Elevated tricuspid regurgitant jet velocity in children and adolescents with sickle cell disease: association with hemolysis and hemoglobin oxygen desaturation

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ABSTRACT

Background

Elevation of echocardiography-determined tricuspid regurgitant jet velocity predicts high systolic pulmonary artery pressure and early mortality in adults with sickle cell disease. The definition, prevalence and clinical correlates of elevated jet velocity have not been established in pediatric patients. The present study tested the hypotheses that elevated jet velocity affects 10% of pediatric patients, is associated with both hemolysis and hypoxia, and has clinical correlates with acute chest syndrome, stroke, transfusion requirement and abnormal 6-minute walk test results.

Design and Methods

A prospective multicenter study of 310 patients aged 3-20 years old with sickle cell disease under basal conditions and 54 matched controls was conducted. A hemolytic index was generated by principal component analysis of the levels of lactate dehydrogenase, aspartate aminotransferase and bilirubin and reticulocyte count.

Results

Elevated jet velocity (defined as ≥2.60 m/sec based on the mean±2 SD in controls) occurred in 32 patients (11.0%) including one child of 3 years old. After adjustment for hemoglobin concentration, systolic blood pressure and left ventricular diastolic function, a 2 SD increase in the hemolytic index was associated with a 4.5-fold increase in the odds of elevated jet velocity (p=0.009) and oxygen saturation ≤98% with a 3.2-fold increase (p=0.028). Two or more episodes of acute chest syndrome had occurred in 28% of children with elevated jet velocity compared to in 13% of other children (p=0.012), more than ten units of blood had been transfused in 39% versus 18% (p=0.017) and stroke had occurred in 19% versus 11% (p=0.2). The distance walked in 6-minute walk tests did not differ significantly, but oxygen saturation declined during the tests in 68% of children with elevated jet velocity compared to in 32% of other children (p=0.0002).

Conclusions

According to a pediatric-specific definition the prevalence of elevated jet velocity in this population of young patients with sickle cell disease was 11%. The study provides evidence for independent associations of elevated jet velocity with hemolysis and oxygen desaturation. Further investigations should address whether elevated jet velocity may indicate future complications and whether early intervention is beneficial.

Key words: sickle cell disease, pulmonary hypertension, hemolysis, oxygen saturation, tricuspid regurgitant jet velocity, children.

Introduction

Echocardiographic estimation of pulmonary artery pressure by measuring the tricuspid valve regurgitant jet velocity has been validated as a useful screening method for pulmonary hypertension in adult patients with sickle cell disease.[1] A jet velocity of 2.5 m/sec or more, which corresponds to a systolic pulmonary artery pressure of 30 mmHg or more, has been used for research purposes to define elevated pulmonary artery pressure in adults with sickle cell disease and the prevalence is about 30%.[1-3] Even though this definition includes mild elevations in pulmonary artery pressure, adult sickle cell disease patients with a regurgitant jet velocity of 2.5 m/sec or more have an increased risk of mortality.[1,4,5] The prevalence and natural history of elevated jet velocity in children with sickle cell disease at steady state are largely unknown. A recent review of published studies comprising over 600 children combined indicated that the prevalence of a jet velocity of 2.5 m/sec or more is about 30%.[6] Most studies were not prospective and some of the children were evaluated during a vaso-occlusive crisis or other exacerbation of sickle cell disease. These studies were, therefore, likely biased toward patients with more severe illness.

Pulmonary hypertension may develop in most forms of hereditary and chronic hemolytic anemia[7-10] suggesting that there is a clinical syndrome of hemolysis-associated pulmonary hypertension. Nevertheless, an association between hemolysis and pulmonary hypertension in sickle cell disease has been questioned because, in most studies thus far, not all markers of hemolysis have had significant associations with estimated pulmonary artery pressure. For example, in a recent study of 62 children and adolescents with hemoglobin SS or Sβ[0] thalassemia,[11] reticulocyte count had a significant association with jet velocity but hemoglobin, lactate dehydrogenase and bilirubin concentrations did not. Humans exposed to chronic hypoxia have a tendency to develop pulmonary hypertension.[12] Patients with sickle cell disease may experience chronic hypoxia due to anemia, upper airway obstruction, chronic hemoglobin oxygen desaturation and repeated episodes of vaso-occlusive pain crisis or acute chest syndrome.[13-18] Hypoxia might, therefore, be a factor in the development of pulmonary hypertension in patients with sickle cell disease.

We conducted a multicenter study to determine prospectively the prevalence of elevated tricuspid regurgitant jet velocity in children and adolescents with sickle cell disease at baseline and to test the hypothesis that markers of hemolysis and hypoxia are both independently associated with increased jet velocity in patients with sickle cell disease.

Design and Methods

Study hypotheses

The prospectively determined study hypotheses were: (i) that elevated tricuspid regurgitant jet velocity occurs in 10-20% of children with sickle cell disease; (ii) that systolic blood pressure, hemolysis, hypoxia and degree of anemia are independent risk factors; (iii) that elevated jet velocity is associated with increased need for blood transfusions, risk of stroke, and risk of acute chest syndrome and with a shorter distance walked in the 6-minute walk test.

Enrollment of participants

The study was prospectively designed to enroll 600 patients with sickle cell disease and 100 controls and the present report presents the results of an analysis conducted shortly after the mid-point. Patients (n=310) 3 to 20 years of age with sickle cell disease confirmed by hemoglobin electrophoresis or high performance liquid chromatography were recruited at the Children's National Medical Center and Howard University Hospital, Washington DC, and University of Michigan, Ann Arbor Michigan. A 10% prevalence of elevated tricuspid regurgitant jet velocity was hypothesized and the analysis of 310 patients provided the power to observe this prevalence with a 95% confidence interval of 6.5% to 14.5%. Controls (n = 54), who were matched by age, sex and ethnicity to every sixth patient recruited at each institution, included relatives and acquaintances of participants, and could not have been hospitalized or have presented to the emergency room with an acute illness in the previous 3 weeks. Children with sickle cell trait (n=12) and hemoglobin C trait (n=5) qualified as controls. Patients were invited to participate on a consecutive basis, as they presented for routine outpatient care; no attempt was made to select them by known or perceived risk factors. At least 3 weeks had to have elapsed since hospitalization, emergency department or clinic visit for acute chest syndrome, a pain crisis, infection or other sickle cell disease-related complication. The study was approved by the Institutional Review Board of each participating institution and written informed consent was obtained for all participants.

Clinical evaluation

The medical history was recorded on a standard form designed for this study and in general did not distinguish between active and past problems. An unencouraged 6-minute walk test was conducted according to the guidelines of the American Thoracic Society.[19] Complete blood count and reticulocyte count were measured by a Beckman Coulter LH 750 Analyzer (Fullerton, CA, USA) at Howard University and the University of Michigan, and by a Sysmex 2100QC (Sysmex America, Inc., Mundelein, IL, USA) at the Children's National Medical Center. Serum biochemistry was evaluated by a Beckman Coulter Unicel DXC800 at Howard University, by a RXL 2 Max, Model 973626 (Dade-Behring, Inc., Dover, DE, USA) at the Children's National Medical Center and by a Siemens Advia 2400 (Deerfield, IL, USA) at the University of Michigan. Pulse oximetry was measured by a Criticare Model 506 Series (Waukesha, WI, USA) at Howard University, a Welch Allyn instrument (Beaverton, OR, USA) or a SureSigns VS3 No. 3000 (Philips Medical System, Andover, MA, USA) at the

Children's National Medical Center and by a Masimo Rad 8 Signal Extraction Pulse Oximeter at the University of Michigan.

Echocardiography

Transthoracic echocardiography was performed using a Philips Sonos 5500/7500 or iE33 (Philips Medical Systems, Best, Holland), Acuson Sequoa (Siemans Medical Systems, Mountain View, CA, USA), or General Electric VIVID 7 or VIVID I (General Electric, Milwaukee, WI, USA). All images were recorded digitally and subsequently reviewed on an offline digital work station. Cardiac images were obtained, measurements were performed, and the studies were interpreted according to the guidelines of the American Society of Echocardiography.[20]

Tricuspid regurgitation was assessed in the parasternal long and short-axes, and apical four-chamber views.[21] To standardize across the spectrum of ages and body sizes, left ventricular dimensions was expressed as a standard deviation below or above the mean for body surface area (z-score) and left ventricular mass was indexed to body surface area.[22] Left ventricular diastolic function was assessed by measuring the peak velocities of the mitral inflow E wave[23] and the tissue Doppler E wave at the basilar segments of the left ventricular free wall and interventricular septum. Left atrial (and left ventricular filling) pressures were estimated by calculating the ratio of the mitral inflow E wave to the tissue Doppler E wave.[24] Based on the mean ± 2 SD in the controls of this study, peak regurgitant jet velocities of 2.60 m/sec or more and mitral valve E/tissue Doppler E ratios of more than 9.22 were taken to be elevated. The echocardiograms were centrally reviewed. The study was designed to consider right-sided cardiac catheterization for participants found to have a jet velocity of 3.0 m/sec or more. Only one patient had a jet velocity in this range and the parents refused catheterization.

Statistical analysis

Statistical calculations were made by STATA 10.0 (College Station, TX, USA). Continuous variables were assessed for normality and skewed variables were transformed by the method that most closely approximated normality. Student's t test and the Kolmogorov-Smirnov non-parametric test were used to compare continuous variables between patients with sickle cell disease and control subjects, and Pearson's χ^2 test was used to compare dichotomous variables. Bonferroni adjustments were made for multiple comparisons and for interim analysis. A logistic regression model of tricuspid regurgitant jet velocity less than 2.60 m/sec versus 2.60 m/sec or more was employed to assess the independent associations of prospectively chosen variables with elevated jet velocity.

Principle component analysis was performed to compute a new variable – a hemolytic index – that explained the maximum variance among reticulocyte count and age- and site-adjusted values for lactate dehydrogenase, aspartate aminotransferase and total bilirubin concentrations.[25] Principal component analysis is useful for studying underlying mechanisms reflected in individual bio-logical measurements.[26] The hemolytic index is a normalized factor of the four hemolytic variables with mean of 0 and SD of 1.56. Because of the different reference ranges for the four markers of hemolysis among the three research sites, the computation of the hemolytic index was stratified by research site. The hemolytic index explained 61-64% of total variance of the four factors. It had correlations of r=0.82-0.90 with age-adjusted lactate dehydrogenase concentration, 0.74-0.88 with age-adjusted aspartate aminotransferase concentration, 0.76-0.82 with age-adjusted total bilirubin concentration and 0.57-0.77 with reticulocyte count.

Results

Clinical characteristics of sickle cell disease patients and control participants

Significant differences were observed in systolic blood pressure, measures of hemolysis (reticulocyte count, lactate dehydrogenase, aspartate aminotranserase, total bilirubin), creatinine, hemoglobin oxygen saturation, 6-minute walk test results and left ventricular internal diameter z-score, a reflection of cardiac output. Only 5% of patients versus 4% of controls had a mitral valve E/tissue Doppler E ratio greater than 9.22, a reflection of elevated left atrial pressure (p=0.6) (Table 1).

Prevalence of elevated tricuspid regurgitant jet velocity

The jet velocity could not be measured in 20 (6.5%) of the patients with sickle cell disease and in 6 (11.1%) of the controls (p=0.2). Among those in whom it could be measured, the jet velocity was significantly higher in the patients with sickle cell disease (Table 1, Figure 1A). Based on the mean ± 2SD jet velocity in controls, the upper limit of normal was 2.59 m/sec. Eleven percent (95% confidence interval of 7.3% to 15.8%) of patients with sickle cell disease had elevated jet velocities of 2.60 m/sec or higher. Figure 1b shows the frequency distribution of ages of the patients with elevated jet velocity and that one patient had elevated jet velocity at 3 years of age.

Clinical variables according to jet velocity category in patients with sickle cell disease

The proportion of patients with severe sickling phenotypes (hemoglobins SS, Sβ^0 thal or SD Los Angeles) was higher in the patients with elevated jet velocity. Histories of asthma, sleep apnea, severe pain episodes in the past year and hydroxyurea therapy did not differ significantly. The echocardiogram measurement of mitral valve E/tissue Doppler E ratio was higher (Table 2).

Potential risk factors for pulmonary hypertension according to jet velocity category

We prospectively hypothesized that systolic blood pressure, hemoglobin concentration and markers of hemolysis and hypoxia would be associated with elevated jet velocity (Table 3). Systolic blood pressure was higher in the patients with elevated jet velocity (p=0.030). Hemoglobin concentration, a potential inverse marker of both hemolysis and hypoxia, was lower (p=0.041). Each of the markers of hemolysis was higher in the patients with elevated jet

velocity. The most significant association was with the hemolytic index (Figure 1c; $p<0.0001$), derived by principal component analysis from the reticulocyte count and the concentrations of lactate dehydrogenase, aspartate aminotransferase and bilirubin. The hemoglobin oxygen saturation measured by pulse oximetry was significantly lower in the patients with elevated jet velocity (Figure 1d; $p=0.001$).

Logistic regression analyses of elevated tricuspid regurgitant jet velocity

In a logistic regression analysis of jet velocity that included four variables chosen prospectively at study design, the hemolytic index and an oxygen saturation of 98% or less had significant independent associations with elevated jet velocity while systolic blood pressure and hemoglobin concentration were not associated (Table 4). In a logistic regression analysis that included these same variables plus a measure of left ventricular diastolic dysfunction (mitral E/tissue Doppler E ratio), the hemolytic index and oxygen saturation of 98% or less continued to have significant associations with elevated jet velocity but neither hemoglobin concentration ($p=0.5$) nor mitral E/tissue Doppler E ratio ($p=0.2$) had significant associations (*full analysis not shown*).

Table 1. Clinical characteristics of study participants. Results are median and interquartile range unless otherwise indicated.

	N.	Sickle cell disease	N.	Control	*p* value*
Demographics and phenotype					
Age (years)	310	13 (8-16)	54	13 (8-17)	0.98[2]
Male sex, n. (%)	310	159 (51%)	54	26 (48%)	0.7[1]
Hemoglobin SS, n. (%)	295	214 (72.5%)	46	0	–
Hemoglobin Sβ⁰ thal, n. (%)	295	5 (1.7%)	46	0	–
Hemoglobin SD Los Angeles, n. (%)	295	2 (0.7%)	46	0	–
Hemoglobin SC, n. (%)	295	61 (20.7%)	46	0	–
Hemoglobin Sβ⁺ thal, n. (%)	295	13 (4.4%)	46	0	–
Medical history					
Asthma, n. (%)	298	76 (25%)	54	11 (21%)	0.4[1]
Obstructive sleep apnea, n. (%)	299	27 (9%)	54	0	0.022[1]
Physical examination					
Body mass index (kg/m²)	301	18.2 (16.1-21.8)	53	21.3 (18.0-23.4)	0.006[2]
Systolic blood pressure (mmHg)	310	112 (103-120)	54	113 (107-124)	0.06[3]
Diastolic blood pressure (mmHg)	310	64 (58-70)	54	67 (61-73)	0.022[3]
Laboratory tests					
Reticulocyte count (×10⁹/L)	295	212 (145-309)	42	42.7 (34.5-58.1)	<0.00001[2]
Reticulocytes (%)	294	7.0 (3.9-11.6)	43	1.0 (0.8-1.3)	<0.00001[2]
White blood cells (×10⁹/L)	302	10.3 (7.4-13.5)	53	6.0 (4.9-7.5)	<0.00001[2]
Hemoglobin (g/dL)	301	9.3 (8.1-10.6)	53	12.6 (12.0-13.7)	<0.00001[3]
Mean corpuscular volume (fL)	302	84 (78-91)	53	83 (78-86)	0.001[2]
Creatinine (mg/dL)	303	0.4 (0.3-0.6)	52	0.6 (0.5-0.9)	<0.00001[2]
Alkaline phosphatase (U/L)	303	174 (125-226)	53	228 (80-322)	<0.00001[2]
Asparate aminotransferase (U/L)	303	40 (29-53)	53	22 (18-29)	<0.00001[2]
Alanine aminotransferase (U/L)	303	32 (18-42)	53	31 (17-35)	0.062[2]
Total bilirubin (mg/dL)	303	2.1 (1.4-3.5)	53	0.5 (0.3-0.7)	<0.00001[2]
Lactate dehydrogenase (U/L)	295	370 (274-518)	51	185 (157-215)	<0.00001[2]
Echocardiogram					
Tricuspid regurgitant jet velocity (m/sec)	290	2.3 (2.1-2.5)	48	2.1 (1.9-2.3)	<0.00001[2]
Jet velocity of 2.6 m/sec or higher, n. (%)	290	32 (11.0%)	48	1 (2.1%)	0.05[3]
Left ventricular internal diameter z score	309	1.3 (0.2-2.1)	52	-0.5 (-1.4-0.3)	<0.00001[3]
Mitral valve E/tissue Doppler E	304	6.3 (5.5-7.4)	53	6.2 (5.5-7.3)	0.4[2]
Other measurements					
Oxygen saturation (%)	299	98 (96-99)	52	100 (99-100)	<0.00001[3]
Distance walked in 6-minute walk test (m)	245	448 (402-503)	49	497 (426-550)	0.011[2]

[1]χ^2 test; [2]Kolmogorov-Smirnov exact test; [3]Student's t test *p values <0.001 remained significant after Bonferroni's adjustment for multiple comparisons and interim analysis.

Potential clinical correlates of pulmonary hypertension according to jet velocity category

We prospectively hypothesized that elevated tricuspid regurgitant jet velocity would be associated with increased frequency of acute chest syndrome and stroke, and increased need for blood transfusions. Patients with elevated jet velocity had a medical history of higher numbers of acute chest syndrome episodes (p=0.012) and blood transfusions (p=0.017). A history of stroke was given by 19% of the patients with elevated jet velocity compared to 11% of those with normal jet velocity (p=0.2). We also hypothesized that elevated jet velocity would be associated with a shorter distance walked in 6 minutes, but this did not turn out to be the case. However, a decline in hemoglobin oxygen saturation during the 6-minute walk was greater in the children with elevated jet velocity (p=0.0002) (Table 5).

Discussion

We found the prospectively-determined prevalence of elevated tricuspid regurgitant jet velocity to be 11% among unselected pediatric patients with sickle cell disease under basal circumstances using a definition of 2.6 m/sec or more as derived from the controls. This prevalence is lower than the prevalences in most of the previous studies of pediatric sickle cell disease patients.[6,11] The present study was prospective, included children as young as 3 years of age with mild to severe sickle phenotypes, and avoided effects of exercise or acute vaso-occlusive crisis, known to be confounding factors for the determination of steady state jet velocity in patients.[27] In particular, patients who were hospitalized in the preceding 3 weeks could not be enrolled and this

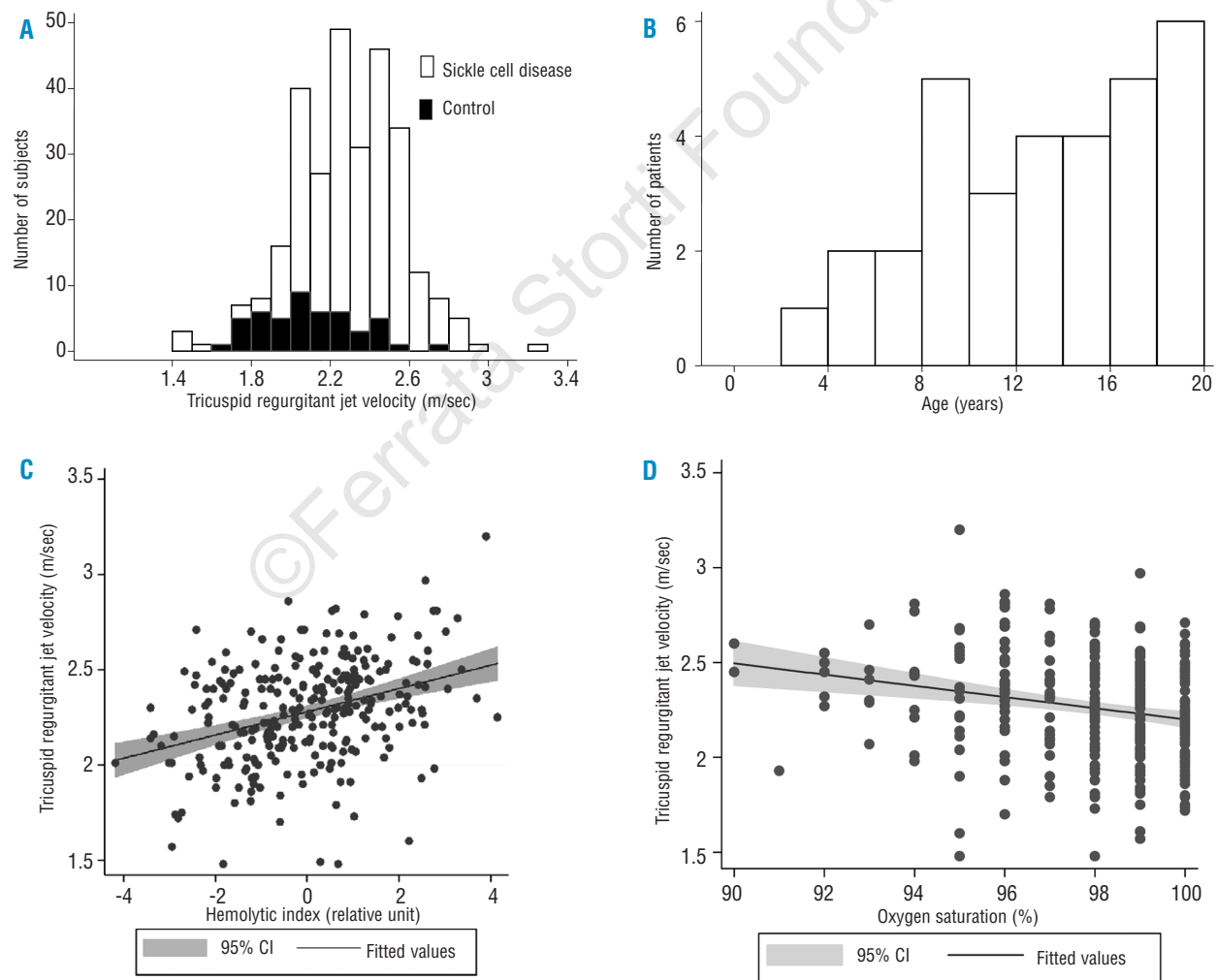Figure 1. (A) Distribution of tricuspid regurgitant jet velocity in sickle disease patients and controls. (B) Distribution of ages in sickle cell disease patients with elevated jet velocity. (C) Correlation of jet velocity and the hemolytic index in sickle cell disease patients (N=290, r=0.35, p<0.0001). (D) Correlation of jet velocity and oxygen saturation in sickle cell disease patients (N=287, r=-0.20, p=0.001)

tended to exclude children with frequent or prolonged hospitalizations, a group that may have a higher prevalence of elevated jet velocity. The threshold for elevated jet velocity was 0.1 m/sec higher than in most previous studies. A relatively high proportion of the participants were on hydroxyurea therapy, but this was not associated with elevated jet velocity.

As prospectively hypothesized, the results of this study support independent associations of markers of hemolysis and hypoxia with elevated tricuspid regur-

gitant jet velocity in children with sickle cell disease. Each of the clinical measurements that are recognized to reflect degree of hemolysis had associations with elevated jet velocity. A hemolytic index, derived by principal component analysis from lactate dehydrogenase, aspartate aminotransferase and bilirubin concentrations and reticulocyte count but not hemoglobin level, correlated with elevated jet velocity with a high degree of statistical significance. This correlation did not appear to merely reflect the degree of anemia, increased blood volume or cardiac output, because the hemolytic index was independently associated with increased odds of elevated jet velocity even after adjustment for hemoglobin concentration. Furthermore, the hemoglobin concentration did not have a significant independent effect on jet velocity in the logistic regression analyses, suggesting against a

Table 2. Clinical characteristics of sickle cell cases according to tricuspid regurgitant jet velocity category. Results are median and interquartile range unless otherwise indicated.

	N.	Tricuspid regurgitant jet velocity <2.6 m/sec	N.	Tricuspid regurgitant jet velocity ≥2.6 m/sec	p value*
Demographics and phenotype					
Age (years)	258	12 (8-16)	32	13 (6-17)	0.9[1]
Male sex, n. (%)	258	131 (51%)	32	19 (59%)	0.4[1]
Hemoglobin phenotype n. (%)	245		30		0.048[1]
SS	245	174 (71%)	30	27 (90%)	
Sβ⁰ thal	245	4 (2%)	30	0	
SD Los Angeles	245	2 (1%)	30	0	
SC	245	56 (22%)	30	2 (7%)	
Sβ⁺ thal	245	9 (4%)	30	1 (3%)	
Medical history					
Priapism, n. (%)	121	23 (19%)	18	2 (11%)	0.4[2]
Asthma, n. (%)	249	63 (25%)	31	8 (26%)	0.2[2]
Obstructive sleep apnea, n. (%)	250	21 (8%)	31	3 (10%)	0.9[2]
Number of severe pain episodes in the past 12 months	249	0 (0-2)	31	1 (0-4)	0.1[2]
Hydroxyurea treatment at the present time, n. (%)	250	103 (41%)	31	15 (48%)	0.95[2]
Chronic transfusion program, n. (%)	248	30 (12%)	31	8 (26%)	0.042[2]
Laboratory tests					
Mean corpuscular volume (fL)	252	84 (78-91)	30	89 (82-98)	0.06[2]
White blood cells (×10⁹/L)	252	10.1 (7.3-13.4)	30	11.2 (8.7-13.8)	0.2[2]
Creatinine (mg/dL)	253	0.4 (0.3-0.6)	30	0.5 (0.4-0.5)	0.4[2]
Alanine aminotransferase (U/L)	253	33 (18-42)	30	33 (22-48)	0.002[2]
Alkaline phosphatase (U/L)	253	177 (128-226)	30	143 (101-218)	0.6[2]
Echocardiogram					
Tricuspid regurgitant jet velocity (m/sec)	258	2.3 (2.0-2.4)	32	2.7 (2.6-2.8)	–
Left ventricular internal diameter z score	257	1.2 (0.2-2.1)	32	1.5 (0.9-2.4)	0.2[2]
Mitral valve E/tissue Doppler E	254	6.3 (5.5-7.4)	32	6.5 (6.0-8.1)	0.023[2]

[1]Unadjusted p value; [2]p value adjusted for age and study site; *P values <0.002 remained significant after Bonferroni's adjustment for multiple comparisons.

Table 3. Distribution of markers prospectively hypothesized to be associated with pulmonary hypertension according to tricuspid regurgitant jet velocity category. Results are median and interquartile range; analyses adjusted for age and study site.

	N.	Tricuspid regurgitant jet velocity <2.6 m/sec	N.	Tricuspid regurgitant jet velocity ≥2.6 m/sec	p value*
Marker of systemic blood pressure					
Systolic blood pressure (mmHg)	258	112 (103-120)	32	118 (109-128)	0.030
Marker of anemia					
Hemoglobin (g/dL)	251	9.3 (8.2-10.6)	30	9.1 (7.6-10.0)	0.041
Markers of hemolysis					
Reticulocytes (%)	244	6.8 (3.9-11.5)	30	9.6 (4.4-14.60)	0.006
Reticulocyte count (×10⁹/L)	245	210 (148-309)	30	248 (120-352)	0.050
Lactate dehydrogenase (U/L)	247	356 (270-496)	29	457 (342-570)	0.009
Total bilirubin (mg/dL)	253	2.1 (1.3-3.3)	30	2.8 (2.3-5.3)	0.005
Asparate aminotransferase (U/L)	253	38 (29-52)	30	47 (35-64)	0.001
Hemolytic index (relative unit)**	237	-0.08 (-1.22-0.91)	28	0.91 (-0.10-2.50)	0.0002
Marker of hypoxia					
Oxygen saturation (%)	250	99 (97-99)	30	97 (96-98)	0.005

*p values <0.003 remained significant after Bonferroni's adjustment for multiple comparisons and interim analysis. **Derived from reticulocyte count and lactate dehydrogenase, total bilirubin, and asparate aminotransferase concentrations.

Table 4. Independent associations of prospectively chosen clinical variables with elevated tricuspid regurgitant jet velocity in logistic regression analysis.

	Odds ratio (95% confidence interval)	p value
Hemolytic index (increase of 2 SDs)	4.5 (1.5-14.4)	0.009
Oxygen saturation ≤98%	3.2 (1.1-9.5)	0.028
Systolic blood pressure (10 mm Hg increase)	1.3 (0.9-2.0)	0.09
Hemoglobin concentration (increase of 1 g/dL)	1.1 (0.8-1.5)	0.6

Area Under Curve for ROC is 77% and p value for Homsher-Lemeshow goodness of fit is 0.7.

Table 5. Prospectively chosen potential clinical correlates of pulmonary hypertension according to tricuspid regurgitant jet velocity category. Results in n. (%) unless otherwise stated; analyses adjusted for age and study site.

	N.	Tricuspid regurgitant jet velocity <2.6 m/sec	N.	Tricuspid regurgitant jet velocity ≥2.6 m/sec	p value*
Medical history					
Acute chest syndrome (two or more episodes in lifetime)	258	33 (13%)	32	9 (28%)	0.012
Stroke	249	27 (11%)	31	6 (19%)	0.2
Blood transfusion (>10 units in lifetime)	247	45 (18%)	31	12 (39%)	0.017

*p values ≤0.017 remained significant after Bonferroni's adjustment for multiple comparisons.

	N.	Tricuspid regurgitant jet velocity <2.6 m/sec	N.	Tricuspid regurgitant jet velocity ≥2.6 m/sec	p value*
Six-minute walk test					
Distance (median and interquartile range in meters)	202	441 (399-494)	29	457 (433-514)	0.2
Decline in hemoglobin oxygen saturation during the test	202	65 (32%)	28	19 (68%)	0.0002

p values ≤0.013 remained significant after Bonferroni's adjustment for multiple comparisons and interim analysis.

strong primary role of anemia in addition to the association with hemolysis. This observation is consistent with the view that a hemolytic vasculopathy contributes to pulmonary hypertension in sickle cell disease.[28,29]

Low hemoglobin oxygen saturation is associated with markers of hemolysis[16,30,31] and increased risk of stroke in sickle cell disease.[32] In this study, lower oxygen saturation correlated significantly with increased jet velocity even after adjustment for the hemolytic index. This finding is consistent with our study hypothesis that hypoxia itself, in addition to hemolysis, contributes to sickle cell-related pulmonary hypertension.

Left ventricular diastolic dysfunction is associated with mortality in sickle cell disease and may contribute to elevated pulmonary artery pressures,[33] and elevated mitral valve E/tissue Doppler E ratio is a marker of left ventricular diastolic dysfunction.[24] Only 5.3% of the patients with sickle cell disease had left ventricular diastolic dysfunction, as defined by a mitral valve E/tissue Doppler E ratio of greater than 9.22, and there was not a significant association of this ratio with elevated jet velocity in multivariate logistic regression analysis. Thus, left ventricular diastolic dysfunction seemed to be a relatively minor factor in the development of elevated jet velocity in this study.

As prospectively hypothesized, we obtained histories of significantly increased numbers of acute chest syndrome episodes and units of blood transfused in the patients with elevated jet velocity in this study.

Although almost twice as many patients with elevated jet velocity had a history of stroke, this difference was not statistically significant. About one-third of the children and adolescents in our data set who received more than ten transfusions were being treated for stroke, and it is conceivable that similar mechanisms are at play in cerebral and pulmonary vasculopathy.[34] It is also possible that chronic transfusion therapy for stroke may have prevented the development of elevated jet velocity in some patients.

In contrast to our hypothesis and results of studies in adults, the elevated jet velocity in the children and adolescents in this study was not associated with a shorter distance covered in the 6-minute walk test. The reason for this lack of association is not clear, but it may be that limitation in the 6-minute walk test occurs only after systolic pulmonary artery pressure has been elevated for a certain length of time. Another potentially adverse physiological parameter related to the 6-minute walk test, a decrease in oxygen saturation of hemoglobin during the walk, was significantly more common in the patients with elevated jet velocity. Other studies have shown that oxygen desaturation during the 6-minute walk test is associated with mortality in patients with primary pulmonary hypertension[35] and reflects pulmonary disease severity in those with secondary pulmonary hypertension.[36]

The associations of high jet velocity with a history of acute chest syndrome or blood transfusions and with oxygen desaturation during the 6-minute walk test suggest that children with elevated jet velocity may be at high risk of increased complications in later decades of life. Further investigations are, therefore, needed to clarify the clinical consequences of elevated jet velocity in children and adolescents, and to determine whether early intervention may prevent morbidity and early mortality.

Authorship and Disclosures

CPM participated in designing the study, data collection and preparing the manuscript; CS participated in data collection and preparing the manuscript; AC participated in data collection and preparing the manuscript; SR participated in data collection and preparing the manuscript; GE participated in data collection and preparing the manuscript; ND participated in data collection; OO participated in data collection and preparing the manuscript; DD participated in data collection and preparing the manuscript; MN participated in data analysis and preparing the manuscript; GJK participated in designing the study and preparing the manuscript; MTG participated in designing the study and preparing the manuscript; OLC participated in designing the study, data collection and preparing the manuscript; VG participated in designing the study, data collection, data analysis and prepared the manuscript.

The authors reported no potential conflicts of interest.

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