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A Simple Index Using Age, Hemoglobin, and Aspartate Transaminase Predicts Increased Intracerebral Blood Velocity as Measured by Transcranial Doppler Scanning in Children With Sickle Cell Anemia

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**ABSTRACT**

**OBJECTIVE.** Increased intracerebral blood velocity measured by transcranial Doppler scanning identifies children with sickle cell anemia who are at increased risk of stroke. We have tried to develop an index based on routine clinical measurements that also predicts increased intracerebral blood flow.

**METHOD.** Routinely collected clinical and laboratory data were correlated with transcranial Doppler measurements on children with sickle cell anemia seen in a single institution in 2006. The index produced was validated on a second independent data set from children with sickle cell anemia.

**RESULTS.** The time-averaged mean of the maximum velocity in centimeters per second in the middle cerebral artery circulation correlated significantly with age, hemoglobin, lactate dehydrogenase, and aspartate transaminase levels, white blood cell count, and creatinine level. On multiple regression, hemoglobin and aspartate transaminase levels maintained their significance, whereas age had borderline significance, and an index was developed linked to a time-averaged mean of the maximum velocity of 220 \( \times \) (8 \( \times \) hemoglobin) \( - (1.4 \times \) age) \( + (0.4 \times \) aspartate transaminase). This detected a time-averaged mean of the maximum velocity of \( >170 \text{ cm/second} \) with 100% sensitivity and 58% specificity. The index was validated on the second data set and again showed 100% sensitivity with 73% specificity.

**CONCLUSION.** This simple index has the potential to identify children who are at higher risk of cerebrovascular disease to allow them to be prioritized for transcranial Doppler scanning and other intracerebral imaging.

**INFARCTIVE STROKE** is one of the major complications affecting children with sickle cell anemia (SCA). In the United States and many European countries, SCA is now the most common cause of pediatric stroke. The peak incidence of 1.02 per 100 patient-years occurs between the ages of 2 and 5 years, with \( \sim 11\% \) having suffered an overt stroke by the age of 20 years. The Cooperative Study for Sickle Cell Disease found that the risk of first infarctive stroke was increased by previous transient ischemic attacks, lower steady-state hemoglobin, previous acute chest syndrome, and systolic hypertension. Adams et al\textsuperscript{2} also showed that increased blood velocity as measured by transcranial Doppler (TCD) scanning in the middle cerebral artery circulation was predictive of stroke with velocities of \( >170 \text{ cm/second} \), conferring a relative risk of stroke of 44 compared with those with lower velocities. A subsequent randomized, controlled trial, referred to as the Stroke Prevention in Sickle Cell Disease Study, showed that regular blood transfusions in those with TCD velocities of \( >200 \text{ cm/second} \) led to a 90% reduction in the incidence of stroke for the duration of the transfusion. Various guidelines have been written recommending the introduction of TCD...
screening for increased risk of stroke in children with SCA and primary prevention by using blood transfusion. Observational studies have also suggested a decline in stroke rates after the introduction of TCD screening and primary prevention.

However, the development of TCD screening programs is patchy in both the United States and Europe, despite the potential availability of appropriate health resources. A survey in the United States suggested that only 42% of children between the ages of 2 and 12 years with SCA and hemoglobin S/ββ thalassemia had been screened using TCD in the preceding year, mainly because of long distances to travel to the nearest vascular laboratory. Similarly, surveys in the United Kingdom suggest that fewer than half of the children with SCA have access to TCD. Many centers across Europe and the United States are in the process of establishing TCD screening programs, and we have, therefore, tried to develop a simple way of identifying children most likely to have abnormal TCD scans to allow these children to be prioritized for early scanning. This approach has also identified laboratory parameters correlated with TCD measurements with potential implications for the pathogenesis of cerebrovascular disease in SCA.

METHODS

Patients

The study was performed at King’s College Hospital, which is a teaching hospital in south London. Approximately 450 children with sickle cell disease attend the Pediatric Hematology Clinic, and ~320 of these have SCA. Since 2004, all of the children between the ages of 2 and 16 years have been offered TCD scanning every 1 to 2 years. All of the children have annual steady-state blood and urine tests. Initial analysis was performed on the results of all of the children with SCA who had TCD scanning in 2006, excluding those on regular transfusion and those for whom steady-state data were not available in that year. The index developed was then validated in a second data set consisting of children who had scans in 2007 (excluding those who also had TCD scans in 2006) and those with abnormal or conditional scans in 2005 (again excluding those in the 2006 data set). All of the tests were performed as part of routine clinical care, and the results were recorded and analyzed anonymously. All of the patients who had not been screened in the preceding 12 months were offered TCD screening at their routine clinic appointment, with no attempt to select patients.

Laboratory Analysis

Routine hematologic and biochemical analyses were performed in the accredited hospital laboratories using standardized automated techniques. The lower limit of urine albumin detection was 6 mg/L. The diagnosis of SCA was confirmed in all of the subjects by high-performance liquid chromatography of hemoglobin, with family and DNA studies where appropriate. Single, steady-state measurements were analyzed from samples taken in 2006. The upper limit of normal for lactate dehydrogenase (LDH) was 240 IU/L, and the reference range for aspartate transaminase (AST) was 10 to 50 IU/L.

TCD Scanning

All of the studies were performed in the vascular laboratory at King’s College Hospital by using TCD imaging and following the protocols in the Stroke Prevention in Sickle Cell Disease Study. Color duplex scanners (Siemens Sequoia and Aspen ultrasound duplex scanners, Munich, Germany) were used with a 2-MHz transcranial transducer to record the time-averaged maximum velocity for the middle cerebral artery, anterior cerebral artery, bifurcation, distal internal carotid artery, and posterior cerebral artery on both sides. Previous evaluation of TCD imaging versus TCD scanning had shown that both methods gave the same results when used according to strict, established protocols. The maximum velocity recorded in the middle cerebral artery, bifurcation, or distal internal carotid was recorded as the time-averaged mean of the maximum velocity (TAMMV) in centimeters per second.

Statistical Analysis

Correlation analysis was performed for each laboratory parameter against TAMMV for the 2006 data set. Multiple regression analysis was performed on those factors found to correlate significantly against TAMMV, and an index was developed from the significant results. This index was then analyzed against the TAMMV readings by using scatter plots and receiver-operator characteristic (ROC) curves.

The index was then validated against the second data set by using scatter plots and ROC curves. All of the statistics were obtained by using SPSS 14.0 (SPSS Inc, Chicago, IL).

RESULTS

TCD Scans

The mean TAMMV for the 2006 data set was 125 cm/second, with a median of 122 cm/second and a range from 78 to 215 cm/second. This is similar to the mean of 129 cm/second found by Adams et al in 167 children with SCA and no evidence of cerebrovascular disease.

Laboratory Parameters and 2006 Data

TCD scans were performed on 96 children with SCA who were in the steady state and not receiving blood transfusions. Nine patients were receiving hydroxyurea, and their exclusion from analysis did not significantly alter the findings. Correlation analysis was performed between TAMMV and the following measurements in these children: age, hemoglobin, neutrophils, white blood cell (WBC) count, platelets, mean corpuscular hemoglobin concentration, reticulocyte number, creatinine, vitamin D, calcium, phosphate, alkaline phosphatase, total bilirubin, alanine transaminase, AST, LDH, and urine microalbumin/creatinine ratio. The following parameters showed a significant correlation with TAMMV: age ($R = -0.269; P = .008$), hemoglobin ($R = -0.413; P < .001$), LDH ($R = 0.207; P = .048$), creati-
nine ($R = -0.293; P = .004$), AST ($R = 0.289; P = .005$), and WBC count ($R = 0.236; P = .021$).

Linear regression was then performed by using the significant factors (hemoglobin, LDH, age, creatinine, and AST) against TAMMV, with the results shown in Table 1. Analysis of variance was highly significant ($F = 6.839; P < .001$), with hemoglobin and AST maintaining their significance and age approaching significance.

Development of Predictive Index for TAMMV

Using the 2006 data, linear regression gave the equation of $\text{TAMMV} = 208.7 - (8.025 \times \text{hemoglobin}) - (1.356 \times \text{age}) - (0.376 \times \text{AST})$, with TAMMV measured in centimeters per second, hemoglobin in grams per deciliter, age in years (to 1 decimal place), and AST in international units per liter. A scatter plot of this function against TAMMV suggested that all of the conditional and abnormal TAMMV readings ($\geq 170\text{ cm/second}$) were detected if a threshold of 160 was used for this index. To make the index simpler and the abnormal threshold coincide with the abnormal-conditional TAMMV reading, the constant in the formula was rounded up to 220 (ie, $10$ greater to take the abnormal threshold from 160 to 170), and the other figures corrected to 1 decimal place. Thus, the index was calculated as $220 - (8 \times \text{hemoglobin}) - (1.4 \times \text{age}) - (0.4 \times \text{AST})$. A scatter plot of this index against TAMMV confirmed that all 5 of the subjects with conditional and abnormal TCD results also had an index of $\geq 170$ (Fig 1). The index was analyzed by plotting an ROC curve, with a TAMMV of $\geq 170$ being classified as a positive result (Fig 2). The area under the curve was 0.84 (95% confidence interval: 0.73–0.96). The index threshold of 170 again detected all of the conditional-abnormal scans (100% sensitivity) with a specificity of 73% (Fig 4).

DISCUSSION

There is good evidence that TCD screening and regular blood transfusion of children with increased blood flow are effective means of primary stroke prevention, reducing the risk of stroke by 90%.$^3$ The majority of children in the world with sickle cell disease do not have access to...
safe and regular blood transfusions, and this has to be established before a program of TCD screening becomes a priority. However, the majority of children with SCA in developed countries have not had TCD scans, and screening programs are only just being established in many hospitals and countries. This index, based on 2 routinely measured laboratory parameters, offers a simple way of identifying those children most likely to have an abnormal TCD and allows these children to be scanned more urgently. The index identified children with TCD readings of >170 cm/second with 100% sensitivity when a threshold of 170 was used. To achieve 100% sensitivity, the specificity is inevitably lower at 60% to 70%, although this is sufficient to allow effective targeting of children most likely to have cerebrovascular disease.

Both AST and LDH show significant correlation with TAMMV, although only the relationship with AST maintains its significance on linear regression. Both of these are increased in hemolysis, and the relationship suggests that intravascular hemolysis may be implicated in the pathogenesis of cerebrovascular disease in children. There is good evidence that intravascular hemolysis has contributed to the development of vascular complications in adults with SCA, including a strong association with pulmonary hypertension, and less well established links with priapism and chronic leg ulceration. In vitro evidence suggests that free plasma hemoglobin released by intravascular hemolysis binds avidly to and causes a functional deficiency of nitric oxide. Using this information, it is reasonable to suggest that increased hemolysis results in lower hemoglobin and increased free plasma hemoglobin, which binds avidly to nitric oxide and inactivates it; this results in a chronic shortage of nitric oxide, causing a vasculopathy and progressive narrowing of large intracerebral blood vessels, which causes higher blood velocity as measured by TCD. An alternative explanation for our findings is that increasing anemia because of hemolysis results in increases in cardiac output and intracerebral blood flow, which increases vascular endothelial damage and results in progressive narrowing of intracerebral vessels. Our study also confirms the findings of the Cooperative Study for Sickle Cell Disease that increasing anemia is an important risk factor for stroke, with linear regression suggesting that this is independent of any effect of hemolysis. The association found in this study suggests that hemolysis is important in the development of cerebrovascular disease and that some of the promising treatments for pulmonary hypertension might also be of potential benefit in cerebrovascular disease, including sildenafil. This possibility could be investigated in clinical trials, and there is as yet no evidence to support the use of sildenafil in treating or preventing cerebrovascular disease.

Age correlated significantly with TAMMV on bivariate analysis (P = .008), although this significance was reduced on linear regression (P = .079). An equation using only AST and hemoglobin was developed, although this was less sensitive and specific than that which also included age (data not shown). The inclusion of age as a parameter is further supported by previous studies showing that the rate of stroke is highest in younger children, with a peak incidence of 1.02/100 patient-years in those aged 2 to 5 years. The index was developed by using TCD imaging as
opposed to nonimaging TCD; some studies suggest that these methods both give similar readings, whereas others have found that TCD imaging measurements are ~10% lower. It seems possible that other centers may need to adjust the threshold for abnormality depending on the TCD methods used. Similarly, differences in hemoglobin and AST measurements between different hospitals and countries may mean that the exact parameters of the equation will need to be modified to optimize sensitivity and specificity. The index has been developed and validated for children between the ages of 2 and 16 years because of the practical difficulties of performing TCD scans in younger children; however, there is evidence that some infants have neurodevelopmental problems, and it has the potential to be used in young children before TCD is possible. It is also possible that those children with an abnormal index but normal TCD are also at an increased risk of stroke compared with those with a normal index. Both of these questions should be addressed by additional prospective studies.

CONCLUSIONS
It is possible to predict which children with SCA are likely to have abnormal TCD results using simple, routinely measured laboratory parameters and age. This is of practical significance in that it allows these children to be screened for cerebrovascular disease preferentially. It also suggests that anemia and hemolysis are the important factors contributing to the pathology of cerebrovascular disease in SCA.

REFERENCES
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