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INTRA-INDIVIDUAL VARIATION IN BLOOD FLOW VELOCITIES IN CEREBRAL ARTERIES OF CHILDREN WITH SICKLE CELL DISEASE

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Abstract

Background—Children with sickle cell disease are at elevated risk of stroke. Risk increases with blood flow velocity in selected cerebral arteries, as measured by transcranial Doppler (TCD) ultrasound and use of TCD to screen these patients is widely recommended. Interpretation of TCD results should be based on knowledge of intra-individual variation in blood flow velocity, information not currently available for sickle cell patients.

Procedures—Between 1995 and 2002, 4141 subjects, 2–16 years old, with homozygous sickle cell disease or Sβ^0-thalasemmia and no history of stroke were screened with TCD, including 2018 subjects screened in one clinical trial (STOP), 1816 screened in another (STOP 2) and 307 screened in an interval ancillary prospective study. The 812 subjects with ≥2 examinations <6 months apart were selected for analysis, including 242 (29.8%) subjects with normal average velocities (i.e. <170 cm/sec), 350 (43.1%) subjects with conditional velocities (i.e. 170–199 cm/sec) and 220 (27.1%) subjects with abnormal velocities (i.e. >200 cm/sec). The intra-subject standard deviation of TCD velocity was estimated from the difference between velocities at the first two eligible examinations on each subject.

Results—An intra-subject standard deviation of 14.9 cm/sec was obtained. Seven (0.9%) subjects had unusually large and unexplained differences between velocities at the two examinations (range of absolute differences: 69–112 cm/sec).

Conclusions—While stroke risk is well demonstrated to increase with increasingly abnormal TCD velocity, given the relatively large intra-subject variability, one TCD examination is generally not sufficient to characterize stroke risk in this patient population.

Keywords

sickle cell disease; stroke; transcranial Doppler ultrasound

Introduction

It is well established that the risk of stroke by 20 years of age is approximately 10% in patients with homozygous sickle cell disease (SCD-SS) [1]. Adams and his colleagues showed that the risk of stroke increases with blood flow velocity in selected cerebral

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arteries, as measured by transcranial Doppler (TCD) ultrasound [2] [3]. In the Stroke Prevention Trial in Sickle Cell Anemia (STOP) chronic transfusion sufficient to maintain the level of circulating sickle hemoglobin at <30% reduced the annual incidence of stroke by 90% in high risk children [4]. A follow-up trial, STOP 2, demonstrated that cessation of transfusion therapy in children whose screening TCD exam had normalized was associated with return to abnormal TCD and stroke [5]. Screening with TCD has become part of evidence-based recommendations for care of SCD patients [6][7] and primary stroke prevention guidelines [8]. Chronic transfusion of TCD-abnormal patients offers the prospect of preventing many first time strokes in SCD [9]. Preliminary data indicate that the STOP approach may be reducing the incidence of stroke in the community [10].

The level of confidence in the results of a TCD examination as a measure of risk for an individual patient is inversely related to the variability of TCD results within that patient. Variability includes both measurement error and intra-patient variation in blood flow velocity; total variability is at issue in inferring stroke risk from TCD results. Intra-patient variability of TCD results has not been addressed. During STOP and STOP 2, nearly 10,000 TCD's were performed on similar equipment using a standardized protocol. The examinations were read by personnel blinded to clinical data [11]. Here we present an analysis of intra-patient variability based on repeated TCD screening of patients in the two clinical trials and an interim ancillary study.

Methods

Patients

Data for this analysis were obtained during the screening phases of STOP and STOP 2, and during an ancillary screening study that was run while STOP was in progress and STOP 2 was in development. Screening in the three studies took place between 1995 and 2002. Subjects were screened at 14 clinical sites in STOP, 12 sites in the ancillary study and 26 sites in STOP 2. In all three studies, screening was limited to patients who were 2–16 years old, had SCD-SS or $S\beta^0$-thalasemmia, and had no history of stroke. Study recommendations during screening called for a follow-up TCD examination within one month of an abnormal exam, within three months of a conditional and six to twelve months after a normal exam.

Patients with at least two interpretable TCD examinations within six months were included in this analysis. Examinations performed over longer intervals were excluded to avoid contaminating intra-subject variation around a subject's steady state mean with systematic changes in that mean over time.

The study procedures were approved by the Institutional Review Boards at all participating sites. Written informed consent for screening was obtained from the parents or guardians of all subjects.

TCD Screening Procedures

TCD screening procedures have been described in detail elsewhere [11][12][13]. Briefly, a standard TCD protocol developed for children with SCD was used at all study sites. TCD examiners were all rigorously trained for this protocol and all examinations were read centrally by one of two individuals to reduce inter-examiner variability. Subject ID numbers were masked before examinations were sent to the readers. Identical equipment and software were used (2-Mhx pulsed Doppler; Nicolet EME TC 2000 or Nicolet Pioneer; Nicolet, Madison, WI). Stroke risk was evaluated by measuring blood flow velocities in 2 mm increments in the middle cerebral and internal carotid arteries. Measurements were also obtained from other cerebral arteries but were not used in evaluating stroke risk. The maximum time-averaged mean velocity (MTAMV) recorded during an examination was

used to assess stroke risk. Velocities were considered normal if MTAMV was <170 cm/sec, conditional if 170–199 cm/sec, and abnormal if ≥200 cm/sec. A TCD examination was considered not interpretable if velocities were not obtained from both middle cerebral arteries and the patient could not be classified as abnormal based on the TCD data that was obtained.

Statistical Methods

The intra-subject standard deviation of TCD velocity was estimated from the variance of the differences between velocities on repeated examinations of the same subjects. If the difference between velocities at two examinations on the same patient has variance $2\sigma^2$, then the intra-subject standard deviation is σ. A single estimate of σ was obtained using data combined across subjects, on the assumption that intra-subject variation was the same for all subjects. Because the variability of many biological measurements increases with the mean and with time between examinations, the assumption of homogeneous intra-individual variation was tested by classifying the subjects into six subgroups and estimating the standard deviation within each subgroup from the variance of the difference as just described. The six subgroups were created by averaging each subject's MTAMVs over the examinations and classifying the average as normal, conditional or abnormal using the velocity ranges above, and dichotomizing time between examinations (≤3 months vs >3 and <6 months). A more detailed look at the relationship between intra-individual variation and average MTAMV velocity was undertaken by dividing the averages of the MTAMVs used to assess stroke risk at the two examinations of each subject into into 21 ordered categories and calculating the intra-subject standard deviation within each category from the variance of the difference. The number of categories was chosen to produce a fairly narrow range of intra-subject mean TCD velocities within each category and a sample size large enough to obtain a reasonably stable estimate of the standard deviation in each one. The correlation between the standard deviation and category-specific median velocity provided a measure of the relationship between intra-subject variation and mean velocity.

The Kolmogorov-Smirnov test was employed to determine if the assumption of a normal distribution for the intra-subject differences was reasonable. The relationship between the absolute difference between two velocities from the same patient and the interval between measurements was also examined to determine if the variability of measurements was related to the interval between them.

Results

A total of 4141 subjects were screened, including 2018 in STOP, 1816 in STOP 2 and 307 in the ancillary study. The total for STOP 2 does not include 829 subjects who were screened in STOP 2 after having been screened in STOP and/or the ancillary study because only the results obtained from these patients in the two earlier studies were used in this analysis. Eligible exam pairs were available from 812 subjects: 507 from STOP, 35 from the ancillary study and 264 from STOP 2. Subjects ranged from 2 to just under 17 years of age at the first examination (Table 1). The average of the velocities at the two examinations was normal in 242 (29.8%) subjects, conditional in 350 (43.1%) and abnormal in 220 (27.1%). The percentages of subjects with conditional or abnormal velocities in this subset exceeded the corresponding percentages in the entire group of screened patients (conditional: 17.7%; abnormal: 8.3%) due to the result-based re-screening schedule described earlier. Descriptive statistics for time between examinations and intra-subject average velocity is provided in Table 1.

The extent to which a single TCD examination adequately represents a patient's status with regard to the risk of stroke was explored by comparing the outcomes – normal, conditional

or abnormal – at the two examinations used in this analysis (Table 2). Classification of the two examinations agreed for 505 (62%) of subjects but disagreed for 307 (38%) others. Fourteen subjects had one normal and one abnormal examination each.

The intra-subject differences between TCD velocities at the first two examinations failed to fit a normal distribution ($p \leq 0.01$) mainly because of 7 subjects, all from STOP, who had large differences between the velocities at their first two interpretable examinations (range of absolute differences: 69–112 cm/sec). Excluding these subjects improved the fit to a normal distribution ($p=0.073$). In five cases, the large differences were caused by single TCD velocities that departed substantially from all other results from the same subject (4–11 total examinations per subject). In two cases, the extreme value was considerably higher than all other values but in three cases it was lower. In the sixth case, with seven interpretable examinations, high TCD velocities (212–230 cm/sec) were obtained on the first, third and fourth examinations, while lower TCD velocities (108–138 cm/sec) were obtained on the other four. Additional interpretable examinations were not obtained from the seventh subject. Examinations that were not interpretable were obtained considerably more frequently from these seven patients than from the patients screened in STOP as a whole (20% of 49 examinations vs. 4.4% of 4,055 examinations).

Several explanations for the large differences were considered but the reasons for these extreme values remain unclear. Available data on these patients were examined for evidence of unreported transfusions, such as large changes in hemoglobin concentration, but no such evidence was found. However, data obtained during screening were not sufficiently detailed to completely rule out unreported transfusions.

Intra-individual standard deviations for the six subgroups and for the entire analysis cohort are provided in Table 3. There is little evidence that the standard deviations vary with time between examinations or average TCD velocity. The differences among the standard deviations are small enough that the standard deviation for the entire group is probably a suitable estimate for most patients. Given the well-understood impact of extreme values on the estimated standard deviation, intra-subject standard deviations were also calculated after excluding the seven large absolute differences (Table 3). Time between examinations was <3 months for all but one of the excluded subjects. The exclusions reduced the intra-subject standard deviation of TCD velocity from 14.9 cm/sec to 13.7 cm/sec. The standard deviations were smaller with ≤3 months than with 3–6 months between examinations but the differences were slight.

When the 812 subjects were classified into 21 ordered categories based on intra-subject mean TCD velocities, the highest standard deviation, 31.2 cm/sec, was obtained from 21st category. This subset only included 13 (1.6%) subjects with mean velocities of 267–356 cm/sec. Aside from this small group, there was no evidence that the standard deviation increased with median velocity for the remaining 20 categories ($r=0.13$, $p=0.59$). Thus, aside from a few subjects with very high velocities, a single estimate of the intra-subject standard deviation should be applicable over a broad range of TCD velocities.

Discussion

In children with sickle cell disease, the risk of stroke clearly increases as flow velocity on transcranial Doppler ultrasonography increases at velocities above 140 cm/sec. In STOP, a velocity of 200 cm/sec was chosen as the minimum that would justify the risks associated with chronic transfusion. The risk of stroke over 30 months was estimated to be 47 percent for patients with velocities above 200 cm/sec [12]. The actual stroke rate in the standard care arm of STOP was approximately 10 percent per year over three years and lower after that.

Results of STOP 2 indicate that transfusion may need to be continued indefinitely to assure optimal reduction of risk. Even so, TCD screening has been embraced as an effective means of risk assessment, and transfusion therapy is well demonstrated to prevent stroke. Not only is periodic screening of asymptomatic patients to asses risk indicated, but ongoing screening to document response to therapy (or lack of) and consequent risk adjustment may also be useful.

The cutoff of 200 cm/sec used in STOP has largely been adopted in clinical practice as the threshold for TCD results requiring intervention. The data presented here suggest that clinical decisions based on TCD results must be interpreted carefully. The level of intra-subject variability of TCD velocity is high enough that the result of a single TCD examination may not be sufficient to reliably assign a patient's steady state mean velocity to any of the three risk categories; in fact, in our study population, risk group classification would have been different based on the second TCD examination in 38% of our subjects. A patient with a blood flow velocity of 185 cm/sec on a single examination could have a steady state mean velocity in any of the three risk categories; this may argue for prompt repeat examination in those with conditional velocities, especially in the higher range. Patients with abnormal velocity should have confirmatory testing as soon as feasible to reduce the risk of intervening stroke. . If the initial velocity is >230 cm/sec, then a confirmatory examination is less critical because it is highly likely that the steady state mean is in the abnormal range.

Repeat exams will improve the confidence in risk assessment. Suppose, for example, that a single examination has been performed on a subject. If V is TCD velocity at that examination and s is the estimated intra-individual standard deviation, the 95% confidence limits for steady state mean velocity are V ± 1.96s. Assuming a standard deviation of 14.9 cm/sec, the confidence limits are V ± 29 cm/sec. On the other hand, suppose the results of two examinations are available. The 95% confidence limits around the average of the two are $\overline{V} \pm 1.96\, s/\sqrt{2}$ or $\bar{V}$ ± 20.7 cm/sec where $\bar{V}$ is the average of the two velocities.

The estimated intra-subject standard deviations obtained here are based on examinations performed with a specific ultrasound machine. The extent to which estimates of intra-subject variation will be affected by differences in measurement variation among types of machines, if at all, is not clear. For purposes of this discussion, total intra-subject variation can be divided between biological variation, which captures fluctuations in TCD velocity within a subject, and measurement variation, which captures machine variation, operator variation and possibly other sources. If measurement variation is a small component of the total, then differences in variation among types of machines will have little impact on the intra-subject standard deviation. Suppose, for example, that measurement variance is 20% greater on a given model than it is on the machines used in this study and that measurement variation is 50% of total intra-subject variance. Then, the greater measurement variance would increase the intra-subject standard deviation from 14.9 cm/sec to 15.6 cm/sec, or only 4.6%. Thus, intra-individual variation should not be affected much by type of TCD machine unless there are very large differences in measurement variation between machines and/or measurement variation is a large fraction of total variation.

Larger problems can arise if a subject is examined on two different TCD machines. For example, TCD examinations performed using color imaging produce velocities that are usually approximately 10% lower than those on the equipment used to generate the data in this report [14]. If two examinations of a patient are performed on machines that produce different velocities, then, the difference between the results will include both intra-subject variation and the difference between the machines. The difference between the results of the two examinations may not be interpretable if information on the difference between the two

machines is not available. In examining a patient's history of TCD results, it is clearly important to know which machines were used to obtain previous results.

The rather high degree of intra-individual variability in TCD velocity also has implications for assessing stroke risk in cohort studies, such as those at the Medical College of Georgia and in STOP [2][4]. The subset of patients considered TCD-abnormal in those studies likely included some patients with steady-state mean velocities below the abnormal range. Thus, the risk of stroke in patients with steady state mean velocities in the abnormal range was likely underestimated in both studies

As noted earlier, the reasons for the few large differences between velocities at two examinations in STOP are not clear. It is possible that the cause of the large variation differs among patients. For example, a single value that is considerably lower than all other values on a patient could reflect a temporary decline in velocity or a failure to obtain a measurement from the portion of the artery in which the highest velocity occurred. A single high value could reflect a temporarily elevated velocity. The elevated rate of examinations of these patients that were not interpretable may be evidence that TCD examinations were, for various reasons, more difficult to perform in this subset than in the other patients who were screened. This increased difficulty could explain the high level of intra-individual variation in some of these patients. Unreported transfusions, which may temporarily reduce blood flow velocity, also cannot be ruled out. In any case, the small number of individuals with large differences between the velocities at the first two readable examinations should reinforce the idea that the results of a single TCD examination should be interpreted with caution.

TCD screening remains an important means of stroke prediction and prevention. Screening should be offered to all children with sickle cell disease. Families need to be informed as precisely as possible regarding the significance of one or several exams, and risk presented in the context of other clinical and laboratory factors as well as patient, family and physician values and priorities.

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TABLE I

Descriptive statistics for subjects included in the analysis of intra-individual variation in TCD velocity.

	MIN	PERCENTILE					MAX
		5%	25%	50%	75%	95%	
AGE (YEARS)	2.0	2.7	4.6	6.5	9.3	14.2	16.9
TIME BETWEEN TCD EXAMINATIONS (MONTHS)	0.20	0.46	1.06	1.81	3.70	6.0	6.0
MEAN TCD VELOCITY (CM/SEC)	89	125	167	182	202	237	356

TABLE II

Classification of TCD results at the two examinations used to estimate intra-individual variation. Normal: TCD velocity <170 cm/sec; conditional: TCD velocity 170–199 cm/sec; abnormal: TCD velocity ≥200 cm/sec.

	SECOND TCD			
FIRST TCD	**NORMAL**	**CONDITIONAL**	**ABNORMAL**	**TOTAL**
NORMAL	136	21	2	159
CONDITIONAL	150	195	56	401
ABNORMAL	12	66	174	252
TOTAL	298	282	232	812

TABLE III

Intra-individual standard deviations of blood flow velocity (cm/sec) on TCD given time between examinations and tcd classification

ALL SUBJECTS			
TIME BETWEEN EXAMINATIONS	**TCD CLASSIFICATION**	**SUBJECTS**	**STANDARD DEVIATION (CM/SEC)**
≤3 MONTHS	NORMAL	99	14.8
	CONDITIONAL	270	14.2
	ABNORMAL	199	15.2
>3, <6 MONTHS	NORMAL	143	14.6
	CONDITIONAL	84	13.8
	ABNORMAL	17	14.1
ALL SUBJECTS	--	812	14.9

EXCLUDING 7 SUBJECTS WITH DIFFERENCES BETWEEN VELOCITES ON TWO EXAMINATIONS ≥69 CM/SEC.			
TIME BETWEEN EXAMINATIONS	**TCD CLASSIFICATION**	**SUBJECTS**	**STANDARD DEVIATION (CM/SEC)**
≤3 MONTHS	NORMAL	98	13.5
	CONDITIONAL	268	12.7
	ABNORMAL	196	13.4
>3, <6 MONTHS	NORMAL	142	13.8
	CONDITIONAL	84	13.8
	ABNORMAL	17	14.1
ALL SUBJECTS	--	805	13.7