








RESEARCH ARTICLE

Tricuspid regurgitation velocity and other biomarkers of mortality in children, adolescents and young adults with sickle cell disease in the United States: The PUSH study

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Abstract

In the US, mortality in sickle cell disease (SCD) increases after age 18-20 years. Biomarkers of mortality risk can identify patients who need intensive follow-up and early or novel interventions. We prospectively enrolled 510 SCD patients aged 3-20 years into an observational study in 2006-2010 and followed 497 patients for a median of 88 months (range 1-105). We hypothesized that elevated pulmonary artery systolic pressure as reflected in tricuspid regurgitation velocity (TRV) would be associated with mortality. Estimated survival to 18 years was 99% and to 25 years, 94%. Causes of death were known in seven of 10 patients: stroke in four (hemorrhagic two, infarctive one, unspecified one), multiorgan failure one, parvovirus B19 infection one, sudden death one. Baseline TRV ≥ 2.7 m/second (>2 SD above the mean in age-matched and gender-matched non-SCD controls) was observed in 20.0% of patients who died vs 4.6% of those who survived ($P = .012$ by the log rank test for equality of survival). The baseline variable most strongly associated with an elevated TRV was a high hemolytic rate. Additional biomarkers associated with mortality were ferritin ≥ 2000 $\mu\text{g/L}$ (observed in 60% of patients who died vs 7.8% of survivors, $P < .001$), forced expiratory volume in 1 minute to forced vital capacity ratio (FEV1/FVC) < 0.80 (71.4% of patients who died vs 18.8% of survivors, $P < .001$), and neutrophil count $\geq 10 \times 10^9/\text{L}$ (30.0% of patients who died vs 7.9% of survivors, $P = .018$). In SCD children, adolescents and young adults, steady-state elevations of TRV, ferritin and neutrophils and a low FEV1/FVC ratio may be biomarkers associated with increased risk of death.

1 | INTRODUCTION

Sickle cell disease (SCD) is one of the most common monogenic diseases in the United States and worldwide. Characterized by the homozygous Glu6Val mutation in *HBB*, the beta-globin gene, SCD is associated with progressive multisystem organ damage and early mortality. In recent decades, interventions such as newborn screening, penicillin prophylaxis, immunizations, screening for risk of stroke with transcranial Doppler, and disease-modifying therapies such as hydroxyurea and chronic blood transfusion have led to a decline in mortality and morbidity for children and adolescents with SCD, especially those living in developed countries.¹ A Dallas, TX cohort study of 711 newborns diagnosed with sickle cell anemia between 1983 and 2007 reported that 95% survived the first decade of life and that the patterns of mortality are evolving.² Mortality increases in adolescents and young adults after transition to adult care had causes including acute chest syndrome, multi-organ failure syndrome, sepsis and other complications.^{3,4} Studies of SCD cohorts from Europe have reported sepsis, stroke and acute anemia as the leading causes of mortality in children with SCD.^{1,5-7}

Despite advances in care, mortality in children, adolescents and young adults with SCD in the US continues to be unacceptably high. In one recent US study, mortality up to age 18 years was 6.1% in patients with sickle cell anemia and 1.6% with milder forms of SCD.³ It is important to identify young patients at high risk of death so that early interventions can be developed to reduce this high mortality. The goal of the present analysis was to determine the frequency and causes of death in a prospective, multi-institutional contemporary cohort of children, adolescents and young adults with SCD and to determine biomarkers associated with mortality in this cohort.

2 | METHODS

The Pulmonary Hypertension and the Hypoxic Response study (PUSH) is a multi-institutional observational investigation that prospectively followed a cohort of children, adolescents and young adults with SCD. The study postulated that elevated systolic pulmonary pressure as reflected in an elevated tricuspid regurgitation velocity (TRV) would be a risk factor for mortality.

2.1 | Clinical evaluation of SCD patients and controls

The study enrolled 510 patients with SCD and 75 control subjects 3 to 20 years of age from 2006 to 2010 at four institutions. The institutional review boards of all four participating institutions approved the study protocol. The subjects and/or their parent or legal guardian provided written informed consent to participate in accordance with the Declaration of Helsinki. The trial is registered with clinicaltrials.gov (NCT00495638). The controls were matched by age, sex and ethnicity

to approximately every sixth patient enrolled. SCD was confirmed by hemoglobin electrophoresis, high performance liquid chromatography or genetic analysis in some cases. The SCD 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. Before enrollment, at least 3 weeks had elapsed since hospitalization, emergency department or clinic visit for a pain crisis, acute chest syndrome, infection or other SCD related complication.⁸ Baseline clinical assessments including routine blood work, echocardiography and pulmonary function testing were performed.⁸⁻¹³ Standardized medical history forms that included questions regarding stroke, asthma and blood transfusions were filled out by a clinical research nurse who interviewed the patient or a parent and confirmed the findings by discussion with the patient's physician and by chart review. At the time of enrollment in PUSH, it was the policy of all participating institutions to perform transcranial Doppler (TCD) screening on all eligible SCD patients. Follow-up for survival was available for 497 of the participants at a median of 88 months (range of 1-105 months) after enrollment.

2.2 | Definition of cutoff values for biomarkers

A TRV ≥ 2.7 m/second is more than 2 SD above the mean value in the non-SCD controls in this study as described in the results section. A serum ferritin of ≥ 2000 $\mu\text{g/L}$ was used to define transfusional iron overload in SCD patients by the Multi-Center Iron Overload Research Group.¹⁴ A forced expiratory volume in 1 minute to forced vital capacity ratio (FEV1/FVC) $< 80\%$ has been used to define pathological airway obstruction in childhood asthma.^{15,16} An absolute neutrophil count $\geq 10 \times 10^9$ per liter is a cut-off that suggests the possibility of a bacterial infection in otherwise healthy children and adolescents.¹⁷ N-terminal pro-brain natriuretic peptide (NT-proBNP) ≥ 160 ng/L has been used to define an elevated value in numerous previous studies.¹⁸⁻²¹ Hemoglobin oxygen saturation $\leq 95\%$ has been suggested to identify SCD children at risk for hypoxia.²² Glomerular filtration rate (GFR) < 90 mL/minutes/1.73 m² is the cutoff that defines Stage I decline in GFR by Kidney Disease: Improving Global Outcomes (KDIGO) guidelines.²³ Systolic blood pressure > 115 mmHg is the cut-off that requires further evaluation or intervention in early adolescents.²⁴ A left atrial diameter ≥ 3.68 cm, a lateral transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity (E/e') ratio ≥ 8.84 , and a left ventricular internal diameter in diastole (LVIDD) z-score ≥ 1.8 are more than 2 SD above mean values in the non-SCD controls of this study.

2.3 | Statistical analysis

We applied principal component analysis to create a hemolytic component from age and site-adjusted values of aspartate amino transferase

(AST), lactate dehydrogenase (LDH), total bilirubin and reticulocyte count. Statistical comparisons were made using the Fisher exact test for categorical variables and the Student *t* test for continuous variables (with normally transformed data). The independent relationship of serum ferritin concentration with baseline variables was assessed with multiple linear regression. The survival of patients over the study period was calculated by Kaplan-Meier estimate. The association of historical variables and biomarkers with mortality was determined by the log rank test for equality of survivor function. We did not perform multi-variate analysis of mortality using the identified risk factors because there were too

few deaths to justify this approach. Analyses were performed in Stata 15.0 (StataCorp, College Station, TX).

3 | RESULTS

3.1 | Comparison of SCD patients and controls

The genotypes of the 497 SCD patients with follow-up information were HbSS in 372 (74.8%), HbSC in 91 (18.4%), Hb S β^+ -thalassemia in

TABLE 1 Clinical characteristics of SCD patients and controls. Results expressed in median and interquartile range unless otherwise indicated. Statistical comparison by *t* test or Fisher's exact test

	N	SCD	N	Controls	P
Demographics and genetics					
Age (years)	497	12 (7-16)	75	14 (9-17)	.021
Female gender in no. (%)	497	243 (48.9%)	75	40 (53.3%)	.5
Alpha-thalassemia in no. (%)	466		66		.5
No alpha-globin gene deletion		316 (67.5%)		47 (71.2%)	
Single alpha-globin deletion		138 (29.5%)		19 (28.8%)	
Double alpha-globin deletion		14 (3.0%)		0 (0%)	
Medical history					
Asthma	493	119 (24.1%)	75	14 (18.7%)	.38
Stroke	492	52 (10.6%)	75	0	.001
Acute chest syndrome or pneumonia	492	239 (48.6%)	75	2 (2.7%)	<.001
Physical examination					
Body mass index (kg/m ²)	494	17.8 (15.7-21.5)	74	21.3 (17.8-24.3)	<.0001
Systolic blood pressure (mmHg)	480	112 (104-119)	73	116 (109-125)	.001
Diastolic blood pressure (mmHg)	480	64 (59-71)	73	68 (62-75)	.008
Echocardiogram and PFT					
Tricuspid regurgitation velocity (m/sec)	441	2.3 (2.1-2.5)	67	2.1 (2.0-2.3)	<.0001
Left ventricular internal diameter z score	469	1.3 (0.2-2.2)	71	-0.5 (-1.3-0.1)	<.0001
Left atrial diameter (cm)	477	2.9 (2.5-3.3)	73	2.7 (2.4-3.0)	.002
Lateral E/e'	476	6.4 (5.5-7.4)	73	6.1 (5.4-7.0)	.036
FEV1/FVC (%)	156	84 (80-89)	34	85 (83-90)	.30
Laboratory tests					
Oxygen saturation (%)	472	98 (97-99)	71	99 (99-100)	<.0001
eGFR (Schwartz)* (mL/min/1.73 m ²)	475	135 (111-165)	73	95 (80-110)	<.0001
Absolute neutrophils (10 ⁹ per L)	478	4.5 (3.3-6.8)	74	2.7 (2.0-3.7)	<.001
Hemoglobin (g/L)	480	91 (80-104)	75	126 (121-138)	<.0001
Reticulocytes (%)	463	7.6 (4.0-11.6)	63	1.0 (0.8-1-1.4)	<.0001
Ferritin (μ g/L)	420	178 (85-620)	70	43 (28-62)	<.0001
Alanine aminotransferase (U/L)	492	31 (18-42)	75	22 (14-34)	.0005
Asparate aminotransferase(U/L)	480	42 (31-54)	75	21 (17-29)	<.0001
Total bilirubin (μ mol/L)	480	37.6 (23.9-56.4)	75	8.6 (5.1-12.0)	<.0001
Lactate dehydrogenase (U/L)	437	398 (287-561)	73	180 (152-209)	<.0001
NT-proBNP (ng/L)	456	85 (47-155)	68	36 (20-48)	<.0001

Abbreviations: FEV1/FVC, forced expiratory volume in 1 minute to forced vital capacity ratio.

*eGFR (estimated glomerular filtration rate) = 0.413 \times (height in cm/creatinine in mg/dL).

18 (3.6%), Hb S β^0 -thalassemia in 9 (1.8%), and other genotypes including Hb SD_{Los Angeles} and Hb SO_{Arab} in 7 (1.4%). The SCD patients and control subjects are compared in Table 1. The BMI, systemic blood pressure, and oxygen saturation were lower in the SCD patients, while TRV and LVIDD z score were higher. Among laboratory tests, creatinine and hemoglobin were lower in the SCD patients while neutrophils, reticulocytes, ferritin, AST, total bilirubin, LDH and NT-proBNP were higher. Twenty four percent of SCD patients vs 19% of healthy non-SCD controls had a history of asthma. Blood eosinophil counts were significantly higher in subjects with history of asthma (mean 0.43×10^9 per liter vs 0.32×10^9 per liter, $P = .022$) and, in the subgroup of participants with pulmonary function tests (N = 190), FEV1/FVC was significantly lower in subjects with history of asthma (median 0.82 vs 0.86, $P < .001$).

3.2 | Definition of elevated TRV in SCD patients

Most studies of children and adolescents with SCD have defined an elevated TRV to be ≥ 2.5 m/second,²⁵⁻²⁹ but this cut-off represents 2 SD above the mean value in adult rather than pediatric non-SCD controls.³⁰ For the present study, we performed echocardiography in 75 control children without SCD, 67 of whom had a measurable TRV. The control children and adolescents were studied during the same time period as the SCD subjects and TRV was determined using the same equipment and by the same investigators. As previously published in a subgroup of the PUSH study,⁸ we used TRV values greater than 2 SD above the mean in the control subjects to define elevated TRV in patients with SCD. The mean ± 2 SD TRV in the control subjects was 2.1 ± 0.5 m/second. Therefore, we defined elevated TRV to be ≥ 2.7 m/second. Twenty-three (4.9%) of the SCD subjects had an elevated TRV at baseline using this definition.

3.3 | Serum ferritin in SCD patients

The serum ferritin concentration in SCD patients ranged from 7-12,700 ng/mL. In linear regression analysis of serum ferritin in the

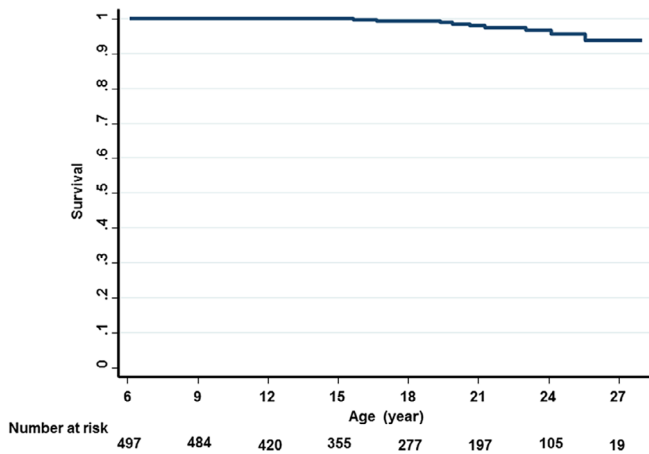


FIGURE 1 Kaplan-Meier estimated survival by age of follow-up [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Baseline characteristics of 10 SCD patients who died

Gender	Hemoglobin genotype	Age at recruitment (yrs)	Age at death (yrs)	Hydroxyurea therapy	Transfusion program	Iron chelation	History of stroke	TRV (m/sec)	Ferritin ($\mu\text{g/L}$)	Neutrophils (10^9 per L)	FEV1/FVC (%)	Circumstance of death
Female	SC	9	17	No	No	No	No	2.3	54	4.0	79	Parvovirus B19 infection and severe anemia
Male	SC	13	16	No	No	No	No	2.3	147	2.0	Not done	Sudden death at home
Female	SS	17	21	Yes	Yes	No	Yes	2.4	12 696	11.8	Not done	Unkown
Male	SS	17	20	Yes	Yes	No	No	2.9	1043	5.1	76	Multi-organ failure during admission for VOC
Male	SS	18	19	Yes	Yes	Yes	Yes	Not able to be measured	9296	6.4	80	Thrombotic stroke
Female	SS	18	24	No	No	No	No	1.9	165	7.2	73	Unkown
Male	SS	19	21	No	Yes	Yes	Yes	1.5	3568	10.9	89	Hemorrhagic stroke
Male	SS	20	23	Yes	No	Yes	Yes	2.0	2164	4.1	Not done	Stroke, unspecified
Female	SS	20	28	No	Yes	Yes	No	2.5	5923	20.7	72	Thrombotic stroke
Male	SS	20	26	No	Yes	Yes	Yes	2.9	5923	9.0	78	Unkown

PUSH data set, transfusion history, absolute granulocyte count and the liver function test, ALT, accounted for 56% of the variation in serum ferritin. Transfusion history reflecting the iron burden of transfused red blood cells had the strongest independent relationship with higher serum ferritin concentration (partial $r = 0.61$, $p < 0.000001$). Higher absolute granulocyte count reflecting inflammation (partial $r = 0.17$, $p = 0.000006$) and higher ALT reflecting hepatocellular dysfunction (partial $r = 0.11$, $p = 0.003$) also had significant independent relationships with serum ferritin concentration.

3.4 | Estimated survival and causes of death in SCD patients

Hydroxyurea was prescribed for 36.7% of the SCD subjects at baseline and 14.3% were on a chronic transfusion program. The mean corpuscular volume (median 92 fL vs 81 fL; $P < .001$) was significantly

greater and the absolute neutrophil count (median $4.2 \times 10^9/L$ vs $4.8 \times 10^9/L$; $P = .03$) was significantly lower in patients receiving hydroxyurea, suggesting compliance with the regimen at baseline. A history of stroke was obtained in 52 patients with a median age of 16 years (range 4-20 years); 29 (55.8%) were on a chronic transfusion program and 21 (40.4%) were on hydroxyurea therapy. This history of stroke seems high compared to the Dallas newborn cohort² and the Cooperative Study of Sickle Cell Disease Cohort,³¹ possibly reflecting that our study was conducted at tertiary care centers where patients with special management needs are referred. There was a history of recurrent acute chest syndrome in 73 of the patients, 41 (56.2%) of whom were treated with hydroxyurea at baseline.

The median follow up was 88 months (interquartile range 78-95 months). Ten of 497 patients (2.0%) died during the follow-up period. Eight had hemoglobin SS and two had hemoglobin SC. The median age at the time of death was 21 years (range 16-28 years). The overall survival at 18 years of age was 99% (95%CI: 98%-99%)

TABLE 3 Relationship of baseline medical history with death. *P* values were calculated from log rank test for equality of survival

	N	Alive	N	Died	<i>P</i> (log rank test)
Chronic transfusion program, no. (%)	479	64 (13.4%)	10	6 (60.0%)	<.001 ^a
History of stroke, no. (%)	482	47 (9.8%)	10	5 (50.0%)	.001 ^a
Age ≥ 13 years, no. (%)	487	227 (46.6%)	10	9 (90.0%)	.008 ^a
History of asthma, no (%)	483	113 (23.4%)	10	6 (60.0%)	.009 ^a
3+ Severe pain episodes past year, no. (%)	487	91 (18.6%)	10	5 (50.0%)	.013 ^a
α -thalassemia gene deletion, no. (%)	459	147 (32.0%)	9	5 (55.6%)	.09
History of ACS or pneumonia, no (%)	482	232 (48.1%)	10	7 (70.0%)	.17
Hemoglobin SS genotype, no (%)	487	364 (74.7%)	10	8 (80.0%)	.70
Male gender, no (%)	487	248 (50.9%)	10	6 (60.0%)	.64
Hydroxyurea treatment, no (%)	483	177 (36.6%)	10	4 (40.0%)	.88

^aSignificant after adjusting for multiple hypothesis testing using False Discovery Rate < 0.05 .

TABLE 4 Relationship of baseline TRV and other biomarkers with death. *P* values were calculated from log rank test for equality of survival

	N	Alive	N	Died	<i>P</i> (log rank test)
TRV ≥ 2.7 m/sec, no. (%)	462	21 (4.6%)	10	2 (20.0%)	.012 ^a
Ferritin ≥ 2000 $\mu\text{g/L}$, no. (%)	410	32 (7.8%)	10	6 (60.0%)	<.001 ^a
FEV1/FVC < 0.80 , no (%)	149	28 (18.8%)	7	5 (71.4%)	<.001 ^a
Absolute neutrophils $\geq 10 \times 10^9$ per L, no. (%)	468	37 (7.9%)	10	3 (30.0%)	.018 ^a
NT-proBNP ≥ 160 (ng/L), no (%)	446	103 (23.1%)	10	5 (50.0%)	.044
eGFR (Schwartz) ≤ 90 mL/min/1.73 m ²	465	33 (7.1%)	10	2 (20.0%)	.08
Oxygen Saturation $\leq 95\%$, no (%)	462	67 (14.5%)	10	3 (30.0%)	.24
Systolic blood pressure > 115 mm Hg, no. (%)	470	165 (35.1%)	10	4 (40%)	.72
Hemolytic component* over the mean, no. (%)	403	206 (51.1%)	10	5 (50%)	.83
Left atrial diameter ≥ 3.68 cm, no. (%)	457	52 (11.4%)	10	0 (0%)	.23
Lateral E/e', ≥ 8.84 no. (%)	454	36 (7.9%)	10	0 (0%)	.37
LVIDD z score ≥ 1.8 , no. (%)	459	170 (37.0%)	10	3 (30.0%)	.60

Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-prohormone B-type natriuretic peptide; TRV, Tricuspid regurgitation velocity.

^aSignificant after adjusting for multiple hypothesis testing using False Discovery Rate < 0.05 .

^bPrincipal component 1 of reticulocyte count, LDH, AST and total bilirubin.

and at age 25 years was 94% (95%CI: 87%-97%) (Figure 1). The baseline age, age of death and cause of death in the 10 patients who died are summarized in Table 2. The causes of death in the hemoglobin SS patients were de novo stroke in one, recurrent stroke in three, multi-organ failure in one and unknown in three. The causes of death in the hemoglobin SC patients were parvovirus B19 infection in one and sudden death at home in one. Seven of the patients who eventually died were on a chronic transfusion program and/or received treatment with hydroxyurea at the time of enrollment.

3.5 | Relationship of baseline medical history to death in SCD patients

The prevalence of baseline medical history variables according to death status are shown in Table 3. Older age, a chronic transfusion program, a history of stroke, a history of asthma and 3+ severe pain episodes in the past year were the strongest baseline medical history variables associated with increased risk of death. As expected there was a high degree of overlap among patients on a chronic transfusion program and those with a history of stroke: 41.7% of the patients on a chronic transfusion program had a history of stroke vs 5.6% of those not on such a program. Hemoglobin SS genotype and baseline hydroxyurea treatment status were not significantly associated with mortality in this cohort.

3.6 | Relationship of baseline TRV to risk of death in SCD patients

Baseline TRV ≥ 2.7 m/second was observed in 20.0% of the patients who died vs 4.6% of survivors ($P = 0.012$ by the log rank test for equality of survival) (Table 4). Death occurred in two (8.7%) of 23 patients with TRV ≥ 2.7 m/second compared to eight (1.8%) of 449 patients with lower TRV values.

3.7 | Relationship of other biomarkers with death in SCD patients

Additional biomarkers associated with mortality were ferritin ≥ 2000 $\mu\text{g/L}$, FEV1/FVC < 0.80 and absolute neutrophil count $\geq 10 \times 10^9$ per L (Table 4). Death occurred in six (15.8%) of 38 patients with ferritin ≥ 2000 $\mu\text{g/L}$ vs four (1.1%) of 382 patients with lower ferritin values ($P < .001$ by the log rank test for survival). The FEV1/FVC ratio was determined in a subset of 156 patients. Death occurred in 5 (15.2%) of 33 patients with FEV1/FVC < 0.80 vs five (4.1%) of 123 patients with higher FEV1/FVC values ($P < .001$). Death occurred in three (7.5%) of 40 patients with absolute neutrophil count $\geq 10 \times 10^9$ /L vs seven (1.6%) of 428 patients with lower neutrophil counts ($P = .018$). Left atrial diameter, lateral E/e', LVIDD z-score, NT-proBNP and a hemolytic component were not significantly associated with mortality in these analyses.

3.8 | Relationship of baseline variables with the prognostic biomarker categories

The strongest association of TRV ≥ 2.70 m/second was with a high degree of hemolysis as shown by the hemolytic component ($P < .001$). The strongest associations of ferritin ≥ 2000 $\mu\text{g/L}$ were with older age, a chronic transfusion program, a history of stroke, and an elevated neutrophil count ($P < .001$). FEV1/FVC < 0.8 was associated with a history of asthma ($P < .001$) and a higher eosinophil count ($P = .006$). The strongest associations of neutrophil $\geq 10 \times 10^9$ /L were with a higher degree of hemolysis as reflected in the hemolytic component, an elevated serum ferritin, and a chronic transfusion program ($P < .001$).

4 | DISCUSSION

In this report we describe certain biomarkers associated with mortality, predominantly after transition to adulthood, in a cohort of children, adolescents and young adults with SCD who were followed prospectively in the Pulmonary Hypertension and the Hypoxic Response in SCD (PUSH) study. Stroke was the cause of death in four of 10 cases and in four of seven in whom the cause of death was known. There was a history of previous stroke in three of the four who died of stroke. Three of the four patients who died of stroke were on a chronic transfusion program and two of the three with a past history of stroke were on a chronic transfusion program. Baseline historical variables that were significantly associated with death over a median of almost 7 years of observation of this cohort included a chronic blood transfusion program, a history of stroke, older age, and histories of asthma and three or more severe pain episodes in the past year.

In the PUSH cohort we were particularly interested in biomarkers of mortality, with a focus on the TRV. The echocardiography-determined TRV is considered to be a valid estimate for the systolic pulmonary artery pressure.^{32,33} An elevated TRV can reflect pulmonary arterial hypertension or an increased pulmonary wedge pressure secondary to pulmonary venous hypertension or cardiac diastolic dysfunction as reflected in the echocardiogram parameters, left atrial diameter and the lateral E/e' ratio.³⁴ The TRV, left atrial diameter and lateral E/e' were all increased in the SCD patients in this study, as shown in Table 1. A number of studies have defined an elevated TRV to be values ≥ 2.5 m/second, which is more than 2 SD above the mean in non-SCD adult controls, and have reported that an elevated TRV by this definition is associated with increased mortality in SCD adults.^{30,35-37} Some studies have found that a higher TRV cut-off of 3.0 m/second is more strongly associated with mortality than the 2.5 m/second cut-off.^{38,39} The definition of elevated TRV of ≥ 2.5 m/second has been adopted for studying children with SCD,²⁵⁻²⁹ although it is not clear that the same definition of elevated TRV would apply to both adults and children. The PUSH study enrolled a non-SCD control group contemporaneous with the SCD patients, and we utilized this control group to define an elevated TRV to be ≥ 2.7 m/second, or more than 2 SD above the mean in non-SCD

controls. The TRV values in this range were found in 4.9% of the SCD patients and proved to be significantly associated with death. An elevated hemolytic rate was the strongest baseline variable associated with TRV ≥ 2.70 m/second in the present study. Left atrial diameter and lateral E/e' did not have significant positive associations with TRV ≥ 2.70 m/second.

Three additional biomarkers measured at baseline proved to be even more significantly associated with mortality, namely serum ferritin ≥ 2000 $\mu\text{g/L}$ (found in 9.1% of the patients), FEV1/FVC < 0.8 (found in 21.2% of the patients), and absolute neutrophils $\geq 10^9/\text{L}$ (found in 8.4% of the patients). The serum ferritin was ≥ 300 $\mu\text{g/L}$, the upper limit of the reference range in adults, in 38.8% of the SCD patients in this study and it was ≥ 2000 $\mu\text{g/L}$ in 9.1% of them. Serum ferritin is an indirect indicator of iron stores⁴⁰ that increases in relation to the amount of blood transfused in sickle cell disease and other conditions.⁴¹⁻⁴³ Serum ferritin also increases with inflammation or with hepatocellular damage in the absence of increased iron stores.⁴⁴ In sickle cell anemia patients that elevated serum ferritin concentration reflects increased iron stores because of the frequency of blood transfusions for non-blood loss indications.⁴¹ In the present study, 71% of subjects with ferritin ≥ 2000 $\mu\text{g/L}$ were on a chronic transfusion program while 10% of subjects with a lower serum ferritin were on such a program. Overall in the PUSH cohort, serum ferritin appears to be a composite marker that reflects increased iron stores related to blood transfusions, the presence of inflammation (independent relationship with absolute neutrophil count), and the potential organ toxicity of iron overload (independent relationship with increased ALT).

Low FEV1/FVC is a marker of airway obstruction that affects about one in five children with SCD.^{13,45} Reduction in lung growth,⁴⁶ higher hemolysis¹³ and/or anemia,⁴⁷ and increased pulmonary capillary blood volume⁴⁸ may contribute to airway obstruction in SCD children. A history of asthma and higher blood eosinophil counts were associated with a low FEV1/FVC ratio in the present study, consistent with a potential allergic component as well. Our present observation of a relationship between low FEV1/FVC ratio and death is in contrast to another recent study,⁴⁵ but is consistent with reports that measures of lower airway obstruction are associated with more frequent hospitalizations for pain or acute chest syndrome in children with SCD⁴⁹ and with higher mortality in adults with SCD.³⁵

In SCD, intravascular hemolysis, free heme, inflammation and ischemia-reperfusion injury accelerate neutrophil activation and binding to red bloods, platelets and endothelium.⁵⁰⁻⁵² Neutrophil activation is believed to play an important role in vaso-occlusive crisis.⁵³ In our study, a neutrophil count $\geq 10 \times 10^9/\text{L}$ was a significant prognostic factor of mortality, and markers of hemolysis and iron overload were associated with higher neutrophil count.

Hydroxyurea use at baseline was not significantly different between the patients who died and survived in the PUSH study. Although lower neutrophil count and higher mean corpuscular volume suggested compliance with hydroxyurea at baseline, we did not evaluate adherence to hydroxyurea therapy during the study. Other limitations are that only one TRV measurement was made at inclusion into the study, that the cohort was recruited starting at age 3 years and the

mortality rate reflects this characteristic of the study, that the exact cause of death was not known for all of the subjects who died, and that we did not focus on certain potential risk factors for mortality, such as a past history of splenic sequestration and hypercoagulation. It would have been ideal to follow the PUSH cohort for a longer period of time but we were unable to secure funding to do so.

In summary, the estimated survival of the PUSH cohort of children, adolescents and young adults with SCD was 99% to 18 years and this declined to 94% at 25 years. Stroke was the most common cause of death. Elevated systolic pulmonary artery pressure as reflected in TRV ≥ 2.7 m/second, high serum ferritin concentration, low FEV1/FVC and high neutrophil count were biomarkers of increased risk of death, predominantly after transition to adulthood. These biomarkers are easily obtainable and potentially can help identify patients in need of novel new therapies as well as established approaches such as the use of hydroxyurea to prevent vaso-occlusive complications, the need for blood transfusions and the use of iron chelation therapy for iron overload.

CONFLICT OF INTEREST

None to report.

AUTHOR CONTRIBUTIONS

VRG and MN performed the statistical analysis. OLC, MTG, GJK, VRG and CPM designed the study. MA, AC, OLC, DSD, GE, ND, VRG, LLJ, CPM, SN, SR, CS and JGT collected data. All authors participated in writing the manuscript and approved it.

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