Screening Newborns for Hemoglobinopathies—Enduring Challenge

In 1987, the National Institutes of Health published the results of a Consensus Developmental Conference on screening newborns for hemoglobinopathies; in this summary [1] they recommended universal screening of neonates for evidence of abnormal hemoglobins, regardless of ethnic background. The major impetus for this recommendation was the reported mortality rate of 25–30% in children younger than 5 years who have sickle cell anemia [2]. The majority of deaths in this group occur secondary to fatal bacterial infections, acute splenic sequestration, or aplastic crises [2–6]. Functional hypo- or asplenism in this population often occurs within the first few months postpartum and results from splenic sequestration of irreversibly sickled erythrocytes with subsequent splenic infarction. Because the ability to produce antibodies specific to encapsulated organisms such as Streptococcus pneumoniae or Hemophilus influenzae is limited in the first 2 to 3 years of life, a lack of splenic function often leads to overwhelming bacterial sepsis and rapid demise in children so infected. Aplastic crises may be devastating in individuals with sickle cell anemia because of the combined effects of shortened erythrocyte survival in the periphery and lack of compensatory erythrocyte production in bone marrow during the aplastic phase. Severe anemia and congestive heart failure may ensue. Acute splenic sequestration—which may lead to peripheral circulatory collapse, shock, and rapid demise—is one of the most common causes of death in children under age 2 years with sickle cell anemia.

Prolactin antibiotic therapy can significantly decrease the morbidity and mortality associated with infection with encapsulated bacteria in young children with sickle cell anemia [7]. Vaccination for S. pneumoniae in asplenic children has also been thought to contribute to improved survival. The NIH Consensus Panel recommended institution of penicillin prophylaxis at age 3 months in this population; continued treatment with this antibiotic was recommended until age 3–4 years. In addition to penicillin prophylaxis, neonatal detection of sickle cell anemia, coupled with parental education regarding risks and potential complications of this disorder, was believed to promote early and aggressive intervention for a variety of hematologic and infectious problems unique to this patient population [8, 9]. The documented utility of this type of interventional program was felt to justify the expense of broad-based neonatal screening programs.

Differences of opinion regarding universal vs directed screening of newborns for hemoglobinopathy center principally around the issues of cost and practicality [10, 11]. Although the technical costs of directed screening are significantly less, the time cost of obtaining accurate historical and ethnic information, together with the documented fact that targeted screening programs miss a substantial number of infants with hemoglobinopathies, mitigates the advantages of this type of approach [12, 13]. Although debate continues regarding these assumptions, most states have mandated universal screening programs for detection of hemoglobinopathies in addition to other inborn errors of metabolism. A rapid, accurate, and efficient method of identifying hemoglobinopathies would make universal screening programs more cost effective and acceptable to the public in an environment where cost containment for healthcare-related issues makes programs such as this open to scrutiny and at potential risk for continued financial support.

Cord-blood specimens obtained at delivery represent one possible source of blood sample for hemoglobinopathy screening. Alternatively, capillary blood obtained by heelstick and dried on filter paper is readily obtainable and is already used by the majority of large-scale screening programs in this country.

Because of relative simplicity and ease of quantification, many hematology laboratories use electrophoresis on cellulose acetate gels at alkaline pH in conjunction with citrate agar gel electrophoresis at acid pH. A disadvantage of this approach is the necessity of using two different types of gels, which is cumbersome and time-consuming for large-scale hemoglobinopathy screening [1]. Moreover, this method is subject to potential loss of fast hemoglobins when the electrophoresis is poorly monitored and these hemoglobins are run off the gel.

An alternative method of hemoglobin determination used in some newborn screening programs is isoelectric focusing. This method allows unequivocal identification of a large number of both common and uncommon hemoglobin variants, which may be more difficult to accurately identify on combined cellulose acetate/citrate agar gels [14]. A major advantage of isoelectric focusing is the ability to separate hemoglobin A₂ (Hb A₂) from both Hb C and Hb E, thus allowing discrimination of the homozygous condition for either of these hemoglobins from double heterozygosity for the hemoglobin variant and β thalassemia. Disadvantages include high cost of equipment, relatively long electrophoretic time, and difficulty with quantification of hemoglobins. Isoelectric focusing is the method currently used by the Minnesota Department of Health in its newborn hemoglobinopathy screening program. Although neither normal nor variant hemoglobin concentrations are quantified in this program, Hb Barts is reported as either “low” or “high” based on visual inspection of the gels.

Automated HPLC represents another method for rapid screening of dried-blood specimens for evidence of hemoglobinopathies. Cation-exchange HPLC allows for unambiguous separation of common structural hemoglobin variants as well as separation of fast-migrating hemoglobin such as Hb Barts from Hb F [15, 16]. Small amounts of normal and variant hemoglobins can be accurately quantified with this method. In addition, use of an algorithm that compares the amount of an abnormal hemoglobin with the summed amounts of Hb A and the abnormal hemoglobin allows for discrimination of double heterozygosity for β⁺ thalassemia and the normal hemoglobin from heterozygosity for the abnormal hemoglobin. This is important in screening programs because double heterozygosity for Hb S and β thalassemia is a sickling disorder, the severity of which may approach that of homozygous Hb S, whereas Hb S trait is clinically benign. The combined advantages of rapid turnaround time, superior separation of small quantities of normal or abnormal hemoglobins from the large amount of Hb F present in newborns’ blood, and the ability to quantify these hemoglobins make automated cation-exchange HPLC an eminently suitable method for screening neonatal blood samples.

The report by Eastman et al. [17] in this issue describes a commercially available automated HPLC system in which software-based algorithms are utilized to derive presumptive hemoglobin phenotypes on the basis of quantitative determinations of the hemoglobin species present in routinely obtained dried-blood samples from newborns. Two shortfalls the authors
identify in the system are lack of barcode identification and lack of automated pipetting of sample into microplate wells. Although Eastman et al. note that they can use this system to identify and quantify Hb Barts, they point out that their laboratory does not report this hemoglobin because the California newborn hemoglobinopathy program does not mandate screening for it. Perhaps this decision will change in the future, given that identification and quantification of this hemoglobin are straightforward with the described methodology and the information may be important to patient management and counseling in a subset of individuals at risk for a potentially serious hematologic disorder.

The incidence of α thalassemia in black and Southeast Asian populations approaches 30%, making this one of the most commonly encountered abnormalities in any hematology laboratory that routinely cares for populations with these ethnic backgrounds [18]. The most frequent cause of α thalassemia is deletion of one or more α globin genes [19]. This relative lack of α chains results in formation of Hb Barts, a γ chain tetramer in newborns with α thalassemia. Reports in the literature [20, 21] suggest that many but not all individuals with α-thalassemia exhibit Hb Barts at birth in amounts that correspond roughly with the number of deleted α globin genes. Correlation of complete blood count (CBC) data with the presence of Hb Barts on newborn screening in populations with a high incidence of α thalassemia has been extremely helpful in defining the spectrum of presentation of this heterogeneous disorder. Although Hb H disease (deletion of three α globin genes) and hydrops fetalis (deletion of four α globin genes) are unusual in black populations, they occur with significant frequency in Southeast Asian populations and represent severe manifestations of α globin chain deletion. Hb H disease is characterized by an unusually large amount of Hb Barts at birth, which could easily be quantified by the method described by Eastman et al. [17]. Severe anemia with hemoglobin of 40–50 g/L may occur in association with infection, iron deficiency, or pregnancy in individuals with Hb H disease [18]. Thus, although not yet mandated by legislation, failure to provide this information to families at risk together with a recommendation for follow-up genetic counseling appears to represent a lack of optimum use of data generated by the screening program.

One of the shortfalls of any type of methodology for screening blood from newborns is the inability to accurately assess this population for evidence of β thalassemia. In adults or in children beyond early infancy, the presence of specific changes in the red cell indices (erythrocytosis, microcytosis, normal hemoglobin, and mean cell hemoglobin concentration) suggests the possibility of thalassemia. Confirmatory hemoglobin electrophoresis may show an increase in the amount of Hb A2, which is diagnostic of β thalassemia. As noted by Eastman et al. [17], Hb A2 concentrations in newborns are so low that, for practical purposes, they are not interpretable for the diagnosis of β thalassemia in the newborn period. Heterozygous β thalassemia is associated with relatively little clinical morbidity, so that detection in the newborn period is usually not of critical importance. Of the entities associated with β thalassemia that are likely to be encountered in newborn screening programs, only double heterozygosity for β thalassemia and either Hb S or E may be associated with significant morbidity and (or) mortality.

One of the advantages of automated HPLC is the ability in many cases to differentiate homozygosity for Hb S from double heterozygosity for Hb S and β thalassemia. The form of β thalassemia most common in the black population results in only partial loss of β globin chain production from the affected gene (βâ′ thalassemia); therefore, differential diagnosis for double heterozygosity for Hb S/βâ′ thalassemia includes sickle cell trait. Accurate differentiation of these two entities is important. Sickle trait is clinically innocuous, whereas Hb S/βâ′ thalassemia is a sickling disorder that may approach that of sickle cell anemia in severity. Discrimination of homozygous Hb S (classic sickle cell anemia) from double heterozygosity for Hb S and βâ′ thalassemia in the newborn period is difficult but is less critical because both entities are severe sickling disorders that require early identification and intervention.

Because the retention times of Hb E and A2 are similar in cation HPLC [15–17], a potential disadvantage of this method is the inability to differentiate homozygous Hb E from Hb E/βâ′ thalassemia. Use of an algorithm comparing the amount of Hb A with the amount of Hb E to derive presumptive evidence for the presence of a β thalassemia is less useful in Southeast Asian populations, given that βâ′ thalassemia is much less common in this ethnic group than in the black population. A similar disadvantage is encountered in using combined cellulose acetate and citrate agar gels: Hb E and A2 comigrate on cellulose acetate gels, whereas both comigrate with Hb A on citrate agar gels. Differentiation of these entities is an important issue in patient care because homozygous Hb E is characterized by erythrocytosis and microcytosis without anemia and is clinically benign, whereas Hb E/βâ′ thalassemia is a severe hemolytic disorder with marked anemia and significant morbidity in most cases [22, 23]. Given the frequency of Hb E in some populations, the incidence of double heterozygosity for Hb E and βâ′ thalassemia is presumably greater than either homozygous Hb E or homozygous βâ′ thalassemia. Of the methods currently available, the most accurate means of differentiating homozygous Hb E from Hb E/βâ′ thalassemia is isoelectric focusing, which allows for separation of Hb E from Hb A2. Practically speaking, this type of determination requires reassessment of the CBC data and repeat hemoglobin electrophoresis after the newborn period. Appropriate follow-up of individuals with hemoglobinopathies identified in newborn screening programs, with institution of early aggressive intervention in children with sickling disorders, is the final critical pathway in legitimizing the societal cost of this type of screening program [11]. Review of data derived from newborn screening programs suggests that hemoglobinopathies represent the single most common congenital disorder detected in these programs [8]. Confirmatory testing should be evaluated in the context of CBC data with review of red cell morphology on a peripheral blood smear to optimize interpretation of the electrophoretic data. Those individuals with sickling disorders require early confirmation and intervention. For other types of abnormalities, confirmatory testing is optimal after about age 6 months, by which time Hb F will have declined to relatively low amounts while the Hb A2 in infants with β thalassemia will have increased to values that will usually allow reliable diagnosis of this disorder [24]. In children without significant anemia, confirmatory testing might be optimal at about age 2 years.

References


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