Early Reticulocytosis and Anemia Are Associated with Abnormal and Conditional Transcranial Doppler Velocities in Children with Sickle Cell Anemia

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Objective To improve prediction of sickle cell anemia severity at an early age, we evaluated whether absolute reticulocyte count (ARC) or hemoglobin (Hb) levels during early infancy (2-6 months of age) in patients with sickle cell anemia predict the risk of later developing an abnormal (abTCD) or conditional (cdTCD) Transcranial Doppler (TCD).

Study design We used chart review to identify 121 consecutive patients who underwent TCD screening and had steady state ARC and Hb levels recorded between 2 and 6 months of age. Cox regression analysis was used to determine the relationship between ARC, Hb levels, and risk of developing cdTCD/abTCD over time.

Results Mean ARC in early infancy was highest and mean Hb lowest in those children with abTCDs and cdTCDs. Cox regression analysis revealed that those subjects with an ARC ≥200 K/μL in early infancy had nearly 3 times the risk of having an abTCD/cdTCD than the group with an ARC <200 K/μL, and patients with a Hb <8.5 g/dL had 2.7 times the risk of having an abTCD/cdTCD.

Conclusions These data suggest that both elevated ARC and low baseline Hb during early infancy are associated with an increased risk of developing a cdTCD or abTCD later in childhood. *(J Pediatr 2016;169:227-31)*.

Although all patients with sickle cell anemia ([SCA], hemoglobin [Hb] SS genotype) share the same genetic mutation, a single amino acid substitution (Glu → Val) at the sixth codon of the beta globin gene on chromosome 11, their clinical phenotype is highly variable and currently very difficult to predict early in life.[1] Neurologic complications are common in children with SCA; silent cerebral infarcts occur in over one-third of patients with SCA by age 15 years.[2] Historically, prior to the onset of routine screening with transcranial Doppler (TCD), 10% of children with SCA under the age of 20 years suffered an overt stroke.[3] The institution of annual TCD screening and appropriate therapeutic interventions for abnormal results has decreased the rate of overt stroke to 1% in children with SCA.[4] Reticulocytosis immediately prior to TCD screening was associated with an increased rate of abnormal or conditional velocities.[5]

The first erythropoietic stress in all term infants, including those with hemoglobinopathies like SCA, usually occurs between the ages of 2 and 6 months following the abrupt increase in oxygenation that occurs at birth. Recovery from the resulting physiologic nadir[6] in healthy infants involves the production of erythrocytes with almost entirely healthy adult hemoglobin, while the fetal Hb (HbF)-containing erythrocytes of infants with SCA, are replaced almost entirely by sickle Hb (HbS)-containing erythrocytes.[7] HbS-containing erythrocytes have a shortened half-life and impair blood flow. Infants with SCA have an exaggerated reticulocyte response to the physiologic nadir when compared with healthy 3-month-old infants without SCA (mean absolute reticulocyte count [ARC] 199 K/μL vs mean ARC 83 K/μL, respectively).[8,9]

In term infants with SCA, the physiologic nadir period precedes the onset of clinical signs and symptoms. Our prior studies have compared hematologic data measured at ages 2-6 months and the clinical course of SCA during the first 3 years of life. The mean ARC (140 ± 63 K/μL) for the 23 subjects who were not hospitalized during the first 3 years of life was lower than that of the remaining 36

abTCD	Abnormal TCD
ARC	Absolute reticulocyte count
AUC	Area under the curve
cdTCD	Conditional TCD
CBC	Complete blood count
Hb	Hemoglobin
HbF	Fetal Hb
HbS	Sickle Hb
nlTCD	Normal TCD
SCA	Sickle cell anemia
TCD	Transcranial Doppler

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subjects who required hospitalization (204 ± 94 K/μL, $P = .0054$). However, the mean Hb was not different between the 2 groups (not hospitalized 9.0 ± 1.1 g/dL vs hospitalized 8.8 ± 1.4 g/dL, $P = .53$). Higher ARCs were also associated with a markedly shorter time to first hospitalization and a 3-fold higher cumulative frequency of clinical events.[10] We have also reported that reticulocytosis during early infancy is associated with a higher rate of overt stroke and death in a historical cohort of pediatric patients with SCA.[11] The objective of this study was to ascertain whether ARC between ages 2 and 6 months is a predictive marker for conditional (cdTCD) or abnormal TCD (abTCD), the only validated predictor of a severe SCA complication. We hypothesized that patients with cdTCD or abTCD will have higher ARC levels between age 2 and 6 months.

Methods

Following Children's National Institutional Review Board approval, including a waiver of consent, consecutive patients with SCA born after December 31, 1998, who had TCD screening were identified by searching the electronic radiology database at Children's National (Montage, Philadelphia, Pennsylvania). Chart review for each included patient was performed to collect the steady state ARC and accompanying complete blood count (CBC) between the ages of 2 and 6 months. All steady state CBCs and ARCs recorded during that time frame were used to determine the most appropriate cut point to predict future TCD velocities. Steady state was defined as a sample collected at least 30 days from an acute illness and at least 60 days after receipt of a blood transfusion. We excluded all data points (hematologic and TCD velocities) that were collected within 60 days of a packed red blood cell transfusion to minimize the effects of transfused erythrocytes on study variables.[12] We also excluded patients with SCA who were born prematurely because prematurity can affect timing of the physiologic nadir and the severity of anemia during the nadir period. Steady state ARC and CBC data were collected longitudinally until either the time of initiation of SCA-modifying therapy (hydroxyurea, monthly blood transfusions, or hematopoietic stem cell transplant) or July 1, 2014.

TCD

Imaging TCDs (LOGIQ E9 GE Healthcare, Waukesha, Wisconsin) were performed per the clinical guidelines of the Children's National Sickle Cell Program, where TCD screening is initially attempted at age 2 years in the Radiology Department at Children's National, per the Stroke Prevention Trial in Sickle Cell Anemia recommendations.[4] Results were classified as normal if the time averaged mean maximum velocity in the middle cerebral artery or distal internal carotid artery was less than 170 cm/s (normal TCD [nlTCD]), conditional if time averaged mean maximum velocity was 170-199 cm/s (cdTCD), and abnormal if the velocity in either artery was greater than 200 cm/s (abTCD). Nonimaging TCD values are used in our center for classification of normal, conditional, and abnormal velocities because of angle correction that is performed when analyzing the studies. nlTCDs are repeated annually, and cdTCDs are repeated every 3 months.

Statistical Analyses

All analyses were conducted in Stata 11 (StataCorp, College Station, Texas).[13] Based on our previous findings,[10,11] study subjects were divided into groups: ARC less than 200 K/μL (ARC <200) and ARC greater than or equal to 200 K/μL (ARC ≥200), or Hb less than 8.5 g/dL (Hb <8.5) and Hb greater than or equal to 8.5 g/dL (Hb ≥8.5). Initially, frequencies of categorical variables across the 3 groups were compared using χ^2 analysis and means of continuous variables were compared in 1-way ANOVA. Kaplan-Meier estimates of TCD event rates were calculated and compared among the groups using the log-rank test. Subsequently, Cox regression analysis was used to adjust for age and sex. A value of $P < .05$ was considered statistically significant. Data are presented as mean ± SD unless otherwise indicated.

Results

Consecutive patients with SCA (n = 121) were included in this study and ranged in age from 2-15 years (mean age of cohort at most recent TCD was 5.8 ± 3.0 years). Sixty-five (53.7%) patients had nlTCD velocities; 36 (29.8%) had conditional velocities, and 20 (16.5%) had abnormal velocities (Table I). Females represented nearly 60% of the patients in the abnormal and cdTCD groups compared with 45% of the nlTCD group. The overwhelming majority of patients were African American. None of the patients received a blood transfusion during early infancy (2-6 months of age). Mean age at the time of first TCD test did not differ between groups (nlTCD: 2.9 ± 1.4 years vs cdTCD:

Table I. Demographic information

	nlTCD, N = 65	cdTCD, N = 36	abTCD, N = 20	*P* value
Male sex (%)	36 (55.4%)	11 (30.6%)	6 (30.0%)	.022
Ethnicity/race				.376
African American/African	65 (98.5%)	31 (93.9%)	19 (95.0%)	
Hispanic	1 (1.5%)	2 (6.1%)	1 (5.0%)	
Mean age (y) at first TCD (±SD)	2.9 (±1.4)	3.5 (±2.3)	3.0 (±1.1)	.214
Mean age (y) at most recent TCD (±SD)	5.3 (±2.8)	7.2 (±3.1)	4.9 (±2.4)	.002
Mean age (y) at first abTCD or cdTCD (±SD)	NA	4.6 (±2.3)	4.4 (±2.2)	.646

NA, not applicable.