An Overview of Hemoglobinopathies and the Interpretation of Newborn Screening Results

Shanmuganathan Chandrakasan, MD; and Deepak Kamat, MD, PhD, FAAP

Sickle cell disease (SCD), thalassemias, and other hemoglobinopathies are major health problems in the United States. It is estimated that there are approximately 100,000 patients with SCD in the United States,[1] with approximately 2,000 babies born with SCD every year.[2] Historically, infection from capsulated organisms caused high mortality and morbidly in children with SCD. The introduction of penicillin prophylaxis in 1986 for infants with SCD has resulted in a dramatic decrease in infection-related mortality.[3] Based on this observation, universal screening for SCD was recommended by the National Institutes of Health in 1987.[4] Currently, all 50 states and the District of Columbia perform newborn screening (NBS) for SCD. Although the initial focus of NBS was to identify newborns with SCD, identification of beta (β) and alpha (α) thalassemia has gained importance in recent years. With an increase in Asian immigration to the United States, hemoglobin disorders common in Asian populations, such as α thalassemia (hemoglobin H [HbH] disease and HbH constant spring [HbHCS]), and defects in beta globin, such as β thalassemia, hemoglobin E (HbE), and HbEβ thalassemia, are being seen more frequently.[5]

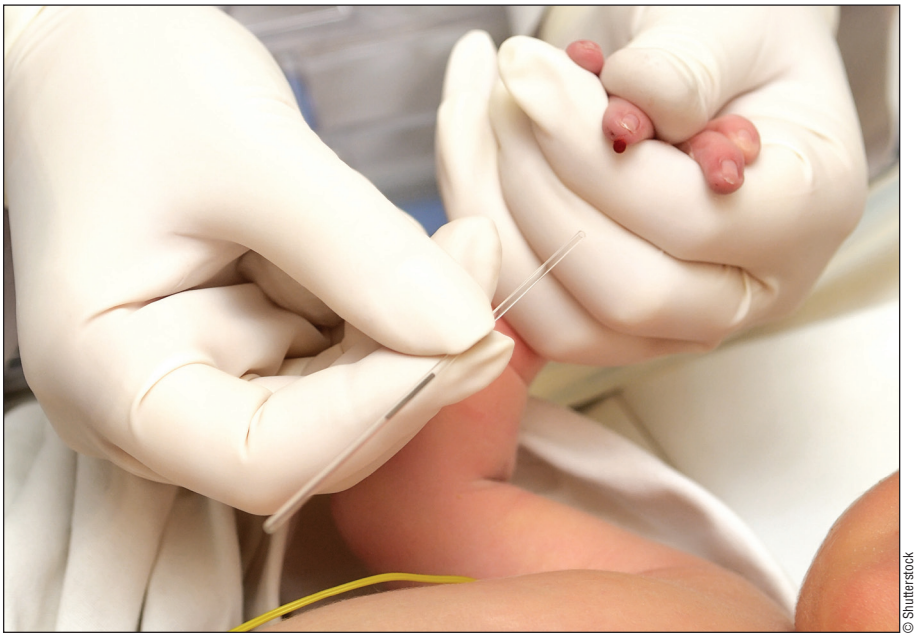
Despite the availability of universal NBS, significant delay in follow-up and initiation of care has been reported.[6] In the United States, the NBS program is decentralized, and guidelines on follow-up after a positive NBS vary across states. Some states have a well-developed support staff, whereas others rely on the primary care provider (PCP) for confirmatory testing, education, and referral to a specialist. In a survey of all NBS programs in the US,[7] it was noted that positive SCD screen results were communicated to 100% of PCPs, 81% of hematologists, and 73% of the hospitals submitting the samples. Additionally, in most states, the PCP is the only person informed about a positive screen for a sickle cell trait and α thalassemia

Shanmuganathan Chandrakasan, MD is Clinical Fellow, Divisions of Hematology, Oncology, and Bone Marrow Transplantation, Cincinnati Children's Hospital. Deepak Kamat MD, PhD, FAAP, is Professor of Pediatrics and Vice Chair of Education, The Carman and Ann Adams Department of Pediatrics, Wayne State University, Designated Institutional Official, Children's Hospital of Michigan.

Address correspondence to: Deepak Kamat MD, PhD, FAAP, The Carman and Ann Adams Department of Pediatrics, Wayne State University, Children's Hospital of Michigan, 3901 Beaubien Boulevard, Detroit, MI 48201; email: dkamat@med.wayne.edu.

Disclosure: The authors have no relevant financial relationships to disclose.

doi: 10.3928/00904481-20131122-11



trait.[7] Thus, a PCP might be the first to contact the family; explain and educate the family on the positive results; and arrange genetic counseling, confirmatory testing, and referral to a hematologist. Although this role is important, up to 10% of pediatricians and one-third of family physicians do not feel competent discussing NBS test results.[8] Hence, knowledge not only of interpretation of NBS, but also basic knowledge of different hemoglobinopathies/thalassemias is essential for providing the patients with better quality of care.

This article gives a basic overview of different hemoglobinopathies/thalassemias, provides clues for identification of abnormal NBS patterns characteristic of different hemoglobinopathies/thalassemias, and discusses indications for follow-up evaluation as well as referral to hematologist and genetic counselor.

TABLE 1.

Normal and Variant Hemoglobin at Birth and in Older Children

Hemoglobin	Structure	Levels at Birth	Levels in Adults	Comments
A	$\alpha_2\beta_2$	20%-25%	97%	Reaches adult levels by 1 year of age
A_2	$\alpha_2\delta_2$	0.5%	2.5%	Elevated in β thalassemia trait
F	$\alpha_2\gamma_2$	75%-80%	< 1%	Reaches adult levels by 1 year of age
HbH	β_4	15%-20% in HbH disease	NA	HbH produces Heinz bodies in the erythrocytes and hemolysis
Hb Bart	γ_4	100% in hydrops fetalis, 15%-25% in HbH disease	NA	Increased in carriers of α thalassemia trait at birth

NA = not applicable.

NORMAL HEMOGLOBIN DEVELOPMENT AND HEMOGLOBIN SWITCH

In order to better understand and interpret NBS results, an understanding of normal hemoglobin development and switch from fetal hemoglobin to adult hemoglobin is essential. Normal adult hemoglobin (HbA) is a tetramer of two α chains and two β chains ($\alpha_2\beta_2$). In humans, there are four α genes on chromosome 16 and two β loci on chromosome 11. During embryonic and fetal development, timed hemoglobin switch from embryonic to fetal and later to adult hemoglobin occurs. In the embryonic stage, ζ- and ε-globin chains are expressed before the γ globin and α globin chains. Between 4 and 14 weeks, three distinct Hbs are produced; ζ_2, ε_2 (Hb Gower-1), $\zeta_2\gamma_2$ (Hb Portland), and $\alpha_2\varepsilon_2$ (Hb Gower-2).[9] After development of the placenta, embryonic hemoglobins are replaced by hemoglobin F (HbF) ($\alpha_2\gamma_2$).[9] At birth, fetal hemoglobin is the predominant hemoglobin (**Table 1**). Depending on the gestation age at birth, its value can range from about 70% in a term newborn to 95% in an extreme preterm newborn. The level of HbF decreases progressively during the first year of life, with a corresponding increase in adult hemoglobin (HbA). The normal adult level is reached by age 1 year. Hemoglobin A2 (HbA_2), a combination of α chain with δ chains ($\alpha_2\delta_2$), is present both in the neonatal period and during the rest of an individual's life. A schematic illustration of hemoglobin switch is shown in **Figure 1**.

INTRODUCTION TO HEMOGLOBINOPATHIES

Sickle Cell Disease

SCD is a common hemoglobinopathy in the US. It is estimated that SCD occurs among 1 in every 500 African-American and 1 in every 36,000 Hispanic-American births.[10] Increased incidence of SCD is also noted in individuals of Mediterranean, Middle Eastern, Indian, Caribbean, and South and Central American ancestry. The sickle hemoglobin results from a point mutation (A-T) in the sixth codon of the β globin gene, which leads to amino acid substitution from glutamine to valine. Clinically, SCD is characterized by varying degrees of anemia, vaso-occulsive pain crisis, acute chest syndrome, stroke, splenic sequestration, priapism, and multiple end-organ damage. In infants, increased susceptibility to pneumococcal infection, dactilitis, and splenic sequestration are common.[9,11] The clinical severity of SCD depends on the genotype of SCD and the associated disease modifiers (**Table 2**). HbSS and $HbS\beta^0$ thalassemia are more severe than $HbS\beta^+$ and HbSC disease.[9,11] Coinheritance of defects in the α chain (found in approximately 35% of patients with SCD) results in decreased HbS levels, which leads to decreased disease severity. Early identification by NBS and initiation of penicillin prophylaxis, pneumococcal vaccination, parental education, and comprehensive care has dramatically decreased the early mortality in SCD.

Sickle Cell Trait

Individuals with sickle cell trait (SCT) are generally asymptomatic and are expected to have normal life ex-

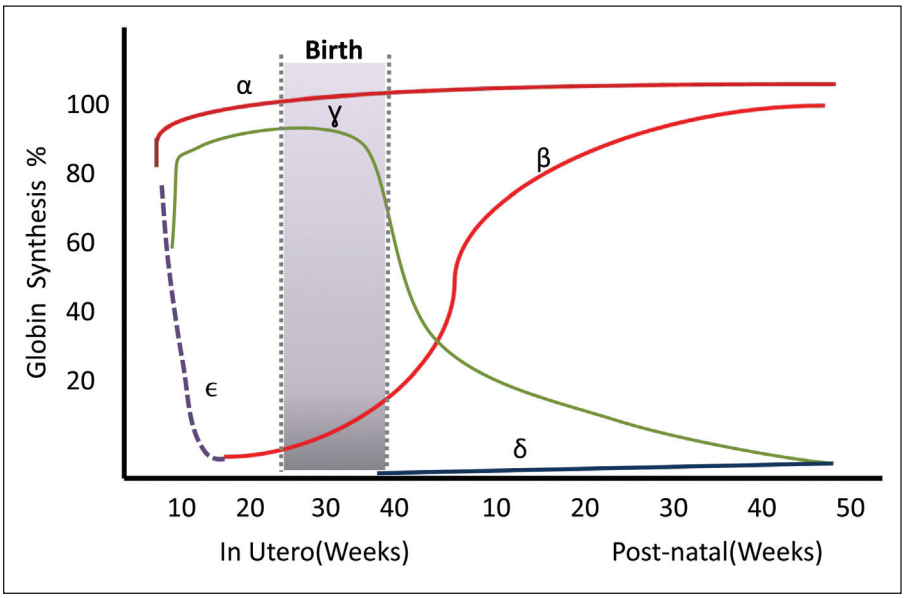


Figure courtesy of Deepak Kamat, MD, PhD, FAAP.

Figure 1. Globin switch during in utero and post-natal life.

pectancy.[9,11] However, certain clinical abnormalities have been identified in patients with SCT. Urine concentrating defect due to microscopic infarction of the renal medulla is one of the most common abnormalities found in SCT.[11] Hematuria from papillary necrosis of the kidney is also associated with SCT.[12] SCT was initially thought to be associated with a high incidence of sudden death on extreme physical exertion,[13] but follow-up studies showed that adequate hydration and training eliminates this risk.[14] Hence, SCT is not a contraindication to participate in competitive sports. Under certain extreme conditions of hypoxia, such as severe pneumonia and exercise at high altitudes, sickling may occur in individuals with SCT. These risk factors need to be highlighted at the time of counseling.

Non-Sickle Hemoglobin Abnormalities

The prevalence of non-sickle cell hemoglobin abnormalities (**Table 3**), such as α and β thalassemias, is being increasingly recognized in the U.S.[5] With the increase in Asian immigration, especially from Southeast Asia and China, the prevalence of HbH disease and HbEβthalassemia has increased compared with classic β thalassemia in parts of the U.S.[5]

α Thalassemia syndrome

In the U.S., α thalassemia is commonly seen in infants of Southeast Asian and southern Chinese descent.[5] In humans, there are four α genes. Loss of one α gene (++/+-) results in a silent carrier state with no anemia or microcytosis. Loss of two α genes (*Cis* --/++ or *Trans* -+/-+) results in the α thalassemia trait characterized by mild anemia and microcytosis. Loss of three genes (--/-+) results in HbH disease, characterized by elevated HbH (β4), leading to hemolytic anemia of varying severity and transfusion requirement. Loss of all four α genes results in Hb Barts (γ4), which usually results in hydrops fetalis, leading to in utero death in most cases. HbHCS is caused by point mutation in the stop codon of the α chain mRNA, leading to a markedly long (α^{CS}) chain and a decreased α chain synthesis. When α^{CS} occurs in the setting of cis α-chain defect, it behaves as HbH disease.[15] As illustrated in **Table 3**, clinically significant α thalassemia defects are common in newborns of Southeast Asian and southern Chinese descent. Although African Americans also have high asymptomatic carrier frequency of α chain defects, they very rarely develop HbH disease. This is because most African Americans carry the α chain defect in the *trans* state (-+/-+) compared with the *cis* α-chain defect (--/++) seen in Southeast Asian and southern Chinese populations.

β Thalassemia

β thalassemia is commonly reported in newborns of Asian Indian, Middle Eastern, and Southeast Asian descent. β thalassemia results from impaired production of β globin; the mutation in β globin could either result in total absence of β globin (β^0) or decreased β globin synthesis (β^+) resulting in some HbA formation. The clinical spectrum can vary from transfusion-independent β thalassemia intermedia phenotype to transfusion-dependent β thalassemia major phenotype. Individuals with the β thalassemia trait are asymptomatic, with mild microcytic hypochromic anemia, target cells, and characteristic HbA_2 elevation on electrophoresis.

Hemoglobin E disease

HbE disease is a common hemoglobinopathy seen in southern Chinese and Southeast Asian populations. It results from single amino acid replacement at 26th amino acid of the β chain (glutamic acid to lysine). Both heterozygous and homozygous individuals with HbE have mild hypochromic microcytic anemia. Combination of HbE and β thalassemia defects leads to HbE β thalassemia; it is seen exclusively in people of Southeast Asian descent. Clinical features of HbE β thalassemia resemble those of thalassemia intermedia.

Hemoglobin C disease

Hemoglobin C (HbC) disease is commonly seen in people of African descent. It is the second most common hemoglobinopathy in the U.S. It results from single amino acid substitution at the sixth

TABLE 2.

Sickle Cell Diseases and Their Salient Features

Hemoglobinopathy	Estimated % of US Patients	NBS Results	Pattern at Age 2 Months	Severity	Hematological Studies at 2 years		
					MCV	HbA_2	HbF
SCD-SS	65	FS	FS	Severe	Normal or increased	< 3.6	< 25
SCD-SC	25	FSC	FSC	Mild	Normal or decreased	NA	< 15
SCD-S β^+ thalassemia	8	FSA	FSA	Mild	Normal or decreased	> 3.6	< 25
SCD-S β^0 thalassemia	2	FS	FS	Severe	Decreased	> 3.6	< 25
S-HPFH	< 1	FS	FS	No anemia	Decreased	< 2.5	< 25

MCV = mean corpuscular volume; N = normal; NBS = newborn screening; SCD = sickle cell disease.

amino acid of the β chain (glutamic acid to lysine). HbC carriers are largely asymptomatic; however, individuals homozygous for HbC have mild anemia. Infants with HbSC disease have milder anemia and less pain than infants with HbSS disease. Infants with HbCβ thalassemia are usually symptomatic, with mild to moderate hemolytic anemia.

BASICS OF NEWBORN SCREENING FOR HEMOGLOBINOPATHY

Isoelectric focusing (IEF) and high-performance liquid chromatography (HPLC) are the two common techniques used in the U.S. for detecting abnormal hemoglobinopathies. These methods have an excellent sensitivity and specificity for detecting abnormal hemoglobins.[16,17]

Generally, hemoglobin identified by neonatal screening is reported as a percentage. At birth, HbF is the more predominant than HbA. Hence, most normal infants show HbFA pattern. Even in most of the patients with hemoglobinopathies, HbF is still the predominant hemoglobin at birth.

On NBS, hemoglobin concentration is reported in decreasing order. For example, a report of "FAS" on NBS indicates that in the given sample the quantity of HbF is greater than that of HbA, and the quantity of HbA is greater than that of HbS.

Prior to interpretation of NBS, the following facts should be kept in mind.

1. Prematurity: The only hemoglobin that can be identified on NBS is HbF, because premature infants might still not have begun the hemoglobin switch from HbF to HbA. Therefore, hemoglobinopathies affecting HbA, such as SCD, HbC, and HbE, could potentially be missed. Hence, infants without HbA on their NBS should have a repeat NBS so that SCD and other hemoglobinopathies

TABLE 3.

Common Non-Sickle Hemoglobinopathies in the United States

α Thalassemia Syndromes	Defect	Ethnicity	Severity
HbH	--/α -	Southeast Asian, southern Chinese	Variable, some transfusion dependent
HbH Constant Spring	--/$\alpha^{cs}\alpha$	Thai/Laotian	Variable, similar to HbH disease
HbH with HbEE	--/α- + $\beta^E\beta^E$	Southeast Asian	Mild phenotype
β Thalassemia syndrome			
β thalassemia	$\beta^0\beta^0$ or $\beta^+\beta^+$	Asian Indian, Southeast Asian	Thalassemia major with $\beta^0\beta^0$, intermedia with $\beta^+\beta^+$
HbE/β^0	$\beta^E\beta^0$	Southeast Asian	Thalassemia intermedia phenotype
Other hemoglobinopathy			
HbCC	$\beta^C\beta^C$	African origin	Asymptomatic
HbEE	$\beta^E\beta^E$	Southeast Asian, southern Chinese	Mild anemia

are not missed. However, one should strongly consider β thalassemia major if persistence of only HbF is identified on repeat testing.

2. Transfused: Packed red cell transfusion or exchange transfusion in neonates makes NBS results uninterpretable because of presence of transfused red blood cells; therefore, repeat testing should be done in 3 months.

3. Elevated Hb Bart's in NBS screen suggests an underlying defect in α chain.

4. Although NBS can help identify β thalassemia major, it is not a good screen for β thalassemia minor and β thalassemia intermedia. However, a follow-up screening and hematologic parameters such as mild anemia, microcytosis, and narrow red cell distribution width in high-risk groups could help identify this condition.

5. The most common abnormality detected in NBS is a carrier state for one of the hemoglobinopathies. In carrier states, the quantity (percentage) of normal adult hemoglobin is more than that of the abnormal variant, thus giving an "FAX" pattern, with X being the abnormal hemoglobin variant. For example, in SCT the NBS pattern would be FAS. Similarly in carriers of HbC and HbE, the patterns would be FAC and FAE, respectively.

TABLE 4.

Patterns of Hemoglobins on Newborn Screening and Their Interpretation

NBS Pattern	Interpretation	Recommendation
FA	Normal	None
F only	Premature infant β thalassemia major	Repeat testing, if persistent only F, needs education, genetic counseling, confirmatory testing and hematology referral
AF	Likely post-blood transfusion	Repeat testing 3-4 months after last transfusion
FS	SCD (HbSS) Sickle β thalassemia Sickle HPFH	Education, genetic counseling, confirmatory testing, and hematology referral
FSA	Sickle β thalassemia SCD with transfusion	Education, genetic counseling, confirmatory testing, and hematology referral. Repeat testing in 3-4 months if transfused.
FAS	Sickle cell trait Sickle β thalassemia	Education and genetic counseling. Repeat testing at 3 months of age – rule out sickle β thalassemia
FSC	Hemoglobin SC disease	Education, genetic counseling, confirmatory testing, and hematology referral
FC	Hemoglobin C disease Hemoglobin C β thalassemia	Education, genetic counseling, confirmatory testing, and hematology referral
FE	Hemoglobin E disease Hemoglobin E β thalassemia	Education, genetic counseling, confirmatory testing, and hematology referral
FA + variant (FAE, FAC, FAD)	Hemoglobin variant trait	Education and genetic counseling
FA Bart's	Silent α thalassemia carrier α thalassemia trait HbH disease HbH Constant Spring disease	If Bart's < 10%, patient will need education and genetic counseling If Bart's > 10%, patient will need further testing for evaluation of HbH disease and hematology referral

SCD = sickle cell disease.

ABNORMAL NBS PATTERNS

In most infants, FS pattern is due to clinically severe SCD (HbSS or HbSβ^0 thalassemia). Other possibilities include less severe phenotypes such as sickle δβ-thalassemia, and sickle hereditary persistence of fetal hemoglobin.[11] Not infrequently, newborns with HbSβ$^+$ thalassemia can also have an FS pattern at birth (**Table 4**). However, a higher hemoglobin concentration and FSA pattern on re-testing at age 6 months could help in differentiating this from other severe phenotypes of SCD.[11] Identification of HbS with another hemoglobin variant such as HbC is not uncommon, and typically, the quantity of HbS is greater than the variant hemoglobin (eg, FSC in HbSC disease). The clinical and hematological characteristics of different sickle cell disease syndromes are highlighted in **Table 2**. The coinheritance of α-thalassemia is common in SCD. In fact, approximately 30% of African Americans have one α gene defect, and approximately 2% have two α gene defects.[9] A characteristic FS pattern with elevated Hb Bart's can be seen in these newborns. Infants with positive NBS tests for SCD, confirmatory testing, and referral to a hematologist should be done by age 2 months so that parental education, prophylactic penicillin, and comprehensive care can be promptly implemented.

Continued on page 508

Continued from page 506

Infants with hemoglobin FE pattern could either have homozygous HbE (HbEE) disease, which is usually asymptomatic with mild microcytic anemia, or have HbEβ^0 thalassemia, which could lead to severe anemia. Further work-up is required, including hematologic evaluation, family studies, and genetic analysis. Similarly, infants with an FC pattern could either have homozygous HbC (HbCC), or have HbCβ^0 thalassemia. However, both of these conditions are associated with only mild anemia.

Hb Bart's is elevated in newborns with α chain defects. The quantity of Hb Bart's is usually 1% to 2% in silent carriers, 3% to 10% in α thalassemia trait carriers, 25% to 35% in HbH disease and HbHCS disease patients, and 80% to 100% in Hb Bart's disease patients. Identification of more than 10% of Hb Bart's in a newborn warrants further work-up and referral to a hematologist to rule out HbH and HbHCS disease. Identification of carriers of α thalassemia is important for genetic counseling to prevent HbH and Hb Bart's diseases.

Once an abnormal hemoglobinopathy screen result is reported to the PCP, the family should be informed and an appointment for follow-up should be made as soon as possible. Getting a baseline complete blood count with evaluation of peripheral smear and reticulocyte count could help in assessment of anemia and give valuable supportive information. On physical examination, attention should be given to presence or severity of pallor, jaundice, and hepatosplenomegaly. A repeat hemoglobin electrophoresis/HPLC testing to confirm the suspected hemoglobinopathy should be sent. After providing basic clinical information, referral to a hematologist and genetic counseling should be done. In certain situations, screening of family members (ie, complete blood count and hemoglobin electrophoresis) may provide rapid and valuable clinical information. When a carrier state is identified, education and genetic counseling should be offered to the family. The affected person should be informed of their carrier status in their adult life so that they are aware of potential risk of having an affected baby.

CONCLUSION

Early initiation of supportive care for infants with hemoglobinopathies such as sickle cell disease and thalassemia have been shown to decrease mortality and morbidity. Hence, accurate interpretation of newborn screening test for hemoglobinopathies/thalassemias is very important. This can be achieved by a basic understanding of hemoglobin structure and systematic approach in interpretation of results of newborn screening tests. Once hemoglobinopathy/thalassemia is suspected, patients should be referred to hematologists as appropriate. ■

REFERENCES

1. Hassell KL. Population estimates of sickle cell disease in the U.S. *Am J Prev Med*. 2010;38(suppl 4):S512-S521.
2. Council of Regional Networks for Genetic Services. *National Newborn Screening Report-1994*. Atlanta, GA: The Council; 1999.
3. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. *N Engl J Med*. 1986;314(25):1593-1599.
4. Consensus Conference. Newborn screening for sickle cell disease and other hemoglobinopathies. *JAMA*. 1987;258(9):1205-1209.
5. Michlitsch J, Azimi M, Hoppe C, et al. Newborn screening for hemoglobinopathies in California. *Pediatr Blood Cancer*. 2009;52(4):486-490.
6. Listernick R, Frisone L, Silverman BL. Delayed diagnosis of infants with abnormal neonatal screens. *JAMA*. 1992;267(8):1095-109.
7. Kavanagh PL, Wang CJ, Therrell BL, Sprinz PG, Bauchner H. Communication of positive newborn screening results for sickle cell disease and sickle cell trait: variation across states. *Am J Med Genet C Semin Med Genet*. 2008;148C(1):15-22.
8. Kemper AR, Uren RL, Moseley KL, Clark SJ. Primary care physicians' attitudes regarding follow-up care for children with positive newborn screening results. *Pediatrics*. 2006;118(5):1836-1841.
9. Orkin SH, Nathan DG. *Nathan and Oski's Hematology of Infancy and Childhood*. 7th ed. Philadelphia: Saunders/Elsevier; 2009.
10. Centers for Disease Control and Prevention. Sickle cell disease. Data and Statistics. Available at: http://www.cdc.gov/ncbddd/sicklecell/data.html. Accessed Nov. 11, 2013.
11. *The Management of Sickle Cell Disease*. National Institutes of Health. Bethesda, MD: National Heart, Lung, and Blood Institute. Division of Blood Disease and Resources; 2002.
12. Ataga KI, Orringer EP. Renal abnormalities in sickle cell disease. *Am J Hematol*. 2000;63(4):205-211.
13. Kark JA, Posey DM, Schumacher HR, Ruehle CJ. Sickle-cell trait as a risk factor for sudden death in physical training. *N Engl J Med*. 1987;317(13):781-787.
14. Pearson HA. Sickle cell trait and competitive athletics: is there a risk? *Pediatrics*. 1989;83(4):613-614.
15. Lal A, Goldrich ML, Haines DA, Azimi M, Singer ST, Vichinsky EP. Heterogeneity of hemoglobin H disease in childhood. *N Engl J Med*. 2011;364(8):710-718.
16. Galacteros F, Kleman K, Caburi-Martin J, Beuzard Y, Rosa J, Lubin B. Cord blood screening for hemoglobin abnormalities by thin layer isoelectric focusing. *Blood*. 1980;5696):1068-1071.
17. van der Dijs FP, van den Berg GA, Schermer JG, Muskiet FD, Landman H, Muskiet FA. Screening cord blood for hemoglobinopathies and thalassemia by HPLC. *Clin Chem*. 1992;38(9):1864-1869.