REVIEW

Identification and Reporting of Common Hemoglobin Disorders: A Review

Jessica A Hemminger[1], Nazih AbuAlsheikh[2], and Samir B Kahwash[2]

[1]Department of Pathology, The Ohio State University, Columbus, Ohio, USA
[2]Department of Pathology and Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio, USA

Abstract

Hemoglobinopathies and thalassemias constitute a major cause of anemia worldwide. Some of these disorders may necessitate chronic red blood cell transfusion therapy, which frequently results in a host of serious clinical sequelae, including iron overload. The following review attempts to offer a simplified approach to the identification of the most commonly encountered hemoglobin disorders. In addition, practical comments on reporting the results of hemoglobin studies and the expected clinical impact of the various findings are discussed.

Introduction

Hemoglobin (Hb) is the vital oxygen-transporting metalloprotein present in erythrocytes. The Hb molecule consists of two main components: an iron-containing porphyrin ring, referred to as a heme group, and a tetramer of globin polypeptide chain (1). The heme functions as an oxygen-carrying group and the globin chains function to protect and render the heme soluble (2). Inherited disorders of the porphyrin ring, termed porphyrias, are rare (3). In contrast, inherited globin chain disorders are common, and it is estimated that 7% of the world population are carriers (4,5). The latter disorders of Hb are categorized as either structural globin chain defects, often referred to as hemoglobinopathies, or a quantitative reduction in synthesis, termed thalassemia (1). Commonly seen structural Hb variants include Hb S, Hb C, and Hb E (5-7). Thalassemias are named after the globin chain affected, the most common thalassemias are α-thalassemia and β-thalassemia (1). The unusually high prevalence of Hb disorders is thought to be a result of a survival advantage for carriers of these traits, particularly Hb S, in areas where malaria is endemic (6,8-10). Due to population movement, Hb disorders are now commonly seen in all parts of the world.

The globin tetramer consists of two dimers of α-like and β-like globin chains (1). The α-like globins are the α- and

Table 1. Characteristic findings in α-thalassemia in infants

	Clinical Finding	MCV/MCH	Cord blood, Hb Bart's (% Total Hb)
α/α+	Asymptomatic	Normal	0-2%
α/α0	Asymptomatic	Low/Normal	2-8%
α+/α0	Anemia (Hb H disease)	Low	10-40%
α0/α0	Hydrops fetalis	Low	80%

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin

Table 2. Characteristic Findings in α-thalassemia after Infancy

	Clinical Finding	MCV/MCH	Primary Hb
α/α+	Asymptomatic	Low	A
α/α0	Mild anemia	Low	A
α+/α0	Moderate to severe anemia	Low	A H (5-30%)
α0/α0	Hydrops fetalis	-----	-----

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin

ζ-chains, which are encoded on chromosome 16. The β-like globins are the β-, γ-, ε-, and δ-chains, which are encoded on chromosome 11. The ζ- and ε-chains constitute the embryonic Hbs, which are seen in normal embryos and fetuses of less than three months gestational age. During fetal life, Hb F (α2γ2) predominates. Fetal red blood cells (RBCs) with Hb F possess an increased oxygen affinity compared to adult RBCs, which is important for proper maternal-fetal oxygen exchange in utero (11). Adult Hb, Hb A, (α2β2) is minimally produced before 20 weeks of gestation and production begins upon the initiation of β-chain synthesis. At birth, Hb A is 15-40% of the total Hb. The proportion of Hb A compared to Hb F continues to increase, and at six months of life Hb F constitutes <8% of the total Hb. Adults normally have approximately 97% Hb A, <1% Hb F, and 1.5-3.5% Hb A2. Hb A2 (α2δ2) synthesis begins late in fetal life, and constitutes a small proportion of total adult Hb due to less efficient transcription of the δ gene compared to

Table 3. Characteristic Findings in β -thalassemia during and after Infancy

	Clinical Finding	Infancy	Age >1 yr
β/β0 β/β+	Asymptomatic	Normal indices* Normal A2	Thalassemic indices* Hb A: 94% Hb A2: 6%
β+/β0 β+/β+ β0/β0	Moderate to severe anemia	Variable indices* Hb F: Increased Hb A: <1-30%	Thalassemic indices* Hb F: 70-95% Hb A: 5-30% Hb A2: 2-5%

*Thalassemic indices defined by MCV/RBC >13 (Metzner formula)

Table 4. Clinical Impact of Interactions between α- and β-thalassemia Haplotypes

	2nd Haplotype				
1st Haplotype	Nl (α/β)	α+ (α-/)	α0 (--/)	β +	β0
α+ (α-/)	0	1	3-4	1	1
α0 (--/)	1	3-4	X	1	1
β +	0	1	1	3	3
β0	1	1	1	3	4

Clinical Impact Score: 0= None to minimal; 1= Minimal to mild anemia;
2= Mild to moderate anemia; 3= Moderate anemia with clinical symptoms;
4= Severe clinical condition; X= Incompatible with life. Nl, normal

the β gene (12).
The diagnosis of hemoglobin disorders has evolved over the past decades, from being mostly initiated by a symptomatic patient, or an abnormal lab test or peripheral blood smear, to the current reliance on universal screening in most advanced health systems. This evolution has mirrored the expansion of our knowledge about these disorders and the development of better diagnostic methods. In 1949, Dr. Linus Pauling linked sickle cell disease to a defect at the molecular level, paving the way for the molecular evaluation of disease (13). Since his pioneering work, over 1,000 Hb variants and over 400 thalassemias have been identified (14). The molecular understanding of Hb disorders has enabled the development and implementation of effective therapies as well as neonatal screening programs (15). The screening programs, which primarily focus on early detection of sickle cell disease, have successfully decreased the frequencies of life-threatening complications that plague affected infants (16-18).

Hb electrophoresis has been utilized since the 1960's and continues to represent a cost effective technique for the identification of Hb disorders; although, cation-exchange HPLC is emerging as the method of choice for Hb variant identification and quantification (19). The leading commercial cation-exchange HPLC system is the Bio-Rad Variant (Bio-Rad Laboratories) (21,24-27). Zone electrophoresis utilizing cellulose acetate at alkaline pH separates the com-

Table 5. Hemoglobin S Sickling/Hemolytic Disorders

Clinical Phenotype	Hb Disorder	MCV/MCH	Hb (Mean % Total Hb)
Asymptomatic	A/S	Normal	A=60%, S=36%, A2=4%
	S/HPFH	Normal	S=70%, F=30%
	A/S and α-thal	Low	A=70%, S=30%
Sickling/hemolytic disorder	S/S	Normal	S=89%, A2=6%, F=5%
	S/C	Normal	S=47%, C=47%, F=6%
	S/β+-thal	Low	S=62%, A=30%, A2=5%, F=3%
	S/β0-thal	Low	S=88%, A2=7%, F=5%
	S/O-Arab	Normal	S>O
	S/E	Low	S=64%, E=35%, F=1%

Table 6. Clinical Impact of Various Inheritance Patterns of Common Hemoglobin Disorders

1st haplotype	2nd haplotype										
	Nl	αGPh thal	α+ thal	α0 thal	β^+ thal	β^0 thal	δβ thal	Hb S	Hb E	Hb C	HPFH
αGPh thal	0-1	3-4	2	3-4	1	1	1	1	0	0	0
α+ thal	0	2	1	3-4	1	1	1	1	0	0	0
α0 thal	1	3-4	3-4	X	1	1	1	1	0	1	0
β+ thal	0	1	1	1	3	3	3	3	3	2	0
β0 thal	1	1	1	1	3	4	3	4	3	3	0
δβ thal	1	1	1	1	3	3	3	2	3	3	0
Hb S	0	1	1	1	3	4	2	4	1	3-4	1
Hb E	0	0	0	0	3	3	3	1	0	1	0
Hb C	0	0	0	1	2	3	3	3-4	1	1-2	0
HPFH	0	0	0	0	0	0	0	1	0	0	0

Clinical Impact Score: 0= None to minimal; 1= Minimal to mild anemia; 2= Mild to moderate anemia; 3= Moderate anemia with clinical symptoms; 4= Severe clinical condition; X= Incompatible with life. Nl, normal; αGPh, Hb G-Philadelphia; Thal, thalassemia; HPFH, hereditary persistence of fetal hemoglobin.

monly encountered variants: Hb S, Hb F, Hb C from the normally present Hb A and Hb A2 (1). In this setting, Hb A2, Hb C, Hb E and Hb O-Arab co-migrate as do Hb S, Hb D, Hb G, and Hb Lepore. Citrate agar electrophoresis is
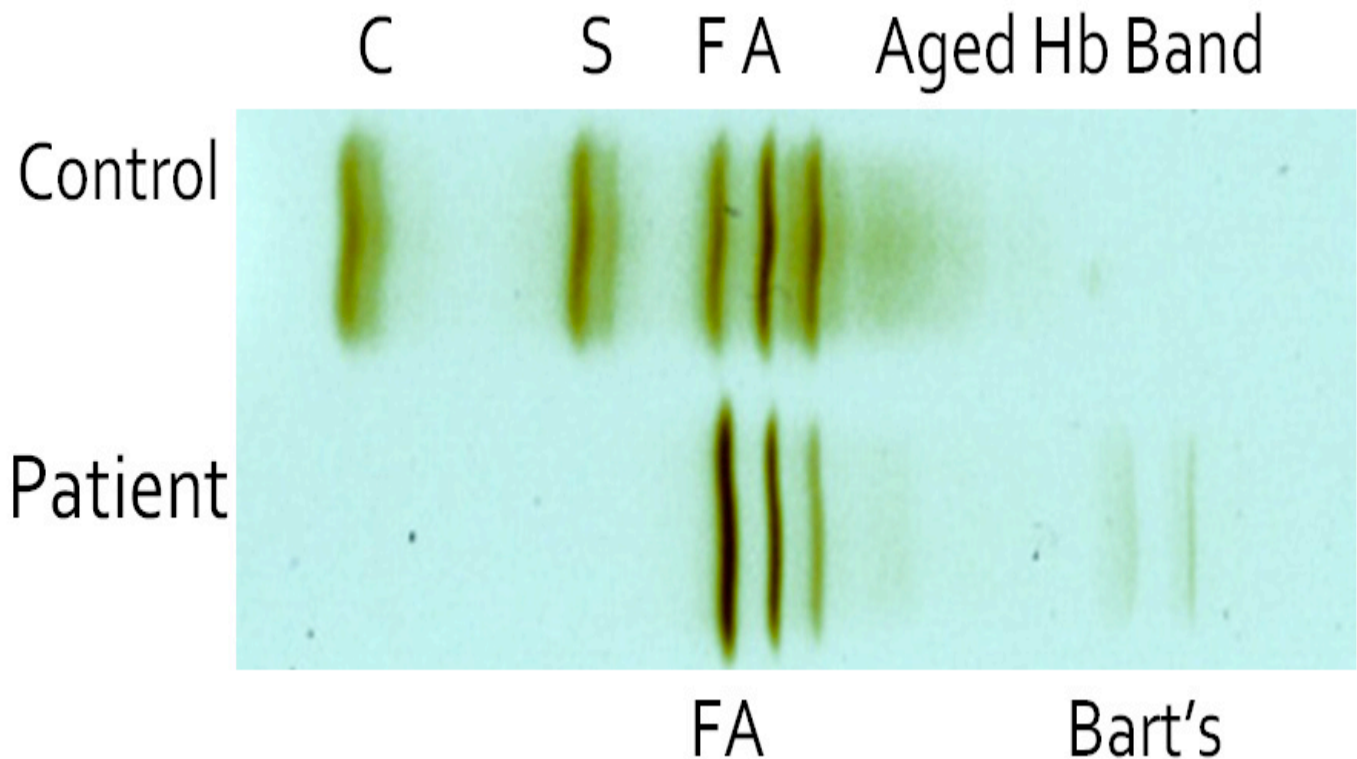
Figure 1. Isoelectric Focusing (IEF) electrophoresis showing hemoglobin Barts.

performed at an acid pH to separate the co-migrating Hbs (Hb S from Hb D and Hb G, and Hb C from Hb E and Hb O). With electrophoresis, quantification of Hb is accomplished by densitometric scanning, which can be inaccurate when variants are present at a low concentration (19). A relatively newer method of high-resolution electrophoresis, isoelectric focusing (IEF), separates hemoglobin variants based on their electric charge in a pH gradient generated by carrier ampholytes present within the medium (20-24). Finally, a method utilizing hemoglobin electrophoresis in a negatively charged capillary system, termed capillary electrophoresis, has been gaining popularity in recent years (21,28-32).

The Thalassemias
The thalassemias are genetic Hb disorders defined by decreased production of one or more globin chains (1). The two most prevalent types are α-thalassemia and β-thalassemia, which involve decreases in the α- and β-globin chains, respectively. Decreases in the minor globin chains as seen in

δβ-thalassemia and γ-thalassemia are also recognized. The α-chain is encoded in duplicate on chromosome 11, while there is only one copy of the β-like genes (10,19). Thus, a diploid cell contains four α genes and two β, δ, and γ genes. In most clinically relevant cases, an individual possesses a deletion or mutation involving either the α or β gene that causes a decrease in α- or β-chain synthesis, ultimately manifesting in decreased Hb A production. Additionally, unbalanced globin synthesis causes intracellular accumulation of unmatched chains that damage the erythrocytes, leading to ineffective erythropoiesis and frequently a hemolytic process (8,10). Thalassemia demonstrates a broad range of clinical phenotypes ranging from no manifestations to symptomatic microcytic, hypochromic anemia to lethal anemia (8). In current practice, thalassemia traits are usually suspected in one of the following clinical settings:

1- Suggestive findings on a newborn screen. This is the usual case for α-thalassemia trait where the finding of Hb Bart's, a tetramers of excess γ-chains Hb, is characteristic

(Figure 1). There is no such indicator for β-thalassemia, which is not excluded by a normal newborn screen.

2- The presence of "thalassemic" indices, which include MCV/RBC >13 (Metzner's formula), on a CBC performed as a screen or as part of another work up (33). "Thalassemic" indices do not manifest in the first few months of life when Hb F predominates.

3- An elevated or decreased level of a normal hemoglobin on Hb electrophoresis. For example, an elevated Hb A2 level is characteristic of β-thalassemia trait (discussed further below).

α-thalassemia
The α-thalassemias encompass all the conditions involving the deletion or mutation of an α gene resulting in decreased α-chain synthesis, and carrier frequencies can be as high as 80-90% in some parts of the world (8). In the vast majority of cases (~95%), α-thalassemia is caused by large deletions in one or both of the α genes on a chromosome (8,10). The specific genetic abnormalities are extremely heterogenous, and the nondeletion forms of α-thalassemia are mostly mutations that result in significant suppression of α-chain production (6,7,10). An uninvolved person has a genotype of αα/αα and a haplotype of α/α, which represent four functioning α genes (10,21). The α-thalassemia haplotypes are denoted as α+ (e.g. α- genotype) or α° (e.g. -- genotype), depending on whether one or both α genes on a chromosome are defective, respectively.

The loss of one α gene (α+/α) is not clinically significant and is referred to as α-thalassemia minor, mild or 'silent' α-thalassemia (8,10). Of note, the most frequent α+ haplotype, affecting a third of African Americans, results from a specific 3.7 kB deletion (designated -α3.7) that spans both α genes on chromosome 16 and produces a functional hybrid α gene (21). Severe α-thalassemia minor, also referred to as α-thalassemia trait, occurs with two defective α genes (α+/α+ or α°/α) and usually causes a minimal to mild microcytic anemia (21). Hb H disease occurs with the deletion or mutation of three α genes (α+/α°). This results in at least a 70% reduction in α-chain synthesis and significant accumulation of unmatched β-chains that form tetramers, Hb H, which are poor oxygen (O2) transporters (8). Hb H disease is characterized by chronic hemolytic anemia (Hb concentration 7-13.8 g/dl), and Hb H comprises 5-40% of the total adult Hb (10,21,34). The homozygous state for α°-thalassemia equates to hydrops fetalis. There is no pro-

duction of α-chain, and abnormal γ tetramers, Hb Bart's (γ4), predominate in the affected fetus with minor components of embryonic Hbs (ζ2γ2 and ζ2β2) and Hb H (8,21). Secondary to high O2 affinity and no subunit cooperativity, Hb Bart's cannot transport O2; thus, oxygen exchange is dependent on ζ2γ2 and ζ2β2 (10). The embryonic Hbs cannot support later stages of fetal growth, and the fetus becomes anemic and dies of anoxia. In both Hb H disease and hydrops fetalis, the inherent structural instability of Hb H and Hb Bart's causes globin chain precipitation within the erythrocytes and results in a prominent hemolytic process (1). Hematologic parameter abnormalities correlate well with the degree of α-chain reduction, and the less α-chain synthesized the greater the microcytic, hypochromic anemia (1,8). Additional peripheral blood smear findings include target cells, varying degrees of anisopoikilocytosis, and occasional characteristic RBC inclusion bodies that are best visualized with Brilliant Cresyl Blue staining, which are Hb H precipitates (1,35). Tables 1 and 2 summarize characteristic findings in α-thalassemia during and after infancy, respectively.

β-thalassemia
The β-thalassemias encompass all the conditions involving the mutation or, less commonly deletion, of a β gene resulting in decreased β-chain synthesis (10,36,37). The specific molecular abnormalities causing β-thalassemia are many (>200), and most mutations cause a defect in transcription, translation or post-translation of the β-chain (7,38). Unlike the α-chain, the β-chain is encoded only once per chromosome. The designations β+ and β° are frequently used to distinguish diminished from absent β-chain synthesis, respectively (36,38). The anemia produced is predominately a result of decreased β-chain synthesis and subsequent accumulation of unmatched α-chains that aggregate and damage the RBC, ultimately leading to significant erythrocyte destruction and prominent ineffective erythropoiesis (1,7,37). Hemolysis can also be present (1,37). Because the γ-chain, not the β-chain, is the main non-α globin chain at birth, the morphologic and clinical manifestations of β-thalassemia trait may not be apparent for a few months after birth. Table 3 lists some of the expected findings in the various β-thalassemia combinations in infancy and beyond.

Clinically, the β-thalassemias are divided into thalassemia minor, major, and intermedia (36). β-thalassemia minor is noted in heterozygotes (β+/β or β°/β) and is often asymptomatic. Hematologic findings include mild microcytic, hypochromic anemia with increased Hb A2 (range:

3.5-8.0%) and Hb F (range: 1-5%) levels (10, 21, 24, 36). Thalassemia major and thalassemia intermedia both require medical management and are mostly homozygotes or compound heterozygotes for β° or β+ genes (36,37). Thalassemia major presents as a progressive anemia during 6 to 24 months of age as γ-chain synthesis diminishes without a concomitant increase in β-chain synthesis due to genetic abnormalities involving both β genes (36,38). The anemia is severe (Hb 2-7 g/dl) and is characterized by microcytic, hypochromic RBCs with extreme polymorphic morphology on the peripheral blood smear. Hb F is the predominant Hb (70 to >94% of the total Hb), the level of which depends on the degree of residual β-chain production (10, 36). The severe, chronic anemia has detrimental effects on most organ systems causing slowed somatic and sexual growth and hepatosplenomegaly in affected children (10, 38). Additionally, those affected acquire characteristic sequelae of increased erythropoiesis, including skeletal abnormalities such as "thalassemic facies" and osteopenia (10, 37, 38). β-thalassemia intermedia refers to β-thalassemia of moderate severity, regardless of genotype, characterized by moderate anemia (Hb 7-10 g/dl) and necessitates no or only infrequent blood transfusions (10,36). The causative β gene abnormalities are diverse, and this subdued phenotype is often produced by increased expression of Hb F, which is associated with certain molecular abnormalities (36-39). Another common etiology for the intermedia phenotype is the coinheritance of both α- and β-thalassemia (36-39). This combination maintains the appropriate balance between α- and β-chain synthesis and avoids the damaging effects of unmatched free chains. Table 3 summarizes the characteristic findings in β-thalassemia. Table 4 depicts the clinical implications of the coinheritance of various α- and β-thalassemia haplotypes.

A variant of β-thalassemias, δβ-thalassemia, is caused by long deletions involving both the β and δ genes (1, 10,12). Interestingly, δβ-thalassemia demonstrates a mild thalassemic phenotype secondary to a compensatory increase in γ-chain production (1,21). Characteristically, heterozygotes have moderately increased Hb F concentrations (5-20%) and develop thalassemia minor, characterized by an asymptomatic, mild hypochromic, microcytic anemia (1). Homozygosity is very rare, and those affected develop thalassemia intermedia with 100% Hb F due to the absence of both β- and δ-chain synthesis (1, 21,40). The peripheral blood smear shows the characteristic thalassemic findings of microcytosis and hypochromia (41). Rare long deletions involving the entire β gene cluster result in εγβδ-

thalassemia, and clinical manifestations of heterozygous individuals are similar to β-thalassemia trait, however, lack increased Hb A2 levels (7,38,42).

γ-thalassemia
A unique deletion involving a portion of the γ gene creates a hybrid gene that maintains γ-chain synthesis (12). Thus, only a mild γ-thalassemia is noted in heterozygous individuals. Homozygosity is rare and despite the partial γ gene deletion, affected individuals are not severely anemic and surprisingly demonstrate 50-60% Hb F on perinatal Hb quantification analysis (12).

Hereditary persistence of fetal hemoglobin
Hereditary persistence of fetal hemoglobin (HPFH) is defined as an increase in adult Hb F levels, and is present in 0.1% of black Americans (1,41). HPFH is usually a result of a deletion in the β or δβ genes that cause decreased β- and/or δ-chain synthesis, or a point mutation in the γ gene promoter that causes increased γ-chain synthesis (1,21,41). Heterozygous individuals, in comparison to δβ-thalassemia heterozygosity, have higher Hb F levels (10-35% of total adult Hb), and the peripheral smear is usually unremarkable (12,41). Additionally, Hb F is characteristically increased in a pancellular pattern, involving all RBCs, while in δβ-thalassemia, Hb F is distributed in a heterocellular pattern and involves only a portion of RBCs (21,41). Despite these differences, clinical and molecular overlap exists between δβ-thalassemia and HPFH (41). In HPFH homozygous individuals Hb A and Hb A2 are not synthesized; thus, Hb quantification analysis demonstrates 100% Hb F.

The Hemoglobinopathies
Sickling/Hemolytic disorders
Hemoglobinopathies are defined by structural defects in the Hb molecule resulting from genetic mutations, and most hemoglobinopathies are of no clinical significance. The most frequent genetic abnormality producing a variant Hb is a single amino acid substitution (10). Mutations resulting in amino acid deletions, insertions, crossovers, and chain elongation have also been documented (10). Genetic mutations can involve any Hb chain; however, most clinically significant hemoglobinopathies contain a variant β-chain since an abnormal β gene affects a large proportion of adult Hb. In general, if one of the two β genes is mutated, the variant will represent approximately half of the total β-chains synthesized compared to variant α-chains, which represent at most 25% of the total α-chains produced (10). Pathologic variant Hbs can potentially cause decreased Hb

synthesis (anemia), imbalance between α- and non-α-chain synthesis, modify the physical characteristics of erythrocytes (i.e. sickling), shorten RBC lifespan through Hb instability, Hb precipitation, and hemolysis, and can affect RBC oxygen carrying capacity by affecting oxygen affinity (10). Usually two abnormal mutant β genes are required to cause significant clinical manifestations (10). Those affected can be either homozygous (i.e. Hb SS) or doubly heterozygous (i.e. Hb SC).

Hemoglobin S

The single base pair substitution A to T involving the β gene results in the insertion of valine for glutamate at the sixth position of the β-chain (10,21). This abnormal substitution produces Hb S, which is the most prevalent hemoglobinopathy and the cause of sickle cell disease. Approximately 9% of African Americans and up to 45% of the population in some parts of equatorial Africa harbor the mutation for Hb S (43,44). Interestingly, recent studies report much lower prevalences in these historically high-prevalence areas (45).

In high Hb S concentrations within a given erythrocyte, as seen in homozygous individuals, deoxygenated Hb S molecules polymerize with each other secondary to the hydrophobic property of the substituted valine residue on the β-chain (10,46). The subsequent polymer formation within the RBC causes severe cellular rigidity and shape deformities that prevent the erythrocyte from traversing the capillary. The misshapen or "sickled" RBCs occlude the microvasculature with resultant ischemia to the tissue. Homozygotes for Hb S have sickle cell disease, which is characterized by chronic hemolytic anemia, vaso-occlusive crises, and vulnerability to infection (10). Symptomatic disease begins upon downregulation of Hb F within the first year of life (47). Hemolysis is predominantly extravascular and Hb levels average 8+/-2 g/dl (10,21). The blood smear reveals misshapen RBCs, including sickled forms termed irreversibly sickled cells, which are permanently sickled-shaped, independent of Hb S polymerization (48). Target cells, immature RBCs, Howell-Jolly bodies, and an increased red cell distribution (RDW) are also common hematologic findings (21). Known triggers of the characteristic painful vaso-occlusive crises afflicting individuals with sickle cell disease include acidosis, hypoxia, and dehydration; however, many painful crises have no known precipitating cause (10). The most common manifestation of a painful crisis is excruciating musculoskeletal pain; however, the crises can affect the bones and joints, including aseptic necrosis of

the femoral and humeral heads, as well as the abdomeninal viscera. Other manifestations of sickle cell disease include hand-foot syndrome, seizures, strokes, cirrhosis, sickle hepatopathy, intrahepatic cholestasis, cholelithiasis, renal dysfunction, acute chest syndrome, priapism, leg ulcers, functional asplenia, acute splenic sequestration, and retinopathy (10,49).

In sickle cell trait, defined by Hb S heterozygosity, the RBCs contain approximately 60% Hb A and 40% Hb S (21). The increased proportion of Hb A is due to faster association rates of the positively charged α-chains with negatively charged non-mutant β-chains compared to the positively charged mutant β-chains that constitute Hb S (50). Hb A in such a concentration effectively inhibits polymerization of Hb S, preventing occlusive episodes in the microcirculation (51). Thus, sickle cell trait is asymptomatic and only exhibits sickling manifestations in the setting of extremely low hypoxemia (O2 saturation <40%) (10, 21). Lab findings are usually unremarkable with normal blood counts and unremarkable red cell morphology.

Hb F (α2γ2) ameliorates sickle cell disease, and mortality from sickle cell disease is inversely related to Hb F levels (46,52,53). Deoxygenated Hb S molecules can polymerize with other Hbs, such as Hb C, Hb O-Arab, and Hb A (54, 55). Hb S can also polymerize with Hb F; however, Hb F inhibits further polymerization, preventing long polymer formation and subsequent RBC rigidity (56). Initially, the beneficial effect of increased Hb F in sickle cell disease was noted in individuals with coinheritance of Hb S and HPFH as well as in individuals with certain genetic clusters associated with increased Hb F production (10,57,58). In the 1980s, hydroxyurea, a ribonucleoside diphosphate reductase inhibitor that was used as a chemotherapeutic agent for chronic myeloproliferative disorders, was shown to increase the Hb F levels in individuals with sickle cell disease (59,60). Since then, multicenter clinical trials have demonstrated that hydroxyurea increases Hb F levels in individuals with sickle cell disease and reduces overall mortality (61). Additionally, hydroxyurea diminishes morbidity by decreasing the frequencies of painful crises and acute chest syndrome, as well as reducing blood transfusion requirements (62).

It is well established that despite harboring the identical point mutation in the β gene, the clinical manifestations of sickle cell disease differ between individuals, ranging from mild to debilitating. The differences in disease phe-

notype are frequently due to varying β gene cluster haplotypes, defined as linked sequence polymorphisms within and around the β-like gene cluster that are identified by restriction enzyme digestion (63,64). These β-chain haplotypes are named for the historical region of prevalence. The most common haplotypes found in the Americas originated in Africa and are known as the Benin, Bantu (also termed Central African Republic or CAR), and Senegal haplotypes (65,66). Homozygotes for the Benin haplotype represent the majority of individuals with sickle cell disease in the Americas (40-50%), followed by Benin/Bantu haplotype heterozygotes (25%), and Senegal/Benin haplotype heterozygotes (13%) (67). The haplotype Arab-India is also recognized (68). It appears that the effect of these polymorphisms on disease severity is secondary to genetic alterations that affect Hb F levels (65,67). The Senegal and Arab-Indian haplotypes are associated with increased levels of Hb F, frequently >20% of total adult Hb, and a milder clinical course (64,69,70). The Benin haplotype is associated with a modest increase in Hb F that demonstrates some clinical benefit (64,71). The Bantu haplotype is associated with low Hb F and an increased risk of organ failure (72).

Given the similar geographic distributions, Hb S is frequently coinherited with other hemoglobin abnormalities including α- and β-thalassemias and variant Hbs such as C and O-Arab. The clinical manifestations produced by double heterozygosity are diverse and depend on the particular Hb abnormalities inherited. Coinheritance of α-thalassemia is frequent and in general ameliorates sickle cell disease by improving cell hydration, which decreases cellular Hb S concentration and prevents polymer formation (68,73-75). Coinheritance of Hb S and β-thalassemia is usually characterized as Hb S/β+ thalassemia in individuals of African descent (21). Only a modest decrease in β-chain synthesis is seen in Hb S/β+ thalassemia; thus, a moderate sickle cell disease is seen with increased Hb A2. Unfortunately, Hb S/β° thalassemia manifests similarly to sickle cell disease (21,76). Table 5 shows the interaction of inheriting a sickle cell gene in combination with other common hemoglobinopathies.

At newborn screening, the finding of only Hb F and Hb S raises the differential diagnosis of the following Hb disorders: Hb SS (most common), Hb S/β° thalassemia (second most common), and Hb S/HPFH. The latter has a benign clinical course, but unfortunately is rare. Hb SS and Hb S/β° thalassemia evolve gradually to a sickling/hemolytic disorder as Hb F levels naturally decline in infancy. Early distinction between these disorders can be facilitated by family studies, correlation with peripheral blood smear findings and/or evaluation of Hb F patterns by flow cytometric analysis (77).

Additional Sickling/Hemolytic Hemoglobin Disorders
Other doubly heterozygous sickling disorders include Hb SC disease, Hb S-O-Arab disease, and Hb SE disease. Coinheritance of Hb variants S and C is logically referred to as Hb SC disease and is present in approximately 0.13% of black Americans (21,43). Hb C is produced by an amino acid substitution of lysine for glutamate at the 6th position of the β-chain. Hb SC disease is a moderately severe sickling syndrome characterized by a moderate hemolytic anemia and vaso-occlusive crises, but to a much lesser degree than seen in sickle cell disease due to slower polymerization rates of Hb S and C compared to Hb S alone (10,78). On peripheral blood smear, some of the red blood cells will contain characteristic Hb C crystals, and many RBCs demonstrate targetoid morphology or a folded configuration (21). Coinheritance of Hb variants S and O-Arab causes Hb S-O-Arab disease, which is characterized by a greater proportion of Hb S compared to Hb O-Arab (79). Hb O-Arab is produced by an amino acid substitution of lysine for glutamate at position 121 on the β-chain. This particular Hb variant co-polymerizes with Hb S avidly, thus, disease manifestations are identical to sickle cell disease (80). Hb E contains a base substitution of lysine for glutamate at position 26 of the β-chain. Double heterozygosity of Hb S and E, or Hb SE disease, is infrequent due to differing ethnic distributions of these two variants (21). In general, the clinical phenotype is mild, characterized by a mild microcytic anemia with infrequent sickling complications (79,81,82). Hemoglobin studies demonstrate approximately 60% Hb S and 30% Hb E. Tables 5 summarizes the characteristic findings in sickling/hemolytic disorders.

Other Selected Hemoglobinopathies
Hemoglobin C
Hb S is the most prevalent variant hemoglobin; however, variant Hb C, Hb E, Hb O-Arab, Hb Hasharon, and Hb G-Philadelphia are common in certain populations. As mentioned earlier, Hb C is produced by the substitution of glutamate by lysine at the 6th position on the β-chain. This particular variant has its origins in Africa where focally up to 26% of the population are carriers (10). Approximately 2.4% of black Americans are heterozygous for Hb C, which has no clinical significance (43). Electrophoresis will demonstrate 30-40% Hb C and the remainder is Hb A with a

slight increase in Hb A2 (10,21). The homozygous state causes Hb C disease, which is mildly symptomatic and characterized by mild to moderate hemolytic anemia and splenic enlargement (83,84). The blood smear of both heterozygous and homozygous individuals will contain target cells, however, to a greater extent in Hb C disease (10,21). Hb C disease also demonstrates spherocytes, microcytosis and occasional characteristic crystals within the RBCs. The crystals are formed by oxygenated Hb C; thus, unlike Hb S polymers, Hb C crystals dissipate upon deoxygenation in the micro-circulation and do not cause vaso-occlusive crises (85). On electrophoresis, Hb C will constitute the majority of hemoglobin, Hb A is absent, and Hb F is slightly increased (10,21).

Hemoglobin E

The hemoglobin variant Hb E originates in Southeast Asia, and it is estimated that 30 million Southeast Asians are heterozygous for the gene (10,21). Hb E contains a base substitution of lysine for glutamate at position 26 of the β-chain. Lysine at this position creates an erroneous splice site resulting in the formation of unstable mRNA and ultimately decreased β-chain synthesis. (86-88). The diminished β-chain synthesis and subsequent unbalanced α:β ratio manifest clinically similar to β-thalassemia minor, and both heterozygous and homozygous individuals are generally asymptomatic (88,89). The blood smear of individuals with Hb E demonstrates microcytic RBCs with targetoid morphology (10,88). Hb E trait individuals are not anemic; however, homozygosity, termed Hb E disease, does cause a mild anemia (21). In the heterozygous and homozygous states, Hb quantification analysis demonstrates 30-35% and 95% Hb E, respectively (21). Logically, the remaining Hb constituents are Hb A for Hb E trait and Hb F for Hb E disease. A Hb E level lower than 30% is likely to result from an associated α-thalassemia (21,88). An associated β-thalassemia would manifest as an increased Hb E level, ranging from 40-90%, the degree of which depends on the severity of the β-thalassemia (i.e. β° versus β+) (21). Additionally, the Hb A level is decreased, and the Hb F level is increased. The clinical phenotype of Hb E/β-thalassemia varies and symptomatology ranges from thalassemia intermedia to resembling thalassemia major, requiring chronic transfusion therapy (90).

Hemoglobin O-Arab, Hemoglobin Hasharon, Hemoglobin G-Philadelphia

Hb O-Arab is a substitution of lysine for glutamate at position 121 of the β-chain and appears to have originated with-

in the Greek Pomaks (91). It is now seen throughout the Mediterranean basin and the Middle East. Both heterozygotes and homozygotes are asymptomatic; however, homozygous individuals can have a mild hemolytic anemia (92). Hb Hasharon originates from the substitution of histidine for asparagine at position 47 of the α2-chain and is classified as an unstable Hb (1,93). It is found among Ashkenazi Jews and in Italian families (94). Heterozygous individuals can demonstrate a mild hemolytic anemia thought to be secondary to inherent instability and subsequent denaturation as well as an element of decreased overall production of the variant Hb (95). The RBC morphology is unremarkable and Hb Hasharon constitutes approximately 16-19% of the total Hb in electrophoretic studies (21,95). Hb G-Philadelphia is a substitution of asparagine for lysine at position 68 of the α-chain and causes no clinical or hematologic abnormalities (21). However, this common α-chain variant occurs in 1 in 5000 African Americans and, is usually associated with α-thalassemia, particularly the -α3.7 haplotype in this particular population (96,97). Hb G-Philadelphia will constitute from 20% to >95% of the total Hb, depending of the degree of associated α-thalassemia (21,98,99). Thus, cases of high Hb G-Philadelphia levels (>35% of total Hb) are caused by concommitent α-thalassemia trait and will manifest hematologically as such with microcytosis and hypochromia (21,100,101).

The Role of Associated Clinical Conditions

1. Iron Deficiency

An associated iron deficiency may complicate the clinical and/or laboratory findings in patients with Hb disorders. For example, in the context of β-thalassemia, it can cause thalassemic indices to become less apparent, and also result in a falsely low level of Hb A2. When an associated iron deficiency is suspected, a final interpretation of Hb studies should be deferred. It is recommended to repeat the studies upon replenishment of the iron stores.

2. Certain Physiologic Conditions and Malignancies

Elevated levels of Hb F may be seen in association with increased bone marrow regeneration secondary to a variety of physiologic processes, such as pregnancy and significant blood loss. Additionally, increased synthesis of Hb F has been described in the context of hematologic malignancies, particularly myeloid neoplasias. The most striking example is juvenile myelomonocytic leukemia, where the Hb F level may reach 60% or higher and increased Hb F is included as a diagnostic criterion (102).

Interpreting and Reporting Results

Abnormal findings on Hb studies include a variant Hb, an abnormal Hb level for age, or a combination of both. Interpretations should be made within the clinical context of age, ethnicity, medical and family histories as well as other pertinent laboratory data. The latter ideally includes a concurrent CBC, examination of a stained peripheral blood smear and a sickle screen test. The interpretive report should list the Hb variants present, the percentage of each variant, and the morphologic findings on the peripheral blood smear, in addition to the overall interpretation and a general statement on the usual clinical impact. As needed, the report should also include recommendations for repeat and/or additional testing, which is common in the pediatric setting where family studies or repeat evaluation at a certain age are often necessary.

The Clinical Impact

Comments on the predicted clinical course and future recommendations should be included in the interpretative reports of Hb studies, especially in the context of newborn screening and in the pediatric population. Caution against the use of dogmatic and blank statements in this setting is advised. This is not only due to the fact that the final phenotype may not manifest until well into the second year of life, but also because the same phenotype can be caused by varying underlying genotypes. For example, a recent review counted more than 200 molecularly different mutations that all result in β-thalassemia (103). On the other hand, the molecular heterogeneity associated with Hb disorders results in a spectrum of clinical presentations for each disease entity. Other factors affecting disease manifestation include the co-inheritance of additional hemoglobinopathies and thalassemic traits as well as the associated nutritional or medical status of the individual. Table 6 depicts a general summary of the usual clinical impacts of the common hemoglobinopathies and thalassemia combinations.

Acknowledgments

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