrombin level (hypoprothrombinemia) or by functionally abnormal prothrombin (dysprothrombinemia). Laboratory testing in homozygous patients shows prolonged PT and PTT; specific prothrombin assays are diagnostic. Mucocutaneous bleeding in infancy and posttraumatic bleeding later are common. Patients are treated with either FFP or PCC at doses of 15-25 mL/kg or 20-40 U/kg, respectively. The half-life of prothrombin is 3-4 days, and once weekly prophylaxis regimens with PCC are reported.

Acquired factor II deficiency can be seen with a small percentage of patients with a lupus anticoagulant and is usually associated with significant bleeding. It is distinguished from inherited prothrombin deficiency by clinical history and the laboratory identification of antiphospholipid antibodies and abnormal PTT mixing studies.

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525.7 Factor V Deficiency

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Deficiency of factor V is an autosomal recessive, mild to moderate bleeding disorder. Factor V circulates with 80% in plasma and 20% in platelet α-granules; this platelet factor V pool has been suggested to ameliorate the bleeding that might be expected from low plasma factor V levels. The most common symptoms are epistaxis, oral cavity bleeding, heavy menstrual periods, and postoperative bleeding; joint, muscle, and intracranial hemorrhages occur rarely.

Laboratory evaluation shows prolonged PT and PTT. Specific assays for factor V show a reduction in factor V levels. FFP is the only currently available therapeutic product that contains factor V. Factor V is lost rapidly from stored FFP. Treatment for major surgery or bleeding is usually 15-25 mL/kg FFP followed by additional doses of 10 mL/kg every 12 hours. Twice weekly FFP prophylaxis regimens are sometimes used for patients with severe bleeding. Platelet transfusions are also an option for acute or prophylactic treatment. Rarely, a patient with a negative family history of bleeding has an *acquired antibody* to factor V, which can be identified in laboratory mixing studies.

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525.8 Combined Deficiency of Factors V and VIII

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Combined deficiency of factors V and VIII is an autosomal recessive bleeding disorder that occurs secondary to the absence of intracellular transporters responsible for the shuttling of factors V and VIII from the endoplasmic reticulum to the Golgi compartments, as the result of pathogenic variants in the genes *LMAN1* or *MCFD2*. Factor V and VIII levels are usually between 5–20% of normal. Bleeding symptoms are often mild and most commonly mucocutaneous, traumatic, surgical bleeding. Treatment for major bleeding or surgery is 15-25 mL/kg of FFP.

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525.9 Congenital Fibrinogen Disorders

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Congenital fibrinogen disorders are a heterogenous group of rare autosomal recessive or dominant diseases because of qualitative **(dysfibrinogenemia)** or quantitative (**hypofibrinogenemia** or **afibrinogenemia**) reductions in functional fibrinogen. Fibrinogen cleavage by thrombin into fibrin is the final step of the coagulation cascade: polymerization follows cleavage and results in fibrin clot formation. Fibrinogen is also the major integrin ligand during platelet aggregation. Conversely, fibrin also sequesters thrombin to the site of clot formation and supports fibrinolysis by enhancing the activation of plasminogen. The multiple roles of fibrinogen and fibrin in hemostasis underlie the phenotypes of inherited fibrinogen disorders, which can have bleeding and clotting clinical manifestations, sometimes in the same patient. The most common bleeding symptoms are mucocutaneous, soft-tissue, joint, genitourinary, traumatic, surgical bleeding, and heavy menstrual periods. Both venous and arterial thrombosis are rare in the quantitative defects but occur in about 20% of cases of dysfibrinogenemia. Some congenital fibrinogen disorders also have nonhematologic manifestations, including liver disease due to retention of misfolded protein, poor wound healing, and splenic rupture.

Diagnosis is based on a decreased fibrinogen activity. Patients usually will also have a prolonged PT and PTT. Dysfibrinogenemia is defined as a decreased ratio of fibrinogen activity compared to fibrinogen antigen. Treatment for bleeding or major surgery is with FFP, cryoprecipitate, or fibrinogen concentrates, depending on local availability. Because of the variable clinical manifestations, including both bleeding and clotting complications, treatment is tailored to the personal and family history. The half-life of fibrinogen is 2-4 days.

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525.10 Factor XIII Deficiency (Fibrin-Stabilizing Factor or Transglutaminase Deficiency)

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Factor XIII deficiency is a rare autosomal bleeding disorder. Factor XIII is a heterotetramer composed of two catalytic A subunits and two carrier B subunits; activation of factor XIII by thrombin frees the activated A subunits, which covalently crosslink fibrin to increase clot strength and constrain fibrinolysis. The different biochemical roles of the A and B subunits result in distinct clinical manifestations. Inherited deficiencies in the factor XIII A-subunit results in soft tissue, umbilical, surgical, joint, and intracranial bleeding; the latter occurs in about a third of cases. Deficiencies in the B-subunit are associated with mild mucocutaneous and surgical bleeding. Because of its role in stabilizing the fibrin clot, factor XIII deficiency often presents with *delayed* abnormal bleeding or bruising symptoms where patients may have mild trauma and then present with an excessive hematoma on the following day. Extrahematologic clinical manifestation of factor XIII deficiency includes delayed umbilical cord separation, poor wound healing, and recurrent miscarriages.

Unlike the other severe inherited coagulation deficiencies, factor XIII deficiency will have normal PTT and PT. Specific activity assays are diagnostic and should be considered in infants with delayed umbilical cord separation, delayed bleeding symptoms, or unexplained intracranial hemorrhage. Treatment for bleeding or major surgery is 20-40 U/kg of factor XIII concentrate. Recombinant A-subunit protein is also available for A-subunit deficiencies. If specific factor XIII replacement therapies are not available, cryoprecipitate is preferred over FFP because of the substantially higher factor XIII content. The half-life of factor XIII is 9-12 days, which facilitates prophylaxis. Because of the high prevalence of intracranial hemorrhage, prophylaxis of 20-40 U/kg factor XIII concentrate every 4 weeks is recommended for patients with <10% normal activity. *Autoimmune acquired* factor XIII deficiency can be differentiated using specific mixing studies or activity assays of platelet lysates.

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525.11 Antiplasmin or Plasminogen Activator Inhibitor Deficiency

Benjamin J. Samelson-Jones, Brian R. Branchford, and Veronica H. Flood

Deficiency of either antiplasmin or plasminogen activator inhibitor, both of which are antifibrinolytic proteins, results in increased plasmin generation and premature lysis of fibrin clots. Affected patients have a mild-to-moderate bleeding disorder characterized by delayed bleeding symptoms, typically associated with trauma or surgery. Mucocutaneous bleeding and heavy menstrual bleeding are the most described symptoms, with rare reports of joint or intracranial hemorrhages.

Because results of the usual hemostatic tests are normal, further workup of a patient with a positive bleeding history (especially delayed bleeding) should include *euglobulin clot lysis time*, which measures fibrinolytic activity and yields a shortened result in the presence of these deficiencies. Specific assays for α_2-antiplasmin and plasminogen activator inhibitor are available, although the lower limit of normative values of these assays are not well defined. Antifibrinolytic therapies such as aminocaproic acid or tranexamic acid are the mainstay of treatments for controlling or preventing bleeding because they mitigate the hyperfibrinolysis. FFP can also be used to replace the deficient proteins.

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Chapter 526

Von Willebrand Disease

Brian R. Branchford and Veronica H. Flood

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, with an estimated prevalence cited at 1:100 to 1:10,000 depending on the criteria used for diagnosis. Patients with VWD typically present with mucosal bleeding. A family history of either VWD or bleeding symptoms and confirmatory laboratory testing are also required for the diagnosis of VWD.

PATHOPHYSIOLOGY

VWD is caused by a defect in, or deficiency of, von Willebrand factor (VWF). VWF has several functions in coagulation. VWF serves to tether platelets to injured subendothelium via binding sites for platelets and for collagen. VWF also serves as a chaperone, or carrier protein, for factor VIII (FVIII), protecting it from degradation in plasma. VWF is stored in endothelial cell Weibel-Palade bodies and platelet dense granules and circulates as a large, multimeric glycoprotein. Shear stress induces a conformational change in VWF that facilitates its ability to bind platelets through an exposed binding site on platelet glycoprotein Ib (GPIb). This enables VWF to recruit platelets to the site of clot formation, a function dependent on the high molecular weight (HMW) multimer forms of VWF.

VWD typically presents with mucocutaneous bleeding, similar to that seen with other platelet defects. **Epistaxis, easy bruising,** and **menorrhagia** in females are common complaints. However, symptoms are variable and do not necessarily correlate well with VWF levels. **Surgical bleeding**, particularly with dental extractions or adenotonsillectomy, is another common presentation. Severe type 3 VWD may present with joint and/or muscle bleeds. Most patients will have a family history of bleeding, but the VWD genetic variant demonstrates incomplete penetrance (not all carriers of the variant express bleeding symptoms) and variable expressivity (different VWF levels can be found in individuals with the same gene variant). Females are more likely to be diagnosed with VWD because of the potential for symptoms with menorrhagia, but males and females are equally likely to have VWD. However, diagnosis based on symptoms may be difficult, since minor bruising and epistaxis are not uncommon in childhood. Significant unexplained bruising, or bruising in unusual areas (stomach, back, face), in infants and toddlers is more often from nonaccidental trauma than from an underlying bleeding disorder. Gastrointestinal bleeding due to VWD may be complicated by associated angiodysplastic lesions anywhere in the gastrointestinal tract.

CLASSIFICATION

VWD may be caused by quantitative or qualitative defects in VWF. Mild to moderate quantitative defects are classified as type 1 VWD, whereas severe quantitative defects, in which there is no detectable VWF protein, are classified as type 3 VWD. The qualitative defects are grouped together as type 2 VWD.

Type 1 VWD is by far the most common type, accounting for 60–80% of all VWD patients. Typical symptoms include mucosal bleeding, such as epistaxis and menorrhagia, as well as easy bruising and potentially surgical bleeding. Diagnostic guidelines use a VWF level, as measured by the VWF antigen assay **(VWF:Ag)**, of <30 IU/dL for a clear diagnosis of VWD. Antigen and activity are usually symmetrically depressed in type 1 VWD. Patients with VWF:Ag <30 IU/dL are most likely to have a genetic defect in VWF. Patients with VWF:Ag between 30 and 50 IU/dL are said to have "low VWF." Whether or not this category truly represents VWD is a subject of some debate, and the joint guidelines suggest that the resolution should be based on presence or absence of bleeding symptoms. Because some patients with VWF levels in this range do experience bleeding, many physicians elect to treat them, especially perioperatively, for surgical procedures such as tonsillectomy.

Patients with type 1 VWD may have low VWF as a result of increased clearance of their VWF, known as **type 1C VWD**. Diagnosis of this subtype is important *because treatment of these patients with desmopressin is likely to be ineffective, necessitating administration of VWF-containing products instead.*

VWF levels can be influenced by external factors. Blood type has long been known to affect VWF, with lower VWF levels seen in people with blood group O. Stress, inflammation, exercise, menstrual cycle, and pregnancy all increase VWF levels; therefore a single normal VWF level does not necessarily rule out the presence of VWD. Certain diseases, such as **hypothyroidism** (see Chapter 603), and medications, such as valproic acid, can lower VWF levels in affected patients. Repeat testing is often required to confidently rule out or confirm a diagnosis of VWD.

Type 3 VWD is the most severe form and presents with symptoms similar to those seen in *mild hemophilia.* In type 3 VWD the VWF protein is completely absent, and the activity and antigen are both essentially zero. Type 3 VWD is seen at a frequency of approximately 1/1,000,000 members of the general population. In addition to mucosal bleeding, patients may experience joint bleeds, muscle bleeds, or central nervous system (CNS) hemorrhage. Some physicians elect to treat patients with prophylaxis, or modified prophylaxis, after injury, given that these patients typically have very low FVIII (<10 IU/dL). Because type 3 VWD is caused by a complete lack of VWF, *desmopressin is ineffective and treatment with VWF-containing concentrates is required.*

Type 2 VWD is often suspected when a discrepancy exists between the VWF antigen level (often normal) and the VWF activity (often low) because this type represents a qualitative defect, in which an abnormal protein is produced in normal amounts. A ratio of activity to antigen of less than 0.7 may raise concern for type 2 VWD, which itself has multiple subtypes. Multimer evaluation or other specialized testing can be used to discriminate the type 2 subtypes.

Type 2A VWD is characterized by a defect in VWF multimerization and decreased VWF activity in terms of platelet binding. It is the most common of the type 2 variants, accounting for approximately 10% of VWD cases. Type 2A VWD can result from variants that affect multimer assembly and processing, or variants that result in increased proteolysis of secreted VWF. Some variants affect both secretion and clearance of the VWF. Regardless of the mechanism, all type 2A VWD patients lack the HMW multimers, and therefore have reduced VWF activity, which results in bleeding. Symptoms are typically more severe than those seen in type 1 VWD. *Desmopressin may have clinical efficacy for treatment of minor bleeding, but significant surgical challenges or major bleeding symptoms generally require a VWF-containing concentrate for treatment.*

Type 2B VWD results from gain-of-function variants that increase the ability of VWF to bind platelets. This leads to increased clearance of both VWF and platelets from circulation and results in the loss of HMW multimers and decreased VWF activity, similar to that seen in type 2A VWD. Special testing is therefore required to diagnose type 2B VWD, either by direct measurement of the increased platelet binding or by an increased response to low dose ristocetin on platelet aggregation testing. Thrombocytopenia is not always present and may be more prominent during times of stress such as surgery or pregnancy. *Desmopressin is relatively contraindicated in type 2B VWD because it may accelerate VWF-platelet binding and clearance, so treatment with VWF-containing product is necessary.*

Platelet-type pseudo-VWD occurs when a variant in platelet GPIb causes spontaneous binding to VWF and also presents with decreased VWF activity, loss of HMW multimers, and thrombocytopenia similar to type 2B VWD. Specific testing is required to distinguish the two conditions. Because the defect involves platelets, treatment generally requires platelet transfusion.

Type 2M VWD includes those patients with decreased VWF activity but normal (or near normal) multimer distribution. This is generally caused by a defect in the ability of VWF to bind platelet GPIb, but this category also includes patients with defects in VWF-collagen interactions. *Some minor bleeding in type 2M VWD may respond to desmopressin, but because type 2M VWD is a functional defect, treatment with VWF-containing concentrates is usually required.*

Table 526.1 Laboratory Testing for von Willebrand Disease (VWD)

TEST	ABBREVIATION	PURPOSE
VWF antigen	VWF:Ag	Measures total amount of VWF protein present.
VWF activity	VWF:RCo*	Assesses interaction of VWF and platelets as mediated by ristocetin.
VWF activity/antigen ratio	VWF:RCo/VWF:Ag	A decreased ratio (<0.7) is found in type 2A, type 2B, and type 2M VWD.
Factor VIII activity	FVIII	Measures circulating FVIII, which will be very low in type 2N and type 3 VWD.
Multimer distribution	VWF multimers	Allows visualization of VWF multimers, used to identify high molecular weight multimers, which will be absent in types 2A and 2B VWD.

*In some laboratories a GPIb-binding assay, the VWF:GPIbM, is available.
VWF, Von Willebrand factor; GPIb, glycoprotein Ib; RCo, ristocetin cofactor.

Table 526.2 Classification of von Willebrand Disease

	TYPE 1	TYPE 3	TYPE 2A	TYPE 2B*	TYPE 2M	TYPE 2N
VWF:Ag	↓	Absent	↓	↓	↓	Normal or ↓
VWF:RCo	↓	Absent	⇊	⇊	⇊	Normal or ↓
FVIII	Normal	⇊	Normal or ↓	Normal or ↓	Normal or ↓	⇊
Multimer distribution	Normal	Absent	Loss of HMWM	Loss of HMWM	Normal	Normal

*Platelet count is also usually decreased in type 2B VWD.
FVIII, factor VIII; HMWM, high molecular weight multimers; VWF:Ag, von Willebrand factor antigen; VWF:RCo, VWF ristocetin cofactor activity.

Type 2N VWD is characterized by a defect in the ability of VWF to bind FVIII. Some patients with type 2N VWD may be misdiagnosed as mild hemophilia; therefore a high index of suspicion for this diagnosis is required in patients with low FVIII and an absent family history of FVIII deficiency. *Because the VWF in this case is dysfunctional, desmopressin is not usually helpful, and VWF-containing product infusion is typically required.*

LABORATORY DIAGNOSIS

There are no reliable screening tests for VWD. Patients with significant bleeding may present with anemia, and some patients with type 2B VWD or platelet-type pseudo-VWD may have thrombocytopenia. The partial thromboplastin time (PTT) may be prolonged if FVIII is low but especially in type 1 VWD it is often normal, precluding use of the PTT as a screening test. Platelet function analysis has been considered as a screening test for VWD, but suboptimal sensitivity and specificity render results difficult to interpret. Bleeding times are similarly unreliable in diagnosis of VWD.

Unfortunately, no single test can reliably diagnose VWD; therefore a panel of tests is usually required (Table 526.1). These include VWF:Ag, which measures the total amount of VWF protein present, and VWF activity test, typically using the *ristocetin cofactor* activity assay **(VWF:RCo)**, which provides a measure of the amount of functional VWF. FVIII activity is also usually included in the workup. Another test measures VWF binding to platelet GPIb without ristocetin **(VWF:GPIbM)**. This test is now FDA approved and its use is increasing. Collagen binding measures an additional function of VWF. Multimer distribution provides an assessment of HMW multimers (Table 526.2; Fig. 526.1).

Additional specialized testing may be employed to help determine the correct diagnosis. Specific testing for type 1C (clearance defects), type 2B, and type 2N VWD can confirm these diagnoses. Genetic diagnosis is not typically performed, partly because of the large size of the VWF gene and the high number of benign sequence variations. Large gene deletions are responsible for some cases of VWD and will not be detected on routine DNA sequencing. However, use of genetic diagnosis is increasing, particularly for types 2A, 2B, 2M, and 2N VWD.

TREATMENT

Treatment of VWD depends on the type of VWD present and the reason for treatment (Table 526.3). In general, type 1 VWD patients may be treated with **desmopressin**, which increases the amount of circulating VWF (and FVIII) by release from storage in endothelial cells. The exceptions are the rare type 1 patient who lacks a response to desmopressin and patients with type 1C VWD who do respond with an increase in VWF levels, but whose rapid clearance of circulating endogenous VWF results in a rapid return to baseline levels. Treatment of types 2 and 3 VWD requires **VWF-containing concentrates,** similar to the treatment of hemophilia. Dosing depends on the type of VWD and the reason for treatment. Careful monitoring of VWF and FVIII levels is recommended to tailor treatment for surgeries and major trauma. For all types of VWD, adjunct therapy should be considered when possible, such as the use of antifibrinolytics for oral surgery or hormonal treatment for menorrhagia.

Alternate treatment strategies should also be considered, particularly for difficult symptoms or severe VWD. Hormonal therapy for females with menorrhagia, although not specific to VWD, can be very helpful in managing symptoms and improving quality of life. Local treatment of epistaxis, such as nasal cautery or packing, may be helpful in some circumstances. Iron therapy for patients with iron-deficiency anemia may also be required.

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	Normal	Type 1	Type 1C (Vicenza)	Type 3	Type 2A	Type 2B	Type 2N	Type 2M	PT-VWD
VWF:Ag	N	↓	↓↓	absent	↓	↓	N or ↓	↓ or N	↓
VWF:RCo	N	↓	↓↓	absent	↓↓↓	↓↓	N or ↓	↓↓	↓↓
FVIII:C	N	N or ↓	↓	2-10 IU/dL	N or ↓	N or ↓	↓↓	N	N or ↓
VWFpp/VWF:Ag ratio	N	N	↑↑	absent	N or ↑	↑	N	N	↑
RIPA	N	often N	↓	absent	↓	often N	N	N or ↓	often N
LD-RIPA	absent	absent	absent	absent	absent	↑↑↑	absent	absent	↑↑↑
PFA'	N	N or ↑	↑	↑↑↑	↑	↑	N	↑	↑
BT'	N	N or ↑	↑	↑↑↑	↑	↑	N	↑	↑
Platelet count	N	N	N	N	N	↓ or N	N	N	↓
VWF multimers	N	N but ↓	N but ↓	absent	abnormal	abnormal	N but ↓	N but ↓	abnormal

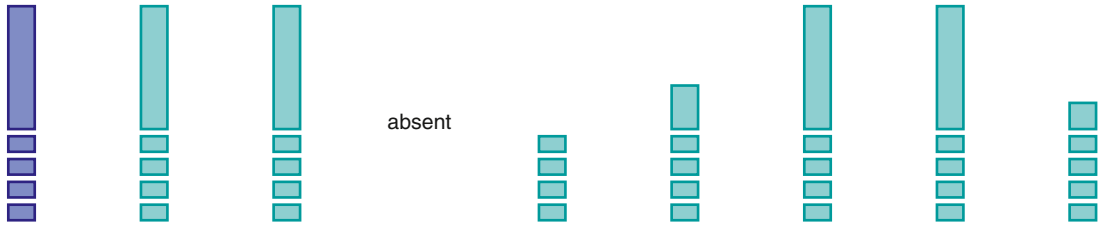

Fig. 526.1 Specialized laboratory testing for von Willebrand disease (VWD). ↓, ↓↓, ↓↓↓, Relative decrease; ↑, ↑↑, ↑↑↑, relative increase; BT, bleeding time; FVIII:C, factor VIII coagulant activity; LD-RIPA, low-dose ristocetin-induced platelet aggregation; N, normal; N but ↓, normal but decreased in intensity; PFA, platelet function analysis; PT-VWD, platelet-type VWD; RIPA, ristocetin-induced platelet aggregation; VWF:Ag, von Willebrand factor antigen; VWF:RCo, VWF activity by ristocetin cofactor; VWF pp, VWF propeptide. *(Courtesy Dr. Robert R. Montgomery.)*

Table 526.3 Treatment of Von Willebrand Disease

TREATMENT	VWD TYPES	ROUTE	DOSING
Desmopressin*	Type 1 VWD Some type 2 VWD (use with caution)	IV or IN	0.3 μg/kg IV† 1 spray IN (<50 kg) 2 sprays IN (>50 kg)
Von Willebrand factor (VWF) concentrates‡	Type 3 VWD Type 2 VWD Severe type 1 VWD (or type 1 clearance defects)	IV	40-60 ristocetin cofactor activity units/kg (adjust dose depending on baseline VWF level and desired peak VWF level). If recombinant VWF used, may need to administer additional recombinant FVIII for emergency treatment.
Antifibrinolytics	Mucosal bleeding, all types of VWD	PO or IV	Aminocaproic acid: 100 mg/kg PO loading dose followed by 50 mg/kg every 6 hr§ Tranexamic acid: 1,300 mg PO 3 times daily for 5 days

*Recommended treatment with Stimate brand nasal spray because this form is concentrated to give 150 μg/spray. Other forms are much more dilute and will not result in desired increase in VWF.
†Maximum recommended dose is 20-30 μg/day.
‡Currently both Humate-P and Wilate are approved for treatment of VWD. Vonvendi is a recombinant VWF that is also approved for treatment of VWD but does not contain FVIII.
§Maximum recommended dose is 24 g/day.
IN, Intranasal; IV, intravenous; PO, oral administration.

Chapter 527

Hereditary Predisposition to Thrombosis

Benjamin J. Samelson-Jones and Leslie J. Raffini

Most children that develop clots have multiple risk factors, which may include inherited predispositions to thrombosis. The strength of the inherited prothrombotic risk factors varies. An estimated 30–60% of individuals with *strong* thrombophilias, such as antithrombin deficiency, protein C deficiency, and protein S deficiency, develop thromboembolic disease by age 60; in contrast, less than 10% of individuals with *weak* thrombophilias, such as heterozygous factor V Leiden variant or prothrombin *G20210A* gene variant, develop thromboembolic disease. The inherited thrombophilias with the best understood pathogenesis, population prevalence, and prothrombotic risks are listed in Table 527.1. Some of these disorders may also be acquired or mixed acquired and genetic (Table 527.2).

The inheritance of other thrombophilias is less well understood. Elevated levels of factor VIII (FVIII) and homocysteine are associated with thrombosis, but their levels are not necessarily genetically determined. Additional alterations in plasma proteins have also been associated with increased thrombotic risk, such as elevated concentrations of factors IX and XI, heparin cofactor II deficiency, elevated lipoprotein (a), and dysfibrinogenemia; however, these abnormalities have not gained widespread acceptance in routine testing of children for inherited thrombophilia.

In general, the prothrombotic tendency conferred by these defects is either a result of an increased procoagulant effect (prothrombin gene pathogenic variant, elevated FVIII, hyperhomocysteinemia) or a decreased anticoagulant effect (factor V Leiden, deficiency of protein C, protein S, or antithrombin). Although numerous inherited risk factors for thrombosis have been identified, the majority of individuals who inherit one of these risk factors, even strong thrombophilias, do not necessarily develop thrombosis during childhood. Before evaluating for these disorders, both the limitations of the testing and potential benefits need to be considered.

The **factor V Leiden pathogenic variant** is the result of a single base pair change at nucleotide 1765 within the factor V gene causing an R506Q amino acid substitution in the encoded protein. This pathogenic variant causes activated factor V to become resistant to inactivation by activated protein C. It is the most common inherited risk factor for thrombosis, although its prevalence varies across ethnicities. Individuals who are heterozygous have a 5-7–fold increased relative risk of venous thrombosis, whereas homozygous individuals have an even higher risk. There appears to be a synergistic prothrombotic risk enhancement with the inherited risk of factor V Leiden and the acquired risk of estrogen-containing contraceptives. The baseline annual risk of thrombosis for females of reproductive age is approximately 1/10,000 and increases to 1/2,500 for those taking estrogen-containing contraceptives. For young females who are heterozygous for the factor V Leiden pathogenic variant and on estrogen-containing contraceptives, the annual risk of venous thromboembolism (VTE) increases to 1 in 200. The **prothrombin 20210 gene pathogenic variant** is a G-to-A transition in the 3' untranslated region of the gene that results in increased levels of prothrombin. It is a weaker risk factor for venous thrombosis than factor V Leiden.

Deficiencies of the natural anticoagulation proteins (protein C, protein S, and antithrombin) are less common than the specific genetic pathogenic variants mentioned previously but are associated with a stronger risk of thrombosis. Although heterozygous deficiencies do not often present during childhood, homozygous defects may be embryonically lethal or result in significant symptoms in infancy. Neonates with *homozygous* deficiencies of protein C or protein S may present with **purpura fulminans**. This rare condition is characterized by rapidly spreading purpuric skin lesions resulting from thromboses of the small dermal vessels, followed by bleeding into the skin. In addition, these infants may also develop cerebral thrombosis, ophthalmic thrombosis, disseminated intravascular coagulation, and large-vessel thrombosis. An infant with purpuric skin lesions of unknown cause should receive empiric replacement with fresh-frozen plasma. Definitive diagnosis can be difficult in the sick premature neonate, who may have undetectable levels of these factors but not have a true genetic deficiency. Protein C and antithrombin concentrates are also available and have been demonstrated to be effective.

Elevated levels of homocysteine have been associated with venous *and* arterial thromboses, although the pathogenic mechanisms for thrombosis in homocystinemia are poorly understood. Thromboembolic complications are well described in children with **homocystinuria,** a rare inborn error of metabolism caused by deficiency of cystathione β-synthase that results in plasma levels of homocysteine

Table 527.1 Clinically Relevant Inherited Thrombophilias and Accompanying Diagnostic Laboratory Studies

THROMBOPHILIA	GENERAL POPULATION PREVALENCE (%)	VTE ADULT PATIENT PREVALENCE (%)	ANNUAL INCIDENCE OF VTE (%)	ODDS RATIO FOR FIRST VTE EPISODE IN CHILDHOOD	ODDS RATIO FOR RECURRENT VTE EPISODE IN CHILDHOOD	LABORATORY STUDIES
Antithrombin deficiency	0.02-0.04	1	1.8	9.4	3.4	Functional coagulation testing‡
Protein C deficiency	0.2	3	1.5	7.7	2.5	Functional coagulation testing‡
Protein S deficiency	0.03-0.13	2	1.9	5.8	3.8	Functional coagulation testing‡
Factor V Leiden variant*	3-7	20	0.5	3.8	0.8	Gene testing
Prothrombin 20210 variant*	1-4	5	0.3	2.6	2.2	Gene testing

*Values refer to heterozygous changes.
‡May be impacted by anticoagulation therapies.
VTE, Venous thromboembolism.

Table 527.2 Classification of Hypercoagulable States

HEREDITARY	MIXED	ACQUIRED
LOSS OF FUNCTION		
Antithrombin deficiency	Hyperhomocysteinemia	Previous venous thromboembolism Hepatic cirrhosis Severe liver disease Nephrotic syndrome L-asparaginase
Protein C deficiency	Obesity	Pregnancy, puerperium
Protein S deficiency	Cancer	
		Drug-induced: Heparin-induced thrombocytopenia Prothrombin complex concentrates L-asparaginase Hormonal therapy
GAIN OF FUNCTION		
Factor V Leiden	Postoperative	
Prothrombin FII G20210A	Myeloproliferative disorders	
Elevated factor VIII, IX, or XI		

Modified from Hoffman R, Benz Jr. EJ, Silberstein LE, et al., eds. *Hematology Basic Principles and Practice*, 7th ed. Philadelphia: Elsevier, 2018: Table 140.1, p. 2077.

that exceed 100 μmol/L. Much more common are mild to moderate elevations of homocysteine, which may be acquired or associated with a polymorphism in the methylenetetrahydrofolate reductase *(MTHFR)* gene. Although moderate elevations of homocysteine have been associated with both venous and arterial thrombotic events, testing for polymorphisms in the *MTHFR* gene is not indicated because these polymorphisms are common and by themselves (without homocystinemia) are not associated with thromboembolism.

Increased plasma concentrations of FVIII appear to be regulated by both genetic and environmental factors and are associated with an increased risk of thrombosis. High FVIII levels are usually polygenic, although rarely specific changes in the FVIII gene have been identified. FVIII is also an acute-phase reactant and may increase during periods of inflammation.

Although interpretation of gene testing is fairly straightforward, several challenges in interpretation of thrombophilia studies are unique to pediatric patients. Neonates have decreased concentrations of protein C, protein S, and antithrombin that increase rapidly over the first 6 months of life; protein C concentrations remain below adult levels throughout much of childhood. It is important to use pediatric normal ranges when evaluating these values and recognize that often the normal range overlaps with heterozygous defects and that retesting may be required, particularly in young children. Several nongenetic factors may also influence the results of inherited thrombophilia testing, including acute thrombosis, infection, inflammation, hepatic dysfunction, nephrotic syndrome, medication, and vitamin K deficiency. In some patients the hereditary nature may be confirmed by testing the parents.

Thrombophilia testing is considered during childhood when a child develops thrombosis or if a child has relatives with thrombosis or thrombophilia. Thrombophilia testing rarely influences the acute management of a child with a thrombotic event. The majority of children who develop thrombosis have multiple, coexistent *acquired* risk factors (see Table 528.1 in Chapter 528). More than 90% of thromboses in children are associated with indwelling intravascular catheters; inherited thrombophilias are uncommon in this scenario, and testing is generally not warranted. However, inherited thrombophilia is more common in an otherwise healthy child or adolescent who develops a blood clot or in a child who develops unusual or recurrent thrombosis. Thrombophilia testing may be useful in these situations because it may help explain why the child developed a blood clot and inform on the duration of therapy. However, current treatment recommendations do not differ based on the presence or absence of an inherited thrombophilia. The identification of an inherited anticoagulant protein defect, such as antithrombin or protein C deficiency, may allow for replacement therapies while off anticoagulation, such as in the perioperative setting.

The decision to perform thrombophilia testing in an otherwise healthy child with a **family history** of thrombosis or thrombophilia should be carefully considered, weighing the potential advantages and limitations of such an approach. Given that the absolute risk of thrombosis in children is extremely low (0.07/100,000), it is unlikely that an inherited thrombophilia will have any impact on clinical decision-making for a young child. The risk of thrombosis increases with age, so identification of a thrombophilic defect in an adolescent may guide primary thromboprophylaxis in high-risk situations (lower-extremity casting or prolonged immobility), inform the discussion about estrogen-based contraceptives, and promote lifestyle modification to avoid behavioral risk factors (sedentary lifestyle, dehydration, obesity, and smoking). Limitations of such testing include the cost as well as the potential for causing unnecessary anxiety or false reassurance.

In most patients with inherited thrombophilia, the treatment is the same as for patients with no inherited disorders (see Chapter 528). In neonates with purpura fulminans caused by homozygous protein C or S deficiency or in patients with warfarin-induced skin necrosis, heparin-induced thrombocytopenias and severe antithrombin deficiency often require factor replacement (protein C, S or antithrombin concentrates or plasma) in addition to unfractionated heparin or low molecular weight heparin. In less severe antithrombin deficiency, higher than usual doses of heparin may be needed.

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Chapter 528

Thrombotic Disorders in Children

Leslie J. Raffini and Brian R. Branchford

Thromboembolic events (TEs) are seen in pediatric tertiary care centers and may result in significant acute and chronic morbidity. TEs in children are rare. Diagnosis and treatment are often extrapolated from adult data because of a current lack of high-quality data in the pediatric setting.

EPIDEMIOLOGY

Although the overall incidence of thrombosis in the general pediatric population is quite low (0.07/100,000), the rate of **venous thromboembolism (VTE)** in hospitalized children is 60/10,000 admissions. Infants <1 year old account for the largest proportion of pediatric VTEs, with a second peak during adolescence.

Most children who develop a TE have multiple risk factors that may be acquired, inherited, or anatomic (Table 528.1). The presence of a central venous catheter (CVC) or peripherally inserted central venous catheter (PICC) is the most important risk factor for VTE in pediatric patients, associated with approximately 90% of neonatal VTE and 60% of childhood VTE. PICC lines may be among the highest-risk CVC subtypes. CVCs are often necessary for the care of premature neonates and children with acute and chronic diseases and are used for intravenous (IV) hyperalimentation, chemotherapy, dialysis, antibiotics, or supportive therapy. CVCs may damage the endothelial lining and/or cause blood flow disruption, increasing the risk of thrombosis. Many other acquired risk factors are associated with thrombosis, including trauma, surgery, infection, inflammation, chronic medical conditions, and certain medications. Cancer, congenital heart disease, and prematurity are the most common medical conditions associated with TEs.

Antiphospholipid antibody syndrome (APS) is characterized by recurrent fetal loss and/or thrombosis (Table 528.2). Antiphospholipid antibodies are associated with venous and, less often, arterial thrombosis. The autoantibodies in APS include lupus anticoagulant, anticardiolipin antibodies, and anti-β_2 glycoprotein I. If all three antibodies are positive **(triple positive)**, the risk of thrombosis is increased. The mechanism by which these antibodies cause thrombosis is not well understood. A diagnosis of APS requires the presence of both clinical and laboratory abnormalities (see "Laboratory Testing"). The laboratory abnormalities must be identified on two separate occasions, at least 12 weeks apart. Because of the high risk of VTE recurrence, patients with APS often require long-term anticoagulation, at least until the antibodies have resolved; the type of antibodies and the presence of arterial thrombosis may have important implications for the type of anticoagulant therapy chosen. Up to 20% of healthy children may have a *transient* lupus anticoagulant, often diagnosed because of a prolonged partial thromboplastin time (PTT) on routine preoperative testing. In this setting, transient antibodies may be associated with a recent viral infection and are not a risk factor for thrombosis. APS is also noted in patients with **systemic lupus erythematosus** (see Chapter 199) and may also be associated with livedo reticularis, neuropsychiatric complications, thrombocytopenia, or anemia; these patients are often persistently positive for the antiphospholipid antibody. **Catastrophic antiphospholipid syndrome** is a rare and potentially fatal disorder characterized by rapid onset of multiorgan thrombosis and/or thrombotic microangiopathies (Table 528.3).

Anatomic abnormalities that impede blood flow also predispose patients to thrombosis at an earlier age. Atresia of the inferior vena cava has been described in association with acute and chronic lower-extremity deep vein thrombosis **(DVT)**. Compression of the left iliac vein by the overlying right iliac artery **(May-Thurner syndrome)** should be considered in patients who present spontaneously with left iliofemoral thrombosis, and thoracic outlet obstruction **(Paget-Schroetter syndrome)** frequently presents with effort-related axillary-subclavian vein thrombosis.

CLINICAL MANIFESTATIONS

Extremity Deep Vein Thrombosis

Children with acute DVT often present with extremity pain, swelling, and discoloration (see Table 524.1). A history of a current or recent CVC in that extremity should be very suggestive. Many times, symptoms of CVC-associated thrombosis are more subtle and chronic, including repeated CVC occlusion (potentially requiring clearance with tissue plasminogen activator [tPA]) or sepsis, or prominent venous collaterals on the chest, face, and neck.

Pulmonary Embolism

Signs and symptoms classically include shortness of breath, pleuritic chest pain, cough, hemoptysis, fever, tachypnea, tachycardia, and/or, in the case of massive pulmonary embolism (PE), hypotension and right-sided heart failure. Based on autopsy studies in pediatric centers, PE is often undiagnosed because young children are unable to describe their symptoms accurately, and their respiratory deterioration may be masked by other conditions (see Chapter 458.1).

Cerebral Sinovenous Thrombosis

Signs and symptoms of cerebral sinovenous thrombosis (CSVT) may be subtle and may develop over many hours or days (see Table 524.1). Neonates with CSVT often present with seizures, whereas older children often complain of headache, vomiting, seizures, visual changes, and/or focal neurologic signs. They may also have papilledema and abducens palsy. Older patients may have a concurrent sinusitis or mastoiditis that has contributed to the thrombosis. Other risk factors may include trauma, meningitis, or dehydration.

Renal Vein Thrombosis

Renal vein thrombosis (RVT) is the most common spontaneous VTE in neonates (see Table 524.1). Affected infants may present with hematuria, an abdominal mass, and thrombocytopenia. Infants of diabetic mothers are at increased risk for RVT, although the mechanism for the increased risk is unknown. Approximately 25% of cases are bilateral.

Portal Vein Thrombosis

Portal vein thrombosis (PVT) often occurs during the neonatal period and is often asymptomatic, only manifesting in those patients who develop symptomatic portal hypertension (e.g., gastrointestinal [GI] bleeding, splenomegaly) after the initial thrombotic event (see Table 524.1). The most common risk factor associated with PVT is an umbilical venous catheter, although sepsis, pancreatitis, cirrhosis, liver transplant, splenectomy, and sickle cell disease are also notable risk factors. A known complication of PVT is cavernous transformation, which confers a risk for variceal bleeding.

Peripheral Arterial Thrombosis

The majority of arterial TEs in children are associated with catheters, often related to umbilical artery lines in neonates or cardiac catheterization via the femoral artery (see Table 524.1). Less common etiologies of arterial thrombosis include homocystinuria and APS. Patients with an arterial thrombosis affecting blood flow to an extremity will present with a cold, pale extremity with poor or absent pulses, which can signify a limb-threatening emergency.

Acute Ischemic Stroke

Acute ischemic stroke (AIS) typically presents with hemiparesis, slurred speech, altered consciousness, or seizures. This condition may occur secondary to pathology that affects the intracranial arteries (e.g., sickle cell disease, vasculitis, vasculopathy, traumatic arterial dissection, or paradoxical embolism across a patent foramen ovale) or may result from venous thrombi that embolize to the arterial circulation (placental

Table 528.1 Risk Factors for Thrombosis

GENERAL
Indwelling catheter, especially PICC lines
Infection
Trauma
Surgery
Cancer
Immobility
Cardiac disease/prosthetic valve
Systemic lupus
Rheumatoid arthritis
Inflammatory bowel disease
Celiac disease
Polycythemia/dehydration
Nephrotic syndrome
Diabetes
Pregnancy
Obesity
Prematurity
Paroxysmal nocturnal hemoglobinuria
Thrombotic thrombocytopenic purpura (acquired)
COVID-19
Antiphospholipid antibody syndrome
INHERITED THROMBOPHILIA
Factor V Leiden pathogenic variant
Prothrombin pathogenic variant
Antithrombin deficiency
Protein C deficiency
Protein S deficiency
Homocystinuria
Elevated factor VIII
Dysfibrinogenemia
GATA-2 deficiency
Hereditary thrombotic thrombocytopenic purpura
ANATOMIC
Thoracic outlet obstruction (Paget-Schroetter syndrome)
Iliac vein compression syndrome (May-Thurner syndrome)
Absence of inferior vena cava
MEDICATIONS
Estrogen-containing contraceptives
Asparaginase
Heparin (heparin-induced thrombocytopenia)
Corticosteroids
Immune checkpoint inhibitors
Hemophilia bypassing agents

PICC, Peripherally inserted central venous catheter.

thrombi, children with congenital heart disease, or patent foramen ovale allowing right-to-left shunting of an embolic venous thrombosis).

Rapidly Progressive Thrombosis (Thrombotic Storm)

Rapid progression or multifocal thrombosis is a rare complication of APS **(catastrophic antiphospholipid syndrome)**, or heparin-induced thrombocytopenia with thrombosis (see Table 528.3). Multiorgan dysfunction develops in the presence of small vessel occlusion and elevated D-dimer levels, and this may progress to disseminated intravascular coagulation. Treatment includes aggressive anticoagulation, often with direct thrombin inhibitors or fondaparinux, followed by prolonged warfarin therapy. In rare cases, plasmapheresis and/or immunosuppression and/or antiinflammatory therapy may be warranted.

DIAGNOSIS

Compression ultrasound with Doppler flow is the most common imaging study for the diagnosis of extremity DVT and chest CT is used most frequently for the diagnosis of PE (Fig. 528.1). Echocardiogram is often used to detect and follow right atrial clots, most often detected in patients with central catheter tips in the right atrium. Other diagnostic imaging options include CT and MR venography, which are noninvasive, although the sensitivity and specificity of these studies is not known. These studies may be particularly helpful in evaluating proximal or abdominal thrombosis. For the diagnosis of CSVT and AIS, the most sensitive imaging study is brain MRI with venography or diffusion-weighted imaging.

Table 528.2 Sydney Investigational Criteria for the Diagnosis of the Antiphospholipid Syndrome

CLINICAL
• Vascular thrombosis (one or more episodes of arterial, venous, or small-vessel thrombosis). For histopathologic diagnosis, there should be no evidence of inflammation in the vessel wall.
• Pregnancy morbidities attributable to placental insufficiency, including: (a) three or more otherwise unexplained recurrent spontaneous miscarriages before 10 weeks of gestation, (b) one or more fetal losses after the 10th week of gestation, (c) stillbirth, and (d) episode of preeclampsia, preterm labor, placental abruption, intrauterine growth restriction, or oligohydramnios that are otherwise unexplained.
LABORATORY
• Medium- or high-titer aCL or anti-β_2GPI IgG and/or IgM antibody present on two or more occasions, at least 12 weeks apart, measured by standard ELISA.
• Lupus anticoagulant in plasma, on two or more occasions, at least 12 weeks apart, detected according to the guidelines of the ISTH SSC Subcommittee on Lupus Anticoagulants and Phospholipid-Dependent Antibodies.
"Definite APS" is considered present if at least one of the clinical criteria and one of the laboratory criteria are met.

aCL, Anticardiolipin; aPL, antiphospholipid; β_2GPI, β_2-glycoprotein I; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.
Modified from Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *Thromb Haemost.* 2006;4:295–306. Table 2.

Table 528.3 Proposed Criteria for the Classification of Catastrophic Antiphospholipid Syndrome

1. Evidence of involvement of three or more organs, systems and/or tissues*
2. Development of manifestations simultaneously or in less than a week
3. Confirmation by histopathology of small vessel occlusion in at least one organ or tissue†
4. Laboratory confirmation of the presence of antiphospholipid antibodies (lupus anticoagulant and/or anticardiolipin antibodies)‡
DEFINITE CATASTROPHIC APS
• All four criteria
PROBABLE CATASTROPHIC APS
• All four criteria, except for only involvement of two organs, systems, and/or tissues
• All four criteria, except for the absence of laboratory confirmation at least 6 weeks apart because of the early death of a patient never previously tested for aPL before the catastrophic APS event
• Criteria 1, 2, and 4
• Criteria 1, 3, and 4 and the development of a third event in more than a week but less than a month, despite anticoagulation

*Usually, clinical evidence of vessel occlusions, confirmed by imaging techniques when appropriate. Renal involvement is defined by a 50% rise in serum creatinine, severe systemic hypertension (≥180/100 mm Hg) and/or proteinuria (≥500 mg/24 h).
†For histopathologic confirmation, significant evidence of thrombosis must be present, although, in contrast with Sydney criteria, vasculitis may coexist occasionally.
‡If the patient had not been previously diagnosed as having an APS, the laboratory confirmation requires that the presence of antiphospholipid antibodies must be detected on two or more occasions at least 6 weeks apart (not necessarily at the time of the event), according to the proposed preliminary criteria for the classification of definite APS.
aPL, Antiphospholipid; APS, antiphospholipid syndrome
Modified from Asherson RA, Cevera R, de Groot PG et al. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus* 2003;12:530–534.

LABORATORY TESTING

All children with a VTE should have a complete blood count and a baseline prothrombin time (PT) and PTT to assess their coagulation status in

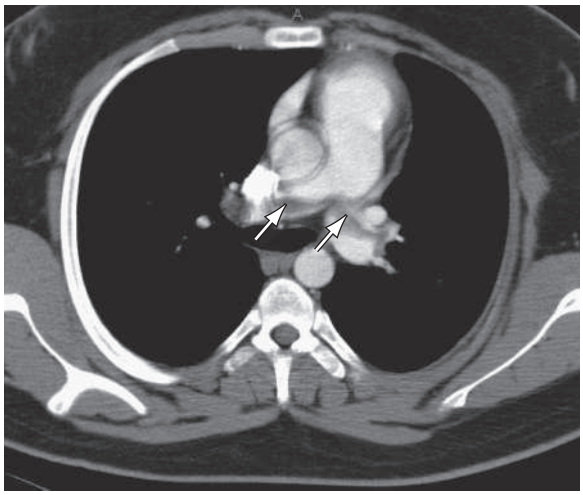

Fig. 528.1 Chest CT scan from a 15-yr-old male with a large pulmonary embolism. Large filling defects are present in the right and left main pulmonary arteries *(arrows)*.

anticipation of anticoagulation treatment. In adults with suspected DVT, the D-dimer level has a high negative predictive value, but the predictive value is not as well established for children. The D-dimer is a fragment produced when fibrin is degraded by plasmin and is a measure of both clot formation and subsequent fibrinolysis. Based on the clinical scenario, other laboratory studies, such as renal and hepatic function, may be indicated. Testing for APS includes evaluation for the lupus anticoagulant as well as anticardiolipin and anti–β_2-glycoprotein antibodies and should be considered in patients with inflammatory disorders, recent trauma, surgery, or infection, and those who present with thrombosis and no other obvious risk factors.

There is debate regarding which patients should have testing for inherited risk factors. Thrombophilia testing rarely influences the acute management of a child with a thrombotic event, and natural anticoagulants like protein C and S or antithrombin (AT) may appear to have low levels due to consumption during the acute phase of thrombosis, even if the patient does not have an existing deficiency of these proteins (see Chapter 527). Identification of an inherited thrombophilia may influence the duration of treatment, particularly for those with a *strong* thrombophilia, and may aid in counseling patients about their risk of recurrence. **Unprovoked** (absence of risk factors) thrombosis and a family history of thrombotic events may be a clue to an inherited thrombophilia, although they may coincide with a known risk factor.

The evaluation and interpretation of coagulation studies in pediatric patients may be complicated by the developing hemostatic system and the differences in normal ranges between infants and adults (see Chapter 527).

TREATMENT

Therapeutic options for children with thrombosis may include observation, anticoagulation, thrombolysis, and surgery. In premature neonates and critically ill children who are at high risk of bleeding, the potential benefits must be weighed against the risks, and close observation with repeat imaging may be an option. The majority of nonneonates with symptomatic thrombosis are treated with anticoagulant therapy. The goal of anticoagulation is to reduce the risk of embolism, halt clot extension, and prevent recurrence (see Chapter 528.1). Systemic or endovascular thrombolysis may be indicated for organ- or limb-threatening thrombosis. Surgery may be necessary for life- or limb-threatening thrombosis when there is a contraindication to thrombolysis. The optimal treatment for a child with AIS depends on the likely etiology and the size of the infarct and may include either anticoagulation or antiplatelet therapy such as aspirin. Children with sickle cell disease who develop stroke are treated acutely with erythrocytapheresis to rapidly lower the percentage of hemoglobin S and may also receive ongoing chronic red blood cell transfusions (or erythrocytapheresis) to reduce recurrence risk.

A summary of management recommendations for various types of VTE can be found in (Tables 528.4 and 528.5)

COMPLICATIONS

Complications of VTE include recurrent thrombosis (local or distant), and development of post-thrombotic syndrome (PTS). Over time, due to venous hypertension or damaged endovascular valves, patients may develop pain, swelling, edema, discoloration, and ulceration of the affected limb. Several prospective studies in adults have shown PTS to be present in 17–50% of patients with a history of thrombosis. The likelihood of developing PTS has been shown to increase with age, thrombus burden, and delay in anticoagulation therapy.

528.1 Anticoagulant and Thrombolytic Therapy

Leslie J. Raffini and Brian R. Branchford

Initial options for anticoagulation in children have generally included unfractionated heparin (UFH) or low molecular weight heparin (LMWH), followed by LMWH or warfarin for outpatient management (see Tables 528.4 and 528.5). Clinical trials of several direct oral anticoagulants (DOACs) have demonstrated the safety and efficacy of these drugs to treat VTE in children, and the landscape of treatment options for children with thrombosis is changing rapidly as these drugs gain regulatory approval. DOACs act by inhibiting factor Xa or thrombin (Table 528.6).

The optimal duration of anticoagulation for children with TEs is not well established. American Society of Hematology (ASH) guidelines recommend that pediatric patients with provoked DVT or PE receive ≤3 months of anticoagulation and those with unprovoked DVT or PE receive 6-12 months of therapy. Patients with strong inherited thrombophilia, recurrent thrombosis, and APS (or other nonmitigatable risk factors) may require indefinite anticoagulation.

UNFRACTIONATED HEPARIN

Both UFH and LMWH act by catalyzing the action of AT. UFH consists of large molecular weight polysaccharide chains that interact with AT, supporting the inhibition of factor Xa and thrombin, as well as other serine proteases.

Heparin Dosing

A therapeutic heparin dose achieves a prolongation of the PTT of 1.5-2.5 the upper limit of normal. A bolus dose of 75-100 units/kg results in a therapeutic PTT in the majority of children. This bolus should be followed by a continuous infusion. Initial dosing is based on age, with infants having the highest requirements. It is important to continue to monitor the PTT closely. In some situations, such as patients with a lupus anticoagulant, those with elevated factor VIII, or neonates, the PTT may not accurately reflect the degree of anticoagulation, and heparin can be monitored using a heparin anti-Xa level of 0.35-0.7 units/mL.

Heparin Complications

Maintaining the PTT in the therapeutic range can be difficult in young children. The bioavailability of heparin is difficult to predict and may be influenced by plasma proteins, including AT level. In many patients, this results in multiple dose adjustments requiring close monitoring with frequent venipuncture. UFH also requires continuous IV access, which may be difficult to maintain in young children.

The most common adverse effect related to heparin therapy is bleeding. There are case reports of life-threatening bleeding in children treated with heparin. The true frequency of bleeding in pediatric patients receiving heparin has not been well established and is reported as 1–24%. If the anticoagulant effect of heparin must be reversed immediately, protamine sulfate may be administered to neutralize the heparin. With an elimination half-life of approximately 30 minutes, the anticoagulant effect of this drug typically wears off in approximately 2-3 hours after stopping the infusion. UFH is cleared by the liver and kidney.

Other adverse effects include osteoporosis (with long-term use) and **heparin-induced thrombocytopenia (HIT)**. Although rare in pediatric populations, HIT is a prothrombotic, immune-mediated

Table 528.4 Management Considerations for Pediatric Thromboembolic Events

VTE	MANAGEMENT RECOMMENDATION	COMMENTS
Symptomatic DVT	Anticoagulation Provoked—treat <3 months (or until provoking factor is resolved) Unprovoked—treat 6-12 months, or longer based on risk/benefit analysis Thrombolysis considered if life- or limb-threatening VTE IVC filter considered if absolute contraindication to anticoagulation	Observation may be necessary or reasonable for premature neonates or critically ill children at high risk of bleeding Recommendations vary regarding utility of radiographic follow-up
Asymptomatic DVT	Anticoagulation or observation	Natural history is unclear, decision may vary based on VTE- and patient-specific factors
Massive PE (hemodynamic compromise)	Thrombolysis followed by anticoagulation	
Submassive PE (no hemodynamic compromise)	Anticoagulation alone	
CVC-related	Anticoagulation Removal of CVC if not functioning or no longer needed	Duration of anticoagulation needed before CVC removal is still being investigated, and recommendations vary
Renal vein thrombosis (RVT)	Unilateral: Anticoagulation alone Bilateral: Consider thrombolysis for bilateral RVT (life- or organ-threatening)	
Portal vein thrombosis (PVT)	Occlusive: Anticoagulation Nonocclusive: Observation (close radiologic follow-up)	Observe for cavernous transformation. Bleeding risk with anticoagulation increases in the setting of portal hypertension (and associated esophageal varices)
Cerebral Sinovenous Thrombosis (CSVT)	Anticoagulation (radiologic follow-up recommended) Acetazolamide if concomitant increased intracranial pressure	Decision in patients with intracranial hemorrhage needs to be individualized, but some patients may benefit from anticoagulation

CVC, Central venous catheter; DVT, deep vein thrombosis; IVC, inferior vena cava; PE pulmonary embolism; VTE, venous thromboembolism

Table 528.5 Comparison of Antithrombotic Agents

	rTPA	UNFRACTIONATED HEPARIN	WARFARIN	LMW HEPARIN (ENOXAPARIN)
Indication	Recent onset of life- or limb-threatening thrombus	Acute or chronic thrombus, prophylaxis	Subacute or chronic thrombosis, thromboprophylaxis for cardiac valves	Acute or chronic thrombus, prophylaxis
Administration	IV continuous infusion	IV continuous infusion	PO once daily	SC injection twice daily
Monitoring	"Lytic state": FDP or D-dimer	PTT	INR	Anti–factor Xa activity
Other	Higher risk of bleeding	Difficult to titrate; requires frequent dose adjustments; higher dose required in newborns	Heavily influenced by other drugs and diet	More stable and easy to titrate; concern of osteopenia with long-term use

FDP, Fibrin degradation products; INR, international normalized ratio; IV, intravenous; LMW, low-molecular-weight; PO, oral; PTT, partial thromboplastin time; rTPA, recombinant tissue-type plasminogen activator; SC, subcutaneous.

complication in which antibodies develop to a complex of heparin and platelet factor-4. These antibodies result in platelet activation, stimulation of coagulation, thrombocytopenia, and in some cases, life-threatening thrombosis. If HIT is strongly suspected, heparin must be discontinued immediately. An alternative parenteral anticoagulant, including the direct thrombin inhibitors argatroban or bivalirudin, may be used in this situation.

LOW MOLECULAR WEIGHT HEPARIN

In contrast to UFH, LMWH contains smaller molecular weight polysaccharide chains. The interaction of the smaller chains with AT results primarily in the inhibition of factor Xa, with less of an effect on thrombin. The several LMWHs available have variable inhibitory effects on thrombin. For this reason, the PTT is not a reliable measure of the anticoagulant effect of LMWH, and the anti–factor Xa activity is used instead. Because of the ease of dosing and need for less monitoring, *LMWH is the most frequently used anticoagulant in pediatric patients.* Although dalteparin was the first (and currently only) LMWH approved by the Food and Drug Administration (FDA) for use in children (older than 1 month of age), enoxaparin is the LMWH that has been studied and used more often in pediatric patients.

Enoxaparin Dosing

The recommended standard starting dose of enoxaparin for infants <2 months old is 1.5 mg/kg/dose subcutaneously every 12 hours and for patients >2 months old, 1 mg/kg every 12 hours, although many

Table 528.6 Pharmacologic Properties of Direct Oral Anticoagulants

	DABIGATRAN	APIXABAN	BETRIXABAN	EDOXABAN	RIVAROXABAN
Target	Thrombin	Factor Xa	Factor Xa	Factor Xa	Factor Xa
Bioavailability, %	6–7%	50%	34%	62%	66%*
Protein binding, %	35%	87%	60%	40–59%	92–95%
Time to maximum concentration, hr	2	1–3	3–4	1–2	2–4
Half-life, hr	12–14	8–15	19–27	9–14	9–13
Renal elimination, %	>80%	25%	6–13%	50%	33%
Metabolism via cytochrome P450 enzymes, %	<2%	<32%	<1%	<5%	57%
Drug interactions	Inhibitors and inducers of P-gp	Dual inhibitors and inducers of CYP3A4 and P-gp	Inhibitors and inducers of P-gp	Inhibitors and inducers of P-gp	Dual inhibitors and inducers of CYP3A4 and P-gp
Specific reversal agents	Idarucizumab	Andexanet alfa	Andexanet alfa†	Andexanet alfa†	Andexanet alfa

*Applies to the 15 mg and 20 mg doses given once a day without food; bioavailability is 80–100% when these doses are given with food.
†Expected to be effective on the basis of its mechanism of action, although not approved for these agents.
P-gp, P-glycoprotein.
From Chan N, Sobieraj-Teague M, Eikelboom JW. Direct oral anticoagulants: evidence and unresolved issues. Lancet 2020;396:1767–1776. Table 1.

centers use slightly higher doses for children <2 years old. In general, peak levels are achieved 3-6 hours after injection. A therapeutic anti–factor Xa level, drawn 4 hours after the second or third dose, should be 0.5-1.0 IU/mL; the dose can be titrated to achieve this range. The elimination half-life of enoxaparin is 4-6 hours. Enoxaparin is cleared by the kidney and should be used with caution in patients with renal insufficiency. It should be avoided in patients with renal failure.

After an initial period of anticoagulation with heparin or LMWH, patients may continue to receive LMWH as an outpatient for the duration of therapy or may be transitioned to an oral anticoagulant such as warfarin.

Direct Thrombin Inhibitors

Argatroban and bivalirudin are IV direct thrombin inhibitors that are used in the setting of HIT, complex heart disease/failure cases with ventricular assist devices, or other relatively uncommon situations in pediatrics. They have short half-lives but cannot be fully reversed. Dosing has not been well established in children.

WARFARIN

Warfarin is an oral anticoagulant that competitively interferes with vitamin K metabolism, exerting its action by decreasing concentrations of the vitamin K–dependent coagulation factors II, VII, IX, and X, as well as protein C and protein S. Therapy should be started while a patient is anticoagulated with heparin or LMWH because of the risk of warfarin-induced skin necrosis. This transient hypercoagulable condition may occur when levels of protein C drop more rapidly than the procoagulant factors.

Dosing

Warfarin therapy is often initiated with a weight-based loading dose, with subsequent dose adjustments made according to a nomogram. When initiating warfarin therapy, UFH or LMWH should be continued until the international normalized ratio (INR) is therapeutic for 2 days. In most patients, this takes 5-7 days. The PT is used to monitor the anticoagulant effect of warfarin. Because the thromboplastin reagents used in PT assays have widely varying sensitivities, the PT performed in one laboratory cannot be compared with that performed in another laboratory. As a result, the INR was developed as a mechanism to standardize the variation in the thromboplastin reagent. The target INR range depends on the clinical situation. In general, a range of 2.0-3.0 is the target for the treatment of VTE. High-risk patients, such as those with mechanical heart valves, APS, or recurrent thrombosis, may require a higher target range.

Polymorphisms in *CYP2C9* and *VKORC1* affect the pharmacokinetics and pharmacodynamics of warfarin. Pharmacogenetic testing can identify wild-type responders, as well as those who are more sensitive (increased risk of bleeding). Genotyping in adults may help select warfarin dose, monitor for bleeding, or choose a DOAC instead of warfarin for patients highly sensitive and at risk for hemorrhage.

Complications

Bleeding is the most common adverse effect of warfarin. The risk of serious bleeding in children receiving warfarin for the treatment of VTE has been reported at 0.5% per year. Children who have supratherapeutic INR are at higher risk. There is considerable interpatient variation in dose. Diet, medications, and illness may influence the metabolism of warfarin, requiring frequent dose adjustments and laboratory studies. Numerous medications can affect the pharmacokinetics of warfarin by altering its clearance or rate of absorption. These effects can have a profound impact on the INR and must be considered when monitoring a patient receiving warfarin.

The strategies used to reverse warfarin therapy depend on the clinical situation and whether there is bleeding. Vitamin K can be administered to reverse the effect of warfarin but takes some time to have an effect. If the patient is having significant bleeding, a nonactivated plasma-derived 4-factor prothrombin complex concentrate (PCC) or fresh-frozen plasma (FFP, 15 mL/kg) should be given along with the vitamin K. PCCs have not been well investigated in children.

Nonhemorrhagic complications are uncommon in children, although hair loss has been reported. Warfarin is a teratogen, particularly in the first trimester. **Warfarin embryopathy** is characterized by bone and cartilage abnormalities known as *chondrodysplasia punctata*. Affected infants may have nasal hypoplasia and excessive calcifications in the epiphyses and vertebrae.

DIRECT ORAL ANTICOAGULANTS

Oral direct thrombin inhibitors (dabigatran) or inhibitors of factor Xa (apixaban, rivaroxaban, edoxaban) are approved for the prevention or treatment of thrombosis in patients >18 years old (see Table 528.6), and dabigatran is FDA-approved for children older than 3 months of age. Fixed dosing, oral administration, no dietary interference with vitamin

Table 528.7 Guidelines for Therapeutic Anticoagulation Treatment Duration

INDICATION	RISK FACTOR	THERAPEUTIC ANTICOAGULATION TREATMENT DURATION
First VTE episode	Provoked, reversible	3 mo
	Provoked, chronic	3 mo, then continue anticoagulation with either therapeutic or prophylaxis regimens until the risk factor is resolved
	Unprovoked	6–12 mo
Recurrent VTE	Reversible	3 mo
	Chronic	If recurrence occurs while the patient was on prophylaxis regimen, after first VTE episode, restart therapeutic regimen until risk factor is resolved
	Unprovoked	Restart therapeutic regimen for at least 3 mo and then switch to lifelong VTE prophylaxis regimen

VTE, Venous thromboembolism.
From Shoag J, Davis JA, Corrales-Medina FF. Venous thromboembolism in pediatrics. *Pediatr Rev.* 2021;42:78–87.

K, and no need to monitor laboratory tests, as well as initial results suggesting noninferiority to warfarin and fewer bleeding episodes, have favored the use of DOACs. Clinical trials have demonstrated the safety and efficacy of dabigatran and rivaroxaban in children. Drugs are available to *reverse* the effects of DOACs if indicated. DOACs should be avoided in patients with APS in the presence of arterial thrombosis.

THROMBOLYTIC THERAPY

Although anticoagulation alone is often effective at managing thrombosis while awaiting natural fibrinolysis, more rapid clot resolution may sometimes be necessary or desirable. In these situations, a thrombolytic agent that can activate the fibrinolytic system is of potential benefit. The pharmacologic activity of thrombolytic agents depends on the conversion of endogenous plasminogen to plasmin. Plasmin is then able to degrade several plasma proteins, including fibrin and fibrinogen. Because of the high risk of bleeding, thrombolytic therapy is generally reserved for patients with life- or limb-threatening thrombosis.

tPA is available as a recombinant product and has become the primary agent used for thrombolysis in children, although proper dose finding studies have not been performed. Depending on the situation, tPA may be used systemically or locally with catheter-directed thrombolysis, sometimes with the addition of mechanical clot lysis devices operated by interventional radiologists.

Dosing

An extremely wide range of doses of tPA has been used for systemic therapy, and no consensus exists as to the optimal dose. Systemic tPA doses of 0.1-0.6 mg/kg/hr were previously recommended, although recent reports indicate successful therapy with fewer bleeding complications using prolonged infusions with very low doses—0.01-0.06 mg/kg/hr.

Monitoring

There is no specific laboratory test to document a "therapeutic range" for thrombolytic therapy. It is important to maintain the fibrinogen >100 mg/dL and the platelet count >75,000 × 10^9/L during treatment. Supplementation of plasminogen using FFP is generally recommended in neonates before initiating thrombolysis because of their low baseline levels.

The clinical and radiologic response to thrombolysis should be closely monitored. The duration of therapy depends on the clinical response. Invasive procedures, including urinary catheterization, arterial puncture, and rectal temperatures, should be avoided.

The role of adjuvant UFH during thrombolytic therapy is controversial. Animal models have demonstrated that thrombolytic therapy can induce a procoagulant state with activation of the coagulation system, generation of thrombin, and extension or reocclusion of the thrombosis. In pediatric patients thought to be at low risk for bleeding, adjuvant UFH should be considered using doses of 10-20 units/kg/hr. The recommended duration of therapy is noted in Table 528.7.

Complications

The most serious complication from thrombolysis is bleeding, which has been reported in 0–40% of patients. Absolute contraindications to thrombolysis include major surgery within 7 days, history of significant bleeding (intracranial, pulmonary, or GI), peripartum asphyxia with brain damage, uncontrolled hypertension, and severe thrombocytopenia. In the event of serious bleeding, thrombolysis should be stopped, and cryoprecipitate should be given to replace fibrinogen.

THROMBOPROPHYLAXIS

There have been no formal trials of VTE prevention in children, although many institutions are starting to develop and use risk-guided algorithms to identify children who may benefit from mechanical (sequential compression devices) or pharmacologic (low-dose anticoagulant) prevention strategies in the absence of concomitant bleeding risk. Hospitalized adolescents with multiple risk factors for thrombosis who are immobilized for a prolonged period are a group that expert consensus would suggest may benefit from prophylactic treatment with enoxaparin, 0.5 mg/kg every 12 hours (maximum 30 mg).

ANTIPLATELET THERAPY

Inhibition of platelet function using agents such as aspirin is more likely to be protective against arterial TEs than VTEs. Aspirin, or acetylsalicylic acid (ASA), exerts its antiplatelet effect by irreversibly inhibiting cyclooxygenase, preventing platelet thromboxane A_2 production. Aspirin is used routinely in children with Kawasaki disease and may also be useful in children with stroke, ventricular assist devices, and single-ventricle cardiac defects. The recommended dose of aspirin to achieve an antiplatelet effect in children is 1-5 mg/kg/day.

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Chapter 529

Postneonatal Vitamin K Deficiency

Brian R. Branchford, Benjamin J. Samelson-Jones, and Veronica H. Flood

Vitamin K deficiency occurring after the neonatal period is usually secondary to insufficient vitamin K intake, malabsorption, or alterations in the intestinal flora as a consequence of antibiotics. The biologic activity of the "vitamin K–dependent factors" (coagulation factors II, VII, IX, and X, as well as the natural anticoagulants protein C and protein S) requires vitamin K for their posttranslational carboxylation. In the absence of vitamin K, only nonfunctional forms of these proteins are synthesized, which are ineffective for hemostasis. Severe vitamin K deficiency will result in a prolonged prothrombin time (PT) and partial thromboplastin time (PTT), although early deficiency may only demonstrate a prolonged PT because of the short half-life of factor VII. Plasma levels of uncarboxylated factor II are measured in the PIVKA-II (proteins induced by vitamin K absence) test and elevated levels are diagnostic of vitamin K deficiency, although clinical history and basic coagulation labs are usually sufficient to make the diagnosis. It can often be clinically helpful to distinguish between deficiencies of the vitamin K–dependent factors and deficiencies in the clotting factors synthesized by hepatocytes due to liver disease (see Chapter 530), which includes the vitamin K–dependent factors as well as factors V and XI.

Intestinal malabsorption of fats may accompany cystic fibrosis, biliary atresia, or other liver diseases and results in a deficiency of fat-soluble dietary vitamins including vitamin K.

Prophylactic administration of water-soluble vitamin K orally is indicated in these patients (2-5 mg/24 hr for children and 5-10 mg/24 hr for adolescents and adults). Vitamin K may also be administered at 1-2 mg intravenously. Broad-spectrum antibiotics can alter the intestinal flora, reducing the vitamin K that is produced in the gastrointestinal tract. Patients have only a few weeks of vitamin K stores. The anticoagulant properties of warfarin (Coumadin) depend on interference with vitamin K, with a concomitant reduction of the vitamin K–dependent clotting factors. **Rat poison** (superwarfarin) produces a similar deficiency that should be considered in young children presenting with bleeding and bruising with a history compatible with ingestion. There are also cases of illicit drugs being adulterated with vitamin K antagonists. Vitamin K is a specific antidote for these substances. Four factor prothrombin complex concentrates contain all the vitamin K–dependent clotting factors; they are available for warfarin reversal and have been used to rapidly replace vitamin K–dependent clotting factors in urgent scenarios. Dosing is based on the international normalized ratio (INR), and vitamin K should also be administered.

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Chapter 530

Coagulopathy in Liver Disease

Brian R. Branchford and Veronica H. Flood

Because all the clotting factors *except* factor VIII (which is synthesized in liver sinusoids and extrahepatic endothelium) are produced exclusively in hepatocytes, coagulation abnormalities are very common in patients with severe liver disease (Table 530.1). Only 15% of such patients have significant clinical bleeding states, possibly because of concomitant reduction in anticoagulation proteins (protein C and S). The severity of the coagulation abnormality appears to be directly proportional to the extent of hepatocellular damage. The most common mechanism causing the hemostasis defect is decreased synthesis of coagulation factors. Patients with severe liver disease characteristically have normal to increased (not reduced) levels of factor VIII activity in plasma. In some instances, **disseminated intravascular coagulation** (DIC; see Chapter 510), hypofibrinogenemia, or hyperfibrinolysis may complicate liver disease, making laboratory differentiation of severe liver disease from DIC-related clotting factor consumption difficult. These entities, as well as vitamin K deficiency (see Chapter 529), may be distinguished by comparing levels of a hepatically synthesized factor (such as factor V), a hepatically synthesized vitamin K–dependent factor (such as factor VII), and a nonhepatically synthesized factor that could be subject to consumption (such as factor VIII).

Treatment of the coagulopathy of liver disease should be reserved for patients with clinical bleeding rather than used to normalize the lab values. Because a reduction in vitamin K–dependent coagulation factors is common in those with acute or chronic liver disease, a trial of vitamin K supplementation can be given. Vitamin K can be given orally, subcutaneously, or preferably intravenously (not intramuscularly) at a dose of 1 mg/24 hr for infants, 2-5 mg/24 hr for children, and 5-10 mg/24 hr for adolescents and adults. Inability to correct coagulopathy with vitamin K indicates that the coagulopathy may be caused by reduced levels of clotting factors that are not vitamin K–dependent and/or by inadequate production of precursor vitamin K proteins. Treatment for bleeding consists of factor replacement with **fresh-frozen plasma** (FFP) or **cryoprecipitate**. FFP (10-15 mL/kg) contains all clotting factors, but replacement of fibrinogen for severe hypofibrinogenemia may require cryoprecipitate at a dose of 1 unit per 5-10 kg body weight, or a fibrinogen concentrate if a patient is unable to tolerate excess fluid volume. In severe liver disease, it is often difficult to attain correction of abnormal clotting studies despite vigorous therapy with FFP and cryoprecipitate, and such attempts are often complicated by volume overload concerns. Some patients with bleeding as a result of liver disease have responded to therapy with desmopressin (DDAVP), whereas others have responded to treatment with recombinant factor VIIa, albeit at a dose lower than typically given for hemophilia. Recombinant factor VIIa is contraindicated in DIC.

Desmopressin (0.3 μg/kg intravenously) is effective in shortening bleeding time, prompts endothelial cell release of von Willebrand factor and factor VIII, promotes platelet activation, and is therefore used effectively to augment hemostasis before diagnostic liver biopsy in the setting of hepatic insufficiency/failure. In clinical trials of adults, recombinant factor VIIa has not been shown to be effective for the treatment of bleeding caused by severe liver disease, possibly because of its short half-life (3-6 hours). Frequently, severe liver disease is associated with moderate prolongation of coagulation disorder screening tests (partial thromboplastin time [PTT]; prothrombin time [PT]) that is not corrected by vitamin K or plasma replacement. Another diagnostic clue may be a low serum albumin level because this protein is also synthesized in the liver.

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Table 530.1 Coagulation Changes in Liver Disease

CHANGES IN PROCOAGULANT PROTEINS	CHANGES IN ANTICOAGULANT PROTEINS	CHANGES IN ANTIFIBRINOLYTIC PROTEINS
Decreased fibrinogen and factors FII, FV, FVII, FIX, FX, and FXI	Decreased protein C	Decreased α_2-antiplasmin and thrombin-activatable fibrinolysis inhibitor
Increased FVIII	Decreased protein S	FXIII decreased in some patients
Increased VWF, decreased ADAMTS-13	Decreased antithrombin	Increased tissue plasminogen activator

ADAMTS-13, A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13.
From Han H, Hensch L, Hui SKR, Teruya J. Evaluation and management of coagulopathies and thrombophilias in pediatric patients. *Clin Lab Med.* 2021;41:83–100. Table 4.

Chapter 531

Acquired Inhibitors of Coagulation

Brian R. Branchford and Veronica H. Flood

Acquired circulating anticoagulants **(inhibitors)** are antibodies that react, or cross-react, with clotting factors or components used in coagulation screening tests (phospholipids), thereby prolonging screening tests, such as prothrombin time (PT) and partial thromboplastin time (PTT), although not all of them result in a clinical bleeding state. Some of these anticoagulants are autoantibodies that react with phospholipid and thereby interfere with clotting in vitro *but not in vivo.* This group comprises anticardiolipin and anti-beta(2)-glycoprotein-Ib antibodies, but the most common subtype of these **antiphospholipid antibodies** has been referred to as the *lupus anticoagulant* (see Chapter 528.1). This unfortunately named antibody is neither found exclusively in patients with systemic lupus erythematosus (SLE; see Chapter 199), nor does its presence consistently signify the concomitant presence of SLE in a patient. It can also be seen in those with other collagen vascular diseases and in association with HIV infection. In otherwise healthy children, spontaneous lupus-like inhibitors have developed transiently after incidental viral infection and can be seen in up to 26% of screening tests in asymptomatic subjects. These transient inhibitors are usually not associated with either bleeding or thrombosis.

Although the classic lupus anticoagulant is more often associated with a predisposition to thrombosis than with bleeding symptoms, bleeding symptoms in a patient with the lupus anticoagulant may be caused by **thrombocytopenia**, which may be a manifestation of the antiphospholipid syndrome or of lupus itself (alone or occasionally in context with Evans syndrome), or, rarely, by a coexistent specific autoantibody against prothrombin (factor II). The antiprothrombin antibody does not inactivate prothrombin but rather causes accelerated clearance of the protein, resulting in low levels of prothrombin and subsequent inadequate hemostasis.

Rarely, antibodies may arise spontaneously against a specific clotting factor, such as *factor VIII* or *von Willebrand factor*, leading to acquired hemophilia A or von Willebrand disease (VWD), but this is usually seen more frequently in adult patients. These patients are prone to excessive hemorrhage and may require specific treatment. In patients with a hereditary deficiency of a clotting factor (factor VIII or factor IX), antibodies may develop after exposure to transfused factor concentrates. These hemophilic inhibitory antibodies are discussed in Chapter 525.1.

LABORATORY FINDINGS

Inhibitors against specific coagulation factors usually affect factors VIII, IX, and XI, or, rarely, prothrombin (factor II). Depending on the target of the antibody and the target's participation in the intrinsic, extrinsic, or common coagulation cascade pathway, the PT and/or PTT may be prolonged. The mechanism by which the inhibitory antibody functions determines whether mixing patient plasma with normal plasma will normalize (correct) the clotting time. Patient plasma that contains antibodies directed against the active site of a clotting factor (factor VIII or factor IX) will not correct on 1:1 mixing with normal plasma, whereas antibodies that lead to increased clearance of the factor (such as antiprothrombin antibodies) will correct on such mixing studies. Specific factor assays are used to determine which factor is involved, and the pattern of abnormalities in PT, PTT, and/or thrombin time (TT) is used to guide initial investigation.

TREATMENT

Management of the bleeding patient with an acquired inhibitory autoantibody against factor VIII or IX is the same as for the patient with congenital hemophilia who has an alloantibody against factor VIII or factor IX. Infusions of recombinant factor VIIa or activated prothrombin complex concentrate may be needed to control significant bleeding. Occasionally, high-dose coagulation factor VIII or IX concentrates may be effective. Immunosuppressive agents have been used "off label" to treat the inhibitor or reduce titers. Acute bleeding caused by an antiprothrombin antibody can often be treated with a plasma infusion and may resolve with a short course of corticosteroid therapy.

Asymptomatic spontaneous inhibitors that arise after a viral infection tend to disappear within a few weeks to months. Inhibitors seen with an underlying disease, such as those associated with SLE, often resolve during the treatment of the underlying disease.

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Chapter 532

Disseminated Intravascular Coagulation

Benjamin J. Samelson-Jones and Leslie J. Raffini

Disseminated intravascular coagulation (DIC) is an acquired clinicopathologic syndrome characterized by the widespread pathologic activation of the coagulation system, which results in both microvascular thrombi and the consumption of clotting factors, anticoagulant proteins, and platelets. Microvasculature injury can contribute to organ dysfunction, while hemorrhage can be life-threatening in a minority of cases. The diagnosis of DIC is based on the laboratory findings and clinical manifestations within the appropriate clinical context. The

simultaneous occurrence of bleeding and clotting is clinically challenging, with management starting from appropriately recognizing this syndrome.

ETIOLOGY

DIC is always a secondary complication of an underlying disorder (Table 532.1) and never an isolated diagnosis. The complex pathogenesis involves the loss of localization and excessive activation of coagulation leading to unregulated thrombin generation and microvascular fibrin deposition. Dysfunction of the vascular endothelium can both precipitate and amplify this process. The excessive activation consumes platelets, procoagulant clotting factors (factor V, factor VIII, prothrombin, and fibrinogen), and anticoagulant proteins (protein C, protein S, and antithrombin). The fibrinolytic system can also become dysregulated with endothelial injury and plasma protein consumption.

Table 532.1 Causes of Disseminated Intravascular Coagulation in Children

INFECTIONS
Meningococcemia (purpura fulminans)
Bacterial sepsis (staphylococcal, streptococcal, *Escherichia coli*, *Salmonella*)
Rickettsia (Rocky Mountain spotted fever)
Viruses (cytomegalovirus, herpes simplex, hemorrhagic fevers)
Malaria
Fungi
TISSUE INJURY
Central nervous system trauma (massive head injury)
Fat embolism
Crush injury
Profound shock or asphyxia
Hypothermia or hyperthermia (heat shock)
Massive burns
MALIGNANCY
Acute promyelocytic leukemia
Acute monoblastic leukemia
Disseminated solid tumors (rhabdomyosarcoma, neuroblastoma)
VENOM OR TOXINS
Toxic shock syndrome
Snakebites
Spider bites
MICROANGIOPATHIC DISORDERS
Severe thrombotic thrombocytopenic purpura or hemolytic-uremic syndrome
Vascular malformations
GASTROINTESTINAL DISORDERS
Fulminant hepatitis
Ischemic bowel
Pancreatitis
HEREDITARY THROMBOTIC DISORDERS
Homozygous/compound heterozygous protein C, protein S, or antithrombin deficiency
PERINATAL
Maternal toxemia
Abruptio placentae
Severe respiratory distress syndrome
Meconium aspiration syndrome
Necrotizing enterocolitis
Erythroblastosis fetalis
Fetal demise of a twin
MISCELLANEOUS
Severe acute graft rejection
Acute hemolytic transfusion reaction
Severe collagen-vascular disease
Kawasaki disease
Heparin-induced thrombosis
Infusion of activated prothrombin complex concentrates
Hyperpyrexia/encephalopathy, hemorrhagic shock syndrome

Adapted from Montgomery RR, Scott IP. Hemostasis: diseases of the fluid phase. In Nathan DG, Oski FA, eds. *Hematology of Infancy and Childhood*, 4th ed. Philadelphia: Saunders, 1993.

LABORATORY FINDINGS

Although DIC is characterized by a number of abnormal laboratory findings, none are specific for the diagnosis. The consumption of coagulation factors and platelets often results in a prolonged prothrombin time (PT), an increased partial thromboplastin time (PTT), and low platelet counts. Low fibrinogen is seen in severe disease, but as an acute-phase reactant, fibrinogen may be high or normal early in the disease process; declining fibrinogen levels, even within the normal range, can suggest DIC. Fibrinogen degradation products and D-dimer levels are frequently highly elevated. Factors V and VIII are usually both reduced in DIC, whereas in acute hepatic disease, factor VIII may be normal or elevated. Thrombi in the microvasculature can lead to red blood cell fragmentation and a **microangiopathic hemolytic anemia** with elevated lactate dehydrogenase and characteristic blood smear morphology, including schistocytes, helmet cells, and microspherocytes.

CLINICAL MANIFESTATIONS

DIC may be subclinical with only laboratory abnormalities, termed *nonovert DIC*. Clinical manifestations include thrombotic and hemorrhagic complications, as well as organ dysfunction. Bleeding frequently first occurs from sites of venipuncture or surgical incision, and the skin may show petechiae, purpura, and ecchymoses. Localized large-vessel arterial or venous thromboembolic events can occur. Tissue necrosis may involve many organs and can be most spectacularly seen as infarction of large areas of skin and subcutaneous tissue. A compromised blood supply can lead to organ dysfunction including organ failure, especially to the lungs, kidneys, liver, and brain.

MANAGEMENT

The primary treatment of DIC is resolving the underlying triggering disease process. Blood components support is recommended for active hemorrhage or in patients requiring invasive procedures; this may consist of platelet infusions (for thrombocytopenia), cryoprecipitate (for hypofibrinogenemia), and fresh-frozen plasma (for replacement of other coagulation factors and anticoagulant proteins). Blood product support in the absence of bleeding should not be based solely on laboratory abnormalities, but clinical practice varies on the importance of minor bleeding symptoms. Hemostatic products with activated clotting factors such as recombinant factor VIIa (FVIIa) and activated prothrombin complex concentrate (aPCC) theoretically may worsen the widespread and unregulated generation of thrombin. Likewise, systemic antifibrinolytics are generally contraindicated except in clinical scenarios associated with hyperfibrinolysis such as acute promyelocytic leukemia and early trauma.

DIC patients with overt thromboembolic complications should receive therapeutic anticoagulation, usually with unfractionated heparin, as outlined in Chapter 528.1; stringent attention to replacement therapy is warranted to maintain an adequate platelet count to limit bleeding. Prophylactic anticoagulation may be carefully used in patients with DIC at high risk of thromboembolic events that are not actively bleeding. Most patients should receive mechanical thromboprophylaxis such as sequential compression devices.

The prognosis of patients with DIC is primarily dependent on the outcome of the treatment of the primary disease and prevention of end-organ damage.

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Chapter 533

Platelet and Blood Vessel Disorders

Brian R. Branchford, Benjamin J. Samelson-Jones, and Veronica H. Flood

MEGAKARYOPOIESIS

Platelets are anuclear cellular fragments produced by megakaryocytes (large polyploid cells) within the bone marrow, lung, and other tissues. When the megakaryocyte approaches maturity, budding of the cytoplasm occurs, and large numbers of platelets are liberated. Platelets circulate with a life span of 7-10 days. **Thrombopoietin (TPO)** is the primary growth factor that controls platelet production (Fig. 533.1). Levels of TPO appear to correlate inversely with platelet number and megakaryocyte mass, with highest expression in the thrombocytopenic states associated with decreased marrow megakaryopoiesis, and may be variable in states of increased platelet production.

The platelet plays multiple hemostatic roles. The platelet surface possesses a number of important receptors for adhesive proteins, including **von Willebrand factor (VWF)** and fibrinogen, as well as receptors for agonists that trigger platelet aggregation, such as thrombin, collagen, and adenosine diphosphate (ADP). After injury to the blood vessel wall, the extracellular matrix containing adhesive and procoagulant proteins is exposed. Subendothelial collagen binds VWF, which then undergoes a conformational change that induces binding of the platelet glycoprotein Ib (GPIb) complex, the VWF receptor. This process is called *platelet adhesion.* Platelets then undergo activation. During the process of activation, the platelets generate thromboxane A_2 from arachidonic acid via the enzyme cyclooxygenase. After activation, platelets release agonists, such as ADP, adenosine triphosphate (ATP), calcium ions (Ca^{2+}), serotonin, and coagulation factors, into the surrounding milieu from dense and alpha granules. Binding of VWF to the GPIb complex triggers a complex signaling cascade that results in activation of the fibrinogen receptor, the major platelet integrin glycoprotein αIIb-β_3 (GPIIb-IIIa). Circulating fibrinogen binds to this receptor on activated platelets, linking platelets in a process called *aggregation.* This series of events forms a hemostatic plug at the site of vascular injury. The serotonin and histamine that are liberated during activation increase local vasoconstriction. In addition to acting in concert with the vessel wall to form the platelet plug, the platelet provides the catalytic phospholipid surface on which coagulation factors assemble and eventually generate thrombin through a sequential series of enzymatic cleavages. Lastly, the platelet contractile proteins and cytoskeleton mediate clot retraction.

THROMBOCYTOPENIA

The normal platelet count is 150-450 × 10^9/L. *Thrombocytopenia* refers to a reduction in platelet count to <150 × 10^9/L, although clinically significant bleeding is not seen until counts drop well below 50 × 10^9/L. Causes of thrombocytopenia include decreased production on either a congenital or an acquired basis, sequestration of the platelets within an enlarged spleen or other organ, and increased destruction of normally synthesized platelets on either an immune or a nonimmune basis (Tables 533.1-533.3, Fig. 533.2, and Chapter 524).

533.1 Immune Thrombocytopenia

Brian R. Branchford and Veronica H. Flood

The most common cause of acute onset of thrombocytopenia in an otherwise-well child is immune thrombocytopenia **(ITP)** (also called immune or idiopathic thrombocytopenic purpura).

EPIDEMIOLOGY

In a small number of children, estimated at 1/20,000, 1-4 weeks after exposure to a common viral infection, an autoantibody directed against the platelet surface develops with resultant sudden onset of thrombocytopenia. A recent history of viral illness is described in 50–65% of children with ITP. The peak age is 1-4 years, although the age ranges from early in infancy to elderly. In childhood, males and females are equally affected. ITP seems to occur more often in late winter and spring after the peak season of viral respiratory illness. Approximately 5–10% of ITP may recur more than 3 months after initial disease resolution.

PATHOGENESIS

The exact antigenic target for most such antibodies in most cases of childhood acute ITP remains undetermined, although in chronic ITP, many patients demonstrate antibodies against αIIb-β_3 and GPIb. After binding of the antibody to the platelet surface, circulating antibody-coated platelets are recognized by the Fc receptor on splenic macrophages, ingested, and destroyed. The most common identifiable viruses that have been described in association with ITP include Epstein-Barr

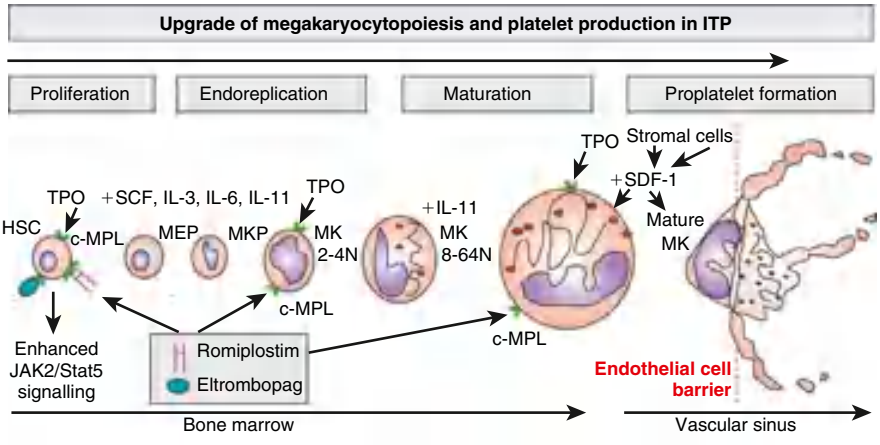

Fig. 533.1 Scheme of megakaryocytopoiesis and platelet production in immune thrombocytopenia (ITP). Hematopoietic stem cells (HSC) are mobilized, and megakaryocyte (MK) and erythroid progenitors (MEP) accumulate with MK-committed progenitors (MKP), giving rise to mature MKs under control of thrombopoietin (TPO) working with chemokines, cytokines, and growth factors, including stem cell factor (SCF) and interleukin (IL)-3, IL-6, and IL-11. Endoreplication results in ploidy changes in MKs and increased chromosome number (up to 64N). Mature MKs migrate to the endothelial cell barrier delimiting the vascular sinus and, under the influence of stromal-derived factor-1 (SDF-1), give rise to proplatelets that protrude into the circulation and produce large numbers of platelets under hemodynamic determinants. Therapeutically given romiplostim and eltrombopag enter the marrow and join with TPO to stimulate megakaryocytopoiesis and platelet production. *(From Nurden AT, Viallard JF, Nurden P. New-generation drugs that stimulate platelet production in chronic immune thrombocytopenic purpura. Lancet 2009;373:1563–1569.)*

Table 533.1 Differential Diagnosis of Thrombocytopenia in Children and Adolescents

DESTRUCTIVE THROMBOCYTOPENIAS
Primary Platelet Consumption Syndromes
Immune thrombocytopenias
- Acute and chronic ITP
- Autoimmune diseases with chronic ITP as a manifestation
  - Cyclic thrombocytopenia
  - Autoimmune lymphoproliferative syndrome and its variants
  - Systemic lupus erythematosus
  - Evans syndrome
  - Antiphospholipid antibody syndrome
  - Neoplasia-associated immune thrombocytopenia
- Thrombocytopenia associated with HIV
- Neonatal immune thrombocytopenia
  - Alloimmune
  - Autoimmune (e.g., maternal ITP)
- Drug-induced immune thrombocytopenia (including heparin-induced thrombocytopenia)
- Posttransfusion purpura
- Allergy and anaphylaxis
- Posttransplant thrombocytopenia

Nonimmune thrombocytopenias
- Thrombocytopenia of infection
  - Bacteremia or fungemia
  - Viral infection
  - Protozoan
- Thrombotic microangiopathic disorders
  - Hemolytic-uremic syndrome
  - Eclampsia, HELLP syndrome
  - Thrombotic thrombocytopenic purpura
  - Bone marrow transplantation–associated microangiopathy
  - Drug induced (quinine, etc.)
- Platelets in contact with foreign material
- Congenital heart disease
- Drug-induced via direct platelet effects (ristocetin, protamine)
- Type 2B VWD or platelet-type VWD

Combined Platelet and Fibrinogen Consumption Syndromes
Disseminated intravascular coagulation
Kasabach-Merritt syndrome
Hemophagocytic lymphohistiocytosis (inherited or acquired)

IMPAIRED PLATELET PRODUCTION
Hereditary disorders (see Table 533.2)
Acquired disorders
- Aplastic anemia
- Myelodysplastic syndrome
- Marrow infiltrative process—neoplasia
- Osteopetrosis
- Nutritional deficiency states (iron, folate, vitamin B12, anorexia nervosa)
- Drug- or radiation-induced thrombocytopenia
- Neonatal hypoxia or placental insufficiency

SEQUESTRATION
Hypersplenism
Hypothermia
Burns

ASSOCIATION WITH OTHER DISEASES
Fanconi anemia
Congenital amegakaryocytic thrombocytopenia
Shwachman-Diamond syndrome
Hemophagocytic lymphohistiocytosis
TAFRO syndrome

HELLP, Hemolysis, elevated liver enzymes, and low platelets; HIV, human immunodeficiency virus; ITP, immune thrombocytopenic purpura; TAFRO, thrombocytopenia, ascites, myelofibrosis renal dysfunction, organomegaly (a variant of multicentric Castleman disease); VWD, von Willebrand disease.
From Wilson DB. Acquired platelet defects. In: Orkin SH, Fisher DE, Ginsburg D, et al., eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed. Philadelphia: Elsevier, 2015: Box 34.1, p. 1077.

virus (EBV; see Chapter 301) and HIV (see Chapter 322), and ITP is also noted as a rare occurrence after measles, mumps, and rubella (MMR) vaccination. EBV-related ITP is usually of short duration and follows the course of infectious mononucleosis. HIV-associated ITP is usually chronic. In some patients, ITP appears to arise in children infected with *Helicobacter pylori* or rarely after vaccines. The SARS-CoV-2 virus responsible for the COVID-19 infection has been associated with development of ITP, as have some of the vaccines for this virus.

CLINICAL MANIFESTATIONS

The classic presentation of ITP is a previously healthy 1–4-year-old child who has sudden onset of generalized petechiae and purpura. The parents often state that the child was fine the previous day but is now covered with bruises and purple dots. There may be bleeding from the gums and mucous membranes, particularly with *profound thrombocytopenia* (platelet count $<10 \times 10^9$/L). There is a history of a preceding viral infection 1-4 weeks before the onset of thrombocytopenia. Findings on physical examination are typically normal, other than petechiae and purpura. *Splenomegaly, lymphadenopathy, bone pain, and pallor are rare.* A simple classification system to characterize the severity of bleeding in ITP on the basis of symptoms and signs rather than platelet count includes:

1. No symptoms
2. Mild symptoms: Bruising and petechiae, occasional minor epistaxis, very little interference with daily living
3. Moderate symptoms: More severe skin and mucosal lesions, more troublesome epistaxis, and menorrhagia
4. Severe symptoms: Bleeding episodes—menorrhagia, epistaxis, melena—requiring transfusion or hospitalization, symptoms interfering seriously with the quality of life

The presence of abnormal findings such as hepatosplenomegaly, bone or joint pain, remarkable lymphadenopathy, other cytopenias, or congenital anomalies suggests other diagnoses (e.g., leukemia, genetic syndromes). When the onset is insidious, especially in an adolescent, chronic ITP or the possibility of a systemic illness, such as **systemic lupus erythematosus** (SLE), is more likely. In addition, presentation at an atypical age (neonates, adolescents) should suggest an underlying disease.

OUTCOME

Severe bleeding is rare (<3% of cases in one large international study). In 70–80% of children who present with acute ITP, spontaneous resolution occurs within 6 months. Therapy does not appear to affect the natural history of the illness. Fewer than 1% of patients develop an **intracranial hemorrhage** (ICH). Proponents of interventional therapy argue that the objective of early therapy is to raise the platelet count to $>20 \times 10^9$/L and prevent the rare development of ICH. There is no evidence that therapy prevents serious bleeding. Approximately 20% of children who present with acute ITP go on to have chronic ITP. The outcome/prognosis may be related more to age; ITP in younger children is more likely to resolve, whereas development of chronic ITP in adolescents approaches 50%.

LABORATORY FINDINGS

Severe thrombocytopenia (platelet count $<20 \times 10^9$/L) is common, and platelet size is normal or increased, reflective of increased platelet turnover (Fig. 533.3). In acute ITP, the hemoglobin value, white blood cell (WBC) count, and differential count are usually normal. Hemoglobin may be decreased in the context of profuse nosebleeds (epistaxis) or menorrhagia. Bone marrow examination, *which is not routinely indicated in this disease*, shows normal granulocytic and erythrocytic series, with characteristically normal or increased numbers of megakaryocytes. Some of the megakaryocytes may appear to be immature and reflect increased platelet turnover. *Indications for bone marrow aspiration/biopsy* include an abnormal WBC count or differential or unexplained anemia, as well as history and physical examination findings suggestive of a bone marrow failure syndrome or malignancy. Other laboratory tests should be performed as indicated by the history and examination. HIV studies should be done in at-risk populations, especially sexually active teens. Platelet antibody testing is seldom useful in acute ITP. A direct antiglobulin test (Coombs) should be done if there is unexplained anemia to rule out **Evans syndrome** (autoimmune hemolytic anemia and thrombocytopenia; see Chapter 506). Evans syndrome may be idiopathic or an early sign of SLE, autoimmune lymphoproliferative syndrome, or common variable immunodeficiency

Table 533.2 Inherited Platelet Disorders

PLATELET DEFECT	GENE DEFECT (CHROMOSOMAL LOCATION)	CLINICAL AND LABORATORY CHARACTERISTICS	BLEEDING TREATMENT
DEFECTS IN PLATELET ADHESION			
Bernard-Soulier syndrome (BSS)	Autosomal recessive: *GP1BA* (17p13) *GP1BB* (22q11) *GP9* (3q21) Autosomal dominant: Ala156Val, GP1BA - Bolzano variant	• Often severe bleeding phenotype • Thrombocytopenia • Large platelets • Platelet aggregation: absent ristocetin-induced response • Flow cytometry: reduced or absent CD42a (GPIX)/CD42b (GP1bα) • *GP1BA* & *GP1BB* gene sequencing	• Supportive care • Platelet transfusion (risk of alloantibodies) • Antifibrinolytics • rFVIIa
Velocardiofacial/DiGeorge syndrome (VCFS)	22q11.2 deletion including *GP1BB*	• Thrombocytopenia • Large platelets and α-granules • Cardiac, thymus, parathyroid, facial, and cognitive abnormalities	• Supportive care
Platelet-type von Willebrand disease (PT-VWD)	Autosomal dominant: gain of function variants in *GP1BA*	• Thrombocytopenia • Large platelets • Platelet clumping • Decreased VWF:Ag, VWF multimers • Platelet aggregation: low dose ristocetin-induced platelet agglutination • *GP1BA* gene sequencing	• Supportive care • Platelet transfusion • Antifibrinolytics • rFVIIa
DEFECTS OF PLATELET AGGREGATION			
Glanzmann thrombasthenia (GT)	Autosomal recessive: *ITGA2B* (17Q21.32) *ITGB3* (17q21.32)	• Often severe bleeding phenotype • Normal platelet count and morphology • Platelet aggregation: absent response to all agonists except ristocetin • Flow cytometry: absent or reduced CD41 and CD61	• Supportive care • rFVIIa (considered the first line) • Platelet transfusion (risk of HPA alloantibodies) • Antifibrinolytics
DEFECTS IN AGONISTS RECEPTORS			
Thromboxane-prostanoid (TP) receptor defects	Autosomal recessive *TBXA2R* (19p13.3)	• Mild bleeding phenotype • Platelet aggregation: abnormal response to arachidonic acid and U46619 • *TBXA2R* gene sequencing	• Supportive care
ADP receptor defects P2Y12	Autosomal recessive *P2RY12* (3q23–25)	• Mild bleeding phenotype • Platelet aggregation: abnormal response to ADP • *P2RY12* gene sequencing	• Supportive care
Collagen receptor defects GPVI	Autosomal recessive *GP6* (19q13.4)	• Mild bleeding phenotype • Platelet aggregation: abnormal response to collagen • *GP6* gene sequencing	• Supportive care
PLATELET GRANULES DEFECTS (α-GRANULES)			
Gray platelet syndrome (GPS)	Autosomal recessive *NBEAL2* (3p21)	• Progressive myelofibrosis • Thrombocytopenia • Large pale platelets on blood smears • Absent α-granules on TEM • *NBEAL2* gene sequencing	• Supportive care • Antifibrinolytics • DDVAP • Platelet transfusion • Splenectomy
Arthrogryposis, renal dysfunction, and cholestasis syndrome (ARC syndrome)	Autosomal dominant *VPS33B* (15q26) *VIPAS39* (14q24)	• Thrombocytopenia • Large pale platelets on blood smears • Absent α-granules on TEM • Lethal early in life • *VPS33B* & *VIPAS39* sequencing	• Supportive care • Platelet transfusion • Antifibrinolytics

Continued

Table 533.2 Inherited Platelet Disorders—cont'd

PLATELET DEFECT	GENE DEFECT (CHROMOSOMAL LOCATION)	CLINICAL AND LABORATORY CHARACTERISTICS	BLEEDING TREATMENT
Quebec platelet disorder (QPD)	Autosomal recessive Tandem duplication of *PLAU* (10q22.2)	• Delayed-onset bleeding not responding to platelet transfusion • Variable thrombocytopenia • Abnormal urokinase in platelets detected with immunoblot or ELISA • *PLAU* duplication testing	• Supportive care • Antifibrinolytics
Paris-Trousseau/Jacobsen syndrome (PTS)	Autosomal dominant Deletion of chromosome 11q23–24 Hemizygous deletion of *FLI1* (11q24.1 – q24.3)	• Thrombocytopenia • Large platelets • Giant α-granules on TEM • Immature megakaryocytes in the bone marrow • Cognitive, cardiac, and facial abnormalities	• Supportive care • Antifibrinolytics • Platelet transfusion
PLATELET GRANULES DEFECTS (δ-GRANULES)			
Hermansky-Pudlak syndrome (HPS)	Autosomal recessive HPS1–10 (*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6,* and *AP3D1*)	• Decreased to absent δ-granules • Lumiaggregometry: decreased/absent ATP release • Whole-mount EM • Gene sequencing of 10 candidate genes • Oculocutaneous albinism	• Supportive care • Antifibrinolytics • Platelet transfusion • DDAVP
Chediak-Higashi syndrome (CHS)	Autosomal recessive *LYST* (1q42 – 1q42.2)	• Giant eosinophilic inclusions in neutrophils • Decreased to absent δ-granules • Lumiaggregometry: decreased/absent ATP release • Hypopigmentation and immunodeficiency	• Supportive care
PLATELET CYTOSKELETAL DEFECTS			
Wiskott-Aldrich syndrome (WAS) / X-linked thrombocytopenia	X-linked *WAS* (Xp11.23 – p11.22) encoding WAS protein	• Thrombocytopenia • Small platelets • Recurrent infections and eczema • Decreased/absent intracellular • WASp per immunoblot/ELISA • *WAS* gene sequencing	• Supportive care • Platelet transfusion • Antifibrinolytics • Splenectomy (not recommended)
ARPC1B deficiency	Autosomal recessive *ARPC1B* (7q22.1)	• Small platelets • Inflammatory disease, recurrent infections, small vessel vasculitis, abnormal platelet function • Decreased/absent intracellular • ARPC1B per immunoblot • *ARPC1B* gene sequencing	• Supportive care • Antifibrinolytics • Platelet transfusion
MYH9-related disease (MYH9-RD)	Autosomal dominant *MYH9* (22q12–13) encoding nonmuscle myosin heavy chain IIA	• Thrombocytopenia • Large platelets • Döhle-like inclusions in neutrophils • Myosin IIA aggregates in neutrophils - immunofluorescence microscopy • Variable degree of renal disease, sensorineural hearing loss, presenile cataract	• Supportive care • Platelet transfusion • Antifibrinolytics • THPO receptor agonists

VWF, Von Willebrand factor; HPA, human platelet antigen; ADP, adenosine diphosphate; TEM, transmission electron microscopy; EM, electron microscopy; ELISA, enzyme-linked immunosorbent assay; DDAVP, desmopressin; THPO, thrombopoietin.
Modified from Al-Huniti A, Kahr WHA. Inherited platelet disorders: diagnosis and management. *Transfu Med Rev* 2020;34:277–285. Table 1.

syndrome. An antinuclear antibody should be considered in adolescents, especially with other features of SLE (see Chapter 199).

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The well-appearing child with moderate to severe thrombocytopenia, an otherwise normal complete blood cell count (CBC), and normal exam findings has a limited differential diagnosis that includes exposure to medication inducing drug-dependent antibodies, splenic sequestration because of previously unappreciated portal hypertension, and, rarely, early aplastic processes, such as Fanconi anemia (see Chapter 517). Other than congenital thrombocytopenia syndromes (see Chapter 533.8), such as thrombocytopenia–absent radius (TAR) syndrome and MYH9-related thrombocytopenia, most marrow processes that interfere with platelet production eventually cause abnormal synthesis of red blood cells (RBCs) and WBCs and therefore manifest diverse abnormalities on the CBC. Disorders that cause increased platelet destruction on a nonimmune basis are usually serious systemic illnesses with obvious clinical findings such as **hemolytic-uremic syndrome** (HUS) and **disseminated intravascular**

Table 533.3	Classification of Fetal and Neonatal Thrombocytopenias*
Fetal	**Alloimmune thrombocytopenia**
	Congenital infection (CMV, toxoplasma, rubella, HIV, syphilis)
	Aneuploidy (trisomy 18, 13, or 21, triploidy, Turner syndrome)
	Autoimmune condition (maternal ITP, SLE)
	Severe Rh hemolytic disease
	Congenital/inherited (Wiskott-Aldrich, Noonan, Cornelia deLange, Jacobsen syndromes)
Early-onset neonatal (<72 hr)	**Placental insufficiency** (PET, IUGR, diabetes)
	Perinatal asphyxia
	Perinatal infection (*Escherichia coli*, GBS, herpes simplex)
	DIC
	Alloimmune thrombocytopenia
	Autoimmune condition (maternal ITP, SLE)
	Congenital infection (CMV, toxoplasma, rubella, HIV)
	Thrombosis (aortic, renal vein)
	Bone marrow disease (congenital leukemia, HLH)
	Kasabach-Merritt syndrome
	Metabolic disease (propionic and methylmalonic acidemia)
	Congenital/inherited (TAR, CAMT)
Late-onset neonatal (>72 hr)	**Late-onset sepsis**
	NEC
	Congenital infection (CMV, toxoplasma, rubella, HIV)
	Autoimmune
	Kasabach-Merritt syndrome
	Metabolic disease (propionic and methylmalonic acidemia)
	Congenital/inherited (TAR, CAMT)

*The most common conditions are shown in **bold**.
CAMT, Congenital amegakaryocytic thrombocytopenia; CMV, cytomegalovirus; DIC, disseminated intravascular coagulation; GBS, group B streptococcus; HIV, human immunodeficiency virus; HLH, hemophagocytic lymphohistiocytosis; ITP, immune thrombocytopenic purpura; IUGR, intrauterine growth restriction; NEC, necrotizing enterocolitis; PET, preeclampsia; SLE, systemic lupus erythematosus; TAR, thrombocytopenia with absent radii.
From Roberts I, Murray NA. Neonatal thrombocytopenia: causes and management. *Arch Dis Child Fetal Neonatal Ed* 2003;88:F359–F364.

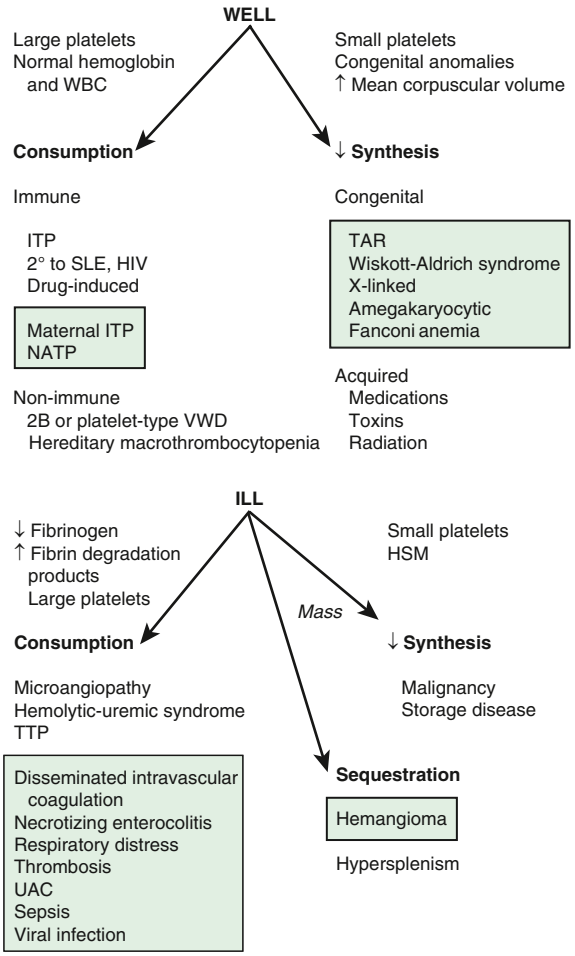

Fig. 533.2 Differential diagnostic algorithm of childhood thrombocytopenic syndromes. The syndromes initially are separated by their clinical appearance. Clues leading to the diagnosis are shown in *italics*. The mechanisms and common disorders leading to these findings are shown in the *lower part* of the figure. Disorders that commonly affect neonates are listed in the *shaded boxes*. HSM, Hepatosplenomegaly; ITP, immune thrombocytopenia; NATP, neonatal alloimmune thrombocytopenic purpura; SLE, systemic lupus erythematosus; TAR, thrombocytopenia–absent radius; TTP, thrombotic thrombocytopenic purpura; UAC, umbilical artery catheter; VWD, von Willebrand disease; WBC, white blood cell. *(From Scott JP. Bleeding and thrombosis. In Kliegman RM, ed. Practical Strategies in Pediatric Diagnosis and Therapy. Philadelphia: Saunders, 1996. p. 849; and Kliegman RM, Marcdante KJ, Jenson HB, et al., eds. Nelson Essentials of Pediatrics, 5th ed. Philadelphia: Saunders, 2006. p. 716.)*

coagulation (DIC) (see Fig. 533.2, and Table 532.1 in Chapter 532). Patients receiving heparin may develop heparin-induced thrombocytopenia. Isolated enlargement of the spleen suggests the potential for hypersplenism caused by liver disease or portal vein thrombosis. Autoimmune thrombocytopenia may be an initial manifestation of SLE, HIV infection, common variable immunodeficiency, and, rarely, lymphoma or autoimmune lymphoproliferative syndrome. Wiskott-Aldrich syndrome (WAS) must be considered in young males found to have thrombocytopenia with *small* platelets, particularly if there is a history of eczema and recurrent infection (see Chapter 165.2). Bernard-Soulier syndrome, on the other hand, involves a macrothrombocytopenia. Gray platelet syndrome is usually associated with splenomegaly and pale-colored platelets on peripheral smear.

TREATMENT

A number of treatment options exist (Table 533.4), but there are no current high-quality data showing that treatment affects either short- or long-term clinical outcome of ITP in children. Many patients with new-onset ITP have mild symptoms, with findings limited to petechiae and purpura on the skin, despite severe thrombocytopenia. Compared with untreated controls, treatment appears to be capable of inducing a more rapid rise in platelet count to the presumed safe level of >20 $\times$ 10^9/L, although no data indicate that early therapy prevents ICH.

Antiplatelet antibodies bind to transfused platelets as well as they do to autologous platelets. Thus platelet transfusion in ITP is usually contraindicated unless life-threatening bleeding is present. Management guidelines for ITP in children and adults reinforce existing emphasis on prioritizing watchful waiting for spontaneous resolution or outpatient therapy in the uncommon cases for which treatment is needed. Initial approaches to the management of pediatric ITP include the following:

1. No therapy other than education and counseling of the family and patient for patients with minimal, mild, and moderate symptoms, as defined earlier. This approach emphasizes the usually benign nature of ITP and avoids the therapeutic roller coaster that ensues once interventional therapy is begun. This approach is much less costly, and side effects are minimal. Observation is recommended for children with no bleeding or *only* mild bleeding symptoms such as bruising or petechiae.
2. Treatment with either intravenous immunoglobulin (IVIG) or corticosteroids, particularly for children who present with *mucocutaneous* bleeding. IVIG at a dose of 0.8-1.0 g/kg/day for 1-2 days induces a rapid rise in platelet count (usually >20 × 10^9/L) in 95% of patients within 48 hours. IVIG appears to induce a response by downregulating Fc-mediated phagocytosis of antibody-coated platelets. IVIG therapy is both expensive and time-consuming and typically requires inpatient admission. Additionally, after infusion, there is a risk of headaches and vomiting, suggestive of IVIG-induced aseptic meningitis.
3. Corticosteroid therapy has been used for many years to treat acute and chronic ITP in adults and children. Doses of prednisone at 1-4 mg/kg/day appear to induce a more rapid rise in platelet count than in untreated patients with ITP. Corticosteroid therapy is usually continued for a short course (approximately 5 days) until a rise in platelet count to >20 × 10^9/L has been achieved to avoid the long-term side effects of corticosteroid therapy, especially growth failure, diabetes mellitus, and osteoporosis.

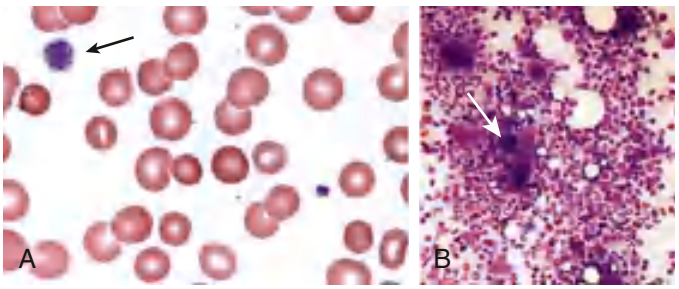

Fig. 533.3 Blood and marrow aspirate from child with immune thrombocytopenia. **A,** Blood smear shows large platelets. **B,** Bone marrow aspirate shows increased numbers of megakaryocytes, many of which appear immature. *(From Blanchette V, Bolton-Maggs P: Childhood immune thrombocytopenic purpura: diagnosis and management. Pediatr Clin North Am 2008;55:393–420. Fig 4.)*

Each of these medications may also be used to treat subsequent ITP exacerbations/recurrences, which usually occur several weeks after an initial course of therapy in 5–10% of patients. In the special case of ICH, multiple modalities should be used, including platelet transfusion (although not recommended in other bleeding manifestations), IVIG, high-dose corticosteroids, and prompt consultation by neurosurgery and general surgery (splenectomy).

Patients who are bleeding significantly (<5% of children with ITP) should be treated. ICH remains rare, and there are no data showing that treatment actually reduces its incidence. *Mucosal bleeding* in particular is the most significant in terms of predicting severe bleeding, but specific predictive indices, such as positive or negative predictive values, are not currently well established.

The role of **splenectomy** in ITP should be reserved for one of two circumstances: (1) the older child (≥4 years) with severe ITP that has lasted >1 year **(chronic ITP)** and whose symptoms are not easily controlled with therapy and/or (2) when life-threatening hemorrhage (e.g., ICH) complicates acute ITP if the platelet count cannot be corrected rapidly with transfusion of platelets and administration of IVIG and corticosteroids. Splenectomy is associated with a lifelong risk of overwhelming postsplenectomy infection caused by encapsulated organisms, increased risk of thrombosis, and the potential development of pulmonary hypertension in adulthood. As an alternative to splenectomy**, rituximab** has been used in children to treat chronic ITP. In 30–40% of children, rituximab has induced a partial or complete remission. TPO receptor agonists have also been used to increase platelet count and are approved for pediatric use.

CHRONIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA

Approximately 20% of patients who present with acute ITP have persistent thrombocytopenia for >12 months and are said to have *chronic* ITP. At that time, a careful reevaluation for associated disorders should be performed, especially for autoimmune disease (e.g., SLE), chronic infectious disorders (e.g., HIV), and nonimmune causes of chronic thrombocytopenia, such as type 2B and platelet-type von Willebrand disease (VWD), X-linked thrombocytopenia, autoimmune lymphoproliferative syndrome, common variable immunodeficiency syndrome, autosomal macrothrombocytopenia, and WAS. The presence of coexisting *H. pylori* and hepatitis C infection should be considered and, if found, treated. Therapy should be aimed at controlling symptoms and

Table 533.4 Treatment Options for Immune Thrombocytopenia (ITP)

	PROS	CONS	COST
Observation	Does not expose patient to unnecessary medications	May increase parent and physician anxiety	Relatively inexpensive
IVIG	Rapid response in most cases	IV administration, side effects	Expensive
Corticosteroids	Oral, effective in 70–80% of patients, minimal side effects with short courses	Side effects, may not affect long term outcome	Inexpensive
Rituximab	Long-term remission in 40–60% of patients	IV administration, immune suppression, potential for reactivation of hepatitis	Very expensive
Splenectomy	Curative in 80% of patients	Requires surgery and anesthesia, lifelong risk of infection	Expensive
Thrombopoietin receptor agonists	Potential for oral administration, 40–60% of patients respond	Not curative, usually required long term, can cause elevated liver enzymes	Very expensive

IV, Intravenous; IVIG, intravenous immunoglobulin.
Adapted from Flood VH, Scott JP. Bleeding and thrombosis. In: Kliegman R, Lye P, Bordini B, et al. eds. *Nelson Pediatric Symptom-Based Diagnosis.* Philadelphia: Elsevier, 2017.

preventing serious bleeding. In ITP, the spleen is the primary site of both antiplatelet antibody synthesis and platelet destruction. Splenectomy is successful in inducing complete remission in 64–88% of children with chronic ITP. This effect must be balanced against the lifelong risk of overwhelming postsplenectomy infection and/or thrombosis. This decision is often affected by quality-of-life issues, as well as the ease with which the child can be managed using medical therapy, such as IVIG, corticosteroids, IV anti-D, or rituximab. Two effective agents that act to stimulate thrombopoiesis, **romiplostim** and **eltrombopag** (see Fig. 533.1), are approved to treat adults and children with chronic ITP. Although these do not address the mechanism of action of ITP, the increase in platelet count may be enough to compensate for the increased destruction and allow the patient to have resolution of bleeding and maintain a platelet count >50 × 10^9/L.

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533.2 Drug-Induced Thrombocytopenia

Brian R. Branchford and Veronica H. Flood

A number of drugs are associated with immune thrombocytopenia as the result of either an immune process or megakaryocyte injury. Some common drugs used in pediatrics that cause thrombocytopenia include valproic acid, phenytoin, carbamazepine, sulfonamides, vancomycin, and trimethoprim-sulfamethoxazole. Most of these drugs may affect platelet function as well as the count itself. Heparin-induced thrombocytopenia (and rarely an associated thrombosis) is seldom seen in pediatrics, but it occurs when, after exposure to heparin, the patient has an antibody directed against the heparin–platelet factor 4 complex. Recommended treatment for heparin-induced thrombocytopenia includes direct thrombin inhibitors such as argatroban or bivalirudin and removal of all sources of heparin, including central venous catheter line flushes.

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533.3 Nonimmune Platelet Destruction

Brian R. Branchford and Veronica H. Flood

The syndromes of DIC (see Chapter 532), HUS (see Chapter 560.5), and thrombotic thrombocytopenic purpura (see Chapter 533.5) share the hematologic picture of a thrombotic microangiopathy in which there exists RBC destruction and consumptive thrombocytopenia caused by platelet and fibrin deposition in the microvasculature. The microangiopathic hemolytic anemia is characterized by the presence of RBC fragments, including helmet cells, schistocytes, spherocytes, and burr cells.

533.4 Hemolytic-Uremic Syndrome

See Chapter 560.5.

533.5 Thrombotic Thrombocytopenic Purpura

Brian R. Branchford and Veronica H. Flood

Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy characterized by the pentad of fever, microangiopathic hemolytic anemia, thrombocytopenia, abnormal renal function, and central nervous system (CNS) changes; TTP is clinically similar to HUS (Table 533.5). Although TTP can be congenital, the acquired form is more common and usually presents in adults and occasionally in adolescents. Microvascular thrombi within the CNS cause subtle, shifting neurologic signs that vary from changes in affect and orientation to aphasia, blindness, and seizures. Initial manifestations are often nonspecific (weakness, pain, emesis), and prompt recognition of this disorder is critical. Laboratory findings provide important clues to the diagnosis and show microangiopathic hemolytic anemia characterized by morphologically abnormal RBCs, with schistocytes, spherocytes, helmet cells, and an elevated reticulocyte count in association with thrombocytopenia. Coagulation studies are usually nondiagnostic. Blood urea nitrogen and creatinine are sometimes elevated. The **treatment** of acquired TTP is plasmapheresis (plasma exchange), which is effective in 80–95% of patients. Treatment with plasmapheresis should be instituted on the basis of thrombocytopenia and microangiopathic hemolytic anemia even if other symptoms are not yet present because of the high mortality (80–90%) in patients without timely intervention. Rituximab, corticosteroids, and splenectomy are reserved for refractory cases. *Caplacizumab,* an anti-VWF humanized immunoglobulin, blocks the interaction of ultralarge (i.e., most likely to bind platelets) VWF multimers with platelets, and many result in rapid resolution of acute TTP.

The majority of cases of TTP are acquired, caused by an autoantibody-mediated **deficiency of ADAMTS13** (a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13) that is responsible for cleaving the high-molecular-weight multimers of VWF and appears to play a pivotal role in the evolution of the thrombotic microangiopathy (Fig. 533.4) that results in a preponderance of ultralarge multimers that more adeptly bind platelets, triggering the microangiopathy. In contrast, levels of ADAMTS13 in HUS are usually normal.

Congenital ADAMTS13 deficiency causes rare familial cases of TTP/HUS, usually manifested as recurrent episodes of thrombocytopenia, hemolytic anemia, jaundice, and renal involvement, with or without neurologic changes, that often present in infancy in the context of an intercurrent illness. Treatment of hereditary TTP with recombinant ADAMTS13 has been successful when patients are refractory to fresh frozen plasma therapy. Abnormalities of the complement system have now also been implicated in rare cases of familial TTP. ADAMTS13 deficiency can be treated by repeated infusions of fresh-frozen plasma.

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533.6 Kasabach-Merritt Syndrome

Brian R. Branchford and Veronica H. Flood

See also Chapter 691.

The association of a giant hemangioma with localized intravascular coagulation causing consumptive thrombocytopenia and hypofibrinogenemia is called Kasabach-Merritt syndrome. In most patients, the site of the hemangioma is obvious, but retroperitoneal and intra-abdominal hemangiomas may require body imaging for detection. Platelet trapping and activation of coagulation occurs inside the hemangioma, with fibrinogen consumption and generation of fibrin(ogen) degradation products. Arteriovenous malformation within the lesions can cause heart failure. Pathologically, Kasabach-Merritt syndrome appears to develop more often as a result of a kaposiform hemangioendothelioma or tufted hemangioma rather than a simple hemangioma. The peripheral blood smear shows microangiopathic changes.

Multiple modalities have been used to treat Kasabach-Merritt syndrome, including propranolol, surgical excision (if possible), laser photocoagulation, high-dose corticosteroids, local radiation therapy, antiangiogenic agents such as interferon-α_2, and vincristine. Over time, most patients who present in infancy have regression of the hemangioma. Treatment of the associated coagulopathy may benefit from a trial of antifibrinolytic therapy with ε-aminocaproic acid (Amicar) or anticoagulation with low molecular weight heparin.

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Table 533.5 ADAMTS13 Deficiency and Thrombotic Thrombocytopenic Purpura

DISEASE	PATHOPHYSIOLOGY	LAB FINDINGS	MANAGEMENT
Thrombotic thrombocytopenic purpura (TTP)	*Acquired*: Ab to ADAMTS13 *Congenital*: Inadequate ADAMTS13 production	Ab to ADAMTS13 ADAMTS13 <10%	*Acquired*: Plasmapheresis with plasma *Congenital*: Scheduled plasma infusions

Autoimmune TTP may be transient, recurrent, drug (ticlopidine, clopidogrel) associated, or seen in some pregnancy-associated cases of TTP.
ADAMTS13 pathogenic variants are often familial and chronic-relapsing RRP.

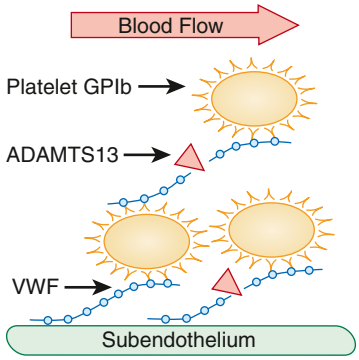

Fig. 533.4 Pathogenesis of thrombotic thrombocytopenic purpura (TTP). The von Willebrand factor (VWF) multimers facilitate platelet adhesion to the subendothelium by binding to exposed connective tissue and then to platelet glycoprotein Ib (GPIb). In flowing blood shear stress unfolds ultralarge VWF multimers in the platelet-rich thrombus and enables ADAMTS13 to cleave a specific Tyr-Met bond in the second of the three A domains in VWF subunits. Cleavage reduces VWF multimer size and limits thrombus growth. In the absence of ADAMTS13, VWF-dependent platelet accumulation continues and eventually results in microvascular thrombosis and TTP. *(Courtesy Dr. J. Evan Sadler, Washington University.)*

533.7 Sequestration

Brian R. Branchford and Veronica H. Flood

Thrombocytopenia develops in individuals with massive splenomegaly, such as those with portal vein thrombosis or liver disease, because the spleen acts as a reservoir for platelets and may sequester a large number of them. Most such patients also have mild leukopenia and anemia on CBC. Individuals who have thrombocytopenia caused by splenic sequestration should undergo a workup to diagnose the etiology of splenomegaly, including infectious, inflammatory, infiltrative, neoplastic, obstructive, and hemolytic causes.

533.8 Congenital Thrombocytopenic Syndromes

Brian R. Branchford and Veronica H. Flood

See Table 533.2.

Congenital amegakaryocytic thrombocytopenia (CAMT) usually manifests within the first few days to week of life, when the child presents with petechiae and purpura caused by profound thrombocytopenia. CAMT is caused by a rare defect in hematopoiesis as a result of pathogenic variants in the *MPL* gene that encodes the stem cell TPO receptor. Other than skin and mucous membrane abnormalities, findings on physical examination are normal. Examination of the bone marrow shows an absence of megakaryocytes. These patients often progress to marrow failure (aplasia). Hematopoietic stem cell transplantation (HSCT) is curative.

Thrombocytopenia–absent radius (TAR) syndrome consists of thrombocytopenia (absence or hypoplasia of megakaryocytes) that presents in early infancy with bilateral radial anomalies of variable severity, ranging from mild changes to marked limb shortening (Fig. 533.5). Many such individuals also have other skeletal abnormalities of the ulna, radius, and lower extremities. *Present thumbs help to differentiate this disorder from Fanconi anemia.* Intolerance to cow's milk formula (present in 50%) may complicate management by triggering gastrointestinal (GI) bleeding, increased thrombocytopenia, eosinophilia, and a leukemoid reaction. The thrombocytopenia of TAR syndrome frequently remits over the first few years of life. The molecular basis of TAR syndrome is linked to *RBM8A*. A few patients have been reported to have a syndrome of **amegakaryocytic thrombocytopenia with radioulnar synostosis** caused by a pathogenic variant in the *HOXA11* gene. In contrast to TAR syndrome, this clinical disorder presents with marrow aplasia.

WAS is characterized by microthrombocytopenia, with tiny platelets, eczema, and recurrent infection as a consequence of immune deficiency (see Chapter 165.2). WAS is inherited as an X-linked disorder, and the gene implicated in WAS has been identified. The WAS protein appears to play an integral role in regulating the cytoskeletal architecture of both platelets and T lymphocytes in response to receptor-mediated cell signaling. The WAS protein is common to all cells of hematopoietic lineage. Molecular analysis of families with X-linked thrombocytopenia has shown that many affected members have a single nucleotide pathogenic variant within the WAS gene, whereas individuals with the full manifestation of WAS have large gene deletions. Examination of the bone marrow in WAS shows the normal number of megakaryocytes, although they may have bizarre morphologic features. Transfused platelets have a normal life span in these patients. Splenectomy often corrects the thrombocytopenia, suggesting that the platelets formed in WAS have accelerated destruction. After splenectomy, these patients are at increased risk for overwhelming infection and require lifelong antibiotic prophylaxis against encapsulated organisms. Approximately 5–15% of patients with WAS develop lymphoreticular malignancies (involving mostly leukemias and lymphomas). Successful HSCT from an unaffected donor cures WAS. **X-linked macrothrombocytopenia** and **dyserythropoiesis** have been linked to pathogenic variants in *GATA1*, which encodes an erythroid and megakaryocytic transcription factor.

MYH9-related thrombocytopenias include a number of diverse hereditary thrombocytopenia syndromes (e.g., Sebastian, Epstein, May-Hegglin, Fechtner) characterized by autosomal dominant macrothrombocytopenia, neutrophil inclusion bodies, and a variety of physical anomalies, including sensorineural deafness, renal disease, and eye disease. These have all been shown to be caused by different pathogenic variants in the *MYH9* gene (nonmuscle myosin-IIa heavy chain 9). The thrombocytopenia is usually mild and not progressive. Some other individuals with recessively inherited macrothrombocytopenia have abnormalities in chromosome 22q11. Pathogenic variants in the gene for glycoprotein Ibβ, an essential component of the platelet VWF receptor, can result in **Bernard-Soulier syndrome** (see Chapter 533.13), a macrothrombocytopenic disease. A diagnostic approach to genetic platelet disorders is noted in Figure 533.6.

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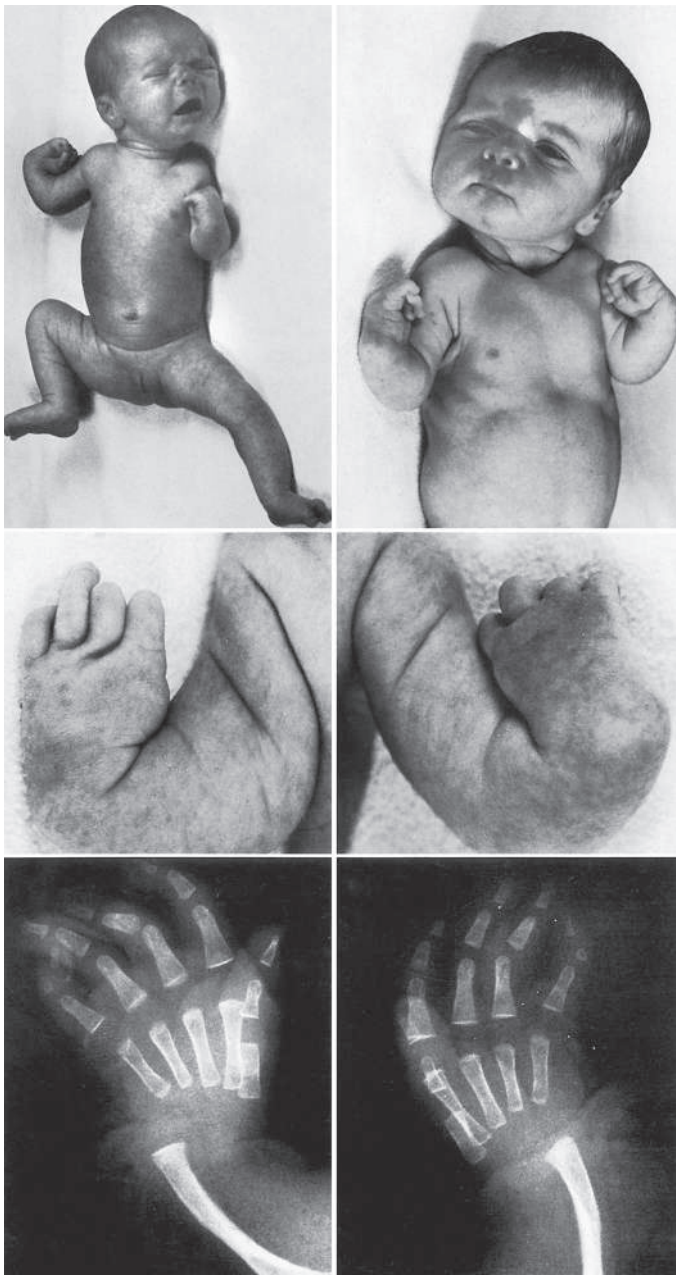

Fig. 533.5 A newborn, the first child of young, healthy parents, with fully expressed thrombocytopenia–absent radius (TAR) syndrome, including hypereosinophilia and anemia. Hypoplasia of the distal humeri and the shoulder girdles, bilateral hip dysplasia, mild talipes calcaneus, and clinodactyly of both little fingers are seen. This patient had a pronounced allergy to cow's milk, with exposure followed by diarrhea, vomiting, and decreased weight and platelet count, making a cow's milk–free diet mandatory. A persistent depressed nasal bridge and development of pronounced bowed legs are seen. *(From Wiedemann H-R, Kunze J, Grosse F-R, eds. Clinical Syndromes, 3rd ed. [English translation]. London: Mosby-Wolfe, 1997. p. 430.)*

533.9 Neonatal Thrombocytopenia

Brian R. Branchford and Veronica H. Flood

Thrombocytopenia in the newborn rarely is indicative of a primary disorder of megakaryopoiesis. It is usually the result of systemic illness or transfer of maternal antibodies directed against fetal platelets (see Table 533.3). Neonatal thrombocytopenia often occurs in association with congenital viral infections, especially rubella, cytomegalovirus, protozoal infection (e.g., toxoplasmosis), and syphilis, and perinatal bacterial infections, especially those caused by gram-negative bacilli. Thrombocytopenia associated with DIC may be responsible for severe spontaneous bleeding. The constellation of marked thrombocytopenia and abnormal abdominal findings is common in necrotizing enterocolitis and other causes of necrotic bowel. Thrombocytopenia in an ill child requires a prompt search for viral and bacterial pathogens.

Antibody-mediated thrombocytopenia in the newborn occurs because of transplacental transfer of maternal antibodies directed against fetal platelets. **Neonatal alloimmune thrombocytopenia (NAIT)** is caused by the development of maternal antibodies against paternally inherited antigens present on fetal platelets that are recognized as foreign by the maternal immune system. The incidence of NATP is 1/4,000-5,000 live births. The clinical manifestations of NAIT are those of an apparently well child who, within the first few days after delivery, has generalized petechiae and purpura. Laboratory studies show a normal maternal platelet count but moderate to severe thrombocytopenia in the newborn. Detailed review of the history should show no evidence of maternal thrombocytopenia. Up to 30% of infants with severe NAIT may have ICH, either prenatally or in the perinatal period. Unlike Rh disease, first pregnancies may be severely affected. Subsequent pregnancies are often more severely affected than the first.

The **diagnosis** of NAIT is made by checking for the presence of maternal alloantibodies directed against the father's platelets. Specific studies can be done to identify the target alloantigen. The most common cause is incompatibility for the platelet alloantigen HPA-1a. Specific DNA sequence polymorphisms have been identified that permit informative prenatal testing to identify at-risk pregnancies. The differential diagnosis of NAIT includes transplacental transfer of maternal IgG antiplatelet autoantibodies (maternal ITP), and, more commonly, viral or bacterial infection.

Treatment of NAIT requires the administration of IVIG prenatally to the mother if the status is known before birth. Therapy usually begins in the second trimester and is continued throughout the pregnancy. Fetal platelet count can be monitored by percutaneous umbilical blood sampling. Delivery should be performed by cesarean section to reduce risk for ICH from vaginal delivery. After delivery, if severe thrombocytopenia persists, transfusion of platelets that share the maternal alloantigens (e.g., washed maternal platelets) will cause a rise in platelet counts to provide effective hemostasis. However, a random donor platelet transfusion is more likely to be readily available. Some centers have units available that may lack the antigens most often involved. After there has been one affected child, genetic counseling is critical to inform the parents of the high risk of thrombocytopenia in subsequent pregnancies.

Children born to mothers with immune thrombocytopenic purpura **(maternal ITP)** appear to have a lower risk of serious hemorrhage than infants born with NAIT, although severe thrombocytopenia may occur. The mother's preexisting platelet count may have some predictive value in that severe maternal thrombocytopenia before delivery appears to predict a higher risk of fetal thrombocytopenia. In mothers who have had splenectomy for ITP, the maternal platelet count may be normal and is not predictive of fetal thrombocytopenia.

Treatment includes prenatal administration of corticosteroids to the mother and IVIG and sometimes corticosteroids to the infant after delivery. Thrombocytopenia in an infant, whether a result of NAIT or maternal ITP, usually resolves within 2-4 months after delivery. The period of highest risk is the immediate perinatal period.

Two syndromes of congenital failure of platelet production often present in the newborn period. In **CAMT** the newborn manifests petechiae and purpura shortly after birth. Findings on physical examination are otherwise normal. Megakaryocytes are absent from the bone marrow. This syndrome is caused by a pathogenic variant in the megakaryocyte TPO receptor that is essential for development of all hematopoietic cell lines. Pancytopenia eventually develops, and HSCT is curative. **TAR syndrome** consists of thrombocytopenia that presents in early infancy, with bilateral radial anomalies of variable severity, ranging from mild changes to marked limb shortening. It frequently remits over the first few years of life (see Chapter 533.8 and Fig. 533.5).

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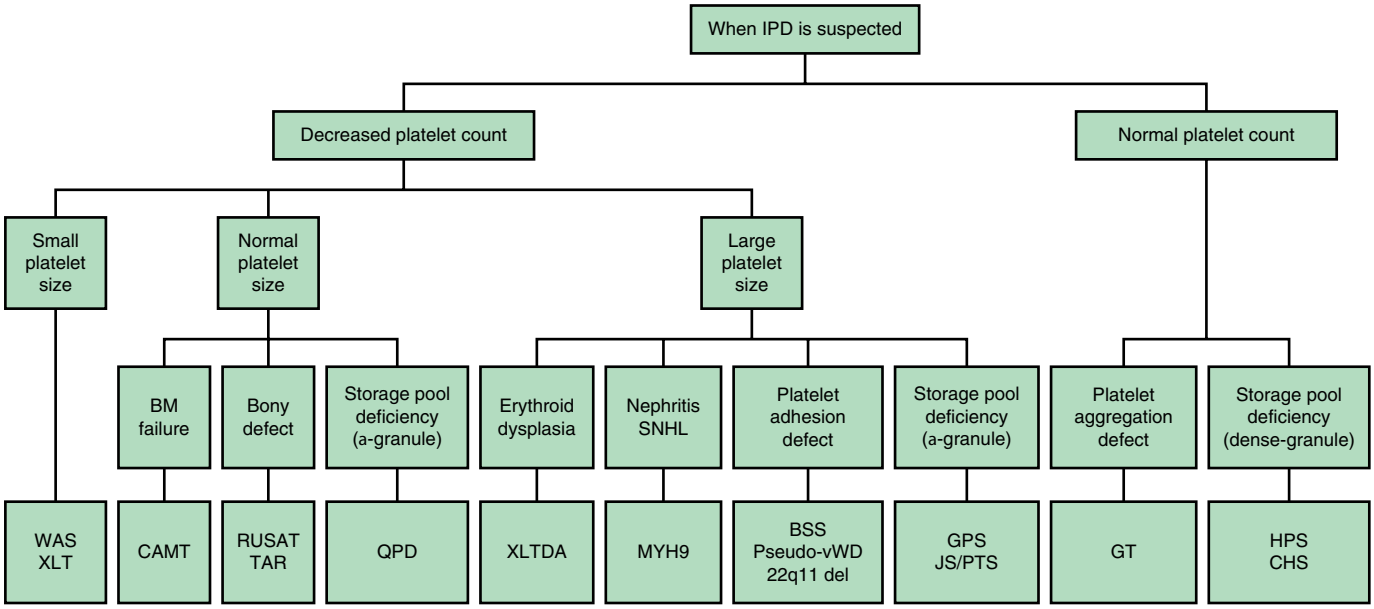

Fig. 533.6 Diagnostic algorithm for sequence and flow in inherited platelet disorders (IPDs). BM, bone marrow; WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia; CAMT, congenital amegakaryocytic thrombocytopenia; RUSAT, radioulnar synostosis with amegakaryocytic thrombocytopenia; TAR, thrombocytopenia-absent radius; QPD, Quebec platelet disorder; XLTDA, X-linked thrombocytopenia with or without dyserythropoietic anemia; MYH9, MYH9-related disorders; BSS, Bernard-Soulier syndrome; vWD, von Willebrand disease; 22q11 del, 22q11 deletion syndrome; GPS, gray platelet syndrome; JS, Jacobsen syndrome; PTS, Paris-Trousseau syndrome; GT, Glanzmann thrombasthenia; HPS, Hermansky-Pudlak syndrome; CHS, Chediak-Higashi syndrome. *(From Shim YJ. Genetic classification and confirmation of inherited platelet disorders: current status in Korea. CEP 2020;63:79–87. Fig. 2.)*

533.10 Thrombocytopenia From Acquired Disorders Causing Decreased Production

Brian R. Branchford and Veronica H. Flood

Disorders of the bone marrow that inhibit megakaryopoiesis usually affect RBC and WBC production. Infiltrative disorders, including malignancies, such as acute lymphocytic leukemia, histiocytosis, lymphomas, and storage disease, usually cause either abnormalities on physical examination (lymphadenopathy, hepatosplenomegaly, or masses), abnormalities of the WBC count, or anemia. Aplastic processes may present as isolated thrombocytopenia, although there are usually clues on the CBC (leukopenia, neutropenia, anemia, or macrocytosis). Children with constitutional aplastic anemia (Fanconi anemia) often (but not always) have abnormalities on examination, including radial and/or thumb anomalies, other skeletal anomalies, short stature, microcephaly, and hyperpigmentation. Bone marrow examination should be performed when thrombocytopenia is associated with abnormalities found on physical examination or on examination of the other blood cell lines.

533.11 Platelet Function Disorders

Brian R. Branchford and Veronica H. Flood

There is no simple and reliable test to screen for abnormal platelet function. Bleeding time and the platelet function analyzer (PFA-100) have been used, but neither has sufficient sensitivity or specificity to rule in or rule out a platelet defect, especially one with mild-moderate phenotypic expression. Bleeding time measures the interaction of platelets with the blood vessel wall and thus is affected by both platelet count and platelet function. The predictive value of bleeding time is problematic because bleeding time is dependent on other factors, including the technician's skill and the patient's cooperation, often a challenge in the infant or young child; it is not recommended in high resource settings. The PFA-100 measures platelet adhesion and aggregation in whole blood at high shear when the blood is exposed to either collagen-epinephrine or collagen-ADP. Results are reported as the closure time in seconds. The use of the PFA-100 as a screening test remains controversial. For patients with a positive history of bleeding suggestive of VWD or platelet dysfunction, specific VWF testing and platelet function studies should be done, irrespective of the results of the bleeding time or PFA-100.

Platelet function in the clinical laboratory is measured using platelet aggregometry. In the aggregometer, agonists, such as collagen, ADP, ristocetin, epinephrine, arachidonic acid, and thrombin (or the thrombin receptor peptide), are added to platelet-rich plasma, and the clumping of platelets over time is measured by an automated machine through either light transmission or electric impedance. At the same time, other instruments measure the release of granular contents, such as ATP, from the platelets after activation. The ability of platelets to aggregate and their metabolic activity can thus be assessed simultaneously. When a patient is being evaluated for possible platelet dysfunction, it is critically important to exclude the presence of other exogenous agents and to study the patient, if possible, off all medications for 2 weeks, especially those with any potential antiplatelet effects. Further evaluation using flow cytometric analysis of surface receptors or molecular and/or genetic testing is often necessary to make a more definitive diagnosis.

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533.12 Acquired Disorders of Platelet Function

Brian R. Branchford and Veronica H. Flood

A number of systemic illnesses are associated with platelet dysfunction, most frequently liver disease, kidney disease (uremia), and disorders that trigger increased amounts of fibrin degradation products. These disorders frequently cause prolonged bleeding time and are often associated with other abnormalities of the coagulation mechanism. *The most important element of management is to treat the primary illness.* If treatment of the primary process is not feasible, infusions of

desmopressin have been helpful in augmenting hemostasis and correcting bleeding time. In some patients, transfusions of platelets and cryoprecipitate have also been helpful in improving hemostasis.

Many medications alter platelet function. The most common drug in adults that alters platelet function is acetylsalicylic acid *(aspirin)*. Aspirin irreversibly acetylates the enzyme cyclooxygenase, which is critical in the formation of thromboxane A_2. Aspirin usually causes moderate platelet dysfunction that becomes more prominent if there is another abnormality of the hemostatic mechanism. In children, common drugs that reversibly inhibit platelet function include other nonsteroidal anti-inflammatory drugs (NSAIDs), valproic acid, and high-dose penicillin. Specific agents to inhibit platelet function therapeutically include those that block the platelet ADP receptor (clopidogrel) and αIIb-β_3 receptor antagonists, as well as aspirin.

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533.13 Congenital Abnormalities of Platelet Function

Brian R. Branchford and Veronica H. Flood

Severe platelet function defects usually present with petechiae and purpura shortly after birth, especially after vaginal delivery (see Table 533.2). Defects in the platelet GPIb complex (the VWF receptor) or the αIIb-β_3 complex (the fibrinogen receptor) cause severe congenital platelet dysfunction. Although laboratory tests of platelet function are available, molecular characterization by genetic testing is rapidly progressing for platelet disorders.

Bernard-Soulier syndrome, a severe congenital platelet function disorder, is caused by absence or severe deficiency of the GPIb complex (VWF receptor) on the platelet membrane. This syndrome is characterized by a macrothrombocytopenia, with giant platelets and greatly prolonged bleeding time (>20 min) or PFA-100 closure time. Patients may have significant mucocutaneous and GI bleeding. Platelet aggregation tests classically demonstrate absent ristocetin-induced platelet aggregation but normal aggregation to all other agonists. Ristocetin induces the binding of VWF to platelets and agglutinates platelets. Results of studies of VWF antigen and activity are normal. The GPIb complex interacts with the platelet cytoskeleton; a defect in this interaction is believed to be the cause of the large platelet size. This receptor deficiency can also be detected by flow cytometry. Bernard-Soulier syndrome is inherited as an autosomal recessive disorder. Causative pathogenic variants are usually identified in the genes encoding the GPIb complex of glycoproteins Ibα, Ibβ, V, and IX.

Glanzmann thrombasthenia is a congenital disorder associated with severe platelet dysfunction that yields prolonged bleeding time and a normal platelet count. Platelets have normal size and morphologic features on the peripheral blood smear, and closure times for PFA-100 or bleeding time are extremely abnormal. Aggregation studies classically demonstrate abnormal or absent aggregation with all agonists used except ristocetin because ristocetin agglutinates platelets and does not require a metabolically active platelet. This disorder is caused by deficiency of the platelet fibrinogen receptor αIIb-β_3, the major integrin complex on the platelet surface that undergoes conformational changes by inside-out signaling when platelets are activated. This receptor deficiency can also be detected by flow cytometry. Fibrinogen binds to this complex when the platelet is activated and causes platelets to aggregate. Glanzmann thrombasthenia is caused by pathogenic variants in the genes for αIIb or β_3 and is inherited in an autosomal recessive manner. For both Bernard-Soulier syndrome and Glanzmann thrombasthenia, the **diagnosis** is confirmed by flow cytometry of the patient's platelet glycoproteins. Bleeding in Glanzmann thrombasthenia may be quite severe and is typically mucocutaneous, including epistaxis, gingival, and GI bleeding. There are reports of curative therapy using stem cell transplant.

Hereditary deficiency of platelet storage granules occurs in two well-characterized but rare syndromes that involve deficiency of intracytoplasmic granules. **Dense granule deficiency** is characterized by absence of the granules that contain ADP, ATP, Ca^{2+}, and serotonin. This disorder is diagnosed by the finding that ATP is not released on platelet aggregation studies and ideally is characterized by transmission electron microscopic studies. **Hermansky-Pudlak syndrome** (with nine subtypes) is a dense granule deficiency caused by defects in lysosomal storage. Affected patients present with oculocutaneous albinism and hemorrhage caused by the platelet defect; some patients also develop granulomatous colitis resembling

Table 533.6 Comparison of Nine Types of Hermansky-Pudlak Syndrome

	1	2	3	4	5	6	7	8	9
Oculocutaneous albinism	Variable, mild-moderate: brown to white hair	Severe: lack of hair and iris pigment	Mild-moderate: light skin pigment	Severe: blonde hair, gray iris	Variable: light-brown hair, brown iris	Variable: iris heterochromia	Variable	Variable: tan skin, silver hair, brown iris	Pale skin, silver-blonde hair, pale-blue iris
Platelet defect/bruising	+	+	+	+	+	+	+	+	+
Granulomatous colitis	+	–	–	+	–	+	+	–	–
Pulmonary fibrosis/ILD	+	+	–	+	–	–	–	–	–
Other symptoms		Neutropenia Failure to thrive Hypothyroidism CAH		Depression	High cholesterol				Cutaneous infections

+, Present; –, absent; CAH, congenital adrenal hyperplasia; ILD, interstitial lung disease.

Crohn disease or pulmonary fibrosis/interstitial lung disease (Table 533.6). **Chédiak-Higashi syndrome** also presents with a dense granule defect, immune dysfunction, and albinism. **Gray platelet syndrome** is caused by the absence of platelet α granules, resulting in large platelets that are large and appear gray on Wright stain of peripheral blood. In this rare syndrome, aggregation and release are absent with most agonists other than thrombin and ristocetin. Transmission electron microscopic studies are diagnostic. Autosomal recessive gray platelet syndrome is caused by defects in the *NBEAL2* gene, while autosomal dominant disease is associated with a pathogenic variant in *GFI1B*. This disorder may also be associated with familial leukemia. **Quebec platelet syndrome** is caused by degradation of platelet α granules caused by defects in *PLAU*, which encodes a urokinase-type plasminogen activator. Treatment usually involves antifibrinolytic therapy.

OTHER HEREDITARY DISORDERS OF PLATELET FUNCTION

Abnormalities in the pathways of platelet signaling/activation and release of granular contents cause a heterogeneous group of platelet function defects that are usually manifested as increased bruising, epistaxis, and menorrhagia. Symptoms may be subtle and are often made more obvious by high-risk surgery, such as tonsillectomy or adenoidectomy, or by administration of NSAIDs. In the laboratory, bleeding time is variable, and closure time as measured by the PFA-100 is frequently, but not always, prolonged. Platelet aggregation studies show deficient aggregation with one or two agonists and/or abnormal release of granular contents.

The formation of thromboxane from arachidonic acid (AA) after the activation of phospholipase is critical to normal platelet function. Deficiency or dysfunction of enzymes, such as cyclooxygenase and thromboxane synthase, which metabolize AA, causes abnormal platelet function. In the aggregometer, platelets from such patients do not aggregate in response to AA.

The most common platelet function defects are those characterized by variable bleeding time/PFA closure times and abnormal aggregation with one or two agonists, usually ADP and/or collagen. Some of these individuals have only decreased release of ATP from intracytoplasmic granules; the significance of this finding is debated.

TREATMENT OF PATIENTS WITH PLATELET DYSFUNCTION

Successful treatment depends on the severity of both the diagnosis and the hemorrhagic event. In all but severe platelet function defects, desmopressin, 0.3 μg/kg intravenously, may be used for mild to moderate bleeding episodes. In addition to its effect on stimulating levels of VWF and factor VIII, desmopressin corrects bleeding time and augments hemostasis in many individuals with mild to moderate platelet function defects. Antifibrinolytic therapy may be useful for mucosal bleeds. For individuals with Bernard-Soulier syndrome or Glanzmann thrombasthenia, platelet transfusions of 0.5-1 unit single donor platelets correct the defect in hemostasis and may be lifesaving. Rarely, alloantibodies develop to the deficient platelet protein, rendering the patient refractory to the transfused platelets. In such patients, the use of recombinant factor VIIa has been effective, and this treatment was recently approved for platelet-refractory Glanzmann's thrombasthenia. In both conditions, HSCT has been curative.

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533.14 Disorders of the Blood Vessels

Brian R. Branchford and Veronica H. Flood

Disorders of the vessel walls or supporting structures mimic the findings of a bleeding disorder, although coagulation studies are usually normal. The findings of petechiae and purpuric lesions in such patients are often attributable to an underlying vasculitis or vasculopathy. Skin biopsy can be particularly helpful in elucidating the type of vascular pathology.

IgA VASCULITIS (HENOCH-SCHÖNLEIN PURPURA)

See Chapter 210.1.

EHLERS-DANLOS SYNDROME

See Chapter 744.

OTHER ACQUIRED DISORDERS

Scurvy, chronic corticosteroid therapy, and severe malnutrition are associated with "weakening" of the collagen matrix that supports the blood vessels. Therefore these factors are associated with easy bruising, and, particularly in the case of scurvy, bleeding gums and loosening of the teeth. Lesions of the skin that initially appear to be petechiae and purpura may be seen in vasculitic syndromes, such as SLE. Leukocytoclastic vasculitis may present with nonthrombocytopenic purpura (see Chapter 210). A unique variant of leukocytoclastic vasculitis is associated with exercise.

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Section 8
The Spleen

Chapter 534
Anatomy and Function of the Spleen

Allison S. Remiker and Amanda M. Brandow

ANATOMY

The splenic precursor is recognizable by 5 weeks of gestation. At birth, the spleen weighs approximately 11 g. Thereafter, it enlarges until puberty, reaching an average weight of 150 g, and then diminishes in size during adulthood. Approximately 15% of patients will have an accessory spleen. The major splenic components are a lymphoid compartment *(white pulp)* and a filtering system *(red pulp)*. The white pulp consists of periarterial lymphatic sheaths (PALS; T-zone) of T lymphocytes with embedded germinal centers containing B lymphocytes. The red pulp has a skeleton of fixed reticular cells, mobile macrophages, partially collapsed endothelial passages (cords of Billroth), and splenic sinuses. A *perifollicular zone (PFZ)* (known as the marginal zone in murine models) is rich in dendritic (antigen-presenting) cells and natural killer cells and separates the red pulp from the white pulp. The splenic capsule contains smooth muscle and contracts in response to epinephrine. Approximately 10% of the blood delivered to the spleen flows rapidly through a closed vascular network. The other 90% flows more slowly through an open system (the *splenic cords*), where it is filtered through 1-5 μm slits before entering the splenic sinuses.

FUNCTION

The unique anatomy and blood flow of the spleen enable it to perform reservoir, filtering, and immunologic functions. The spleen

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FUNCTION

The unique anatomy and blood flow of the spleen enable it to perform reservoir, filtering, and immunologic functions. The spleen

receives 5–6% of the cardiac output but normally contains only 25 mL of blood. It can retain much more when it enlarges, leading to **cytopenias**. Hematopoiesis in the red pulp is a major splenic function at 3-6 months of fetal life but subsequently disappears. *Splenic hematopoiesis* can be resumed in patients with myelofibrosis or severe hemolytic anemia. Factor VIII, iron, plasmablasts, plasma cells, and one third of the circulating platelet mass are sequestered in the spleen and can be released by stress or epinephrine stimulation. **Thrombocytosis** and **leukocytosis** occur with loss of the splenic reservoir function. A high platelet count after the loss of splenic function or splenectomy is not associated with an increased risk of thrombosis in children.

Slow blood flow past macrophages and through small openings in the sinus walls facilitates the filtering functions of the spleen removing any particles >1 micron in size from the circulation. Excess membrane is removed from young red blood cells (RBCs) and loss of this function is characterized by target cells, poikilocytosis, and decreased osmotic fragility. The spleen is the primary site for destruction of old RBCs, and this function is assumed by other reticuloendothelial cells after splenectomy. The spleen also removes damaged/abnormal RBCs (e.g., spherocytes, antibody-coated RBCs) and damaged/senescent platelets. Intracytoplasmic inclusions may be removed from RBCs without cell lysis. Functional or anatomic hyposplenia is characterized by continued circulation of cells containing nuclear remnants **(Howell-Jolly bodies)**, denatured hemoglobin **(Heinz bodies)**, and other debris in RBCs. This debris may appear as "pits" on indirect microscopy.

The spleen plays a large role in host defense against infection. Mechanical filtration occurs removing parasitized erythrocytes, as well as unopsonized bacteria. The spleen is the largest lymphoid organ in the body and contains almost half the body's total immunoglobulin-producing B lymphocytes. The spleen processes foreign material to stimulate production of opsonizing antibody. Upon antigenic challenge, B and T cells are developed in the spleen, with subsequent release of immunoglobulins. Production of immune-mediating proteins important in bacterial clearance occurs in the spleen including complement, opsonins, properdin, and tuftsin. Thus young (nonimmune) or hyposplenic individuals are at increased risk for **sepsis** caused by pneumococci and other encapsulated bacteria. The spleen can also use phagocytosis to trap and destroy intracellular parasites. The spleen has a minor role in antibody response to intramuscularly or subcutaneously injected antigens but is required for early antibody production after exposure to intravenous antigens. Additionally, the spleen is important in the pathogenesis of immune-mediated cytopenias (IMCs). Antibody-coated platelets or erythrocytes are phagocytosed via mechanisms associated with splenic macrophages. The spleen also serves as a reservoir for antibody producing plasma cells involved in IMCs.

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Chapter 535

Splenomegaly

Allison S. Remiker and Amanda M. Brandow

CLINICAL MANIFESTATIONS

A soft, thin spleen is palpable in 15% of neonates, 10% of normal children, and 5% of adolescents. In most individuals, the spleen must be 2-3 times its normal size before it is palpable. The spleen is best examined when standing on the right side of a supine patient by palpating across the abdomen as the patient inspires deeply or with the patient in the right lateral decubitus position. A splenic edge felt more than 2 cm below the left costal margin is abnormal. An enlarged spleen might descend into the pelvis; when splenomegaly is suspected, the abdominal examination should begin at a lower starting point. Superficial abdominal venous distention may be present when splenomegaly is a result of portal hypertension. Patients may also complain of left upper quadrant pain as the spleen enlarges. Radiologic detection or confirmation of splenic enlargement is done with ultrasonography, CT, MRI, positron emission tomography, or technetium-99m sulfur colloid scan. The latter also assesses splenic function.

DIFFERENTIAL DIAGNOSIS

Table 535.1 lists specific causes of splenomegaly. A thorough history with a focus on systemic complaints (e.g., fever, night sweats, malaise, weight loss) and a complete physical examination (with special attention to lymphadenopathy, jaundice, hepatomegaly, rashes, joint swelling, petechiae, ecchymoses), in combination with a complete blood count and careful review of the peripheral smear, can help guide diagnosis.

Pseudosplenomegaly

Abnormally long mesenteric connections may produce a *wandering* or ptotic spleen. An enlarged left lobe of the liver, a left upper quadrant mass, or a splenic hematoma may be mistaken for splenomegaly. Splenic **cysts** may contribute to splenomegaly or mimic it; these may be congenital (epidermoid) or acquired (pseudocyst) after trauma or infarction. Cysts are usually asymptomatic and are found on radiologic evaluation. **Splenosis** after splenic rupture or an accessory spleen (present in 15% of normal individuals) may also mimic splenomegaly; most are not palpable. The syndrome of **congenital polysplenism** includes cardiac defects, left-sided organ anomalies, bilobed lungs, biliary atresia, and pseudosplenomegaly (see Chapter 480.11).

Hypersplenism

Increased splenic function (sequestration or destruction of circulating cells) can result in peripheral blood **cytopenias** (thrombocytopenia, neutropenia, anemia), increased bone marrow activity, and splenomegaly. It is usually secondary to another disease and may be cured by treatment of the underlying condition or, if absolutely necessary, may be moderated by splenectomy. Table 535.1 lists the most common diseases associated with massive splenomegaly.

Congestive Splenomegaly

Splenomegaly may result from obstruction in the hepatic, portal, or splenic veins leading to hypersplenism. Wilson disease (see Chapter 405.2), galactosemia (see Chapter 107.2), biliary atresia (see Chapter 404.1), and α_1-antitrypsin deficiency (see Chapter 405) may result in hepatic inflammation, fibrosis, and vascular obstruction. Congenital abnormalities (absence or hypoplasia) of the portal or splenic veins may cause vascular obstruction. Septic omphalitis or thrombophlebitis (spontaneous or as a result of umbilical venous catheterization in neonates) may result in secondary obliteration of these vessels. Splenic venous flow may be obstructed by masses of sickled erythrocytes leading to infarction. When the spleen is the site of vascular obstruction, splenectomy cures hypersplenism. However, because obstruction usually is in the hepatic or portal systems, **portacaval shunting** may be more helpful since both portal hypertension and thrombocytopenia contribute to variceal bleeding.

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Table 535.1 Differential Diagnosis of Splenomegaly by Pathophysiology

ANATOMIC LESIONS
Cysts, pseudocysts
Hamartomas
Polysplenia syndrome
Hemangiomas and lymphangiomas
Hematoma or rupture (traumatic)
Peliosis

HYPERPLASIA CAUSED BY HEMATOLOGIC DISORDERS
*Acute and Chronic Hemolysis**
Hemoglobinopathies (sickle cell disease in infancy with or without sequestration crisis and sickle variants, thalassemia major, unstable hemoglobins)‡
Erythrocyte membrane disorders (hereditary spherocytosis, elliptocytosis, pyropoikilocytosis)
Erythrocyte enzyme deficiencies (severe G6PD deficiency, pyruvate kinase deficiency)
Immune hemolysis (autoimmune and isoimmune hemolysis)
Paroxysmal nocturnal hemoglobinuria
Chronic Iron Deficiency
Extramedullary Hematopoiesis
Myeloproliferative diseases: CML, juvenile CML, myelofibrosis with myeloid metaplasia, polycythemia vera
Osteopetrosis
Patients receiving granulocyte and granulocyte-macrophage colony-stimulating factors

INFECTIONS†
Bacterial
Acute sepsis: *Salmonella typhi*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Staphylococcus aureus*
Chronic infections: infective endocarditis, chronic meningococcemia, brucellosis, tularemia, cat-scratch disease
Local infections: splenic abscess (*S. aureus*, streptococci, less often *Salmonella* spp., polymicrobial infection), pyogenic liver abscess (anaerobic bacteria, gram-negative enteric bacteria), cholangitis

*Viral**
Acute viral infections
Congenital CMV, herpes simplex, rubella
Hepatitis A, B, and C; CMV
EBV
Viral hemophagocytic syndromes: CMV, EBV, HHV-6
HIV‡

Spirochetal
Syphilis, especially congenital syphilis
Leptospirosis

Rickettsial
Rocky Mountain spotted fever
Q fever
Typhus

Fungal/Mycobacterial
Miliary tuberculosis
Disseminated histoplasmosis
South American blastomycosis
Systemic candidiasis (in immunosuppressed patients)

Parasitic
Malaria‡
Toxoplasmosis, especially congenital
Toxocara canis, *Toxocara cati* (visceral larva migrans)
Leishmaniasis (kala-azar)‡
Schistosomiasis (hepatic-portal involvement)
Trypanosomiasis
Fascioliasis
Babesiosis

IMMUNOLOGIC AND INFLAMMATORY PROCESSES*
Systemic lupus erythematosus
Juvenile idiopathic arthritis
Mixed connective tissue disease
Systemic vasculitis
Serum sickness
Drug hypersensitivity, especially to phenytoin
Graft-versus-host disease
Sjögren syndrome
Cryoglobulinemia
Amyloidosis
Sarcoidosis
Autoimmune lymphoproliferative syndrome‡
Castleman disease‡
Posttransplant lymphoproliferative disease
Large granular lymphocytosis and neutropenia
Histiocytosis syndromes‡
Macrophage activation syndrome
Hemophagocytic lymphohistiocytosis (genetic, acquired)‡

MALIGNANCIES
Primary: leukemia (acute, chronic)‡, lymphoma‡, angiosarcoma, mastocytosis
Metastatic

STORAGE DISEASES
Lipidosis (Gaucher disease‡, Niemann-Pick disease, infantile GM1 gangliosidosis)
Mucopolysaccharidoses (Hurler, Hunter-type)
Mucolipidosis (I-cell disease, sialidosis, multiple sulfatase deficiency, fucosidosis)
Defects in carbohydrate metabolism: galactosemia, fructose intolerance, glycogen storage disease IV
Sea-blue histiocyte syndrome
Tangier disease
Wolman disease
Hyperchylomicronemia type I, IV

CONGESTIVE DISEASE*
Heart failure
Intrahepatic cirrhosis or fibrosis
Extrahepatic portal (thrombosis), splenic, and hepatic vein obstruction (thrombosis, Budd-Chiari syndrome)

*Common.
†Chronic or recurrent infection suggests underlying immunodeficiency.
‡Associated with hypersplenomegaly.
CML, Chronic myelogenous leukemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; G6PD, glucose-6-phosphate dehydrogenase; HHV-6, human herpesvirus 6; HIV, human immunodeficiency virus.

Chapter 536

Hyposplenism, Splenic Trauma, and Splenectomy

Allison S. Remiker and Amanda M. Brandow

HYPOSPLENISM

Congenital absence (asplenia) or underdevelopment (hyposplenia) of the spleen is associated with complex cyanotic heart defects, dextrocardia, bilateral trilobed lungs, and heterotopic abdominal organs (**Ivemark syndrome**; see Chapter 480.11). Patients with isolated congenital asplenia have pathogenic variants in *RPSA* in ~40% of cases. Right atrial isomerism (Ivemark syndrome), a form of heterotaxy, may have asplenia associated with pathogenic variants of *GDF1*. Splenic function is usually normal in children with congenital polysplenia. **Functional hyposplenism** may occur in normal neonates, especially premature infants. Children with sickle cell hemoglobinopathies (see Chapter 511.1) may have splenic hypofunction as early as 6 months of age. In this setting, the spleen eventually autoinfarcts and becomes fibrotic and permanently nonfunctional. Functional hyposplenism may also occur in malaria (see Chapter 334), after irradiation to the left upper quadrant, and when the reticuloendothelial function of the spleen is overwhelmed (as in severe hemolytic anemia or metabolic storage disease). Thrombosis of the splenic/celiac arteries or splenic vein is associated with functional hyposplenism. Splenic hypofunction has been reported occasionally in patients with autoimmune diseases (i.e., juvenile idiopathic arthritis, lupus, sarcoidosis), nephritis, inflammatory bowel disease, celiac disease, chronic hepatitis, Pearson syndrome, Fanconi anemia, those with use of high-dose corticosteroids, and graft-versus-host disease (Table 536.1).

Splenic hypofunction is characterized by **red blood cell (RBC) inclusions** in peripheral blood smears (Howell-Jolly or Heinz bodies), "pits" on interference microscopy, and poor uptake of technetium or other spleen scans (Table 536.2 and Fig. 536.1). Reduced immunoglobulin M memory B cells may also be detected and is a risk factor for overwhelming **sepsis**. Patients with functional hyposplenism or asplenia are at increased risk for sepsis from **encapsulated bacteria** and benefit from antibiotic prophylaxis and urgent evaluation when febrile.

Table 536.1 Diseases Associated with Hyposplenism or Splenic Atrophy

CONGENITAL FORMS	**AUTOIMMUNE DISORDERS**
Normal and premature neonates	Systemic lupus erythematosus
Isolated congenital hypoplasia	Juvenile idiopathic arthritis
Ivemark syndrome	Glomerulonephritis
Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) syndrome	Granulomatosis with polyangiitis
	Goodpasture syndrome
	Sjögren syndrome
Hypoparathyroidism syndrome	Polyarteritis nodosa
Stormorken syndrome	Thyroiditis
Heterotaxia syndromes	Sarcoidosis
GASTROINTESTINAL DISORDERS	**INFECTIOUS DISEASES**
Celiac disease	Pneumococcal meningitis
Inflammatory bowel disease	HIV/AIDS
Whipple disease	Malaria
Dermatitis herpetiformis	**IATROGENIC FORMS**
Intestinal lymphangiectasia	Exposure to methyldopa
Idiopathic chronic ulcerative enteritis	High-dose steroids
	Total parenteral nutrition
	Splenic irradiation
HEPATIC DISORDERS	**ALTERATION IN SPLENIC CIRCULATION**
Active chronic hepatitis	Thrombosis of splenic artery
Primary biliary cirrhosis	Thrombosis of splenic vein
Hepatic cirrhosis and portal hypertension	Thrombosis of celiac artery
Alcoholism and alcoholic hepatopathy	**MISCELLANEOUS**
	Amyloidosis
HEMATOLOGIC AND ONCOLOGIC DISORDERS	
Sickle cell disease (all genotypes)	
Bone marrow transplantation	
Chronic graft-versus-host disease	
Acute leukemia	
Chronic myeloproliferative disorders	
Fanconi syndrome	
Splenic tumors	
Mastocytosis	

From Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. *Lancet* 2011;378:86–97.

SPLENIC TRAUMA

Injury to the spleen may occur with abdominal trauma. Small splenic capsular tears may cause abdominal or referred left shoulder pain as a result of diaphragmatic irritation by blood. Larger tears result in more severe blood loss, with similar pain and signs of **hypovolemic shock**. Previously enlarged spleens (as in patients with infectious mononucleosis) are more likely to rupture with minor trauma. *Patients with splenomegaly should avoid contact sports and other activities that increase the risk of splenic trauma.* CT scan with intravenous contrast is the best imaging modality to assess splenic trauma in a hemodynamically stable patient. In emergent situations extended focused assessment with sonography in trauma (E-FAST) should be considered as an initial evaluation, although the sensitivity and specificity in children is variable.

Treatment of a small capsular injury should include careful observation with attention to changes in vital signs or abdominal findings, serial hemoglobin determinations, and the availability of prompt surgical intervention if a patient's condition deteriorates (see Chapter 80). RBC transfusion requirements should be minimal (<25 mL/kg/48 hr). These patients are usually hospitalized for 10-14 days and have their activities restricted for months. Laparotomy, with or without splenectomy, is indicated for more marked abdominal bleeding, in patients who have clinical instability or deterioration, or when other organ damage is suspected. Partial splenectomy and splenic repair should be substituted for total splenectomy when feasible to maintain some splenic immune function. Nonoperative management is typically possible in >90% of patients. Delayed splenic rupture is quite rare in children.

SPLENECTOMY

Splenectomy should be limited to specific indications where medical therapy is (or has been) ineffective. These include traumatic splenic rupture, anatomic defects, splenic malignancy (i.e., splenic marginal zone lymphoma [SMZL]), severe transfusion-dependent hemolytic anemia, refractory and severe immune-mediated cytopenias, metabolic storage diseases, and secondary hypersplenism. There are certain conditions where splenectomy should be avoided because of increased risk of complications and/or better alternative treatments. These include autoimmune lymphoproliferative syndrome (ALPS), hereditary stomatocytosis (HSt), hereditary xerocytosis (HX), cold agglutinin disease (CAD), paroxysmal cold hemoglobinuria (PCH), Gaucher disease, and thrombocytopenia in hepatic cirrhosis. *The major long-term risk of splenectomy is sudden, overwhelming postsplenectomy infections (sepsis or meningitis).* This risk is especially high in children <5 years old at surgery. The risk of sepsis is less when splenectomy is performed for trauma, RBC membrane defects, and immune thrombocytopenia (2–4%) than when there is sickle cell anemia, thalassemia, or a preexisting immune deficiency (Wiskott-Aldrich syndrome, Hodgkin disease) or reticuloendothelial blockade (storage disease, severe hemolytic anemia) (8–30%). The overall risk is 2-5 per 1,000 asplenic patient-years,

Table 536.2 Diagnostic Techniques for and Features of Spleen Dysfunction

	DESCRIPTION	COMMENTS
Immunoglobulin M memory B cells	Cells dependent on spleen for survival. Produced in marginal zone.	Special tests required.
Technetium-99m–labeled sulfur colloidal scintiscan	Quantitation of splenic uptake of colloidal sulfur particles enables a fairly accurate static assessment of spleen function.	Hypertrophy of the left hepatic lobe might be a limiting factor (this technique does not clearly show whether the mass originated in the liver or the spleen in the presence of an overlapping hypertrophic left hepatic lobe).
Technetium-99m–labeled or rubidium-81–labeled heat-damaged autologous erythrocyte clearance	Measurement of clearance time allows a dynamic evaluation of spleen function.	Preexisting erythrocyte defects, difficult erythrocyte incorporation of the radioisotope, and false-positive or false-negative results in relation to excessive or insufficient heat damage make the test not suitable for clinical practice.
Detection of Howell-Jolly bodies by staining	Erythrocytes with nuclear remnants Flow cytometry	No need for special equipment; inaccurate in the quantitation of splenic hypofunction.
Detection of pitted erythrocytes by phase-interference microscopy	Erythrocytes with membrane indentations (4% upper limit of the normal range)	Need for phase-interference microscopy; counts enable a wide range of measurements and correlate with radioisotopic methods.

From Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. *Lancet* 2011;378:86–97. Table 1.

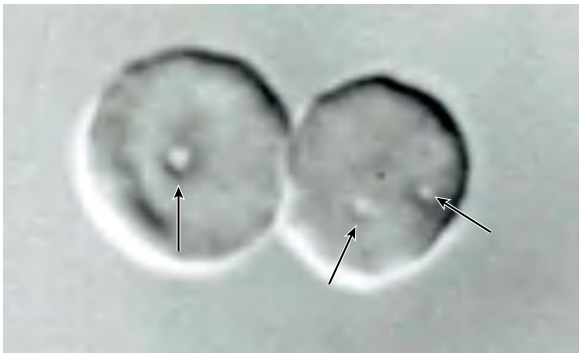

Fig. 536.1 Characteristic pitted erythrocytes in hyposplenism. A pitted erythrocyte is recognizable on phase-interference microscopy by the characteristic "pit" on the cell membrane *(arrows). (From Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97. Fig 2.)*

with a lifelong risk of overwhelming postsplenectomy infections of 5%; more than half occur within 2 years after splenectomy, although the risk remains lifelong. The use of laparoscopic splenectomy has decreased surgical morbidity and hospitalization time.

Encapsulated bacteria, such as *Streptococcus pneumoniae* (>60% of cases), *Haemophilus influenzae,* and *Neisseria meningitidis,* account for >80% of cases of postsplenectomy sepsis. Because the spleen is responsible for filtering the blood and for early antibody responses, sepsis (with or without meningitis) can progress rapidly, leading to death within 12-24 hours of onset. Febrile splenectomized patients should be evaluated and treated promptly with antibiotics to cover the organisms previously mentioned. This treatment should be initiated at home if access to definitive medical care will be delayed. Common empiric antibiotics include amoxicillin-clavulanate or cefdinir. A broad-spectrum cephalosporin (cefotaxime or ceftriaxone) is recommended until specific antibiotic susceptibility and presence, or absence of meningitis is known. Vancomycin (to cover penicillin-resistant pneumococci) should be initiated, depending on the illness severity and susceptibilities of pneumococci at the institution. Splenectomized patients are also at increased risk for contracting protozoal infections, such as malaria and babesiosis. Serious infections may occur after an animal bite or lick (particularly dogs) and is caused by *Capnocytophaga canimorsus* or *C. cynodegmi*. Prophylactic antibiotics should be given after a bite potentially to prevent sepsis caused by these organisms (see Chapter 765).

Preoperative, intraoperative, and postoperative management may decrease the risk of postsplenectomy infection. It is important to be certain of the need for splenectomy and, if possible, to postpone the operation until the patient is ≥5 years of age. *Pneumococcal, meningococcal, and H. influenzae conjugate vaccines given at least 14 days before splenectomy may reduce postsplenectomy sepsis.* The 7-valent (PCV7) was replaced by the 13-valent pneumococcal polysaccharide-protein conjugate vaccine (PCV13). Thus, depending on what primary pneumococcal vaccine was given, a single dose of PCV13 may be recommended. In addition, the 23-valent pneumococcal polysaccharide vaccine (Pneumovax) should be given at age ≥2 years and a second dose 5 years later. Yearly influenza vaccine should also be given because influenza infection is a risk factor for secondary pneumococcal infections. Prophylaxis with oral penicillin VK (125 mg twice daily for children <5 years old; 250 mg twice daily for children ≥5 years) should be given until at least 5 years of age and for at least 2 years after splenectomy. Although the greatest risk is in the immediate postoperative period, reports of deaths occurring years after splenectomy suggest that the risk (and the need for prophylaxis) may be lifelong. Lifelong prophylaxis should be strongly considered in patients who have had an invasive pneumococcal infection or who have an underlying immune deficiency. In children with sickle cell disease, penicillin prophylaxis should be started as soon as the diagnosis is made. Prophylaxis may be continued into adulthood for higher-risk patients, including those with a history of pneumococcal sepsis, but effectiveness in this older group has not been well documented.

In patients with traumatic injury, splenic repair or partial splenectomy should be considered in an attempt to preserve splenic function. Partial splenectomy or partial *splenic embolization* may be sufficient to ameliorate some forms of hemolytic anemia. Up to 50% of children whose spleen is removed because of trauma have spontaneous splenosis; *surgical splenosis* (distributing small pieces of spleen throughout the abdomen) may decrease the risk of sepsis in patients whose splenectomy is necessitated by trauma. However, in both these settings, the splenic tissue that regrows frequently has poor function.

In addition to postsplenectomy sepsis, splenectomized patients may be at risk for **thromboembolic complications**, including arterial and venous thrombosis and pulmonary hypertension. These findings have been reported regardless of the underlying reason for splenectomy and the postsplenectomy platelet count. Proposed mechanisms include loss of filtering function of the spleen, allowing abnormal RBCs to remain in the circulation and activate the coagulation cascade. Portal vein thrombosis has been reported as a complication of laparoscopic splenectomy. The etiology of pulmonary hypertension is not well understood. It has been described in chronic hemolytic conditions including thalassemia, sickle cell disease, and hereditary spherocytosis, indicating there may be a relation to ongoing chronic hemolysis postsplenectomy.

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Section 9

The Lymphatic System

Chapter 537

Anatomy and Function of the Lymphatic System

Michael E. Kelly and Richard L. Tower

The lymphatic system participates in many biologic processes, including fluid homeostasis, absorption of dietary fat, and initiation of specific immune responses. Besides these well-known, classical functions, several novel and unexpected physiologic and pathophysiologic functions of the lymphatics have been recently discovered, including blood pressure regulation, reverse cholesterol transport, association with metabolic diseases and obesity, and an important role in the preparation for neonatal respiration. This system includes circulating lymphocytes, lymphatic vessels, lymph nodes, spleen, tonsils, adenoids, Peyer patches, and thymus. **Lymph** is an ultrafiltrate of blood and is collected by lymphatic capillaries that are present in all organs where blood flows except the bone marrow and retina. Lymphatic capillaries form progressively larger vessels that drain regions of the body. The lymphatic vessels carry lymph to the lymph nodes, where it is filtered through sinuses, particulate matter and infectious organisms are phagocytosed, and antigens are presented to surrounding lymphocytes. These actions stimulate antibody production, T-cell responses, and cytokine secretion (see Chapter 165). Lymph is ultimately returned to the intravascular circulation.

The composition of lymph can vary with the site of lymph drainage. It is usually clear, but lymph drained from the intestinal tract may be milky **(chylous)** because of the presence of fats. The protein content is intermediate between an exudate and a transudate. The protein level may be increased with inflammation and in lymph drained from the liver or intestines. Lymph also contains variable numbers of lymphocytes and antigen-presenting cells.

Embryonic lymphatic development is a stepwise process that starts in the embryonic veins, where lymphatic endothelial cell (LEC) progenitors are initially specified. The differentiation and maturation of these progenitors continues as they bud from the veins to produce scattered primitive lymph sacs, from which most of the lymphatic vasculature is derived. *PROX1* gene expression is important to LEC specification, and studies have shown the critical importance of bone morphogenetic protein (BMP), Wnt, Notch, and vascular endothelial growth factor (VEGF) signaling pathways in lymphatic system development.

Little is known about the establishment of organ-specific lymphatics at later stages. Studies using lineage-tracing technology suggest a venous and nonvenous origin of LECs giving rise to organ-specific lymphatics in the mesentery, skin, and heart. Lymphatic vessels also run parallel to the dural sinuses in the central nervous system. The embryonic origin of the meningeal lymphatics has not been determined.

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Chapter 538

Abnormalities of Lymphatic Vessels

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Developmental lymphatic anomalies including **lymphatic malformations (LMs) and complex lymphatic anomalies (CLAs)** manifest as localized or multifocal lesions of the lymphatic vasculature, respectively (see International Society for the Study of Vascular Anomalies [**ISSVA**] classification; www.issva.org).

Cystic LMs are the most common *congenital* lymphatic anomalies. They occur as solitary lesions of variable size that are classified as macrocystic, microcystic, or mixed cystic LM. LMs commonly infiltrate soft tissues and can be found anywhere on the body, although they occur more frequently on the head, neck, and axilla.

CLAs are multifocal lymphatic vascular lesions involving both soft tissue and bone and often result in disruption of central collecting lymphatic channels. They include **generalized lymphatic anomaly (GLA), Gorham-Stout disease (GSD)** (Fig. 538.1), **kaposiform lymphangiomatosis (KLA), and central conducting lymphatic anomalies (CCLA)** (Fig. 538.2). CLAs are rare diseases with overlapping and variable clinical features. Common features include bone involvement, lymphatic leakage into the thoracic (chylous effusion) and abdominal (chylous ascites) cavities and mesenteric involvement leading to protein losing enteropathy. In addition to common features, GSD is uniquely associated with progressive cortical bone loss (see Fig. 538.1). KLA can be distinguished by the presence of spindle cells on histology, an aggressive clinical course, and evidence of consumptive coagulopathy and hemorrhage. CCLA, or channel type anomaly is characterized by dilation, malformation, and dysfunction of the major abdominal or thoracic lymphatic vessels.

LMs can also occur as combined malformations with other blood vessel types like capillaries (capillary-lymphatic malformation) or veins (lymphatic-venous malformation). LMs are commonly associated with **PIK3CA-related overgrowth spectrum (PROS) disorders**, which include **Klippel-Trenaunay syndrome (KTS), CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and scoliosis/skeletal/spinal anomalies) and Proteus syndrome** (Fig. 538.3).

Genetics

Somatic, activating variants in the *PIK3CA* gene that encodes the p110α catalytic subunit of PI3K are present at variable frequencies in most (80%) isolated LMs and LMs associated with PROS. These activating *PIK3CA* variants are identical to those found in venous malformations and in many human cancers. The genetics of CLAs are far more diverse. Activating *PIK3CA* gene variants have been identified in several patients diagnosed with GLA. In addition, pathologic genetic variants in genes involved in the RAS-MAPK signaling pathway have been implicated in other CLAs. Activating *NRAS* gene variants are associated with KLA while two patients with GSD were found to have activating gene variants in *KRAS*. Somatic, pathologic genetic variants in both the *ARAF* and *CBL* genes have been found in patients diagnosed with CCLA as have inactivating germline pathogenic variants in *EPHB4*. The role of RAS/MAPK pathway activation in CLAs is further reinforced by the observation that patients with known RASopathies, including Noonan and Noonan-like syndrome, can present with central conducting lymphatic anomalies.

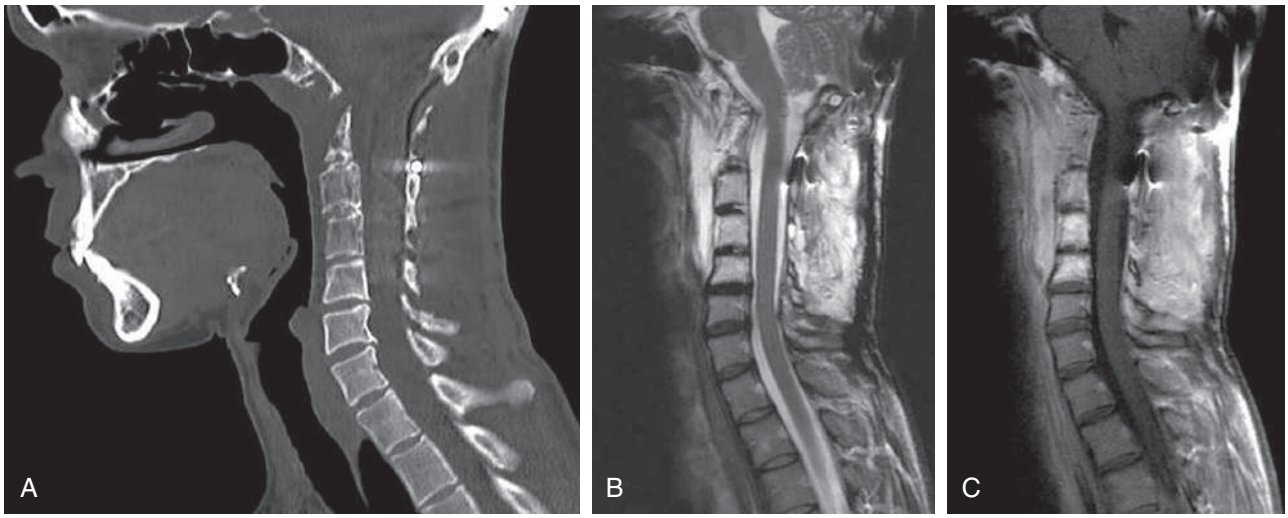

Fig. 538.1 Gorham–Stout disease. **A,** CT sagittal reformat. Multiple erosive changes in the occiput, clivus, and the cervical spine. (**B**) Sagittal T2-weighted fat-saturated (FS) MRI sequence. High signal is seen in the clivus and C 1–5 vertebral and the surrounding soft tissue. **C,** Postgadolinium T1 FS MRI. Enhancement is seen in the areas of osseous and soft tissue abnormalities. *(From Trenor III CC, Chaudry G. Complex lymphatic anomalies. Sem Pediatr Surg 2014;23:186–190. Fig. 3.)*

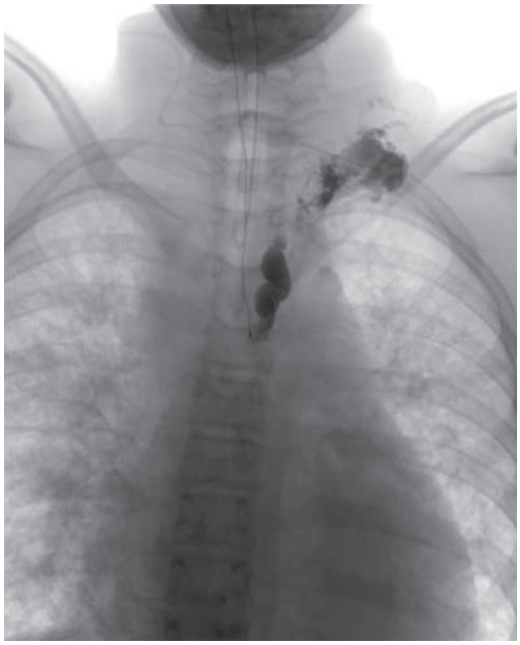

Fig. 538.2 Intranodal lymphangiogram in 15-year-old male with central conducting lymphatic anomaly and recurrent pericardial effusions. Stagnant flow is seen in the patulous superior portion of the thoracic duct. Direct puncture of the terminal portion demonstrates marked dilation with no spontaneous emptying.

Treatment

A decision to treat an LM depends on the anatomic location, involvement of local structures, and symptoms. Referral to a specialized vascular anomalies clinic with the expertise to guide appropriate imaging and treatment decisions is recommended. For localized macrocystic LMs, **interventional radiology (IR) administration of sclerosing agents** (OK432, ethanol, bleomycin) is most effective. For lesions involving skin and mucosa, **laser treatments** may be used.

Most patients with CLAs have extensive and invasive disease, necessitating systemic therapeutic approaches. The mTOR inhibitor **sirolimus** has had excellent effects in patients with vascular anomalies, including those patients with CLAs. A majority of CLA patients showed improvements in functional impairment and quality of life measures, whereas fewer showed evidence of decreased disease burden by imaging. **Combining sirolimus with bisphosphonates** may be even more effective in CLA patients with significant bone disease. **Alpelisib,** a *PIK3CA* inhibitor, has been used with success in patients with PROS and in a growing number of GLA patients with a known *PIK3CA* pathologic variant. Small numbers of patients with KLA who were known to have pathogenic variants in either NRAS or CBL experienced clinical improvement with **trametinib**, a MEK inhibitor. Trametinib also induced remodeling of the central lymphatics and induced symptom resolution in a patient with CCLA who had an *ARAF* pathogenic variant, as well a patient with Noonan syndrome and a *SOS1* pathogenic variant with severe lymphatic abnormalities.

LYMPHEDEMA

Lymphedema is a localized swelling caused by impaired lymphatic flow and can be primary (congenital) or acquired. **Primary lymphedemas** are grouped as LMs because they result from dysgenesis of lymphatic networks during early development. Primary lymphedema may be found in Turner syndrome, Noonan syndrome, autosomal dominantly inherited Milroy disease, and other chromosomal abnormalities. Pathologic variants in multiple genes, including the vascular endothelial growth factor receptor–3 gene *(VEGFR3), GJC2*, *PTPN14*, and *GATA2,* are associated with primary lymphedema (www.issva.org) (Table 538.1). Autosomal recessive, dominant or de novo pathogenic variants of *VEGFR3* produce Milroy disease. Pathologic variants in other genes are associated with specific syndromes: *CCBE1* (Hennekam), *FOXC2* (lymphedema distichiasis), *SOX18* (hypotrichosis-telangiectasia-lymphedema), *KMT2D/MLL2,* and *KDM6A* (Kabuki). Unilateral or bilateral lower extremity lymphedema in an adolescent may be Meige disease.

Acquired obstruction of the lymphatics is much more common than primary disorders and result from tumor, postirradiation fibrosis, and postinflammatory scarring. **Filariasis** is an important cause of lymphedema in Africa, Asia, and Latin America. One third of the 120 million infected persons (primarily older adolescents and adults) have lymphedema or a hydrocele. Injury to the major lymphatic vessels can cause

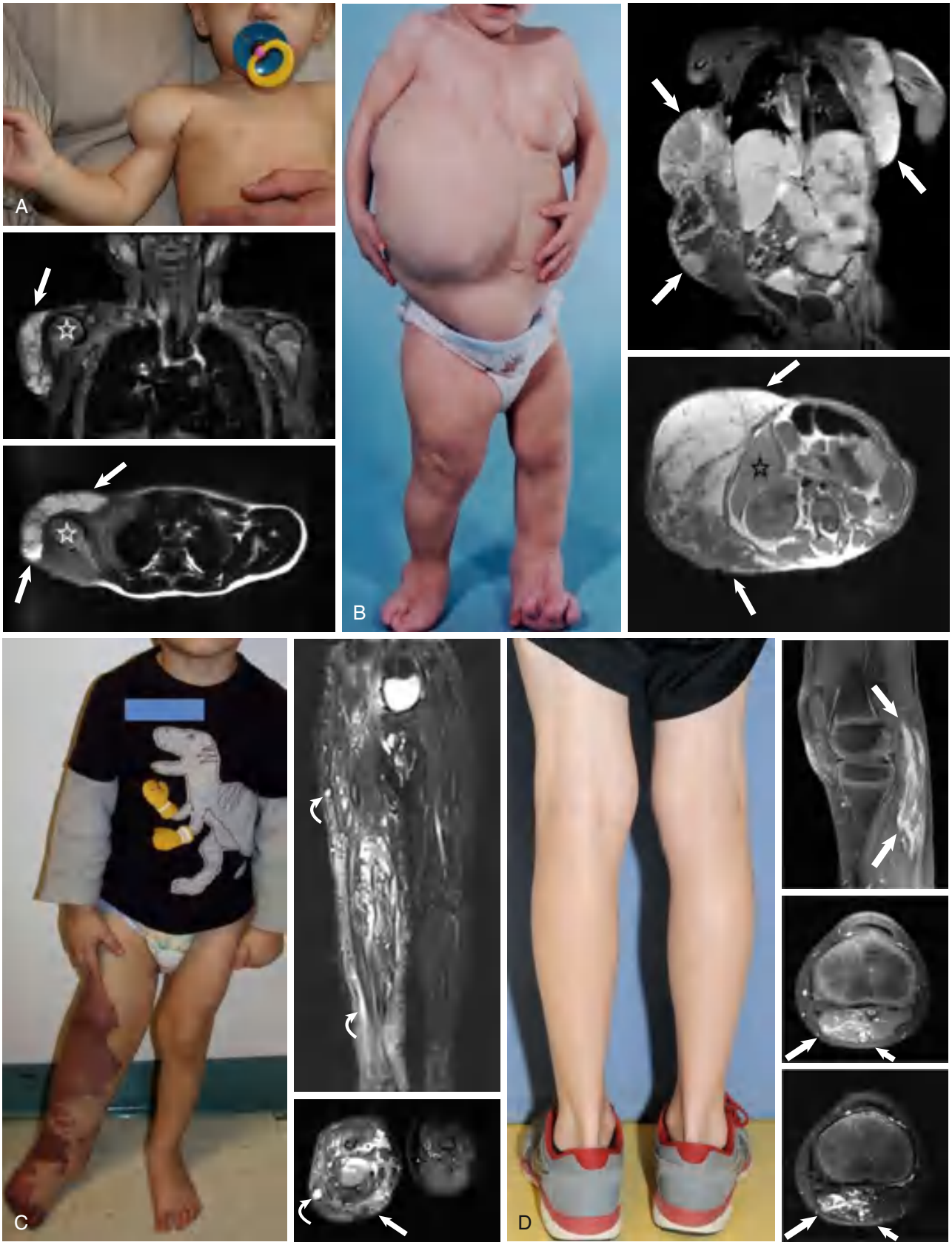

Fig. 538.3 Photographs and MRIs of participants with isolated LM, CLOVES, KTS, and FAVA. **A,** An 8-month-old male with isolated LM. Note swelling in deltoid region without cutaneous vascular signs. Coronal and sagittal fat-saturated T2-weighted MRI demonstrates macrocystic LM (a multilocular cystic mass) involving the anterolateral aspects of the right shoulder without muscular infiltration *(arrows)*; humeral head *star*. **B,** A 19-month-old female with CLOVES syndrome. Note asymmetric distribution of truncal lipomatous masses and bilateral lower extremity involvement. Coronal fat-saturated T1-weighted MRI after contrast administration demonstrates moderate heterogeneous enhancement of the bilateral truncal masses *(arrows)*. Axial T1-weighted MRI without contrast depicts truncal lipomatous overgrowth *(arrows)*; segment VI of the liver *(asterisk)*.

Continued

Fig. 538.3, cont'd **C,** A 3-yr-old male (KT4) with KTS. Note capillary-lymphatic malformation and overgrowth involving right lower extremity. Coronal and axial fat-saturated T2-weighted MRI shows the persistent marginal vein system *(bent arrows)* and marked enlargement of the subcutaneous tissues because of a combination of lymphatic fluid and fat *(straight arrow)*. There are also intramuscular venous malformations. **D,** A 9-yr-old male (F8) with FAVA of the left gastrocnemius muscle; note absence of overgrowth and cutaneous vascular anomalies. Sagittal fat-saturated T1-weighted MRI after contrast administration demonstrates the longitudinal distribution of the diffuse, fibro-adipose vascular anomaly *(arrows)*. Axial fat-saturated T2-weighted MRI with (upper) and without (lower) contrast. Note right head of the gastrocnemius muscle is diffusely replaced by a contrast enhancing heterogeneous soft tissue lesion *(arrows)*. CLOVES, congenital lipomatous overgrowth, vascular malformations, epidermal nevis, spinal/skeletal anomalies/scoliosis; FAVA, fibro-adipose vascular anomaly; KTS, Klippel-Trenaunay syndrome; LM, lymphatic malformations. *(From Luks VL, Kamitaki N, Vivero MP, et al. Lymphatic and other vascular malformative/overgrowth disorders are caused by somatic mutations in PIK3CA. J Pediatr. 2015;166:1048–1054.)*

Table 538.1 Chromosomal, Gene, and Syndromes Associated with Lymphedema

	SYNDROMES	KNOWN GENES, CHROMOSOMAL LOCI, INHERITANCE PATTERN
Primary lymphedema	Milroy disease	*VEGFR3* AR, AD
	Meige disease	Familial lymphedema
		No known gene
Chromosomal aneuploidy	Turner syndrome	45,X
	Klinefelter syndrome	47,XXY
	Trisomy 21	47,XX+21; 47,XY+21
	Trisomy 13	47,XX+13; 47,XY+13
	Trisomy 18	47,XX+18; 47,XY+18
Other genetic and syndromic disorders	Emberger syndrome (primary lymphedema with myelodysplasia)	*GATA2*(3q21.3), AD
	Lymphedema-distichiasis syndrome	*FOXC2*(16q24.1), AD
	Hennekam lymphangiectasia-lymphedema syndrome 1 (HKLLS1)	*CCBE1*(18q21.32), AR
	Hennekam lymphangiectasia-lymphedema syndrome 2 (HKLLS2)	*FAT4*(4q28.1), AR
	Hypotrichosis-lymphedema-telangiectasia ± renal defect syndrome (HLTRS)	*SOX18*(20q13.33), AR/AD
	Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR)	*KIF11*(10q23.33), AD
	Microcephaly and chorioretinopathy, 1 (MCCRP1)	*TUBGCP6*(22q13.33), AR
	Noonan syndrome	*PTPN11*, *SOS1*, *RAF1*, *RIT1*, *KRAS*, AD
	Cholestasis-lymphedema syndrome (Aagenaes syndrome)	*LSC1*, AR
	Ectodermal dysplasia, hypo/anhidrotic, lymphedema and immunodeficiency; immunodeficiency 33	*IKBKG*, (Xq28), XLR

AD, Autosomal dominant; AR, Autosomal recessive; XLR, X-linked recessive.
Modified from Unolt M, Barry J, Digilio MC, et al. Primary lymphedema and other lymphatic anomalies are associated with 22q11.2 deletion syndrome. *Eur J Med Genetics.* 2018;61:411–415. Table 1.

collection of lymph fluid in the abdomen (chylous ascites) or chest (chylothorax).

Untreated lymphedema can be disabling and is associated with immune dysfunction, inflammation, fibrosis, adipose tissue overgrowth, and lymphangiosarcoma. Treatment modalities attempt to reduce localized swelling through massage, exercise, and compression. Lymphatic reconstructive procedures, including lymphovenous bypass and lymphatico-lymphatic anastomosis are increasingly used to achieve lymphatic decongestion, especially in the early stages of disease.

LYMPHANGIOLEIOMYOMATOSIS

Lymphangioleiomyomatosis **(LAM)** is characterized by proliferation of lymphatic endothelial cells and smooth muscle cells in the lungs, leading to airway and lymphatic obstruction, cyst formation, pneumothorax, and respiratory failure. It may initially be mistaken for asthma. LAM occurs in young females and is associated with pathogenic variants in the tuberous sclerosis tumor-suppressor gene *TSC2* in one third of cases. **Sirolimus** stabilizes lung function, reduces symptoms, and improves life quality. Lung transplantation may be required.

LYMPHANGITIS

Lymphangitis is an inflammation of the lymphatics that drain an area of infection. Tender, erythematous streaks extend proximally from the infected area. Regional nodes may also be tender. Group A streptococci and *Staphylococcus aureus* are the most common pathogens, and therapy should include antibiotics that treat these organisms.

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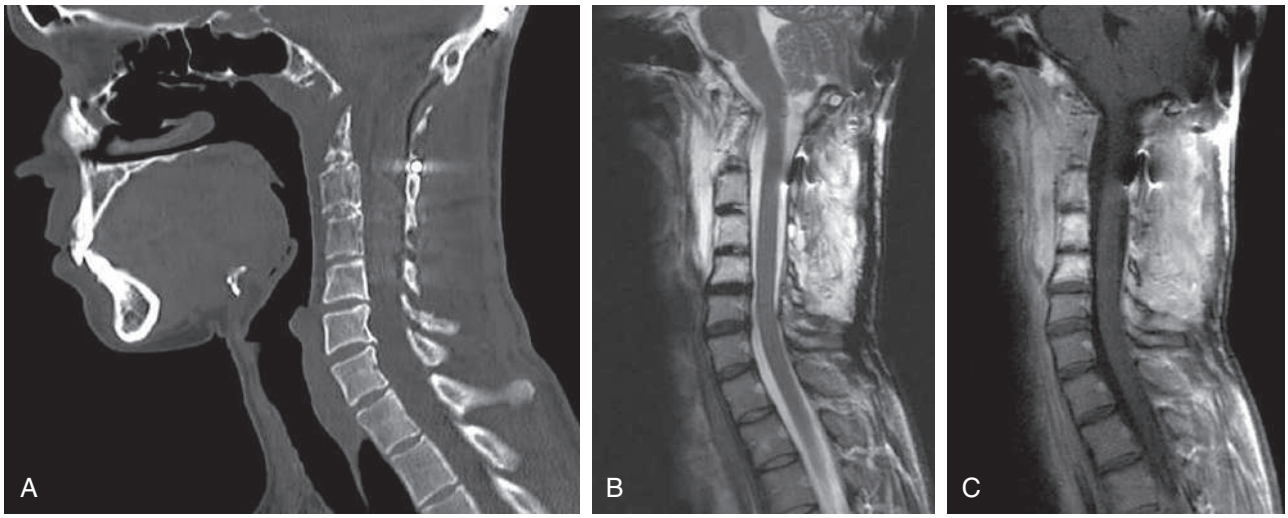

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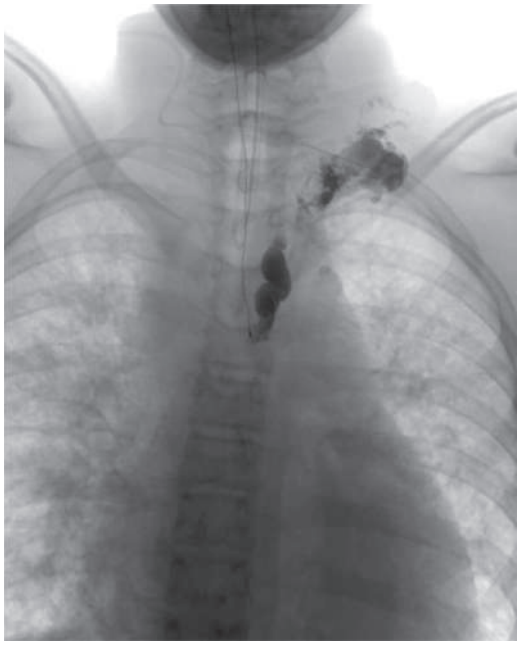

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Most patients with CLAs have extensive and invasive disease, necessitating systemic therapeutic approaches. The mTOR inhibitor **sirolimus** has had excellent effects in patients with vascular anomalies, including those patients with CLAs. A majority of CLA patients showed improvements in functional impairment and quality of life measures, whereas fewer showed evidence of decreased disease burden by imaging. **Combining sirolimus with bisphosphonates** may be even more effective in CLA patients with significant bone disease. **Alpelisib,** a *PIK3CA* inhibitor, has been used with success in patients with PROS and in a growing number of GLA patients with a known *PIK3CA* pathologic variant. Small numbers of patients with KLA who were known to have pathogenic variants in either NRAS or CBL experienced clinical improvement with **trametinib**, a MEK inhibitor. Trametinib also induced remodeling of the central lymphatics and induced symptom resolution in a patient with CCLA who had an *ARAF* pathogenic variant, as well a patient with Noonan syndrome and a *SOS1* pathogenic variant with severe lymphatic abnormalities.

LYMPHEDEMA

Lymphedema is a localized swelling caused by impaired lymphatic flow and can be primary (congenital) or acquired. **Primary lymphedemas** are grouped as LMs because they result from dysgenesis of lymphatic networks during early development. Primary lymphedema may be found in Turner syndrome, Noonan syndrome, autosomal dominantly inherited Milroy disease, and other chromosomal abnormalities. Pathologic variants in multiple genes, including the vascular endothelial growth factor receptor–3 gene *(VEGFR3), GJC2*, *PTPN14*, and *GATA2,* are associated with primary lymphedema (www.issva.org) (Table 538.1). Autosomal recessive, dominant or de novo pathogenic variants of *VEGFR3* produce Milroy disease. Pathologic variants in other genes are associated with specific syndromes: *CCBE1* (Hennekam), *FOXC2* (lymphedema distichiasis), *SOX18* (hypotrichosis-telangiectasia-lymphedema), *KMT2D/MLL2,* and *KDM6A* (Kabuki). Unilateral or bilateral lower extremity lymphedema in an adolescent may be Meige disease.

Acquired obstruction of the lymphatics is much more common than primary disorders and result from tumor, postirradiation fibrosis, and postinflammatory scarring. **Filariasis** is an important cause of lymphedema in Africa, Asia, and Latin America. One third of the 120 million infected persons (primarily older adolescents and adults) have lymphedema or a hydrocele. Injury to the major lymphatic vessels can cause

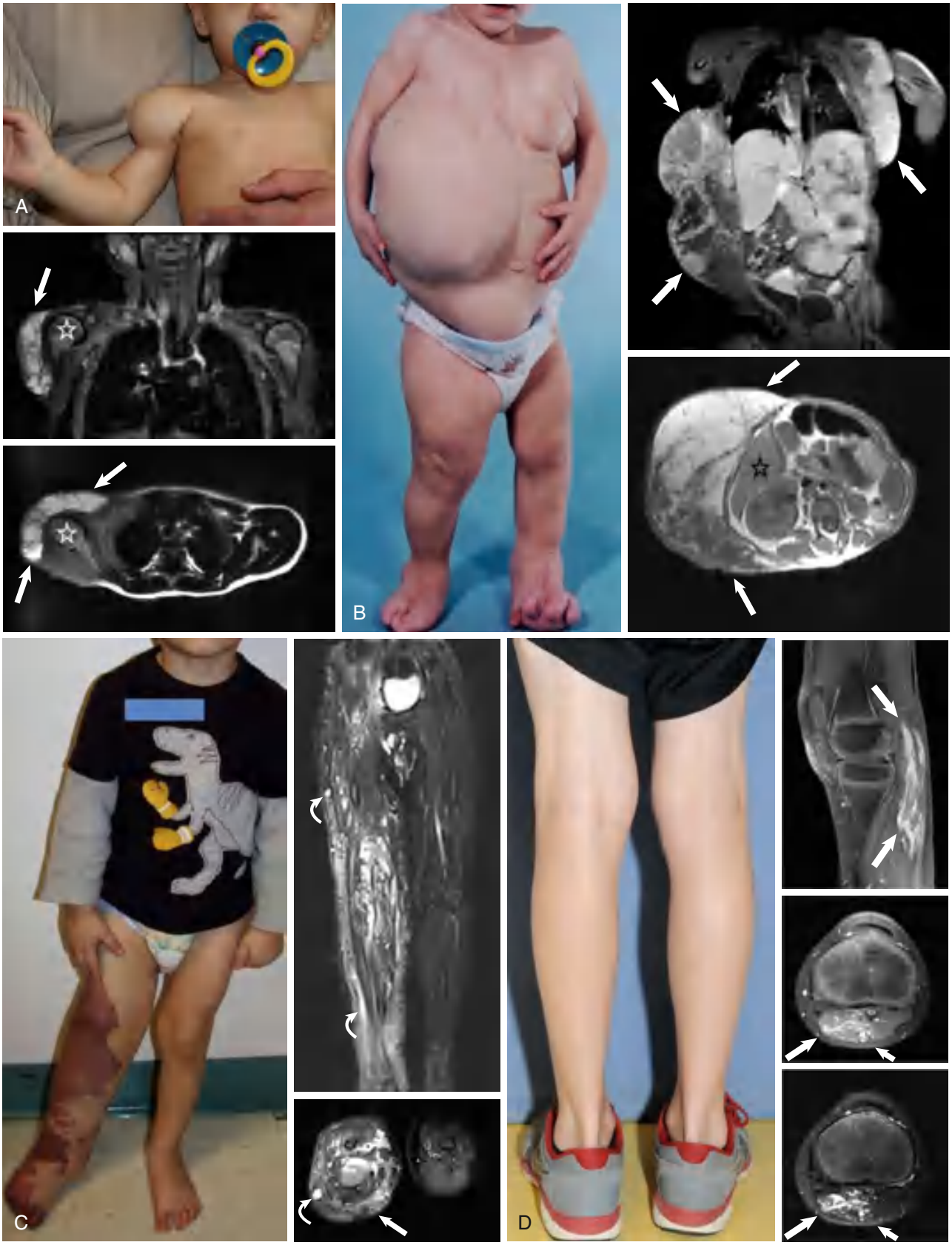

Fig. 538.3 Photographs and MRIs of participants with isolated LM, CLOVES, KTS, and FAVA. **A,** An 8-month-old male with isolated LM. Note swelling in deltoid region without cutaneous vascular signs. Coronal and sagittal fat-saturated T2-weighted MRI demonstrates macrocystic LM (a multilocular cystic mass) involving the anterolateral aspects of the right shoulder without muscular infiltration *(arrows)*; humeral head *star*. **B,** A 19-month-old female with CLOVES syndrome. Note asymmetric distribution of truncal lipomatous masses and bilateral lower extremity involvement. Coronal fat-saturated T1-weighted MRI after contrast administration demonstrates moderate heterogeneous enhancement of the bilateral truncal masses *(arrows)*. Axial T1-weighted MRI without contrast depicts truncal lipomatous overgrowth *(arrows)*; segment VI of the liver *(asterisk)*.

Continued

Fig. 538.3, cont'd **C,** A 3-yr-old male (KT4) with KTS. Note capillary-lymphatic malformation and overgrowth involving right lower extremity. Coronal and axial fat-saturated T2-weighted MRI shows the persistent marginal vein system *(bent arrows)* and marked enlargement of the subcutaneous tissues because of a combination of lymphatic fluid and fat *(straight arrow)*. There are also intramuscular venous malformations. **D,** A 9-yr-old male (F8) with FAVA of the left gastrocnemius muscle; note absence of overgrowth and cutaneous vascular anomalies. Sagittal fat-saturated T1-weighted MRI after contrast administration demonstrates the longitudinal distribution of the diffuse, fibro-adipose vascular anomaly *(arrows)*. Axial fat-saturated T2-weighted MRI with (upper) and without (lower) contrast. Note right head of the gastrocnemius muscle is diffusely replaced by a contrast enhancing heterogeneous soft tissue lesion *(arrows)*. CLOVES, congenital lipomatous overgrowth, vascular malformations, epidermal nevis, spinal/skeletal anomalies/scoliosis; FAVA, fibro-adipose vascular anomaly; KTS, Klippel-Trenaunay syndrome; LM, lymphatic malformations. *(From Luks VL, Kamitaki N, Vivero MP, et al. Lymphatic and other vascular malformative/overgrowth disorders are caused by somatic mutations in PIK3CA. J Pediatr. 2015;166:1048–1054.)*

Table 538.1 Chromosomal, Gene, and Syndromes Associated with Lymphedema

	SYNDROMES	KNOWN GENES, CHROMOSOMAL LOCI, INHERITANCE PATTERN
Primary lymphedema	Milroy disease	*VEGFR3* AR, AD
	Meige disease	Familial lymphedema
		No known gene
Chromosomal aneuploidy	Turner syndrome	45,X
	Klinefelter syndrome	47,XXY
	Trisomy 21	47,XX+21; 47,XY+21
	Trisomy 13	47,XX+13; 47,XY+13
	Trisomy 18	47,XX+18; 47,XY+18
Other genetic and syndromic disorders	Emberger syndrome (primary lymphedema with myelodysplasia)	*GATA2*(3q21.3), AD
	Lymphedema-distichiasis syndrome	*FOXC2*(16q24.1), AD
	Hennekam lymphangiectasia-lymphedema syndrome 1 (HKLLS1)	*CCBE1*(18q21.32), AR
	Hennekam lymphangiectasia-lymphedema syndrome 2 (HKLLS2)	*FAT4*(4q28.1), AR
	Hypotrichosis-lymphedema-telangiectasia ± renal defect syndrome (HLTRS)	*SOX18*(20q13.33), AR/AD
	Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR)	*KIF11*(10q23.33), AD
	Microcephaly and chorioretinopathy, 1 (MCCRP1)	*TUBGCP6*(22q13.33), AR
	Noonan syndrome	*PTPN11*, *SOS1*, *RAF1*, *RIT1*, *KRAS*, AD
	Cholestasis-lymphedema syndrome (Aagenaes syndrome)	*LSC1*, AR
	Ectodermal dysplasia, hypo/anhidrotic, lymphedema and immunodeficiency; immunodeficiency 33	*IKBKG*, (Xq28), XLR

AD, Autosomal dominant; AR, Autosomal recessive; XLR, X-linked recessive.
Modified from Unolt M, Barry J, Digilio MC, et al. Primary lymphedema and other lymphatic anomalies are associated with 22q11.2 deletion syndrome. *Eur J Med Genetics.* 2018;61:411–415. Table 1.

collection of lymph fluid in the abdomen (chylous ascites) or chest (chylothorax).

Untreated lymphedema can be disabling and is associated with immune dysfunction, inflammation, fibrosis, adipose tissue overgrowth, and lymphangiosarcoma. Treatment modalities attempt to reduce localized swelling through massage, exercise, and compression. Lymphatic reconstructive procedures, including lymphovenous bypass and lymphatico-lymphatic anastomosis are increasingly used to achieve lymphatic decongestion, especially in the early stages of disease.

LYMPHANGIOLEIOMYOMATOSIS

Lymphangioleiomyomatosis **(LAM)** is characterized by proliferation of lymphatic endothelial cells and smooth muscle cells in the lungs, leading to airway and lymphatic obstruction, cyst formation, pneumothorax, and respiratory failure. It may initially be mistaken for asthma. LAM occurs in young females and is associated with pathogenic variants in the tuberous sclerosis tumor-suppressor gene *TSC2* in one third of cases. **Sirolimus** stabilizes lung function, reduces symptoms, and improves life quality. Lung transplantation may be required.

LYMPHANGITIS

Lymphangitis is an inflammation of the lymphatics that drain an area of infection. Tender, erythematous streaks extend proximally from the infected area. Regional nodes may also be tender. Group A streptococci and *Staphylococcus aureus* are the most common pathogens, and therapy should include antibiotics that treat these organisms.

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Chapter 539

Lymphadenopathy

Zachary T. Graff and Richard L. Tower

Palpable lymph nodes are common in pediatrics and often pose a diagnostic challenge. Lymph node enlargement can be caused by proliferation of normal lymphoid elements or by infiltration with malignant or other phagocytic cells. In most patients, a careful history and a complete physical examination suggest the proper diagnosis.

DIAGNOSIS

What Is the Site of the Mass?

The differential diagnosis of a mass varies greatly based on anatomic location. Although pathologic masses can occur in any site, masses occurring in the supraclavicular and lower half of the neck are more likely to reflect pathologic lymphadenopathy than in other sites of the body.

Is the Mass a Lymph Node?

Nonlymphoid masses (cervical rib, thyroglossal cyst, branchial cleft cyst or infected sinus, cystic hygroma, goiter, thyroiditis, sternomastoid muscle tumor of infancy, neurofibroma) occur frequently in the neck and less often in other areas.

Is the Node Enlarged?

Lymph nodes are not usually palpable in the newborn. With antigenic exposure, lymphoid tissue increases in volume. They are not considered enlarged until their diameter exceeds 1 cm for cervical and axillary nodes, 1.5 cm for inguinal nodes, and epitrochlear >0.5 cm. Other lymph nodes usually are not palpable or visualized with plain radiographs.

What are the Characteristics of the Node?

Acutely infected nodes are usually tender. There may also be erythema and warmth of the overlying skin. Fluctuance suggests abscess formation. *Tuberculous* nodes may be matted. With chronic infection, many of these signs are not present. A firm, fixed, nonmobile (to adjacent or underlying tissues), nontender node should always raise the question of malignancy, regardless of the presence or absence of systemic symptoms or other abnormal physical findings. Tumors or tumor-involved nodes are often present for >2 weeks and may be associated with local extension (voice change, dysphagia, otalgia) or systemic signs (fever, weight loss, night sweats).

Is the Lymphadenopathy Localized or Generalized?

Generalized adenopathy (enlargement of >2 noncontiguous node regions) is caused by systemic disease (Table 539.1) and is often accompanied by abnormal physical findings in other systems. In contrast, *regional adenopathy* is most frequently the result of infection in the involved node and/or its drainage area (Table 539.2). When caused by infectious agents other than bacteria, adenopathy may be characterized by atypical anatomic areas, a prolonged course, a draining sinus, lack of prior pyogenic infection, and unusual clues in the history (cat scratches, tuberculosis exposure, venereal disease). The histopathologic pattern may also help with a differential diagnosis (Table 539.3).

Table 539.1 Differential Diagnosis of Generalized Lymphadenopathy

NEONATE	CHILD	ADOLESCENT
COMMON CAUSES		
CMV	Nonspecific viral infections	Viral infections
HIV	EBV	EBV
Syphilis	CMV	CMV
Toxoplasmosis	HIV	HIV
	Toxoplasmosis	Measles
	Measles	Toxoplasmosis
		Syphilis
RARE CAUSES		
Chagas disease (congenital)	Serum sickness	Serum sickness
Congenital leukemia	SLE, JIA	SLE, JIA
Congenital tuberculosis	Leukemia/lymphoma	Leukemia/lymphoma
Reticuloendotheliosis	Tuberculosis (miliary)	Tuberculosis
Metabolic storage disease	Sarcoidosis	Sarcoidosis
Histiocytic disorders	DRESS	DRESS
Listeria sepsis	Fungal infections	Fungal infections
	Plague	Plague
	Leptospirosis	Leptospirosis
	Brucellosis	Brucellosis
	Langerhans cell histiocytosis	Drug reaction (immune)
	Macrophage activation syndrome	Castleman disease
	Hemophagocytic lymphohistiocytosis	Rickettsial infection
	Castleman disease (very rare in this age group)	
	Chronic granulomatous disease	
	Sinus histiocytosis (Rosai-Dorfman disease)	
	Kikuchi-Fujimoto disease	
	Autoimmune lymphoproliferative disease (ALP)	
	Rickettsial infection	

CMV, Cytomegalovirus; DRESS, drug reaction, eosinophilia, systemic symptoms; EBV, Epstein-Barr virus; JIA, juvenile idiopathic arthritis (as Still disease); SLE, systemic lupus erythematosus.

From Kliegman RM, Toth H, Bordini BJ, Basel D, eds. *Nelson Pediatric Symptom-Based Diagnosis: Common Diseases and Their Mimics*, 2nd ed. Philadelphia: Elsevier, 2023: Table 48.3, p. 891.

TREATMENT

Evaluation and treatment of lymphadenopathy is guided by the probable etiologic factor, as determined from the history and physical examination. Many patients with **cervical adenopathy** have a history compatible with viral infection and are observed without intervention. If bacterial infection is suspected, antibiotic treatment covering at least streptococci and staphylococci is indicated. Those who do not respond to oral antibiotics, as demonstrated by persistent swelling and fever, require intravenous (IV) antistaphylococcal antibiotics. If there is no response in 1-2 days, or if there are signs of airway obstruction or significant toxicity, ultrasound, CT, or MRI of the neck with contrast should be obtained. If *pus* is present, it may be aspirated, or if it is extensive, it may require incision and drainage. Gram stain and culture of the pus should be obtained. The sizes of involved nodes should be documented before treatment. Failure to decrease in size within 14 days also suggests the need for further evaluation. This evaluation may include a complete blood cell count (CBC) with differential; Epstein-Barr virus, cytomegalovirus, *Toxoplasma,* and *Bartonella henselae* titers; anti–streptolysin O or anti-DNase B serologic tests; tuberculin skin test or gamma interferon assay; and chest radiograph to evaluate for mediastinal adenopathy. If these are not diagnostic, consultation with an infectious diseases or oncology specialist may be helpful. Biopsy should be considered if there is persistent or unexplained fever, weight loss, night sweats, supraclavicular location, mediastinal mass, hard nodes, or fixation of the nodes to surrounding tissues. Biopsy may also be indicated if there is an increase in size over baseline in 2 weeks, no decrease in size in 4-6 weeks, no regression to "normal" in 8-12 weeks, or if new signs and symptoms develop.

Table 539.2 Sites of Regional Lymphadenopathy and Associated Diseases

CERVICAL
Oropharyngeal infection (viral, group A streptococcal, staphylococcal, or fusobacterial)
Scalp infection (tinea)
Mycobacterial lymphadenitis (tuberculous and nontuberculous mycobacteria)
Viral infection (EBV, CMV, HHV-6, measles)
Cat-scratch disease
Kawasaki disease
Multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19
Thyroid disease
Kimura disease
Rosai-Dorfman (sinus histiocytosis)
Periodic fever, aphthous stomatitis, pharyngitis, cervical adenopathy (PFAPA) syndrome
Kikuchi-Fujimoto disease
Unicentric Castleman disease
ANTERIOR AURICULAR
Conjunctivitis or other eye infections
Oculoglandular tularemia, cat-scratch disease, EBV, adenovirus
POSTERIOR AURICULAR
Otitis media
Viral infection (especially rubella, parvovirus)
SUPRACLAVICULAR
Malignancy or infection in the mediastinum (right)
Metastatic malignancy from abdomen (left)
Lymphoma
Tuberculosis
EPITROCHLEAR
Hand infection, arm infection*
Lymphoma†
Sarcoidosis
Syphilis
EBV
HIV
INGUINAL
Urinary tract infection
Sexually transmitted infection (especially syphilis or lymphogranuloma venereum)
Lower extremity suppurative infection
Plague
HILAR (NOT PALPABLE, FOUND ON CHEST RADIOGRAPH OR CT) (SEE TABLE 539.1)
Tuberculosis†
Histoplasmosis†
Blastomycosis†
Coccidioidomycosis†
Leukemia/lymphoma†
Hodgkin disease†
Metastatic malignancy*
Sarcoidosis†
Castleman disease
AXILLARY
Cat-scratch disease
Arm infection
Malignancy of chest wall
Leukemia/lymphoma
Brucellosis

*Unilateral.
†Bilateral.
CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, human herpesvirus 6.
From Kliegman RM, Toth H, Bordini BJ, Basel D, eds. *Nelson Pediatric Symptom-Based Diagnosis: Common Diseases and Their Mimics*, 2nd ed. Philadelphia: Elsevier, 2023: Table 48.2, p. 890.

Differentiating benign disorders from a malignancy may initially be difficult. Hard, nontender, nonerythematous nodes involving multiple regions (including mediastinum and abdomen), hepatic or splenic enlargement, fever, night sweats, and weight loss suggest malignancy or a granulomatous process. Persistence of symptoms and lymphadenopathy >2 weeks and certain locations (supraclavicular, mediastinal, abdominal) also suggest malignancy. Cytopenias and elevated blood lactate dehydrogenase are associated with malignancy and certain infectious and inflammatory disorders. Ultrasound is useful in distinguishing malignancy from reactive nodes. CT with contrast is helpful in identifying other affected nodes and organs. Although fine needle aspiration (FNA) is sometimes used as an initial approach to diagnosis, excisional biopsies are often needed and are the preferred diagnostic approach to fully appreciate the lymph node architecture and genomic profiling when malignant conditions are being considered.

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539.1 Kikuchi-Fujimoto Disease (Histiocytic Necrotizing Lymphadenitis)

Zachary T. Graff and Richard L. Tower

Kikuchi-Fujimoto disease is a rare, usually self-limiting disease that is seen in all ethnic groups; children and adults may be affected. Familial cases have been reported. The etiology is unknown, although infectious and autoimmune diseases such as systemic lupus erythematosus (SLE) have been associated. The differential diagnosis includes infectious lymphadenitis, lymphoma, tuberculosis, and SLE.

Presentation is varied but most often presents as firm, painful unilateral posterior cervical adenopathy evolving over several weeks. Fever is frequently reported. Labs are often normal; however, elevated erythrocyte sedimentation rate (ESR), elevated CRP, atypical lymphocytosis, and leukopenia can be seen. Nodes range in size from 0.5-6.0 cm, are painful or tender in only 50% of cases, may be multiple, and must be differentiated from lymphoma. Node involvement may occasionally be bilateral or rarely present in axillary, supraclavicular, or intraabdominal lymph nodes. Ultrasound usually shows multiple conglomerated, unilateral cervical lymphadenopathy with perinodal fat swelling and even size distribution. Aseptic meningitis is an uncommon associated feature.

The diagnosis is made by lymph node biopsy. Histologic features include paracortical lymph node expansion with patchy, well-circumscribed areas of necrosis showing karyorrhexis, an absence of neutrophils and eosinophils, a CD68+/MPO+ histiocytic infiltrate, and CD123+/TCL1+ plasmacytoid dendritic cells. Kikuchi-Fujimoto disease usually resolves within 6 months, although relapses have occurred up to 16 years later. Treatment is typically symptomatic including rest and analgesics. Therapy with systemic steroids is reserved for patients with severe symptoms. Recurrences occur in ~5-20% depending on site (higher recurrences with bone, CNS, or skin involvement). Patients should be followed for the possible development of systemic lupus erythematosus. Rarely, the disease has been fatal.

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539.2 Sinus Histiocytosis with Massive Lymphadenopathy (Rosai-Dorfman Disease)

Zachary T. Graff and Richard L. Tower

The uncommon, benign, and usually self-limited Rosai-Dorfman disease (RDD) is a non-Langerhans cell histiocytosis that has a worldwide distribution but is more common in individuals of African descent. The etiology is unknown, but immune dysfunction is suspected. Lesional histiocytes are $S100^+$, $CD68^+$, and $CD1a^-$. It is not a single entity, but a

Table 539.3 Differential Diagnosis for the Histologic Patterns Seen in Pediatric Lymphadenopathies

HISTOLOGIC PATTERN	DIFFERENTIAL DIAGNOSIS	KEY FEATURES
Isolated follicular hyperplasia	Nonspecific reactive pattern Viral infections HIV lymphadenitis Measles Follicular lymphoma Primary immunodeficiencies	Multinucleated Warthin-Finkeldey cells seen in both HIV and measles Expansile irregular follicles in acute HIV Normal polarization of germinal center cells and phenotyping helpful to rule out follicular lymphoma
Follicular hyperplasia with PTGC	Nonspecific reactive pattern Nodular lymphocyte predominant Hodgkin lymphoma	Cytologically atypical cells seen in nodular lymphocyte predominant Hodgkin lymphoma are absent in PTGC
Paracortical hyperplasia	EBV infectious mononucleosis HSV1/2 CMV Varicella Measles Classic Hodgkin lymphoma Drug-induced lymphadenopathy Primary immunodeficiencies Non-Hodgkin B-cell and T-cell lymphoma	Polymorphic with numerous immunoblasts RS-like cells express CD20, unlike RS cells of classic Hodgkin lymphoma Warthin-Finkeldey cells may be seen in measles and HSV lymphadenitis Well-delineated necrosis in HSV Intranuclear inclusions may be seen in CMV, HSV, and varicella Associated monocytoid B-cell hyperplasia seen in CMV lymphadenitis
Suppurative	Bacterial lymphadenitis, Staphylococcus, Streptococcus, Haemophilus, Yersinia, *Francisella tularensis*, Brucella HSV lymphadenitis Kawasaki	Poorly formed granulomas also may be seen in bacterial infections Necrotic foci beneath the capsule seen in Kawasaki
Necrotizing granulomas	Mycobacteria, tuberculous, and nontuberculous Fungal infections CSD Kikuchi-Fujimoto lymphadenitis/SLE	Acellular (caseating) necrosis with numerous giant cells seen in mycobacterial and fungal infections CSD palisading histiocytes with neutrophilic microabscesses Kikuchi-Fujimoto lymphadenitis/SLE necrosis with nuclear debris and absence of neutrophils; C-shaped histiocytes; clusters of immunoblasts and plasmacytoid dendritic cells
Nonnecrotizing granulomas	Sarcoidosis; infections; nonspecific	
Histiocytoses	HLH, Rosai-Dorfman disease	
Miscellaneous	Toxoplasmosis • Triad: follicular hyperplasia, monocytoid B-cell hyperplasia, epithelioid histiocytes extend into lymphoid follicles • Kimura • Follicular hyperplasia and interfollicular eosinophils	

CMV, cytomegalovirus; CSD, cat-scratch disease; EBV, Epstein Barr virus; HLH, hemophagocytic lymphohistiocytosis; HSV, herpes simplex virus; PTGC, progressive transformation of germinal centers; SLE, systemic lupus erythematosus.
From Faraz M, Rosando FGN. Reactive lymphadenopathies. *Clin Lab Med.* 2021;41:433–451. Table 1.

pattern, and can be associated with neoplasia or autoimmune disease. It can be sporadic or associated with inherited conditions. Some patients have somatic pathogenic variants in *NRAS*, *KRAS*, and *MAP2K1*. Familial RDD has been noted in patients with pathogenic variants in *SLC29A3*. RDD may coexist with SLE, JIA, and autoimmune hemolytic anemia.

Patients can be classified as classical (nodal) RDD or extranodal RDD. **Classical RDD** patients present with massive *bilateral*, painless, mobile cervical adenopathy with or without fever, night sweats, and weight loss. Other lymph node involvement can include mediastinal, axillary, and inguinal, although retroperitoneal involvement is rare. **Extranodal RDD** occurs in 43% of cases. Soft tissue involvement has been reported in many organ systems. The most common sites are the skin, followed by the nasal cavity and sinuses, palate, orbit, bone, and central nervous system. Lab abnormalities can include normocytic anemia, leukocytosis (typically neutrophilia), elevated ESR, and polyclonal elevation of immunoglobulin G (hypergammaglobulinemia). Initial imaging studies are determined by symptoms and sites of suspected disease.

A biopsy that demonstrates histiocytes with hypochromatic nuclei with pale cytoplasm containing engulfed erythrocytes, plasma cells, and lymphocytes (emperipolesis), and immunoreactivity to S100 protein, in conjunction with expected clinical features, is diagnostic. IgG4-positive cells are often abundant. Occasionally, autoantibodies to erythrocytes or synovium may be present. The differential diagnosis includes Langerhans cell histiocytosis, myeloproliferative disorders, lymphoma, and hyper-IgG4 syndrome. After diagnosis, whole-body imaging with MRI or PET scan is recommended to evaluate for extranodal RDD.

Observation alone is a reasonable treatment choice for patients with uncomplicated nodal/cutaneous disease, as spontaneous remissions are seen in 20–50% of these patients. Symptomatic patients may benefit from surgical resection or debulking with the presence of single-site disease. Systemic therapy is typically reserved for severe, refractory, multifocal, or unresectable disease. Steroids are helpful in reducing nodal size; however, durable responses are often not seen with steroids alone. Various immunomodulatory (sirolimus) and chemotherapy regimens have been used successfully in severe disease, with treatment courses ranging from 6-12 months followed by close observation. Targeted therapies, including MEK inhibitors, have shown promising early results with studies ongoing. Radiation therapy has modest efficacy in RDD but has been used successfully in emergent therapy with visual or airway compromise. RDD may recur for many years with an unpredictable clinical course.

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539.3 Castleman Disease (Angiofollicular Lymph Node Hyperplasia)

Zachary T. Graff and Richard L. Tower

Castleman disease (CD) is an uncommon nonmalignant B-cell lymphoproliferative disorder and is also called **angiofollicular lymph node hyperplasia**. CD causes lymph node enlargement and is classified as either unicentric (involving a single lymph node or lymph node region) or multicentric (involving multiple lymph node regions). **Unicentric CD** typically presents with enlargement of a single bulky lymph node, most often in the mediastinum, neck, or abdomen, which can be asymptomatic or present with locally compressive symptoms. Patients may have fever, night sweats, weight loss, and fatigue. Diagnosis is made by an excisional lymph node biopsy. Staging imaging is performed with CT scan of chest/abdomen/pelvis or PET-CT scan to evaluate for multicentric disease. Management includes surgical resection when possible and is associated with a benign course, although patients should be educated on an increased risk of lymphoma.

Multicentric CD is subclassified based on the etiologic driver of the disease, either human herpes virus-8 (HHV-8), peripheral neuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, skin changes (POEMS) syndrome, or idiopathic. An underlying immunocompromised state is the primary risk factor for HHV-8 CD, particularly patients with HIV. Other forms of multicentric CD have no known risk factors. HHV-8 and idiopathic multicentric CD are driven by the excessive production of interleukin-6 (IL-6). Lymphadenopathy is often small volume and is present in peripheral and central lymph node chains. Constitutional symptoms such as fever, night sweats, and weight loss are more common at presentation compared to unicentric CD. Fluid collections including ascites, cytopenias, and liver/kidney dysfunction are also seen and indicate more aggressive disease. Diagnosis is again made by excisional lymph node biopsy as well as consistent clinical features. To determine type of multicentric CD, testing for HIV, HHV-8, and evaluation for POEMS (polyneuropathy, organomegaly, edema/endocrinopathy, monoclonal-protein, skin) syndrome is needed. Serum inflammatory markers are often tested at baseline and used to track disease. The differential diagnosis is broad even after lymph node biopsy, including infection, autoimmune disease, and malignancy. There is no standard treatment for multicentric Castleman disease. For symptomatic HHV-8+ multicentric CD, rituximab +/- etoposide with antiretroviral therapy for HIV+ patients have achieved excellent relapse-free survival. Idiopathic multicentric CD is managed with anti-IL-6 directed therapy (siltuximab) and steroids. Even after initial responses, symptoms can return after stopping therapy. Rituximab, steroids, and additional immunomodulatory medications (particularly the mTOR inhibitor sirolimus) are used next in patients that have had an inadequate response to therapy. Cytotoxic chemotherapy can be used for severe disease with organ dysfunction after failure of first-line therapy.

TAFRO (thrombocytopenia, anasarca, fever, reticulum fibrosis, organomegaly) syndrome is thought to be a subtype of multicentric Castleman disease based on the histologic appearance of the lymph node. Although there are overlapping features, TAFRO may be a distinct entity (Fig. 539.1).

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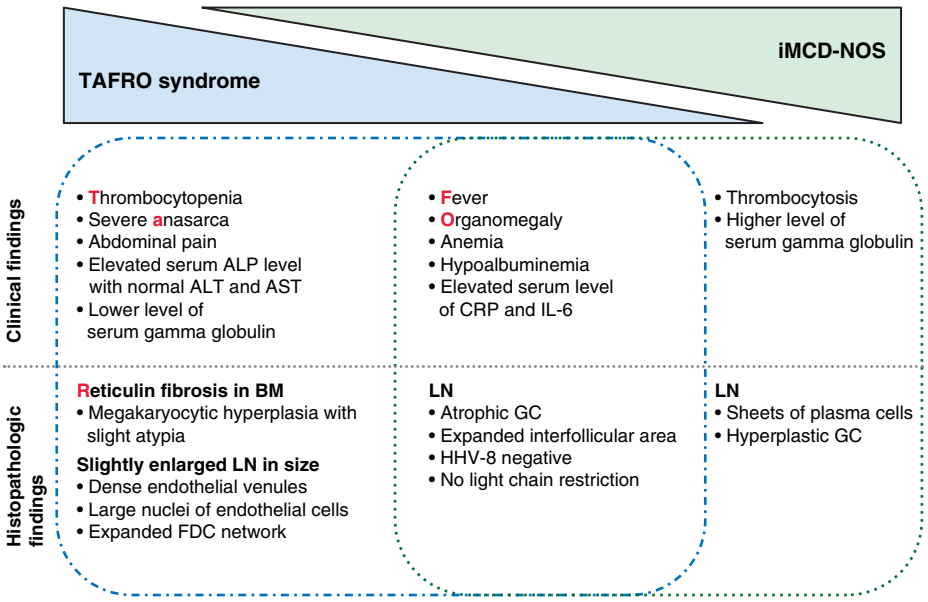

Fig. 539.1 Summary of the clinical and histopathologic features of TAFRO syndrome and iMCD-NOS. Although TAFRO syndrome and iMCD-NOS have clinicopathologic features in common, TAFRO syndrome has unique characteristics that can distinguish it from iMCD-NOS. ALP, alkaline phosphatase; ALT, Alanine transaminase; AST, aspartate transaminase; BM, bone marrow; CRP, C-reactive protein; FDC, follicular dendritic cell; GC, germinal center; HHV, human herpes virus; LN, lymph node; iMCD, idiopathic multicentric Castleman disease; NOS, not otherwise specified; TAFRO, thrombocytopenia, anasarca, fever, reticulin fibrosis, organomegaly. *(From Igawa T, Sato Y. TAFRO syndrome. Hematol Oncol Clin N Am. 2018;32:107–118. Fig. 4.)*