Original Article

A simple method of screening newborn infants for hereditary spherocytosis

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We sought to develop a simple screening test for hereditary spherocytosis (HS) among neonates using the erythrocyte indices. This concept was based on the fact that the mean corpuscular hemoglobin concentration (MCHC) in children with HS is generally elevated and on our observation that many neonates (but not older children) with HS have a low mean corpuscular volume (MCV). Erythrocyte indices of neonates with confirmed HS were compared with those of a control group without HS. Thirty-three newborns with HS had 1–22 complete blood counts (CBCs) during their first 90 days of life; 24 of these newborns had a parent with HS, while 9 had no family history of HS. The MCHC of the 33 newborns with HS averaged 36.2 (95% confidence interval [CI], 34.8–37.8), and the MCV averaged 89.9 (95% CI, 81.1–99.3). In the control group (n = 40,946), the MCHC averaged 34.0 (95% CI, 32.3–35.7) and the MCV averaged 106.7 (95% CI, 100.0–113.5). For each CBC, we calculated a "Neonatal HS Index" by dividing the MCHC by the MCV. The index was 0.41 ± 0.03 in those with HS and 0.32 ± 0.01 in the controls (P = 0.0000). An index of >0.36 had 97% sensitivity, >99% specificity, and a >99% negative predictive value for identifying HS. Thus, the Neonatal HS Index can be used for rapid and simple screening of newborn infants for hereditary spherocytosis.

KEY WORDS: neonate, infant, screening test, hereditary spherocytosis, erythrocyte indices

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Hereditary spherocytosis (HS) is the most common cause of hereditary hemolytic jaundice among neonates of Northern European ancestry, with a prevalence of 1 case per 2,000–4,000 births (1,2). HS is a heterogeneous condition, in which various mutations in the erythrocyte membrane proteins result in spherocytic erythrocytes (1-3). Neonates with HS frequently develop significant hyperbilirubinemia, which can result in kernicterus if complicated by infection, dehydration, or other hemolysis-associated conditions (4,5).

Sometimes, HS is suspected in a neonate because this disorder, which is principally inherited in an autosomal dominant fashion, was previously diagnosed in a parent. However, 25–30% of cases involve either *de novo* mutations or autosomal recessive mutations (6). In such cases, the diagnosis of HS can easily be missed during the neonatal period. Moreover, a parent with chronic anemia and jaundice may not have been diagnosed with HS; thus, a diagnosis of HS may not be suspected in the neonate (7).

We previously reported that among neonates with a serum bilirubin level of >20 mg/dL, an erythrocyte mean corpuscular hemoglobin concentration (MCHC) of >36.0 might be a useful screening test for HS (8). However, the statistical performance of the MCHC as a screening tool for HS during the neonatal period has not been reported thus far. The mean corpuscular volume (MCV) is generally normal in adults with HS (1,2,9), but in our experience, neonates with HS often have a low MCV. Since neonates with HS can have a high MCHC but a low MCV, we reasoned that a simple equation (calculated by dividing the MCHC by the MCV [MCHC ÷ MCV]) may be used for rapid and inexpensive screening of neonates for HS.

Methods

The study was approved by the institutional review board of Intermountain Healthcare as a retrospective de-identified analysis not requiring individual consent. This was a cross-sectional, multihospital, retrospective data analysis of individuals born between January 1,

2002 and August 31, 2010 using the Intermountain Healthcare databases. Intermountain Healthcare is a not-for-profit organization that owns and manages 21 hospitals with labor and delivery services. The study consisted of 2 parts. The first identified children with a diagnosis of HS confirmed at any time during childhood (arbitrarily taken as the first 8 years of life) for whom 1 or more MCHC and MCV values obtained in the first 90 days of life were present in our database. Erythrocyte indices were obtained using a Beckman Coulter Hematology Analyzer (Fullerton, CA, USA) at McKay-Dee Hospital, LDS Hospital, Intermountain Medical Center, LDS Hospital, Utah Valley Regional Medical Center, Logan Regional Hospital, American Fork Hospital, Alta View Hospital, Orem Community Hospital, Valley View Medical Center, Dixie Regional Medical Center, and Cassia Regional Medical Center as well as using a Sysmex Hematology Analyzer (Mundelein, IL, USA) at Primary Children's Medical Center. The MCHC was expressed as "g hemoglobin/dL red blood cells (RBC)" and MCV as "femtoliters (fL; 10–15L)." All erythrocyte indices of the patients with HS obtained during their first 90 days were recorded, but those obtained within 3 weeks after an RBC transfusion were excluded from the data analysis. Post-transfusion values were excluded due to the possible confounding effects of the transfused erythrocytes.

The second part of the study identified MCHC and MCV values of the reference population in their first 90 days after birth using the same hospital system and dates of birth during the same period of time as that for those with HS. For the reference population, only 1 complete blood count (CBC) value from each subject was included in the dataset. Moreover, only neonates delivered at 35–41 weeks (the gestational age at which the HS patients were born) who had no diagnosis of HS, no diagnosis of any type of anemia, and no RBC transfusions were included.

MCHC and MCV values of the HS and reference populations were compared using histogram analysis. Means, standard deviations, and 95% confidence interval (CI) ranges were used to express the values. We calculated a term named the "Neonatal HS Index" for those with and those without a diagnosis of HS by dividing the MCHC by the MCV.

Results

Characteristics of neonates with HS

One or more MCHC/MCV measurements of 33 children with a confirmed diagnosis of HS and who were born during the stipulated period were recorded in our databases during their first 90 days after birth. The average gestational age of these 33 patients at birth was 39 weeks (range, 35–41 weeks), while the average birth weight was 3,250 g (range, 1835–4660 g). Twenty-six of these children were diagnosed with HS during their first month, while the other 7 were diagnosed between 2 months and 6 years of age (Table 1).

A total of 24 of the 33 patients had a father or mother with HS. Each of these 24 patients was suspected of having HS during the first days following birth, when all were treated with phototherapy for hyperbilirubinemia. Nine of the 33 did not have a family history of HS (although the diagnosis of HS in 2 of these 9 patients, who were twins, subsequently led to a diagnosis of HS in their mother, as detailed in a previous report (6)). Similar to those with a positive family history of HS, these 9 with no family history were all treated with phototherapy for neonatal hyperbilirubinemia. The diagnosis of HS was suspected sooner when a family history was positive; the diagnosis was confirmed sooner in these children as well (Table 1).

A diagnosis of HS in the 9 patients with no family history was confirmed on the basis of persistent spherocytosis and polychromasia on the blood film as well as increased erythrocyte osmotic fragility and reticulocyte counts in the absence of other diagnoses to explain the chronic hemolytic anemia. Four of the 9 patients did not have G6PD and pyruvate kinase deficiencies. A diagnosis of HS in the 24 patients with documented HS in a parent was confirmed on the basis of persistent spherocytosis on the blood film and the absence of other diagnoses to explain the chronic hemolytic anemia. Seven of these patients had also been given an erythrocyte osmotic fragility test that showed increased fragility.

In total, 2 of the 33 patients with HS moved from the state of Utah within the first 2 months following birth; follow-up data were not available for these patients. Of the 31 patients for whom follow-up data were available, RBC transfusions were administered during the first 6 months to 23 patients: 12 patients received 1, 5 received 2, 4 received 3, and 2 received 4 transfusions.

MCHC and MCV measurements

In our database, each of the 33 children with HS had 1–22 CBC values, which were obtained during their first 90 days after birth. The 182 values that were obtained included the following: MCHC, 36.2 ± 0.1 (95% CI, 34.8–37.8) and MCV, 89.9 ± 6.7 (95% CI, 81.1–99.3).

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Table 1. Characteristics of the 33 neonates with hereditary spherocytosis who had 1 or more mean corpuscular hemoglobin concentration and mean corpuscular volume values in the Intermountain Healthcare databases during the first 3 months of their life

Year of Birth	Fam Hist	Race	RBC Osmotic Fragility	Sphero on the blood film	Age when HS was suspected	Age when HS was confirmed	PK	G6PD	Tx in first 6 mo
2002	F	W	-	Marked	Birth	1 mo	-	-	3
2002	No	H	Increased	Marked	1 y	7 y	21.7 (9–22)	15.3 (4.6–13.5)	1
2003	M	W	-	Marked	Birth	1 mo	-	-	1
2003	F	W	-	Marked	Birth	2 mo	-	-	1
2003	F	W	Increased	Marked	Birth	1 wk	-	-	1
2004	No	W	Increased	Marked	4 wk	4 wk	nl	Nl	1
2004	M	W	-	Marked	Birth	1 mo	-	-	1
2004	M	W	Increased	Marked	Birth	1 mo	-	-	1
2004	No	W	Increased	Marked	1 mo	1 mo	-	-	2
2004	M	W	Increased	Marked	Birth	8 mo	-	-	0
2005	M	W	-	Marked	2 mo	1 y	-	-	0
2005	No	W	-	Marked	1 y	2 y	-	22 (7–20.5)	0
2005	No	H	sl inc	Rare	6 mo	2 y	10.9 (9–22)	26.9 (7–20.5)	1
2005	M	W	-	Marked	4 d	1 mo	-	-	2
2005	M	W	-	sl inc	2 d	2 mo	-	-	1
2006	F	W	sl inc	Increased	Birth	1 mo	-	-	2
2007	M	W	-	-	Birth	-	-	-	Moved
2007	F	W	-	Increased	2 mo	4 mo	-	-	3
2008	F	W	-	Marked	Birth	14 mo	-	-	0
2008	F	W	Increased	Increased	3 wk	3 wk	-	-	Moved
2008	F	W	Increased	Increased	Birth	3 mo	-	-	0
2008	M	W	-	Marked	?	2 y	-	-	0
2008	F	W	Increased	Increased	Birth	1 mo	-	-	4
2008	F	W	-	Increased	Birth	15 mo	-	-	0
2008	No	W	sl inc	Occasion	2 mo	3 mo	-	-	0
2009	F	W	-	Marked	Birth	2 wk	-	-	1
2009	No	W	Increased	Increased	2 wk	4 wk	-	-	4
2009	F	W	-	-	Birth	2 mo	-	-	2
2009	F	W	-	sl inc	Birth	2 mo	-	-	1
2009	F	W	-	sl inc	Birth	3 mo	-	-	1
2010	M	W	-	Increased	Birth	1 mo	-	-	2
2010	No	H	Increased	Increased	1 wk	2 wk	-	-	3
2010	No	H	Increased	Increased	1 wk	2 wk	-	-	3

HS, hereditary spherocytosis; F, father; M, mother; W, white; H, Hispanic; sl inc, slightly increased; d, days; wk, weeks; mo, months; y, years; nl, reports as "normal"; PK pyruvate kinase; G6PD, glucose-6-phosphate dehydrogenase deficiency; Tx, RBC transfusion

During this time period, the MCHC and MCV values of 40,946 reference patients were recorded in their first 90 days. The MCHC values of the reference group were: 5th percentile, 32.7; mean, 34.0; and 95th percentile, 35.3. As seen in Figure 1, the MCHC values of the 33 neonates with HS were higher than that of the reference group, but with overlapping histograms. If an MCHC value > 36.0 was selected as a screening test for HS, the sensitivity of the screen would be 46%, specificity would be >99%, positive predictive value would be 21%, and negative predictive value would be >99%.

The MCV values of the reference group (Figure 2) had a 5th percentile of 100 fL, a mean of 107 fL, and a 95th percentile of 114 fL. As seen in Figure 1, the MCV values obtained from neonates with HS were lower than that of the reference group, but with overlapping histograms. Selecting a MCV value of <90 fL as a screening test for HS performed similarly to a MCHC value of >36.0 resulted in a sensitivity of 54%, specificity of >99%, positive predictive value of 57%, and a negative predictive value of >99%.

Histograms of the Neonatal HS Index from the reference and HS groups are shown in Figure 3. Less

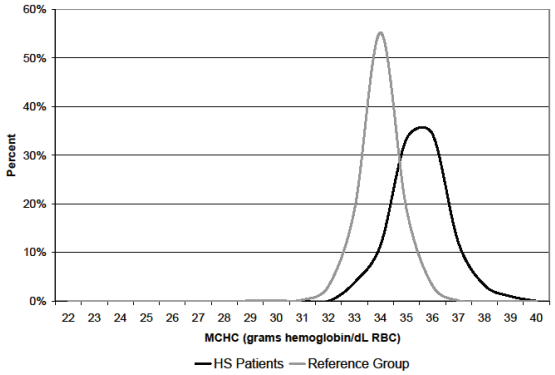

Figure 1. The histogram shows the distribution of erythrocyte mean corpuscular hemoglobin concentration (MCHC) values (g hemoglobin/dL red blood cells [RBC]) recorded during the first 90 days in 2 populations of neonates and young infants (reference group = grey line; HS = black line). The reference population (n = 40,946 individuals) included individuals born after 35–41 weeks' gestation, who had never received transfusions, and who had no diagnosis of anemia. Only the first value obtained was recorded (one per individual). The HS population (n = 33) included individuals who had a confirmed HS diagnosis by 8 years of age and had at least 1 CBC in the first 90 days of life in the Intermountain Healthcare database. All values of the patients with HS were recorded, and multiple values were included from individuals. Values obtained within 3 weeks following a RBC transfusion were excluded.

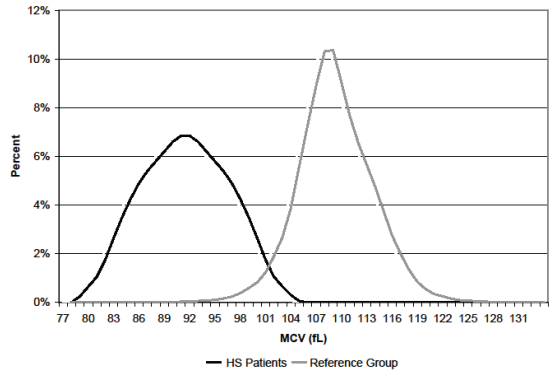

Figure 2. The histogram shows the mean corpuscular volume (MCV; fL) values recorded during the first 3 months of life in 2 populations of neonates and young infants (reference group = grey line; HS = black line). The reference population included individuals born after 35–41 weeks' gestation, who had never received transfusions, and who had no diagnosis of anemia. Only the first value obtained was recorded (one per individual). The HS population all had a confirmed HS diagnosis by 8 years of age and had 1 or more complete blood count in the first 90 days of life in our database. All values of the patients with HS were recorded, including multiple values from individuals. Values obtained within 3 weeks following a red blood cell transfusion were excluded.

overlap is seen in this than in the MCHC (Figure 1) or the MCV histograms (Figure 2). On analyzing various "cutoff" values for the sensitivity and specificity of the Neonatal HS Index, a screening test with an index

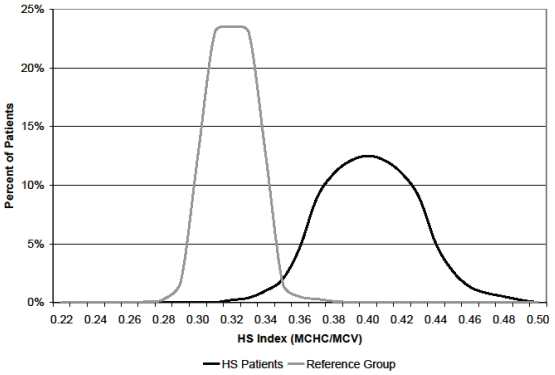

Figure 3. Histogram of the "Neonatal HS Index" (calculated by dividing the MCHC value by the MCV value) recorded during the first 90 days of life in 2 populations of neonates and young infants (reference group = grey line; HS = black line). The reference population included individuals born after 35–41 weeks' gestation, who had never received transfusions, and who had no diagnosis of anemia. Only the first value obtained was recorded (one per individual). The HS population all had a confirmed HS diagnosis by 8 years of age and had 1 or more CBC in the first 90 days of life in our database. All values of patients with HS were recorded, including multiple values from individuals. Values obtained within 3 weeks following a red blood cell transfusion were excluded.

of ≥0.36, which had 97% sensitivity, >99% specificity, a 7% positive predictive value, and a >99% negative predictive value was judged to be the best.

Discussion

Diagnosing HS during the neonatal period can be challenging because the presence of spherocytes on a neonate's blood film can be artifactual (10) or it can accompany conditions other than HS, such as ABO hemolytic jaundice (11). Moreover, not all spherocytes seen on a neonate's blood film are necessarily a result of HS. Identifying increased erythrocyte osmotic fragility can be helpful in diagnosing HS, but this test is less accurate in neonates and young infants than in older children; in many institutions, the test must be sent to a reference laboratory (12). Additionally, neonates generally have a low haptoglobin, confounding the quantification of haptoglobin in confirming hemolysis (13). In addition, neonates have high reticulocyte counts and a high RBC distribution width (RDW), which sometimes hinders the identification of hemolysis (14). Quantitative erythrocyte membrane protein electrophoresis and the sequencing of relevant genes can be confirmative of HS, but these are not generally used outside of research applications (1,2).
Even when a father or mother has HS, it is difficult to know initially whether the newborn infant is affected. This problem was seen in 2 cases of our 33 in which,

although a parent had HS, the families were told that the baby did not have HS because spherocytes observed in the blood film were very few for confirming HS. In both cases, the infants subsequently became anemic and required 1 or more RBC transfusions, and HS was eventually diagnosed.

The present study was undertaken to assess neonatal HS screening using 2 electronic elements of the CBC, both of which can be perturbed in predictable ways in neonates with HS. As a potential screening method, we reasoned that the elevated MCHC value, which is well described among most neonates (8), children (15), and adults with HS (1,2), could be divided by the MCV value, which were low in many of our neonates with HS. To test our hypothesis, we obtained each CBC that was obtained during the first 90 days after birth of those children who were diagnosed with HS in our multihospital system since January 2002. We then compared the MCHC and MCV measurements of these with a large contemporary reference group from the same hospital system.

We previously reported that the MCV values obtained on the day of birth is associated with the gestational age (16). As suspected, MCV values of neonates with HS tended to be below the reference range. Reticulocytes generally have a higher MCV than non-reticulocytes (17), and neonates with HS tend to have an elevated reticulocyte count (1,6,7). Despite this, the MCV of neonates with HS tends to be low, while the MCHC values tend to be high. Taking advantage of these 2 trends, we calculated the Neonatal HS Index (obtained by diving the MCHC values by the MCV values) and found that it performed better than either the MCHC or the MCV for identifying HS.

As a screening test for HS in neonates, we judged the sensitivity and negative predictive values to be particularly useful features, despite the fairly high false positive rate. We reasoned that a false positive screen would not subject neonates to harm. Rather, normal neonates identified falsely by the test to be at risk for HS would be carefully observed for hyperbilirubinemia and anemia, and the specific HS testing could be arranged beyond the neonatal period if the clinical course warranted such. In contrast, if the screening missed true positives, such missed patients might indeed be at risk on the basis of less vigorous monitoring for hyperbilirubinemia and anemia. An index cutoff of ≥0.36 identified all of the patients with HS in our series.

Other screening tests for HS have been proposed, but they are not specific for use in neonates and young infants. For instance, Michaels et al. used the MCHC and the RDW as a means of screening older children for HS (15). While caring for neonates with HS, the RDW is less informative than the MCHC or MCV because it tends to be high in normal neonates and does not seem to be significantly elevated in neonates with HS. It is possible that another equation that includes RDW and possibly other measurements would perform better than our Neonatal HS Index. Broséus et al. examined a "mean sphered corpuscular volume" for HS screening in adults, but it has not been evaluated in neonates (18). Cyanober et al. examined adults with HS for associations between clinical severity and erythrocyte indices, but no such associations were obvious in our series (19).

We recognize that the Neonatal HS Index described in this report is subject to counting, sampling, and reporting errors. Moreover, the reference range index values might possibly vary according to the electronic cell counting equipment used and the reference population sampled. Therefore, a cutoff value of ≥0.36, which performed well in the present study, should not be considered a universal or absolute screening cutoff level for HS in neonates. We emphasize that the index is intended to be a screening test, and not a diagnostic test. Despite these limitations and issues, we predict that this simple, rapid, and inexpensive way of using information already available in the CBC results can be of value to clinicians while they consider the possible diagnosis of HS in neonates.

Authorship Contributions and Disclosure of Conflicts of Interest Statement:

The authors have no conflict of interests to disclose relative to this study or manuscript.

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