

Acquired and Congenital Hemolytic Anemia

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Educational Gaps

1. Pediatricians and other general practitioners may not be aware of the significance of central nervous system disease in children who have sickle cell disease, particularly the more subtle silent infarct.
2. Pediatricians frequently fail to order a reticulocyte count or detect splenomegaly on physical examination before referring a patient with anemia. These findings are critical to diagnosing hemolytic anemia.

Objectives After completing this article, the reader should be able to:

1. Recognize clinical features of hemolysis, including reticulocytosis and splenomegaly.
2. List the different types of acquired autoimmune hemolytic anemias that can manifest throughout childhood.
3. Understand the role of transfusion in the management of neurologic disease in patients who have sickle cell disease.
4. Review the spectrum of disease of α - and β -thalassemias.
5. Recognize clinical findings associated with hereditary spherocytosis.
6. Determine when to suspect glucose-6-phosphate dehydrogenase deficiency and how to counsel families on triggers to avoid.

INTRODUCTION

Hemolytic anemia (HA) affects a substantial proportion of the pediatric population globally. Many children are hospitalized every year due to sequelae of this heterogeneous disease. Clinicians should be facile in recognizing its presentation.

PATHOPHYSIOLOGY: EXTRAVASCULAR VERSUS INTRAVASCULAR HEMOLYSIS

HA may be defined as increased destruction of red blood cells (RBCs). RBCs are cleared from the circulation via extravascular or intravascular mechanisms (Figure). HA can be caused by congenital or acquired RBC abnormalities (Table 1).

AUTHOR DISCLOSURE Dr Noronha has disclosed no financial relationships relevant to this article. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.

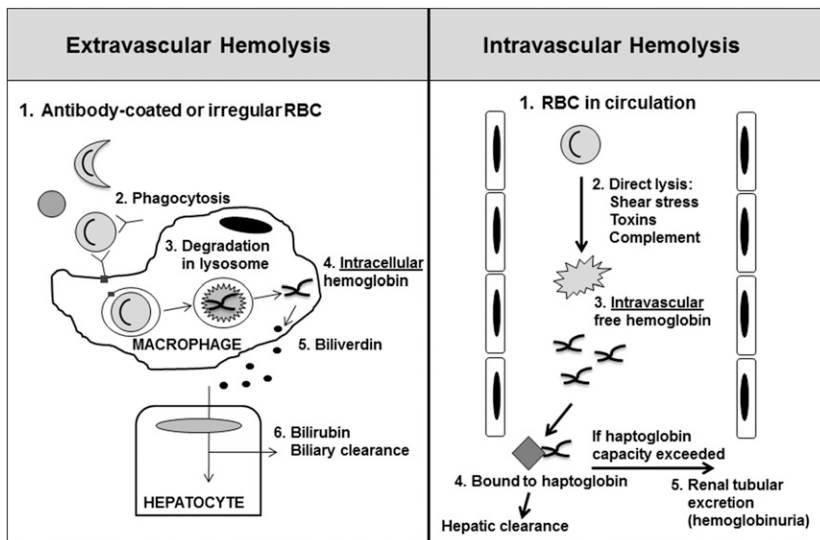


Figure. Mechanism of extravascular versus intravascular hemolysis. RBC=red blood cell. Courtesy of Jessica Shand, MD.

Extravascular hemolysis is mediated by the reticuloendothelial system (RES) of the spleen and liver. Most HAs, such as warm autoimmune hemolytic anemia (AIHA), sickle cell disease (SCD), and hereditary spherocytosis (HS), are characterized by extravascular hemolysis. The hallmark of extravascular hemolysis is phagocytosis of erythrocytes by splenic macrophages or hepatic Kupffer cells, followed by sequestration and removal. Heme, released from free hemoglobin in the phagocytosed cells, is converted to biliverdin within the phagocyte. Biliverdin is subsequently converted to bilirubin.

Intravascular hemolysis is defined as damage incurred by the RBC membrane directly within the vasculature due to shear stress, toxins, or complement-mediated lysis. Examples include mechanical valve-induced hemolysis, Shiga toxin-associated hemolytic-uremic syndrome, and cold agglutinin disease. Whereas hemoglobin clearance occurs

within the macrophage in extravascular hemolysis, during intravascular hemolysis, circulating free hemoglobin is bound irreversibly to the plasma haptoglobin and cleared by the liver. If free hemoglobin exceeds the binding capacity of haptoglobin, hemoglobinemia occurs. Unbound hemoglobin dimers are reabsorbed by the proximal renal tubule until the absorptive capacity is exceeded. Free hemoglobin is subsequently excreted in the urine, which appears dark.

CLINICAL FINDINGS AND DIFFERENTIAL DIAGNOSIS

Children may present with acute or insidious onset of pallor, fatigue, and lightheadness as a consequence of anemia. New-onset or recurrent jaundice may result from unconjugated hyperbilirubinemia. Parents may describe dark urine, which is due to hemoglobinuria from intravascular hemolysis. Acrocyanosis may occur, tachycardia and/or a flow

TABLE 1. Classification of Common Hemolytic Anemias

ORIGIN	DISORDER	MECHANISM OF DESTRUCTION
Acquired	Hemolytic disease of newborn	Extravascular
	Warm AIHA	Extravascular
	Mechanical valve HA	Intravascular
	Hemolytic-uremic syndrome	Intravascular
	Cold agglutinin disease	Intravascular
	Malaria	Intravascular/extravascular
Congenital	Sickle cell disease	Extravascular
	Thalassemia	Extravascular
	Hereditary spherocytosis	Extravascular
	G6PD deficiency	Intravascular/extravascular

AIHA=autoimmune hemolytic anemia, G6PD=glucose-6-phosphate dehydrogenase, HA=hemolytic anemia

murmur may be appreciated on physical examination, and splenomegaly may be observed due to sequestration of RBCs.

Adenopathy or hepatosplenomegaly should prompt investigation for malignancy or lymphoproliferative disorders. Lymphomas, in particular, can manifest with immune cytopenias, including AIHA and immune thrombocytopenia. In these cases, additional evaluation for hyperuricemia and examination of a peripheral blood smear by a hematologist is recommended. This type of paraneoplastic autoimmunity may be accompanied by constitutional symptoms suggestive of malignancy (eg, fevers, night sweats, weight loss, and fatigue).

In contrast to malignancy-associated immune hemolysis, children are typically healthy before the onset of isolated AIHA, although they may have experienced nonspecific viral symptoms or fever within several weeks of diagnosis. If the hemolytic anemia is congenital, the stigmata of chronic hemolysis may be noted, such as pigmented gallstones and related sequelae, due to excess production of bilirubin.

LABORATORY FINDINGS

Reticulocytosis is an important distinguishing feature of hemolysis and usually exceeds 2%, with the absolute reticulocyte count greater than $100 \times 10^3/\mu\text{L}$ ($100 \times 10^9/\text{L}$). However, transient reticulocytopenia can occur in up to 33% of patients, due to *in vivo* hemolysis of reticulocytes, nutritional deficiencies, concurrent parvovirus infection, toxin exposure, or underlying marrow dysfunction. (1)(2)

Peripheral Smear Findings

Microspherocytes are characteristic of AIHA due to membrane changes that occur when immunoglobulins are bound to the RBC surface. Fragment forms, such as schistocytes or helmet cells, may result from toxin- or shear stress-mediated hemolysis. Polychromasia, related to the increase in circulating reticulocytes, may also be reported.

Chemistry Panel

Unconjugated bilirubin, lactate dehydrogenase, and aspartate aminotransferase values may be elevated. The latter two intracellular enzymes are released into the plasma with cell destruction. As the plasma carrier for free hemoglobin, haptoglobin is often decreased. However, this is not a useful marker in infants younger than age 3 months.

Hemoglobin Electrophoresis

This test identifies sickle cell and β -thalassemia variants. Blood for hemoglobin electrophoresis must be collected before transfusion because it reflects the donor hemoglobin profile if performed within 3 months posttransfusion.

Direct Antiglobulin Testing

Once hemolysis is identified, further management is based on whether the hemolysis is antibody-mediated. The direct Coombs or antiglobulin test (DAT) is the primary method to detect *in vivo* coating of patient erythrocytes by autoantibodies. In the DAT, a nonspecific antihuman globulin is added to the patient's RBCs. If this antibody recognizes immunoglobulin bound to the RBC surface, as in AIHA, it binds or crosslinks other bound antibodies and agglutinates the antibody-bound RBCs. Subsequently adding antihuman antibodies for complement or immunoglobulin (Ig)G identifies the type of immunoglobulin bound to the RBC surface. Binding of anticomplement antibodies usually implies bound IgM. The specific antibody binding patterns can help to differentiate between warm-reactive (IgG) and cold-reactive (IgM) AIHA.

Indirect Antiglobulin Testing (Indirect Coombs)

Patient serum (rather than RBCs) is incubated with healthy donor RBCs. If RBC autoantibodies in the patient's serum bind to the donor RBCs, agglutination occurs, indicating the presence of circulating antibody. This may be performed if AIHA is suspected but the DAT result is negative.

NEONATAL ALLOIMMUNE HEMOLYTIC DISEASE

Maternal antibodies to incompatible fetal RBC antigens, such as Rhesus (Rh)D, A, or B, can cause hemolytic disease *in utero*. In the postnatal period, infants may exhibit mild anemia to hydrops fetalis. Before the introduction of anti-D Ig prophylaxis and intrauterine transfusions, Rh disease of the newborn was the predominant cause of neonatal AIHA, which is associated with 50% mortality and often lifelong morbidity. Rh disease remains a significant global burden. Today the most common cause of neonatal AIHA in Western countries is ABO incompatibility, ie, infants with A or B antigen born to group O mothers with high-titer IgG antibodies. These infants may demonstrate isolated unconjugated hyperbilirubinemia rather than hyperbilirubinemia and anemia, which is more typical of Rh disease. Hyperbilirubinemia may be exacerbated by coexistent glucose-6-phosphate dehydrogenase (G6PD) deficiency or Gilbert syndrome. In addition to ABO incompatibility, prenatal alloimmunization to minor RBC antigens such as Kell, Fy, Jk, C, and E is also rising in relative frequency and may lead to severe disease.

Onset of jaundice within the first postnatal day or prolonged or severe hyperbilirubinemia should prompt investigation of hemolytic disease. Infants with alloimmunization usually are DAT-positive. If the DAT result is negative, the

laboratory performs an indirect antiglobulin test with the infant's serum. If agglutination occurs, maternal antibody is present in the serum. If the indirect antiglobulin test result is negative, a search for nonimmune or congenital causes of HA is warranted.

Intensive phototherapy is often sufficient to address ABO-associated hemolytic disease, although exchange transfusion may be necessary. The use of intravenous immunoglobulin to prevent exchange transfusion is controversial, as demonstrated in a recent meta-analysis. (3) After discharge from the nursery, late-onset anemia may ensue 1 to 3 weeks after birth. Anemia results from continued immune-mediated destruction of RBCs and RBC progenitors as well as antibody-associated suppression of erythropoiesis. Neonates should be closely followed after discharge to determine the need for transfusion.

CHILDHOOD AUTOIMMUNE HEMOLYTIC ANEMIA

Beyond the neonatal period, AIHA is rare in children, with an annual incidence of 0.2 per million individuals younger than age 20 years. A recent French national cohort study suggests the incidence may be as much as 10 to 20 times higher. (2) AIHA may be classified as primary or secondary.

Primary Autoimmune Hemolytic Anemia

Primary AIHA, for which a cause is not identified, accounts for 30% to 40% of pediatric cases. Primary AIHA is further categorized by thermal reactivity or the temperature at which the RBC autoantibody is most reactive and causes agglutination. Agglutination at 37°C (98.6°F) constitutes warm AIHA, while agglutination below 30°C (86°F) is defined as cold AIHA (Table 2).

Sixty percent of adult and pediatric patients with AIHA are diagnosed with warm agglutinins, which are almost always IgG but sometimes involve complement. Most of these patients were previously healthy but may have had

nonspecific fever or viral symptoms. At diagnosis, patients present with jaundice, splenomegaly, and laboratory findings consistent with HA. If DAT is negative for anti-IgG but positive for anticomplement, further testing for cold agglutinins and paroxysmal cold hemoglobinuria (PCH) should be pursued.

Cold agglutinin disease is caused by IgM autoantibodies, which bind below 37°C (98.6°F) and are maximally reactive at 4°C (39.2°F). IgM autoantibodies trigger complement deposition *in vitro*, resulting in agglutination with anticomplement. Patients may display acrocyanosis when cold, which results from autoagglutination of RBCs in the skin capillaries, causing localized stasis. Although bedside autoagglutination of the blood can be observed in the test tube as the sample cools, this may be a result of clinically insignificant cold autoantibodies. Further testing includes initial screening at room temperature (20°C [68°F]), which should induce agglutination in the presence of cold agglutinin disease. Subsequently, agglutination of the RBCs in saline and albumin is observed in a staged manner from 0° to 30°C (32° to 86°F). If agglutination occurs at 30°C (86°F), pathogenicity is inferred.

PCH is a rare, self-limited AIHA caused by the Donath-Landsteiner (DL) antibody. Recurrence is unusual. Once commonly associated with syphilis, it is now seen predominantly in children, often preceded by an upper respiratory tract infection. The DL antibody is a cold-reactive IgG, which is considered biphasic because of the 2-step nature of its *in vitro* characteristics. The DL test involves incubation of the patient's blood at 4°C (39.2°F) for 1 hour to allow maximal binding of the IgG, followed by a second incubation at 37°C (98.6°F) to activate complement and induce hemolysis. The DL test is not universally available and may become negative after a few days into the clinical course of the condition, making diagnosis of PCH a challenging proposition. (4)

DAT-negative AIHA may be present, particularly if cold antibodies are involved. Antibody may not be tightly bound

TABLE 2. Classification and Features of Autoimmune Hemolytic Anemia

THERMAL REACTIVITY	CLINICAL SIGNS, SYMPTOMS	DAT FINDINGS	TREATMENT OPTIONS
Warm	Jaundice Splenomegaly	+IgG, -C3	First-line: corticosteroids Second-line: rituximab, splenectomy
Cold	Acrocyanosis Hemoglobinuria	-IgG, +C3	Avoidance of cold
Biphasic	Hemoglobinuria, self-limited	-IgG, +C3	Supportive care

C=complement, DAT=direct antiglobulin test, Ig=immunoglobulin

to RBCs and may be eliminated with the eluate in vitro. DAT-negative AIHA may also involve IgA or natural killer (NK) cells, which are not routinely screened for by the standard DAT. (4) More detailed immunologic evaluation of the RBCs using flow cytometry, gel card diagnostics, or more sensitive Coombs reagents may be available through select reference laboratories.

Secondary Autoimmune Hemolytic Anemia

A triggering cause was identified in 63% of cases of AIHA in the French national cohort study, characterizing the condition as secondary. A defined infection was diagnosed in 22% of patients, and almost 50% of these individuals ultimately were diagnosed with an immune disorder. Associated organisms included Epstein-Barr virus, cytomegalovirus, *Mycoplasma*, pneumococcus, and parvovirus. (2)

Although less common than in adults, pediatric autoimmune cytopenias can be associated with malignancies such as Hodgkin lymphoma. AIHA can be seen with autoimmune lymphoproliferative syndrome (ALPS), common variable immunodeficiency, systemic lupus erythematosus, and after solid organ and allogeneic stem cell transplantation. Disordered immune regulation through multifactorial mechanisms such as altered regulatory T-cell function, abnormal complement activity, and abnormal apoptosis predisposes these individuals to autoimmunity.

An abrupt decline in hemoglobin values after initiation of certain medications should prompt consideration of drug-associated immune hemolytic anemia (DAIHA). The incidence of DAIHA is estimated at 1 per 1 million pediatric and adult patients, but this is likely an underestimate. Recognizing this potentially severe complication allows discontinuation of the drug and resolution of hemolysis. One proposed mechanism asserts that certain drugs bind covalently to RBC antigens, stimulating hapten-dependent antibodies that activate macrophages and Fc-mediated extravascular destruction. Another mechanism involves direct stimulation of RBC autoantibody production. Cefotetan, ceftriaxone, piperacillin, fludarabine, and diclofenac have been implicated. Specialized reference laboratories can perform drug-independent and -dependent assays to facilitate diagnosis.

MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA

Corticosteroids (prednisone 1 mg/kg per day) are first-line therapy for warm AIHA and are associated with an 80% response rate. Improvement usually occurs within 24 to 72 hours of initiation. Once anemia is corrected, corticosteroids are weaned over several months to avoid relapse. Even

after recovery, the DAT may remain positive for years or indefinitely. Recurrence is more likely if an underlying autoimmune disease or immunodeficiency exists. If the patient does not tolerate reduction of corticosteroids or if the agents are ineffective, second-line treatment is indicated. Both rituximab and splenectomy have been used but have not been compared in clinical trials.

Splenectomy has been used as second-line therapy for refractory warm AIHA since the 1950s. There is no role for splenectomy in cold agglutinin disease because hemolysis in those cases is intravascular. There are surprisingly few data on the efficacy of splenectomy in children with refractory warm AIHA. Several large case series of adult patients undergoing splenectomy for benign hematologic diseases show that it is relatively safe, particularly if the spleen can be removed laparoscopically. Patients have an increased risk of thrombosis, especially affecting the portal or mesenteric veins, perhaps exacerbated by postsplenectomy thrombocytosis. However, the risk of thrombotic events may be more a function of the underlying hematologic disorder; thalassemia intermedia and major confer hypercoagulability. (5) The most feared complication is postsplenectomy sepsis due to encapsulated organisms. Splenectomy should be delayed in children younger than age 5 years because the risk of sepsis is greatest in this age group. Vaccination against encapsulated organisms, including the pneumococcal conjugate series and *Haemophilus influenzae* type b (Hib) series should be completed by 15 months of age. Pneumococcal polysaccharide and meningococcal polysaccharide can be administered after age 2 years and should be provided at least 2 weeks before splenectomy. Postsplenectomy, the patient should continue to receive antibiotic prophylaxis, usually penicillin, for at least 5 years or through age 18 years. The family must be educated about fever as an indicator of bacterial sepsis.

Rituximab, a chimeric monoclonal antibody specific to the B-lymphocyte CD20 antigen, may supplant splenectomy as an alternative for corticosteroid-refractory AIHA. Rituximab efficiently eliminates B lymphocytes and has been used to treat other autoimmune diseases. The typical regimen is 375 mg/m² weekly for 3 to 4 weeks. In a case series, 87% of patients had a sustained response at 13 months. Three of the 15 patients suffered recurrence but responded to a second course of rituximab. (6) The aggregate results of other small studies support a durable response to rituximab with few adverse effects. Infusion reactions include fever, hypotension, respiratory distress, and rash; these complications respond to infusion rate reduction and antihistamine use. Premedication with antipyretics, antihistamines, and corticosteroids usually prevents such reactions.

Increased susceptibility to infection and viral reactivation are theoretical concerns but are infrequent and usually seen in patients undergoing stem cell transplant. (7) Rituximab has been used with success in some patients who have cytopenias associated with underlying immune disorders, (8)(9) although patients with AIHA associated with ALPS did not respond to rituximab. (10)

Most cases of cold agglutinin disease result in chronic mild HA. Patients are advised to avoid the cold. Immunosuppressive therapy, such as corticosteroids, cyclophosphamide, chlorambucil, fludarabine, and rituximab, has been used without significant efficacy or durability of response. (11)

Regardless of classification, if a patient ultimately diagnosed with AIHA presents with severe anemia that may cause cardiovascular compromise (hemoglobin <5 g/dL [50 g/L]) or severe anemia with reticulocytopenia, transfusion is necessary. Communication with the blood bank about the clinical scenario is imperative because autoantibodies often obscure the RBC phenotype and make crossmatching difficult. If the child has not been transfused before, it is reasonable to proceed with transfusion despite positive crossmatching tests because alloimmunization is rare. If the patient has received a previous transfusion, the blood bank performs specialized testing to clarify the presence of alloantibodies.

CONGENITAL HEMOLYTIC ANEMIA

Inherited molecular defects that affect the stability of the RBC, including its shape (SCD), hemoglobin content (thalassemia), membrane stability (spherocytosis), or metabolic stability (G6PD deficiency), can cause hemolysis. In this section, we review the pathophysiology, clinical presentation, and management recommendations for these congenital hemolytic anemias.

HEMOGLOBINOPATHIES

Qualitative Defects: Sickle Cell Disease

SCD occurs in 1 in 300 to 400 African American births in the United States. The spectrum of SCD encompasses multiple sickling variants, which are diagnosed on newborn screen. The most common and most severe type is homozygous SS disease, in which both parents contribute the sickle hemoglobin mutation. The remaining genotypes are compound heterozygotes. Coinheritance of hemoglobins S and C as well as hemoglobin S and β -thalassemia occur to a lesser degree. In people of Asian descent, coinheritance of hemoglobins S and E is rising in incidence.

Expression of sickle hemoglobin results from a point mutation in the β -globin gene. Deoxygenation causes abnormal polymerization of sickle hemoglobin, transforming the erythrocyte into the characteristic crescent or sickle shape. Vaso-occlusion by these poorly deformable RBCs is the hallmark of the disease, but there is extensive literature describing the molecular and cellular changes that contribute to SCD pathophysiology. (12)

The spleen is also subject to multifactorial injury, invariably leading to splenic dysfunction early in life and ultimately autoinfarction. Before autoinfarction, the spleen can sometimes sequester blood cells. Splenic sequestration tends to occur in early childhood in those who have SS disease and later in patients with milder disease. Sequestration may vary from self-limited, with a mild drop in hemoglobin and splenomegaly, to severe, in which the volume of sequestered blood is life-threatening and patients present with cardiovascular collapse. Parents are taught to palpate their children's spleens because they may be the first to detect this complication. Small-aliquot transfusions may be considered for severe anemia. Recurrent severe splenic sequestration may warrant splenectomy in select cases. However, no robust data indicate survival benefit or decrease in morbidity with splenectomy.

Early impairment in splenic function underlies patients' vulnerability to infection with encapsulated organisms such as Hib and *Streptococcus pneumoniae*. Before advances in prophylaxis, children younger than age 3 years with SS disease frequently contracted pneumococcal bacteremia with significant mortality. The advent of universal newborn screening, prompt initiation of penicillin prophylaxis in infancy, and introduction of Hib and pneumococcal vaccinations have dramatically decreased rates of sepsis and death in infants with SCD. Breakthrough pneumococcal disease still occurs, (13) so fevers in those who have SCD must be promptly evaluated. Clinicians should order, at a minimum, a complete blood cell (CBC) count, reticulocyte count, and blood culture and administer parenteral antibiotics with effective antipneumococcal activity in the setting of fever.

Although survival has improved for children who have SCD, quality of life is frequently compromised by pain and multiorgan dysfunction. Recurrent ischemic injury and chronic inflammation can eventually lead to widespread irreversible organ damage in patients with the most severe disease (Table 3). (14) The greatest progress has been made in understanding neurologic disease in SCD.

The Cooperative Study of Sickle Cell Disease, the largest natural history study of SCD in the United States, reported that stroke occurred in 10% of those who had SS disease by

age 20 years. Ischemic stroke accounted for most events. (15) The risk of recurrence, if SCD is untreated, ranges from 47% to 66%, (16)(17) but this risk is substantially decreased with chronic transfusions.

The cause of stroke in SCD is multifactorial and remains incompletely understood. Abnormal adherence of the sickle RBCs to the vascular endothelium, recurrent endothelial injury, a hypercoagulable state, nitric oxide deficiency, altered vasomotor tone, and poor cerebrovascular reserve in the setting of chronic anemia all likely contribute to the pathogenesis of stroke. (18)

Transcranial Doppler (TCD) ultrasonography is a common, noninvasive radiologic technique for assessing the patency of intracranial vessels. Use of TCD ultrasonography is predicated on the fact that flow velocity is inversely proportional to arterial diameter, with high flow velocity suggesting arterial stenosis. Elevated cerebral arterial flow velocity (>200 cm/second) on TCD ultrasonography is highly predictive of stroke in those who have SCD. (19) (20) The landmark randomized, controlled Stroke Prevention Trial in Sickle Cell Anemia (STOP) study showed that in patients with abnormal TCD ultrasonography findings, chronic transfusion to reduce sickle hemoglobin to less than 30% was effective as primary prevention of stroke. (21) Annual TCD ultrasonography imaging, starting at age 2 years, is now a critical component of health care maintenance for those who have severe SCD (ie, hemoglobin SS,

hemoglobin S- β -null-thalassemia). Once identified, affected patients are started on monthly RBC transfusions.

Cessation of transfusions, even after TCD findings normalize, was associated with reversion to abnormal TCD velocities as well as increased rate of stroke in the STOP 2 study. (22) The current standard of care is to continue chronic transfusions indefinitely with concomitant iron chelation.

Silent cerebral infarcts (SCIs) are emerging as a risk factor for neurocognitive deficit in those who have SCD. Childhood prevalence increases with age and is estimated to be 12% to 37%, by age 14. (23) SCIs are more common among those who have SS disease but are also detected in other sickle variants. Defined as ischemic cerebral lesions on magnetic resonance imaging (MRI) without corresponding neurologic deficits, SCIs have been associated with lower reading, math, and IQ scores as well as a higher risk for overt strokes. Schools should be informed of these findings, so that affected students may receive academic support. From a medical standpoint, recent results from the Silent Cerebral Infarct Multi-Center Clinical Trial indicated that chronic transfusions could halt progression of SCIs. (24) However, it is unclear yet how this will affect practice, given the short duration of the study (3 years) and the lack of uniform guidelines for MRI screening in this population.

Quantitative Defects: The Thalassemias

The thalassemias are among the most common genetic disorders worldwide. Almost 5% of the global population carries a globin mutation related to α - or β -thalassemia. The thalassemias represent a spectrum of disorders of decreased or absent globin chain production due to hundreds of point mutations and less commonly, deletions. In addition to ineffective erythropoiesis, the resulting excess of unpaired globins produces insoluble tetramers that inflict oxidant injury on RBC membrane lipids and proteins.

Hemoglobin E is a β -globin variant common in many Asian populations around the world. It produces a thalassemia-like picture, so it will be reviewed here.

α -Thalassemia. Understanding α -thalassemia can be aided by familiarity with developmental hemoglobin expression. In healthy individuals, 2 α -globin gene loci on each chromosome 16 are expressed. The 4 genes express 2 α -globin molecules, which partner with 2 γ -globin molecules to form fetal hemoglobin *in utero* and for several months after birth. Hemoglobin production undergoes a progressive developmental switch from 6 to 12 months of age, which leads to preferential expression of β -globin rather than γ -globin. The β -globin molecules then partner with the 2 α -globin molecules to form normal adult hemoglobin.

TABLE 3. **Clinical Manifestations of Sickle Cell Disease**

ORGAN SYSTEM	CLINICAL MANIFESTATIONS
Neurologic	Overt strokes, silent infarcts, neurocognitive deficit
Retina	Sickle retinopathy
Musculoskeletal	Acute and chronic pain, avascular necrosis
Cardiac	Cardiomegaly, pulmonary hypertension
Pulmonary	Acute chest syndrome, chronic lung disease, nighttime hypoxia
Renal	Hyposthenuria, renal papillary necrosis, sickle nephropathy
Spleen	Splenic sequestration, functional asplenia, hypersplenism
Liver/gallbladder	Jaundice, cholelithiasis, sickle hepatopathy
Coagulation	Hypercoagulability
Genitourinary	Nocturnal enuresis, delayed puberty, priapism

α -Thalassemias demonstrate a remarkably close correlation between genotype and phenotype. Deletion of 1 gene is designated silent carrier because it is clinically asymptomatic. Two-gene deletion represents α -thalassemia minor or trait. Affected individuals may exhibit mild microcytic anemia that does not impair growth or development. Compound heterozygosity with α -thalassemia trait and sickle cell anemia can ameliorate the severity of SCD.

Hemoglobin H disease, or 3-gene deletion, can be diagnosed at birth or later in childhood. At birth, 3-gene deletion leads to decreased α -globin production as well as insoluble tetramers of γ -globin, which is identified as hemoglobin Barts on newborn screen. After the developmental switch, insoluble tetramers of β -globin are identified as hemoglobin H on hemoglobin electrophoresis. The oxidant injury produced by hemoglobin H results in chronic hemolysis. Patients may require RBC transfusions during their second decade of life. Despite only episodic need for transfusions, patients with hemoglobin H disease are at risk for iron overload due to increased gastrointestinal iron absorption.

Four-gene deletion has historically been incompatible with life due to the inability to express α -globin and, thus, fetal hemoglobin. Profound tissue hypoxia and consequent congestive heart failure develop *in utero*, leading to anasarca and a hydropic state. However, intrauterine transfusions have allowed some patients to survive the perinatal period, albeit with the sequelae of congenital anomalies and developmental delay. They require chronic transfusions after birth and eventually bone marrow transplantation.

β -Thalassemia. In contrast to α -globin, β -globin is expressed by a single gene locus on chromosome 11. More than 200 point mutations and, rarely, deletions can cause β -thalassemia, with variable severity. Inheritance of 1 mutation leads to β -thalassemia trait or minor, a benign condition characterized by mild microcytic anemia. Clinicians may encounter β -thalassemia trait when evaluating a child who has mild microcytic anemia unresponsive to iron supplementation. Elevated hemoglobin A₂ on hemoglobin electrophoresis and normal results of iron studies confirm the diagnosis.

β -Thalassemia intermedia results from inheritance of 2 β -globin mutations, 1 of which yields a mild phenotype. Affected patients are not transfusion-dependent but may be at long-term risk for high-output cardiac failure and pulmonary hypertension from persistent tissue hypoxia. Thus, chronic transfusion may be warranted. Patients with thalassemia intermedia may also develop iron overload due to inappropriately brisk gastrointestinal iron absorption, and they benefit from chelation.

The inheritance of 2 severe β -globin mutations in a homozygous or compound heterozygous pattern causes β -thalassemia major. Irritability, lethargy, and failure to thrive develop in the second 6 months after birth, as fetal hemoglobin expression declines and anemia develops. If the disease remains unrecognized, the stigmata of ineffective erythropoiesis emerge, including frontal bossing as the marrow compartment expands to compensate, hepatosplenomegaly and paravertebral pseudotumors due to extramedullary hematopoiesis, and growth failure due to chronic anemia.

Severe microcytic anemia in an older infant who has normal iron stores and poor growth should prompt investigation for β -thalassemia major. Hemoglobin electrophoresis assists in diagnosis. The child should be referred to pediatric hematology for initiation of chronic transfusions, which suppress endogenous erythropoiesis. Transfusions improve oxygen-carrying capacity, growth, and cardiac status. Patients are transfused every 2 to 4 weeks to maintain a pretransfusion hemoglobin of 9 to 10 g/dL (90-100 g/L).

Because each unit of blood introduces 200 to 250 mg of elemental iron, most of which cannot be actively excreted, iron overload inevitably develops. The RES sequesters excess iron. When the capacity of the RES is exceeded, iron is deposited in organ parenchyma, causing toxicity. Without effective chelation, patients are at risk for liver dysfunction, cardiac failure, hypogonadism, and other endocrinopathies. (25)

The gold standard measurement of iron overload is via liver biopsy. Most institutions use ferritin as a surrogate marker for iron stores, with MRI of the liver and heart as an accurate, noninvasive method of confirmation. Most children are started on deferasirox or deferoxamine at age 2 years. These agents bind iron and facilitate its urinary and biliary excretion.

If a matched sibling donor is available, some children with β -thalassemia may be candidates for curative hematopoietic stem cell transplant. Emerging therapies include induction of hemoglobin F expression and gene therapy. In the interim, lifelong chronic transfusion remains the mainstay of therapy for β -thalassemia.

Hemoglobin E. The frequency of the hemoglobin E allele ranges from 15% to 60% on the Indian subcontinent and in Southeast Asia. The product of a splice site mutation in the β -globin gene, hemoglobin E mRNA is transcribed at reduced levels and is translated into an abnormal β -globin molecule. The reduced expression of this abnormal β -globin yields clinical findings equivalent to β -thalassemia trait in patients with homozygous E disease or hemoglobin E trait.

β -Thalassemia variants are common in these geographic regions, so coinheritance of β -thalassemia and hemoglobin E occurs frequently. The clinical spectrum of hemoglobin E/ β -thalassemia ranges from moderate anemia to severe transfusion-dependent anemia, similar to β -thalassemia major. These diagnoses may be suspected when a patient of Asian ancestry presents with microcytic anemia and normal iron stores. Hemoglobin electrophoresis can elucidate these variants. Hemoglobin E/ β -thalassemia of moderate severity may result in iron overload through increased iron absorption, and affected patients benefit from chelation. Patients with severe hemoglobin E/ β -thalassemia should be referred to a hematologist early in life to start chronic transfusion therapy.

MEMBRANOPATHIES: HEREDITARY SPHEROCYTOSIS

The RBC cytoskeleton interacts with the outer lipid bilayer through horizontal spectrin molecules and vertical proteins 4.1 and 4.2 as well as band 3 and ankyrin molecules. Mutations in 1 or more of these proteins are responsible for HS, which demonstrates autosomal dominant inheritance in 75% of cases. The remaining 25% result from spontaneous mutation or autosomal recessive inheritance. Defects of any of these proteins lead to membrane instability and RBC destruction via extravascular hemolysis. This is the most common cause of inherited anemia in individuals of northern European ancestry but has been observed in all races and ethnicities.

HS may present at any age but is usually diagnosed in childhood. Children may present with unexplained anemia, reticulocytosis, jaundice, and/or splenomegaly. Aplasia due to parvovirus B19 infection may be the initial presentation for HS, even in adulthood. The CBC count may reveal elevated red cell hemoglobin concentration (mean corpuscular hemoglobin concentration ≥ 34.5 g/dL [345 g/L]) due to relative cellular dehydration. RBCs may be normocytic or mildly microcytic.

HS should be considered in a neonate who has early jaundice requiring phototherapy or exchange transfusion. The degree of hyperbilirubinemia does not predict future severity of disease. Some neonates develop severe anemia within the first few postnatal weeks that necessitates RBC transfusion, but they become transfusion-independent during their first year after birth. A subset of patients suffers anemia *in utero* and requires transfusion at birth; these patients typically exhibit severe disease throughout their lives. (26)

The diagnosis of HS is straightforward in the child who has a positive family history and nonimmune spherocytic hemolytic anemia. Further testing should be pursued for individuals who have evidence of chronic nonimmune hemolytic anemia but no family history. The studies used

most often are the osmotic fragility test and eosin-5-maleimide (EMA) flow cytometry. Osmotic fragility testing takes advantage of the fact that HS erythrocytes lyse more readily in hypotonic solutions than normal erythrocytes. However, because the sensitivity and specificity of osmotic fragility testing are not optimal, EMA flow cytometry is increasingly being used. EMA is a fluorescent dye that binds to band 3 and indirectly to other RBC membrane proteins. The degree of fluorescence can be used to identify reductions in band 3, spectrin, or ankyrin. This testing method has a sensitivity and specificity of greater than 90%.

Most patients who have HS exhibit mild hemolytic anemia, but moderate and severe disease exists, as defined by the degree of anemia and compensation for hemolysis. Patients with the most severe HS almost always have autosomal recessive disease and are transfusion-dependent. Individuals with mild disease have well-compensated hemolysis and no evidence of anemia.

Management depends on disease severity. Patients with mild HS are asymptomatic. Patients with moderate-to-severe HS benefit from folate supplementation. Cholecystectomy may be required for symptomatic cholelithiasis. Splenectomy was formerly a standard recommendation for moderate-to-severe HS; short-term benefits of splenectomy include improved hemoglobin, reduction in transfusion number, and decreased cholelithiasis. Postsplenectomy sepsis due to encapsulated organisms is a substantial risk for patients, even after appropriate immunization. In addition, a growing body of literature describes long-term risks of vascular disease after splenectomy, including thrombosis, pulmonary hypertension, and atherosclerosis. Although no randomized, controlled trials have been conducted, the general consensus still supports splenectomy for patients with severe HS in whom growth failure, skeletal abnormalities, and exercise intolerance are typical. The decision regarding splenectomy in moderate HS is made on a case-by-case basis. Patients should be vaccinated against encapsulated organisms at least 2 weeks before splenectomy.

ENZYMOPATHIES: GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD is critical for RBC defense against oxidative injury due to its role in the replenishment of nicotinamide adenine dinucleotide phosphate hydrogen via the pentose phosphate pathway. Deficiency of this enzyme predisposes individuals to the development of intravascular HA when their erythrocytes are under oxidative stress.

Worldwide, G6PD deficiency has a prevalence of 400 million cases, making it the most common RBC enzymopathy.

G6PD deficiency is X-linked, affecting predominantly males, although female carriers can exhibit decreased expression of G6PD due to lyonization. Hundreds of G6PD mutations are known, leading to a diverse spectrum of disease. The World Health Organization (WHO) developed a classification of mutations based on enzyme activity and severity of hemolysis. Class I variants express the lowest enzyme activity and cause the most severe disease, resulting in chronic extravascular HA even without exposure to oxidative stress. Class II variants, including G6PD Mediterranean, are characterized by severe deficiency and intermittent hemolysis. Class III variants such as G6PD A-, which is most common in individuals of African descent, demonstrate moderate enzyme deficiency and intermittent hemolysis.

G6PD deficiency is probably responsible for approximately 33% of cases of neonatal jaundice in male infants and a smaller fraction of female patients. (27) Jaundice usually develops in the first postnatal days. Hemolysis of G6PD-deficient cells does not appear to be a major component of neonatal jaundice; it may instead relate to immature bilirubin metabolism. Screening for G6PD deficiency should be considered in neonates with early or severe unconjugated hyperbilirubinemia. (28)

Beyond infancy, most G6PD-deficient individuals are asymptomatic and unaware of their diagnosis. They may come to medical attention with acute HA indicated by fatigue, back pain, dark urine, and jaundice. In the medical history, patients may identify an oxidative stressor, such as illness, fava bean ingestion, or certain medications. Infections associated with hemolysis in these patients include hepatitis A and B and cytomegalovirus. Laboratory testing may reveal anemia, reticulocytosis, high unconjugated bilirubin and lactate dehydrogenase, and decreased haptoglobin. Diagnosis is made by quantitation of enzyme activity when patients are well. G6PD activity may be falsely elevated immediately after a hemolytic episode, when the most G6PD-deficient RBCs are cleared from circulation. Because immature RBCs have greater G6PD activity than older RBCs, reticulocytosis also elevates overall G6PD activity. Enzyme activity in a neonate may be deceptively high because the RBC population is relatively immature.

Hemolytic episodes are typically self-limited, but some individuals require transfusion for severe anemia. G6PD-deficient patients benefit from counseling regarding avoidance of oxidative triggers. Several support organizations maintain updated lists of drugs and chemicals that can induce hemolysis, such as primaquine, sulfa drugs, nitrofurantoin, and naphthalene. Different G6PD variants are sensitive to different medications, so delineating a patient's WHO classification can facilitate more individualized guidance.

COMMON CLINICAL MANAGEMENT POINTS FOR ALL CHRONIC HEMOLYTIC DISORDERS

Parvovirus B19 Aplasia

All patients with chronic hemolysis are at risk from transient parvovirus red cell aplasia. Parvovirus B19 preferentially infects erythroid precursors that, in a patient with increased RBC turnover, can cause life-threatening anemia. Fevers in this patient population should be evaluated with CBC and reticulocyte counts, which will be substantially below baseline in the presence of parvovirus infection. Patients may demonstrate fatigue, lethargy, pallor, or signs of congestive heart failure. As viremia declines, erythropoiesis recovers in 1 to 2 weeks.

Gallbladder Disease

Upper abdominal pain in patients with hemolytic disorders should trigger evaluation for pigmented gallstones. The prevalence of cholelithiasis in this population approaches 50% by adulthood. Cholecystectomy is often recommended because recurrence is common and may be complicated by cholecystitis, choledocholithiasis, pancreatitis, and ascending cholangitis.

ACKNOWLEDGEMENTS

The author would like to thank Drs. Jessica Shand and Gary Noronha for reviewing this manuscript.

Summary

- On the basis of strong research evidence, hemolytic anemia affects all age groups and may be classified as acquired or congenital. It is characterized by genetic and clinical heterogeneity, even within individual disorders. (2)(14)(26)
- Recognition of hemolytic anemia is critical for proper treatment. A thorough history, physical examination, complete blood cell count, reticulocyte count, and direct antiglobulin test can help to narrow the differential diagnosis.
- Stroke causes significant morbidity and mortality in those who have sickle cell disease. On the basis of strong research evidence, stroke risk can be predicted using transcranial Doppler ultrasonography. (19)(20) Both primary and secondary stroke prevention can be achieved with a chronic transfusion program. (21)
- On the basis of strong research evidence, chronic transfusions play an important role in the management of several congenital hemolytic anemias. (21)(24) The resultant iron overload must be managed. (25)

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1. A 3-year-old boy was diagnosed with autoimmune hemolytic anemia due to a warm antibody. The child rapidly responded to corticosteroids within 1 week. However, any attempt to decrease the prednisone dose below 0.5 mg/kg per day resulted in a recurrence of hemolysis during the ensuing 8 weeks. His current hemoglobin is 8.5 g/dL (85 g/L). Which of the following is the most appropriate next therapeutic step?
 - A. Continue the full dose of prednisone for 4 weeks.
 - B. Exchange transfusion.
 - C. Packed red blood cell transfusion.
 - D. Rituximab administration.
 - E. Splenectomy.
2. A 10-month-old girl with known homozygous sickle cell anemia (SS disease) presents to your office with a history of a temperature of 40.1°C (104.2°F) at home. Before coming to the office, her parents gave her acetaminophen. Upon arrival at your office 40 minutes later, her temperature is down to 38.6°C (101.5°F). The girl has nasal congestion but no cough and is comfortable without distress. Which of the following is the most appropriate next step in management?
 - A. Ensure the parents are giving the prophylactic penicillin and send her home.
 - B. Have the family watch her at home and return if there is clinical worsening.
 - C. Observe her in the office for 4 hours and send her home if she looks well.
 - D. Obtain a complete blood cell count, hemoglobin, reticulocyte count, and blood culture and give intravenous antibiotics.
 - E. Obtain a complete blood cell count and blood culture and send the child home if the former is at baseline.
3. A 7-year-old boy with sickle cell anemia (SS) undergoes his annual transcranial Doppler ultrasonography study and is found to have a cerebral arterial flow velocity of 205 cm/second. The study is repeated and the result confirmed. His physical examination yields normal results, and he has no history suggestive of neurologic complications. He has one full sibling who also has sickle cell disease and one half-sibling. The most appropriate next step in management is to:
 - A. Initiate a chronic transfusion program.
 - B. Order cerebral magnetic resonance imaging.
 - C. Perform a hematopoietic stem cell transplant.
 - D. Prescribe sumatriptan.
 - E. Wait 6 months and repeat the transcranial Doppler ultrasonography study.
4. You are examining a 4-year-old who recently emigrated from Burma for the first time in your practice. Physical examination results are normal. A complete blood cell count is normal except for a hemoglobin of 9.5 g/dL (95 g/L) and mean corpuscular volume of 59 μm^3 (59 fL) with a normal red cell distribution width of 13.3%. Results of iron studies are normal. Hemoglobin electrophoresis reveals an elevated hemoglobin A₂ of 4.9%. Which of the following is the most likely diagnosis?
 - A. α -Thalassemia trait.
 - B. β -Thalassemia trait.
 - C. Hemoglobin E trait.
 - D. Homozygous hemoglobin E disease.
 - E. Early iron deficiency anemia.
5. An 11-year-old boy was well until 48 hours ago, when he developed fever, nasal congestion, and mild bilateral cervical adenopathy. Twenty-four hours later, he developed fatigue, jaundice, and very dark urine. Complete blood cell count reveals hemoglobin of 6.1 g/dL (61 g/L), mean corpuscular volume of 83 μm^3 (83 fL), and mean corpuscular

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hemoglobin concentration of 32 g/dL (320 g/L). Reticulocyte count is more than twice normal at $250 \times 10^3/\mu\text{L}$ ($250 \times 10^9/\text{L}$). Other results include an unconjugated bilirubin of 4.1 mg/dL ($70.1 \mu\text{mol/L}$) and conjugated bilirubin of 0.54 mg/dL ($9.2 \mu\text{mol/L}$). Lactate dehydrogenase is elevated to 550 U/L ($9.2 \mu\text{kat/L}$). Peripheral blood smear reveals polychromasia without spherocytes or fragmented erythrocytes. Hemoglobin electrophoresis results are normal. Direct antiglobulin test is normal. The most likely diagnosis is:

- A. Autoimmune hemolytic anemia.
- B. E β -thalassemia.
- C. Hemoglobin glucose-6-phosphate dehydrogenase deficiency.
- D. Hereditary spherocytosis.
- E. Sickle cell (SS) disease.