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# Natural History Of Tricuspid Regurgitant Jet Velocity And A New Association With Proteinuria In Children With Sickle Cell Disease

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NATURAL HISTORY OF TRICUSPID REGURGITANT JET VELOCITY  
AND A NEW ASSOCIATION WITH PROTEINURIA  
IN CHILDREN WITH SICKLE CELL DISEASE

A Thesis Submitted to the  
Yale University School of Medicine in  
Partial Fulfillment of the Requirements for the  
Degree of Doctor of Medicine

By

Suzanne Jaqueth Forrest

Yale University School of Medicine, Class of 2013

**NATURAL HISTORY OF TRICUSPID REGURGITANT JET VELOCITY AND A NEW ASSOCIATION WITH PROTEINURIA IN CHILDREN WITH SICKLE CELL DISEASE.** Suzanne J. Forrest, Judith L. Carbonella, Farzana D. Pashankar, Section of Hematology and Oncology, Department of Pediatrics, Yale University, School of Medicine, New Haven, CT.

Given the morbidity and mortality associated with pulmonary hypertension and progressive renal failure in sickle cell disease, recognition of both organ dysfunctions during childhood should allow for improved management of patients and early treatment for both. The definitive diagnosis of pulmonary hypertension is made on cardiac catheterization, however measurement of tricuspid regurgitant jet velocity (TRV) on echocardiogram can be used to estimate pulmonary artery systolic pressures and can be used as a screening modality for pulmonary hypertension. Pulmonary hypertension is defined as  $TRV \geq 2.5$  m/sec and occurs in 16-30% of children with sickle cell disease on screening echocardiograms. Two studies were conducted on a cohort of children from the sickle cell program at Yale New Haven Children's Hospital. The first study was conducted to determine the longitudinal natural history of TRV in untreated children with sickle cell disease (N=100 patients). A detailed retrospective chart review was conducted on all sickle cell patients screened with echocardiograms from June 2005 to November 2012. Patients aged 6-21 years were included, 87 with HbSS and 13 with S $\beta^0$  thalassemia. At baseline, 67% of patients (N=67) had normal TRV <2.5 m/sec and 33% of patients (N=33) had elevated TRV  $\geq 2.5$  m/sec on screening echocardiogram. Follow up echocardiograms were available for 82% of patients (82 of 100), and the median follow up time was 3.59 years. On follow up, 61.40% (N=35) of patients with baseline normal TRV continued to have normal TRV on all subsequent echocardiograms, whereas 38.60% (N=22) of patients had at least 1 echocardiogram with an elevated TRV. Risk

factors associated with TRV conversion were baseline low O<sub>2</sub> saturation, low hemoglobin and high reticulocyte count (all P <0.05). On follow up, 32% (N=25) of patients with elevated TRV at baseline continued to have elevated TRV on all subsequent echocardiograms, whereas 68% (N=17) of patients had at least 1 echocardiogram with normal TRV without intervention. Risk factors associated with persistent TRV elevation were baseline elevated TRV, low hemoglobin, and high white blood count (all P <0.05). Three of the 100 patients died during the follow up period, one with elevated TRV. A second study was conducted to determine whether elevated TRV was associated with proteinuria in children with sickle cell disease (N=85 patients). A detailed chart review was conducted on sickle cell patients screened with both echocardiograms and urinalysis from June 2005 to July 2010. Longitudinal data from subsequent echocardiograms and urine analyses were also collected. On initial echocardiograms, 32.9% (N=28) had an elevated TRV  $\geq 2.5$  m/sec. At initial screening 7.14% (N=2) of these patients with elevated TRV had proteinuria, compared to only 1.75% (N=1) without elevated TRV (P=0.25). On follow up, 19.08% of repeat urinalysis showed proteinuria in patients with elevated baseline TRV compared to 12.35% in patients with normal baseline TRV (P=0.04). Our first study revealed that patients with elevated TRV should be followed longitudinally prior to initiating treatment, as many patients will have normalization of TRV without intervention. The first study also showed that patients with high hemolytic rate as evidenced by low hemoglobin and high reticulocyte count are at high risk of TRV conversion and should be monitored closely. Our second study found a new association between elevated TRV and proteinuria in children with sickle cell disease followed longitudinally.

## **ACKNOWLEDGEMENTS**

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## INTRODUCTION

### *Overview of Sickle Cell Disease*

Approximately 90,000 people live with sickle cell disease in the United States with an estimated 1 in 2,500 children is born with a form of sickle cell disease each year (1, 2). Sickle cell hemoglobinopathy is a heterogeneous set of diseases characterized by the presence of at least one hemoglobin  $\beta$  globin gene affected by the sickle mutation (2). Hemoglobin, the protein within a red blood cell that carries oxygen, is composed of four globin chains, two  $\alpha$  globin and two  $\beta$  globin chains. Sickle cell disease occurs when an offspring inherits either two sickle  $\beta$  globin genes or one sickle  $\beta$  globin gene in combination with another  $\beta$  globin chain mutation (2, 3). Approximately 60-65% of sickle cell disease patients are homozygous for the sickle mutation and have HbSS disease. The most common compound heterozygote state, affecting approximately 25-30% of patients with sickle cell disease is sickle hemoglobin-C disease. Another form of sickle cell disease, representing approximately 5-10% of patients with hemoglobinopathies, is caused by coinheritance of a  $\beta$  thalassemia mutation with the sickle mutation which results in  $S\beta^{\circ}$  thalassemia (2). Patients with HbSS disease and  $S\beta^{\circ}$  thalassemia, who completely lack normal  $\beta$  globin production, are generally more severely affected than patients with SC disease (3). For the purposes of this thesis, sickle cell disease refers to patients with HbSS and  $S\beta^{\circ}$  thalassemia.

Sickle cell disease can affect any organ in the body. The principal clinical manifestations of patients with sickle cell disease are hemolytic anemia, episodes of vaso-occlusion, and increased susceptibility to infection (4, 5). These pathologic

processes are a result of deoxygenation of the hemoglobin S molecule, which forms an intracellular polymer aggregate, ultimately causing the red cell to sickle (6). The sickle cell mutation, a single nucleotide change in the coding region of the  $\beta$  globin gene, results in an amino acid change from glutamic acid to valine. The amino acid change from a hydrophilic glutamic acid to a hydrophobic valine residue is what causes protein polymerization in a deoxygenated state (2). The sickled red blood cells can obstruct the microvasculature and, unlike normal red blood cells are more likely to adhere to the vascular endothelium (7, 8). Both of these processes contribute to and ultimately cause vaso-occlusion, which results in organ injury and pain.

The prognosis for children born with sickle cell disease has drastically changed over the last three decades. In 1967 more than 50% of children with sickle cell disease died before the age of 20 (9, 10). With the advent of newborn screening, pneumococcal prophylaxis, and better comprehensive care, at least 90% of children born with sickle cell disease now survive past their 20<sup>th</sup> birthday (8, 11). A study of 3764 patients with HbSS disease published in the *New England Journal of Medicine* by Platt et al. in 1994 reported a mean life expectancy of 42 years for males and 48 years for females with HbSS disease (12). With more sickle cell disease patients surviving into adulthood, the frequency of end organ complications has increased (11, 13). Thus, it is important to understand the natural history of end organ dysfunction in children with sickle cell disease and develop an approach to identifying children early who may be at increased risk of end organ disease. With early identification and potential intervention, we may be able to decrease the morbidity and mortality these children would face as adults.



### *Elevated Tricuspid Regurgitant Jet Velocity*

Pulmonary hypertension occurs in approximately one third of adults with sickle cell disease and is associated with significantly increased morbidity and mortality (14, 15). A clinical diagnosis of pulmonary hypertension can be hard to ascertain given the non-specific symptoms, which include fatigue, shortness of breath, and increased heart rate. The definitive diagnosis of pulmonary hypertension is made by right heart catheterization with measurement of exact pulmonary artery pressures; however, this is an invasive and expensive procedure with associated risks. Cardiac catheterization is therefore not a suitable method for screening for pulmonary hypertension. Pulmonary artery systolic pressures can be estimated by measurement of tricuspid regurgitant jet velocity (TRV) on echocardiogram with the use of the modified Bernoulli equation (16). Without structural obstruction to pulmonary blood flow, the right ventricular systolic pressure equals the pulmonary artery systolic pressure. Studies have found that estimates of pulmonary artery systolic pressure by Doppler echocardiography are well correlated with more precise measures during cardiac catheterization (17, 18). The specificity of elevated TRV to diagnose pulmonary hypertension increases as TRV increases. A recent study in a small number of adults with sickle cell disease (N=25) found that the specificity of TRV of 2.51 m/sec in diagnosing pulmonary hypertension was 18.8%, whereas with a TRV of 2.88 m/sec the specificity increased to 81% (19). Doppler echocardiography can thus be used as a noninvasive way to identify patients who have or may be at risk for developing pulmonary hypertension.

In adults with sickle cell disease, elevated TRV is the strongest predictor of mortality versus other markers (20). The first study of TRV in adults with sickle cell

disease was published in 1994 in a retrospective longitudinal analysis of 60 patients (21). Over a mean follow up period of 22 months, the study found that mortality was significantly greater in patients with pulmonary hypertension, defined in the study as TRV  $\geq 2.5$  m/sec. Remarkably, 5 of 12 (42%) of the patients with pulmonary hypertension died during the follow up versus only 4 of 48 (8%) of the patients without pulmonary hypertension. The study found a statistically significant increase in mortality in the patients with TRV  $\geq 2.5$  m/sec (P=0.03). In addition, multivariate analysis revealed that elevated TRV was an independent predictor of death, whereas other variables including older age, lower hematocrit, and cardiac symptoms were not independent predictors of death (21). Furthermore, a prospective cross-sectional study published in 2004 by Gladwin and colleagues showed that elevated TRV ( $\geq 2.5$  m/s) had a prevalence of 32%, and predicted approximately a ten-fold increased risk for early mortality in adults with sickle cell disease (16).

Post mortem studies suggest that the pathological changes that result in pulmonary hypertension in adults are chronic and may start during childhood (22-24). One study published in 2001 by Adedeji and colleagues looked at pulmonary pathology in 12 sickle cell patients at autopsy (23). The objective of the study was to investigate the potential role that pulmonary embolism or small vessel arterial thrombosis play in the development of pulmonary hypertension in sickle cell disease (23). The authors found that the most common pulmonary findings were thrombotic arteriopathy of small pulmonary arteries, which were present in 9 of the 12 patients. In all 9 patients they found right ventricular hypertrophy, and in 8 of the 9 cases they found small arterial changes that were consistent with pulmonary hypertension. The authors commented that it was possible the findings

in the small arteries were from recurrent small pulmonary embolisms, but that the histology of fresh thrombi likely showed sickled red blood cells within the fibrinous developing clots (23). They concluded that there appears to be a progression from early pulmonary alterations in childhood to obliterative vasculopathy in adults that is irreversible.

#### *Elevated TRV in Children With Sickle Cell Disease*

Multiple pilot studies of children with sickle cell disease showed that they had a similar prevalence of elevated TRV as adults. Studies in children report a 16–30% prevalence of elevated TRV  $\geq 2.5$  m/sec on screening echocardiograms (25-27). Early diagnosis of increased pulmonary artery pressures and treatment during childhood may help to reverse these changes and reduce the morbidity and mortality correlated with elevated TRV in adults with sickle cell disease. Further characterization of the natural history and progression of TRV in children with sickle cell disease is critical to early diagnosis and treatment.

A prospective study published in 2008 by Dr. Pashankar and colleagues found that low oxygen saturation, high reticulocyte count, and high platelet count were significantly associated with elevated TRV (25). While there have been multiple cross-sectional studies, there have not been any studies to assess the natural history of TRV in children with sickle cell disease followed longitudinally for many years. It is not known what happens to patients with and without elevated TRV on baseline screening echocardiogram over time. Studying the cohort of sickle cell children followed at Yale offered us the ability to track TRV over time, and try to identify what potential factors

place children at risk of having persistent elevation of TRV, or converting from normal to elevated TRV over time. Longitudinal analysis of this patient population can help to guide the timing of performing certain screening tests, and how to predict which children may need to be closely monitored for the development of end organ dysfunction over time.

#### *Treatment of Elevated Tricuspid Regurgitant Jet Velocity*

There is no effective treatment for adults with sickle cell disease who have pulmonary hypertension, and it is thought to be irreversible (28, 29). Sildenafil, a selective pulmonary vasodilator, was investigated in a double blind placebo controlled study as a potential treatment for elevated TRV in adults with sickle cell disease. Unfortunately, the trial was stopped early because of an increase in acute pain episodes in the treatment group (35%) as compared to the placebo group (14%) (29). Other treatments are currently being investigated, but there remain no FDA approved drugs for adults with sickle cell disease related pulmonary hypertension. There have been limited studies in children as to whether elevated TRV is potentially reversible with treatment. It is possible that initiation of treatment earlier in the course of elevated TRV may be more effective.

Hydroxyurea is the only FDA approved medication for the treatment of sickle cell disease in the United States (30, 31). Hydroxyurea is used commonly to treat sickle cell disease patients for indications including frequent vasoocclusive crises, acute chest syndrome, priapism, and severe anemia (30, 32, 33). Hydroxyurea treatment is thought to be effective by increasing the amount of fetal hemoglobin, which then reduces red

blood cell adhesion and decreases hemolysis (34-36).

In 2008, Dr. Pashankar and her team published a small pilot study, which assessed the effect of hydroxyurea on elevated TRV in children with sickle cell disease. Five patients who had persistent elevated TRV  $>2.5\text{m/s}$  were treated with hydroxyurea. Echocardiograms were repeated every 6-12 months for a mean follow up of 24 months. TRV decreased from  $3.16\pm 0.27$  to  $2.64\pm 0.20$  ( $P=0.017$ ) after 3-6 months and to  $2.42\pm 0.26$  mmHg ( $P=0.002$ ) after 9-12 months on treatment (37). Based on these preliminary findings, a prospective clinical trial was started at three centers including Yale New Haven Children's Hospital.

#### *Pathogenesis and Study of Outcomes Associated with Elevated TRV*

The exact pathogenesis of pulmonary hypertension is unknown and likely multifactorial, with hemolysis induced nitric oxide depletion and vascular dysfunction likely playing major role. Potential mechanisms for the development of pulmonary hypertension include hypoxemia, repeated thrombotic embolism, iron overload, and intravascular and parenchymal sequestering of red blood cells (16, 38-41). Recent studies have highlighted the potential importance of inflammation in the pathogenesis of pulmonary hypertension (42, 43).

Patients with sickle cell disease are at risk not only of pulmonary complications, but dysfunction of other organs, as the disease is systemic and can impact any organ in the body. Multiple studies tried to identify risk factors associated with elevated TRV in children with sickle cell disease, though few have looked at clinical correlates or outcomes associated with elevated TRV. The questions remain: are children with

elevated TRV more likely to develop other end organ dysfunction? Do children with elevated TRV, like their adult counterparts, have an increased mortality risk?

### *Sickle Cell Nephropathy*

Like pulmonary hypertension, the development of sickle cell nephropathy is of significant concern in adults with sickle cell disease. Renal failure affects approximately 12–21% of adults with sickle cell disease, and is associated with decreased survival (11, 44, 45). Approximately 25% of patients with sickle cell disease and end stage renal disease die within the first year of starting dialysis (46). Renal involvement in sickle cell disease is first characterized by a reversible urinary concentrating deficit, then a fixed concentrating defect leading to polyuria (45, 47-49). Over time, glomerular hypertrophy with a high GFR and increased renal blood flow leads to focal segmental glomerulosclerosis, microalbuminuria/proteinuria, hematuria, and ultimately renal failure in many patients (45, 50-52).

One of the earliest manifestations of sickle cell nephropathy is microalbuminuria/proteinuria (53). The onset of proteinuria has been reported in early childhood, and studies in children report a prevalence of microalbuminuria of 16-26% and a prevalence of proteinuria of 6.2% (54-56). One study published in 2007 by McKie et al. in children with sickle cell disease aged 3-20 reported that lower hemoglobin and older age were significant associated with the presence of microalbuminuria or proteinuria (54). The mean hemoglobin with microalbuminuria or proteinuria (N=37) was 7.71 g/dL versus 8.54 g/dL in children without any urinary protein (N=154) (P=0.0004) (54). Additional risk factors significantly associated with proteinuria in children with sickle cell disease reported from

other studies include type of sickle cell disease, history of acute chest syndrome, and stroke (55, 56).

#### *Elevated TRV and Proteinuria*

A study published in 2010 in adults with sickle cell disease found a positive association between albuminuria and elevated TRV. The study reported that TRVs were significantly higher (mean 3.15 m/s) in patients with macroalbuminuria (N=15) when compared to patients with microalbuminuria or normalalbuminuria (N=58) (mean 2.45 m/s) (P=0.00068) (57). Prior to our study, there was no data in children on the potential association between kidney dysfunction manifested by proteinuria and elevated pulmonary artery pressures as measured by TRV. We thus conducted our final study to determine if elevated pulmonary artery systolic pressure, as estimated by echocardiogram, was associated with proteinuria in children with sickle cell disease.

## **PURPOSE**

To determine the natural history of TRV in untreated children with sickle cell disease, and to determine if elevated TRV is associated with proteinuria in children with sickle cell disease.

## **HYPOTHESES**

Untreated children with elevated TRV ( $\geq 2.5$  m/sec) will continue to be more likely to have elevated TRV overtime. A proportion of children with sickle cell disease who previously had normal TRV will go on to develop elevated TRV. Children with elevated TRV are more likely to have or develop proteinuria.

## **SPECIFIC AIMS**

1. To determine whether untreated children with elevated TRV ( $\geq 2.5$  m/sec) are likely to have elevated TRV when followed longitudinally.
2. To determine what proportion of children with sickle cell disease who previously had normal TRV will go on to develop elevated TRV, and what risk factors may be associated.
3. To determine if elevated TRV is associated with proteinuria.



## METHODS

The author of this thesis was not involved in designing the yearly assessments, which were conducted on children with sickle cell disease followed by the Yale New Haven Hospital Pediatric Sickle Cell Program since 2005. The author did not collect the primary data used for the studies, which was collected during clinic visits. Pediatric Cardiologists were responsible for reading and interpreting the echocardiograms and determining the TRV (if applicable). The author was responsible for abstracting primary data from the paper and electronic medical records for each patient involved in the studies, creating a de-identified database of all of clinical information, and performing all statistical analysis completed as part of the studies. All aspects of the investigations were supervised by Farzana Pashankar, MD.

### **1. Patient Population**

The patient population for the two studies included children from the sickle cell program at Yale New Haven Children's Hospital, which follows children with sickle cell disease in southern Connecticut. Patients in this program are seen at least annually for a comprehensive assessment, during which a detailed clinical history, a complete physical examination, and annual screening laboratory tests are performed. The clinical history included documenting history of 1) acute chest syndrome 2) stroke 3) chronic blood transfusions 4) treatment with hydroxyurea. The laboratory tests done during these visits are those recommended by the American Academy of Pediatrics (58). The laboratory tests included complete blood count, reticulocyte count, direct and total bilirubin levels,

lactate dehydrogenase, serum urea nitrogen, creatinine level, and urinalysis. Vital signs were also measured during the visits including heart rate, blood pressure, respiratory rate, and oxygen saturation by pulse oximetry. Transcranial Dopplers were performed on patients with HbSS or S $\beta^{\circ}$  thalassemia between the ages of 2 and 14 years old as part of screening for stroke. Starting in 2005, screening echocardiograms were conducted as part of the annual comprehensive assessment for children older than 6 years with HbSS or S $\beta^{\circ}$  thalassemia. Echocardiograms were performed as an outpatient visit at the Pediatric Echocardiography Laboratory when patients were in a steady state, at least 2 weeks after hospital admissions for vasoocclusive crises. The studies were approved by the institutional review board (IRB) at Yale University School of Medicine.

## **2. Echocardiography**

Two-dimensional Doppler echocardiography was performed for all patients using the Philips IE33 ultrasound system (Philips Medical Systems, Bothell, WA) or the Acuson Sequoia Ultrasound System (Siemens Medical Solutions, Malvern, PA). Selection of appropriate transthoracic transducer was made during the evaluation. Cardiac measurements were performed according to the guidelines of American Society of Echocardiography. TRV was measured by pulsed-wave and continuous-wave Doppler echocardiography where applicable. Multiple views (apical 4-chamber, parasternal short axis, parasternal long axis) were obtained to record optimal tricuspid Doppler flow signals, and a minimum of 5 sequential signals were recorded. The right ventricular to right atrial systolic pressure gradient was calculated using the modified Bernoulli equation ( $4 \times V^2$ ). Pulmonary artery systolic pressure was quantified by adding the

Bernoulli-derived right ventricular systolic peak pressure to the estimated mean right atrial pressure (5 mm Hg). Pulmonary artery diastolic pressure was estimated by measurement of the end diastolic velocity of the pulmonary insufficiency jet by similar Doppler techniques. A peak TRV of 2.5 m/sec equating to a pulmonary artery pressure of at least 30 mmHg was considered to be elevated. Peak TRV of 2.5 m/sec has been previously used in studies of pulmonary hypertension in both adults and children with sickle cell disease (16). Patients with no measurable TRV or TRV <2.5 m/sec were considered to have normal pulmonary artery pressures, and these patients were categorized as having normal TRV.

### **3. Study of the Natural History of TRV in Children with Sickle Cell Disease**

For this study, charts including data from June 2005 to November 2012 were reviewed. Patients with HbSS and S $\beta^{\circ}$  thalassemia who had screening echocardiograms were included in the study. Patients with pulmonary stenosis or other structural obstruction to pulmonary blood flow were excluded. Patients who were on chronic transfusions or hydroxyurea prior to their first echocardiogram were included in the study. Follow up echocardiogram data was censored for patients who started hydroxyurea or chronic transfusions after their first screening echocardiogram. If a patient subsequently stopped taking the medication, their echocardiograms continued to be censored and were not included in the analysis. Data collected included baseline demographics, type of sickle cell disease, clinical history, echocardiogram results, and laboratory results. Longitudinal data from all subsequent echocardiograms were collected. A database was created with de-identified data from each visit. The database

included baseline clinical and laboratory data in addition to follow up echocardiograms and treatment data (for example, dates of hydroxyurea treatment). For oxygen saturation data, when there was a range, the average was taken (for example, O<sub>2</sub> sat 90-92% was recorded in the database as 91%). For echocardiogram data, when there was a range for TRV, the lowest number was taken (for example, TRV 2.4-2.7 was recorded in the database as 2.4). Data was analyzed using Chi square test for categorical variables and student t-test for continuous variables. P value less than 0.05 was considered statistically significant.

#### **4. Study to Determine if Elevated TRV is Associated with Proteinuria**

For this study, charts were reviewed from including data from June 2005 to July 2010. Patients who had a screening echocardiogram and a urinalysis within one year were included in the study. Patients with pulmonary stenosis or other structural obstruction to pulmonary blood flow were excluded. Baseline data collected included demographics, type of sickle cell disease, clinical history, echocardiogram results, laboratory results, and urinalysis. Urinalysis was sent for measurement of protein, which was quantified from 0 to 3+. Urine protein 1+ equaled 30 mg/dl, 2+ equaled 100 mg/dl, and 3+ equaled 300 mg/dl in our laboratory. Urine protein of 1+ to 3+ was considered positive, urine protein of 0 was considered negative. Longitudinal data from all subsequent echocardiograms and urinalyses for each subject were also collected. On follow up, urinalysis was repeated at least annually. Echocardiograms were repeated annually on patients with a baseline TRV  $\geq 2.5$  m/sec, and every two years on patients with normal baseline TRV. Data was analyzed using Chi square test for categorical

variables and student t-test for continuous variables. P value less than 0.05 was considered statistically significant.

## RESULTS

### Study 1. Natural history of TRV in Children with Sickle Cell Disease

#### *Baseline Characteristics*

A total of 100 patients aged 6-21 years were included, 87 with HbSS and 13 with S $\beta$ <sup>o</sup> thalassemia. The male to female ratio was 1.22:1. The mean age at initial screening echocardiogram was 11.82 years (standard deviation was 4.16 years). On initial screening echocardiograms, 67 of 100 patients (67%) had normal TRV <2.5 m/sec and 33 of 100 patients (33%) had elevated TRV  $\geq$ 2.5 m/sec. Table I shows the baseline characteristics of patients with and without elevated TRV on initial screening echocardiogram (ECHO).

**TABLE I. Characteristics of Patients With and Without TRV on Baseline Screening Echocardiogram**

| Patient Characteristics                        | Baseline TRV           |                              | P value |
|--|------------------------|------------------------------|---------|
|  | <2.5 m/sec<br>(n = 67) | $\geq$ 2.5 m/sec<br>(n = 33) |         |
| Age at initial ECHO, mean $\pm$ SD, y          | 11.60 $\pm$ 3.83       | 12.33 $\pm$ 4.80             | 0.21    |
| Male gender, n (%)                             | 37 (55.22)             | 18 (54.55)                   | 0.88    |
| Genotype, n (%)                                |                        |                              |         |
| SS   | 55 (82.09)             | 32 (96.97)                   |         |
| S $\beta$ <sup>o</sup>                         | 12 (17.91)             | 1 (3.03)                     |         |
| H/O acute chest syndrome, n (%)                | 23 (34.33)             | 14 (42.42)                   | 0.57    |
| H/O stroke, n (%)                              | 9 (13.43)              | 1 (3.03)                     | 0.20    |
| Hydroxyurea, n (%)                             | 15 (22.39)             | 6 (18.18)                    | 0.82    |
| Blood transfusion, n (%)                       | 11 (16.42)             | 2 (6.06)                     | 0.25    |
| Systolic BP, mean $\pm$ SD, mmHg               | 108.37 $\pm$ 7.63      | 108.63 $\pm$ 8.91            | 0.44    |
| Oxygen saturation, mean $\pm$ SD               | 97.19 $\pm$ 2.38       | 95.83 $\pm$ 3.80             | 0.01*   |
| Hemoglobin, mean $\pm$ SD                      | 8.70 $\pm$ 1.32        | 8.38 $\pm$ 1.01              | 0.12    |
| Hematocrit, mean $\pm$ SD                      | 25.05 $\pm$ 4.30       | 23.43 $\pm$ 3.40             | 0.07    |
| Reticulocyte count, mean $\pm$ SD              | 10.09 $\pm$ 4.78       | 12.22 $\pm$ 5.66             | 0.04*   |
| WBC count ( $\times 10^3/\mu$ ), mean $\pm$ SD | 12.79 $\pm$ 5.04       | 12.48 $\pm$ 3.83             | 0.38    |
| Platelet count, mean $\pm$ SD                  | 414.78 $\pm$ 139.69    | 508.82 $\pm$ 140.76          | 0.001*  |
| LDH, mean $\pm$ SD                             | 602.71 $\pm$ 245.43    | 637.50 $\pm$ 230.38          | 0.38    |
| Creatinine, mean $\pm$ SD, mg/dl               | 0.51 $\pm$ 0.15        | 0.47 $\pm$ 0.16              | 0.09    |

\*P < 0.05.

As shown in Table I, low daytime oxygen saturation, high reticulocyte count, and high platelet count were significantly associated with elevated TRV (all  $P < 0.05$ ). In the group with elevated TRV, 32 of 33 (96.97%) of the patients had HbSS disease, whereas only one of 33 (3.03%) of the patients had  $S\beta^{\circ}$  thalassemia. In the group with normal TRV, 12 of 67 patients (17.91%) had  $S\beta^{\circ}$  thalassemia. History of acute chest syndrome and history of stroke were not significantly different between patients with and without elevated TRV. Similarly, other laboratory measures including hemoglobin, hematocrit, white blood cell count, lactate dehydrogenase, and creatinine were not statistically different between the two groups.

#### *Longitudinal Follow Up of Echocardiograms*

Repeat echocardiograms were available for 82% of patients (82 of 100) in the study. The total follow up time was 283.23 years and the median follow up time per patient was 3.59 years (range 0.11-7.32 years). The total number of repeat echocardiograms was 230 with an average number of 2.80 follow up echocardiograms per patient. As shown in Table II, 61.40% (N=35) of patients with baseline normal TRV continued to have normal TRV on all subsequent echocardiograms, whereas 38.60% (N=22) of patients had at least 1 echocardiogram with an elevated TRV. Follow up echocardiograms were available for 25 of 33 patients (75.76%) with elevated TRV at baseline. On follow up, 32% (N=8/25) of patients continued to have elevated TRV on all subsequent echocardiograms, whereas 68% (N=17) of patients had at least 1 echocardiogram with normal TRV without intervention. Patients with a TRV  $\geq 2.5$  m/sec on initial echocardiogram had a significantly higher chance of having repeat

echocardiograms with elevated TRV compared to patients with normal baseline TRV ( $P < 0.0001$ ). Patients with a TRV  $\geq 2.5$  m/sec on initial echocardiogram also had a significantly higher chance of having persistent elevation of TRV on all follow up echocardiograms ( $P = 0.04$ ).

**TABLE II. Follow Up ECHOs in Patients With and Without Elevated TRV on Baseline Screening ECHO**

| Patient characteristics  | Baseline TRV           |                              | P value  |
|--|------------------------|------------------------------|----------|
|  | <2.5 m/sec<br>(n = 67) | $\geq 2.5$ m/sec<br>(n = 33) |          |
| Patients with follow up ECHO, n (%)  | 57 (85.07)             | 25 (75.76)                   |          |
| Total number of years  | 212.28                 | 70.95                        |          |
| Average number of years per patient  | 3.72                   | 2.84                         |          |
| Total number of ECHOs  | 140                    | 90                           |          |
| Number of ECHOs per patient  | 2.46                   | 3.60                         |          |
| Number of patients with TRV <2.5 m/sec on all repeat ECHOs (%)             | 35 (61.40)             | 3 (12.00)                    | <0.0001* |
| Number of patients with TRV $\geq 2.5$ m/sec on at least 1 repeat ECHO (%) | 22 (38.60)             | 22 (88.00)                   |          |
| Number of patients with TRV $\geq 2.5$ on all repeat ECHOs (%)             | 6 (10.53)              | 8 (32.00)                    | 0.04*    |
| Number of repeat echocardiograms with TRV < 2.5 m/sec, n (%)               | 100 (71.4)             | 29 (32.2)                    | <0.0001* |
| Number of repeat echocardiograms with TRV $\geq 2.5$ m/sec, n (%)          | 40 (28.6)              | 61 (67.8)                    |          |

\* $P < 0.05$ .

Based on the follow up echocardiogram data, patients were divided into four groups. The objective of creating the four groups was to 1) determine potential factors associated with TRV conversion from normal to elevated 2) identify potential factors associated with persistent TRV elevation. The following were the four groups.

- 1) Patients with baseline TRV <2.5 m/sec who continued to have TRV <2.5 m/sec on all repeat echocardiograms (N=35). This group was labeled Low-low.
- 2) Patients with baseline TRV <2.5 m/sec who had at least 1 repeat echocardiogram with TRV >2.5 m/sec (N=22). This patient was labeled Low-high.
- 3) Patients with baseline TRV  $\geq 2.5$  m/sec who continued to have TRV  $\geq 2.5$  m/sec on all repeat echocardiograms (N=8). This group was labeled High-high.
- 4) Patients with baseline TRV 2.5 m/sec who had at least 1 repeat echocardiogram with TRV <2.5 m/sec (N=17). This group was labeled High-low.



Table III shows the characteristics of the each of the four groups: Low-low, Low-high, High-high, High-low. Of note, 62.5% of the patients in the High-high group were started on hydroxyurea after at least two echocardiograms with elevated TRV  $\geq 2.5$  m/sec. Three patients died during the follow up period of the study, one in the low-low group and two in the low-high group. All three patients had HbSS disease.

**TABLE III. Characteristics of Four Longitudinal Follow Up Groups**

|                                       | <b>Low-low<br/>(N = 35)</b> | <b>Low-High<br/>(N = 22)</b> | <b>High-high<br/>(N = 8)</b> | <b>High-low<br/>(N = 17)</b> |
|---------------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|
| Total number of ECHOs                 | 94                          | 103                          | 24                           | 66                           |
| Average number of ECHOs per patient   | 2.69                        | 4.68                         | 3.00                         | 3.88                         |
| Total number of years                 | 108.57                      | 103.71                       | 12.73                        | 58.22                        |
| Average number of years per patient   | 3.10                        | 4.71                         | 1.59                         | 3.42                         |
| Number of ECHOs <2.5                  | 94                          | 63                           | 0                            | 29                           |
| Number of ECHOs $\geq 2.5$            | 0                           | 40                           | 24                           | 37                           |
| # patients with 1 ECHO >2.5           | 0                           | 11                           | 0                            | 4                            |
| # patients with 2 ECHO >2.5           | 0                           | 7                            | 4                            | 7                            |
| # patients with 3 ECHO >2.5           | 0                           | 2                            | 2                            | 5                            |
| # patients with 4 ECHO >2.5           | 0                           | 1                            | 3                            | 1                            |
| # patients with 5 ECHO >2.5           | 0                           | 1                            | 0                            | 0                            |
| # patients started on HU during study | 9                           | 1                            | 5                            | 3                            |
| # patients deceased during study      | 1                           | 2                            | 0                            | 0                            |
| % of patients started on HU           | 25.71%                      | 4.55%                        | 62.5%                        | 17.65%                       |

As shown in Table IV and Table V, baseline factors including clinical and laboratory data were examined for differences between the Low-low group and Low-high group to determine risk factors for TRV conversion. Baseline factors were also examined between the High-high group and the High-low group to determine factors associated with persistent elevation of TRV. Significant risk factors associated with TRV conversion from normal to elevated were baseline low O2 saturation, and high reticulocyte count (all P <0.05). High white blood cell count was trending towards a significance as a risk factor associated with TRV conversion (P=0.07).

**TABLE IV. Risk Factors Associated with TRV Conversion**

| <b>Baseline Characteristics</b>                | <b>Low-low<br/>(N = 35)</b> | <b>Low-high<br/>(N = 22)</b> | <b>P value</b> |
|--|-----------------------------|------------------------------|----------------|
| TRV, mean $\pm$ SD                             | 2.20 $\pm$ 0.17             | 2.27 $\pm$ 0.16              | 0.09           |
| Age, mean $\pm$ SD                             | 11.87 $\pm$ 3.67            | 11.32 $\pm$ 4.05             | 0.30           |
| O2 Saturation, mean $\pm$ SD                   | 97.71 $\pm$ 2.33            | 95.86 $\pm$ 2.59             | 0.004*         |
| Hemoglobin, mean $\pm$ SD                      | 8.80 $\pm$ 1.42             | 7.89 $\pm$ 1.07              | 0.12           |
| Reticulocyte count, mean $\pm$ SD              | 8.47 $\pm$ 3.66             | 13.61 $\pm$ 4.88             | 0.0001*        |
| WBC count ( $\times 10^3/\mu$ ), mean $\pm$ SD | 12.26 $\pm$ 5.64            | 14.47 $\pm$ 4.60             | 0.07           |
| Platelet count, mean $\pm$ SD                  | 401.29 $\pm$ 119.39         | 438.05 $\pm$ 178.63          | 0.18           |

\*P &lt; 0.05.

As shown in Table V, significant risk factors associated with persistent TRV elevation were baseline TRV, hemoglobin, and white blood cell count (all P < 0.05). Lower oxygen saturation was trending towards significance as a factor associated with persistent TRV elevation (P=0.06)

**TABLE V. Risk Factors Associated with Persistent TRV Elevation**

| <b>Baseline Characteristics</b>                | <b>High-high<br/>(N = 8)</b> | <b>High-low<br/>(N = 17)</b> | <b>P value</b> |
|--|------------------------------|------------------------------|----------------|
| TRV, mean $\pm$ SD                             | 2.79 $\pm$ 0.15              | 2.63 $\pm$ 0.15              | 0.02*          |
| Age, mean $\pm$ SD                             | 14.58 $\pm$ 5.13             | 12.07 $\pm$ 4.04             | 0.09           |
| O2 Saturation, mean $\pm$ SD                   | 94.43 $\pm$ 4.79             | 96.91 $\pm$ 2.65             | 0.06           |
| Hemoglobin, mean $\pm$ SD                      | 7.79 $\pm$ 1.01              | 8.83 $\pm$ 0.93              | 0.009*         |
| Reticulocyte count, mean $\pm$ SD              | 11.91 $\pm$ 2.41             | 10.39 $\pm$ 3.94             | 0.18           |
| WBC count ( $\times 10^3/\mu$ ), mean $\pm$ SD | 14.96 $\pm$ 4.83             | 11.66 $\pm$ 3.26             | 0.03*          |
| Platelet count, mean $\pm$ SD                  | 545.38 $\pm$ 212.97          | 489.47 $\pm$ 130.16          | 0.21           |

\*P &lt; 0.05.

## Study 2. Is Elevated TRV Associated with Proteinuria in Children with Sickle Cell Disease?

### Baseline Characteristics

A total of 85 patients aged 6–21 years were included in the study. The baseline characteristics were similar to the Study 1. Seventy-two patients had HbSS disease and 13 patients had S $\beta^{\circ}$  thalassemia. The male to female ratio was 1.36:1. The mean age at initial screening echocardiogram was 12.16 years (standard deviation was 4.15 years). On initial screening echocardiograms, 28 of 85 patients (32.9%) had elevated TRV  $\geq 2.5$  m/sec. Table VI shows the baseline characteristics of patients with and without elevated TRV. As shown in Table I, low daytime oxygen saturation, high reticulocyte count, and high platelet count were significantly associated with elevated TRV. Of the 28 patients with elevated TRV, 2 patients (7.14%) had proteinuria compared to 1 of 57 patients (1.75%) with normal TRV. This was not a statistically significant finding (P=0.25).

**TABLE VI. Characteristics of Patients With and Without High TRV on Baseline Screening ECHO**

| Patient characteristics                 | Baseline TRV           |                              | P value |
|---|------------------------|------------------------------|---------|
|   | <2.5 m/sec<br>(n = 57) | $\geq 2.5$ m/sec<br>(n = 28) |         |
| Age at echocardiogram, mean $\pm$ SD, y | 11.72 $\pm$ 3.93       | 13.04 $\pm$ 4.45             | 0.17    |
| Male gender, n (%)                      | 33 (57.89)             | 16 (57.14)                   | 0.86    |
| Genotype, n (%)                         |                        |                              |         |
| SS                                      | 45 (78.9)              | 27 (96.43)                   |         |
| S $\beta^{\circ}$                       | 12 (21.0)              | 1(3.57)                      | 0.22    |
| H/O acute chest syndrome, n (%)         | 23 (40.3)              | 16 (57.14)                   | 0.15    |
| Hydroxyurea, n (%)                      | 12 (21.0)              | 5 (17.86)                    | 0.78    |
| Blood transfusion, n (%)                | 12 (21.0)              | 2 (7.14)                     | 0.12    |
| Stroke/abnormal TCD, n (%)              | 10 (7.5)               | 1 (3.57)                     | 0.09    |
| Systolic BP, mean $\pm$ SD, mmHg        | 108.42 $\pm$ 8.10      | 110.00 $\pm$ 7.63            | 0.39    |
| Oxygen saturation, mean $\pm$ SD        | 97.28 $\pm$ 2.45       | 95.56 $\pm$ 4.46             | 0.02*   |
| Hemoglobin, mean $\pm$ SD, g/L          | 8.45 $\pm$ 1.30        | 8.30 $\pm$ 1.09              | 0.60    |
| Hematocrit, mean $\pm$ SD               | 24.49 $\pm$ 4.13       | 23.11 $\pm$ 3.50             | 0.13    |
| Reticulocyte count, mean $\pm$ SD       | 10.43 $\pm$ 5.39       | 13.66 $\pm$ 6.73             | 0.040   |
| WBC count (x103/ml), mean $\pm$ SD      | 14.23 $\pm$ 5.34       | 13.53 $\pm$ 4.76             | 0.55    |
| Platelet count, mean $\pm$ SD           | 406.48 $\pm$ 143.08    | 505.39 $\pm$ 143.75          | 0.003*  |
| LDH, mean $\pm$ SD                      | 648.21 $\pm$ 254.30    | 650.50 $\pm$ 255.18          | 0.98    |
| Total bilirubin, mean $\pm$ SD          | 3.25 $\pm$ 2.50        | 3.90 $\pm$ 2.51              | 0.30    |
| Creatinine, mean $\pm$ SD, mg/dl        | 0.52 $\pm$ 0.16        | 0.48 $\pm$ 0.16              | 0.25    |
| Positive urine protein, n (%)           | 1 (1.75)               | 2 (7.14)                     | 0.25    |

\*P < 0.05.

*Longitudinal Follow Up of Echocardiograms and Urinalysis*

As shown in Table VII, when patients were grouped based on their initial screening echocardiograms, follow up urinalysis data was available in 92.86% of patients with elevated TRV and in 94.73% of patients with normal TRV. The number of follow up urinalysis per patient was 6.17 in the high TRV group and 7.24 in the normal TRV group. Of the 173 repeat urine analyses in patients with elevated TRV, 19.08% were positive for proteinuria (P=0.04).

**TABLE VII. Follow Up Urinalysis in Patients With and Without High TRV on Baseline Screening ECHO**

| Patient characteristics                           | Baseline TRV           |                        | P value |
|---|------------------------|------------------------|---------|
|   | <2.5 m/sec<br>(n = 57) | ≥2.5 m/sec<br>(n = 28) |         |
| Patients with follow up urinalysis, n (%)         | 54 (94.73%)            | 26 (92.86%)            |         |
| Total number of urinalysis                        | 413                    | 173                    |         |
| Number of urinalysis per patient                  | 7.24                   | 6.17                   |         |
| Number of urinalysis with positive protein, n (%) | 51 (12.35%)            | 33 (19.08)             | 0.04*   |

\*P < 0.05.

As shown in Table VIII, when patients were grouped based on the presence of any echocardiogram (baseline or follow up) having TRV ≥2.5 m/sec, follow up urinalysis were available in 95.35% of patients with elevated TRV and 92.86% of patients with normal TRV. Of the 302 repeat urinalysis in patients with any elevated TRV, 21.52% were positive for proteinuria compared to 6.69% (19 of 284 urine samples) in patients with every TRV < 2.5 m/sec (P=<0.001).

**TABLE VIII. Follow Up Urinalysis in Patients With and Without High TRV on Any ECHO (Baseline or Follow Up)**

| Patient characteristics                           | Baseline TRV           |                        | P value |
|---|------------------------|------------------------|---------|
|   | <2.5 m/sec<br>(n = 42) | ≥2.5 m/sec<br>(n = 43) |         |
| Patients with follow up urinalysis, n (%)         | 39 (92.86%)            | 41 (95.35%)            |         |
| Total number of urinalysis                        | 284                    | 302                    |         |
| Number of urinalysis per patient                  | 7.28                   | 7.36                   |         |
| Number of urinalysis with positive protein, n (%) | 19 (6.69)              | 65 (21.52)             | <0.001* |

\*P < 0.05.

## DISCUSSION

Our first study showed that on initial screening echocardiogram 33% of patients had elevated TRV  $\geq 2.5$  m/sec. Risk factors associated with increased TRV were similar to the study previously published by Dr. Pashankar and her group at Yale and included low daytime oxygen saturation, high reticulocyte count, and high platelet count, all of which are signs of increased hemolysis (25). Other studies have shown a similar association between low daytime oxygen saturation and elevated TRV (27, 59). It is unclear whether hypoxia plays a direct role in the development of increased TRV in children with sickle cell disease. Chronic hypoxia from multiple conditions predisposes patients to the development of pulmonary hypertension (60). These conditions include chronic obstructive pulmonary disease, pulmonary fibrosis, sleep-disordered breathing, and exposure to high altitude. In children with sickle cell disease and elevated TRV, hypoxia may play a direct role in the development of increased pulmonary artery pressures, as has been suggested in previous studies (27). Alternatively, the relationship between low oxygen saturation and elevated TRV may not be a causative one. Low oxygen saturation may indicate that these are patients with higher rates of hemolysis, who are therefore predisposed to the development of elevated TRV. This indirect relationship is supported by the association between elevated TRV and other markers of hemolysis, including high reticulocyte and high platelet count.

Over a median follow up time of 3.59 years, 61% of patients with normal TRV at baseline continued to have normal TRV, indicating that most patients who have normal TRV will continue to have normal TRV. On follow up, there were a significant

proportion of patients, almost 40%, who had a normal baseline TRV, but subsequently had an elevated TRV  $\geq 2.5$  m/sec. Interestingly, this group of patients was distinct in terms of other clinical measures from their counterparts who remained with normal TRV over time. Risk factors associated with TRV conversion were baseline low oxygen saturation and high reticulocyte count (all  $P < 0.05$ ). These are similar to the risk factors that put patients at increased risk of having elevated TRV at screening and suggest that patients who have laboratory or clinical markers of increased hemolysis may need to be monitored more closely and may need to have screening echocardiograms on an annual basis. The finding that low oxygen saturation was a risk factor for TRV conversion again highlights the importance of monitoring children with lower oxygen saturation closely. Patients with normal TRV and no signs of increased hemolysis (low oxygen saturation, high reticulocyte count) can likely be screened with echocardiograms every 2 years, as opposed to annually, which is currently the suggested practice at the Yale Sickle Cell Program.

For the 33% of patients with elevated TRV at baseline, follow up was available for 75.76% (25 of 33) of the patients. There were ten patients who were started on hydroxyurea treatment after one echocardiogram with elevated TRV, and this substantially affected the follow up data available for this cohort. On follow up of the children with elevated TRV at baseline 32% (N=8/25) of patients continued to have elevated TRV on all subsequent echocardiograms, whereas 68% (N=17) of patients had at least 1 echocardiogram with normal TRV without intervention. This finding highlights that the reproducibility of TRV measurement is limited. This was previously shown by the Dr. Pashankar and other investigators (37, 61). It is therefore important to repeat

echocardiograms and ensure that the elevated TRV is persistent before considering any therapeutic intervention. Notably, despite the small patient size, significant risk factors associated with persistent TRV elevation were baseline TRV, hemoglobin, and white blood count.

In adults with sickle cell disease elevated TRV is associated with multiple important clinical outcomes, of which the most notable is increased mortality (16, 62). In addition, studies in adults with sickle cell disease with elevated TRV have found significant associations with pulmonary hypertension, decreased exercise capacity (Six-minute walk test), leg ulcers, and proteinuria (13, 16, 29, 63-66). There have been limited studies in children with elevated TRV to investigate associations with clinical outcomes. Sickle cell disease children with increased TRV do not appear to have the same increased risk of short-term mortality that adults with elevated TRV have.

Two studies in children with sickle cell disease have looked at the clinical associations with elevated TRV. One study published in 2009 in a cohort of children and adolescents with sickle cell disease (N=230) found that patients with elevated TRV had a decline in oxygen saturation during exercise with the six-minute walk test (P=0.0002) (27). Unlike in adults, the study did not find an association between elevated TRV and decreased exercise tolerance as measured with the six-minute walk test (27). Another study published in 2011 on children aged 3-20 years old found that over a median follow up of 22 months, baseline TRV elevation was associated with a 4.4-fold increase in the odds of a 10% or more decline in exercise tolerance (P=0.015). Our study is the first to examine whether there is an association between elevated TRV and proteinuria in children with sickle cell disease.

In our second study, urine protein was present in 2 (7.14%) patients with TRV  $\geq 2.5$  m/sec compared to 1 (1.75%) patient with normal TRV. This was not statistically significant, due to the small numbers in each group. Interestingly, the patient with normal TRV who had proteinuria at baseline did have a borderline high TRV of 2.4–2.7 m/sec on initial echocardiogram. All of his subsequent echocardiograms showed a high TRV  $>3$  m/sec, hence it is likely that the pulmonary artery pressures were evolving at the time of the initial echocardiogram.

Follow up of urinalysis data showed a significant association between proteinuria and elevated TRV when patients were grouped either based on their initial echocardiogram or on any subsequent echocardiogram showing TRV  $\geq 2.5$  m/sec. A positive association between proteinuria and pulmonary hypertension has been reported previously in adults (13, 57). Ours is the first study showing that proteinuria is associated with elevated TRV in children with sickle cell disease.

The underlying potential pathogenesis linking proteinuria and elevated pulmonary artery systolic pressures is unclear at this time. Both complications occur in childhood with increasing prevalence with age (54, 55, 67, 68). Both complications are risk factors for death in sickle cell disease patients (12, 16). The risk factors for elevated pulmonary artery pressures are markers of hemolysis such as low hemoglobin, elevated reticulocyte count, elevated lactate dehydrogenase, and high hemolytic index. The pathogenesis of elevated pulmonary artery pressures is thought to be multifactorial with hemolysis induced nitric oxide depletion and consequent vascular dysfunction playing a major role. Recently the role of inflammation has been described in the pathogenesis.



Albuminuria is common in sickle cell disease patients and is reported in 16–26% of children. Studies in adults with sickle cell disease report a 42% prevalence of albuminuria. Risk factors associated with albuminuria include older age, type of sickle cell disease, history of acute chest syndrome, and serum creatinine (37, 55). The association of low hemoglobin with albuminuria or proteinuria has varied in different reports. Several investigators showed that albuminuria/proteinuria was associated with low hemoglobin (54, 57, 69, 70). Others have not found an association between low hemoglobin and albuminuria (55, 57, 68). However Ataga et al. did find that hemoglobin levels were lower and lactate dehydrogenase levels were higher in patients with macroalbuminuria compared to patients with microalbuminuria or normoalbuminuria, hence postulating that hemolysis may play a role in albuminuria in sickle cell disease (57). Interestingly, they also found that the levels of sFLT-1, soluble VCAM, and NT pro-BNP were significantly higher in patients with macroalbuminuria as compared to patients with microalbuminuria or normoalbuminuria. sFLT-1, a tyrosine kinase protein which inhibits VEGF, has also been shown to be associated with PHT. The association between albuminuria and elevated pulmonary artery systolic pressures may be in part due to endothelial activation and endothelial dysfunction that is known to follow increased levels of sFLT-1.

Our study of the association between elevated TRV and proteinuria has some limitations. The data was collected retrospectively and in some patients the echocardiograms and urinalysis were not performed simultaneously. There were some missing urinalysis and echocardiogram data points. Additionally, due to the nature of the available data we were only able to examine proteinuria, and were not able to quantify

microalbuminuria in the patient population that we studied. The addition of microalbuminuria data may have strengthened or further revealed important information about the potential association between elevated TRV and urinary protein. Therefore, we recommend that a prospective study be conducted, ideally on a larger patient population across multiple clinical sites to further define this association and potentially examine the role of microalbuminuria in addition to fulminant proteinuria.

In conclusion, our second study shows that elevated TRV is associated with proteinuria in children with sickle cell disease. The underlying pathogenesis linking the two conditions is currently being explored. It is likely that hemolysis or inflammation induced endothelial dysfunction may play a role.

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## Proteinuria is Associated With Elevated Tricuspid Regurgitant Jet Velocity in Children With Sickle Cell Disease

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**Background.** Sickle cell disease (SCD) affects multiple organ systems. Complications of SCD such as pulmonary hypertension (PHT) and sickle cell nephropathy (SCN) are associated with an increased mortality. Both PHT and SCN have some common risk factors. In adults, PHT has been found to be associated with albuminuria, a manifestation of SCN. There is no data on this association in children. We conducted a study to determine if elevated pulmonary artery systolic pressures estimated on echocardiogram was associated with proteinuria in children with SCD. **Methods.** A detailed retrospective chart review was conducted on a cohort of sickle cell patients screened with echocardiograms from June 2005 to July 2010. Patients who had an initial screening echocardiogram and urine analysis within 1 year were included. Longitudinal data from all subsequent echocardiograms and urine

analyses were collected. **Results.** Eighty-five patients were included. On initial echocardiograms 32.9% had an elevated tricuspid regurgitant jet velocity (TRV)  $\geq 2.5$  m/second. On follow up, in the 28 patients with elevated TRV, 49.27% of all repeat echocardiograms showed persistent elevation. In the 57 patients with normal baseline TRV, 73.6% of all repeat echocardiograms continued to have normal TRV. On initial screening 7.14% of patients with elevated TRV had proteinuria compared to 1.75% without elevated TRV. On follow up, 19.08% of repeat urinalysis had proteinuria in patients with elevated baseline TRV compared to 12.35% in patients with normal baseline TRV ( $P = 0.04$ ). **Conclusions.** Elevated TRV  $\geq 2.5$  m/second is significantly associated with proteinuria on longitudinal follow up in children with SCD. *Pediatr Blood Cancer* 2012;58:937–940. © 2011 Wiley Periodicals, Inc.

**Key words:** children; proteinuria; pulmonary hypertension; sickle cell disease

### INTRODUCTION

Pulmonary hypertension (PHT) occurs in approximately one third of adults with sickle cell disease (SCD) and is associated with a significantly increased mortality [1–3]. The definitive diagnosis of PHT is made by right heart catheterization, however this is invasive. Pulmonary artery systolic pressures can be estimated by measurement of tricuspid regurgitant jet velocity (TRV) on echocardiogram. Studies in children report a 16–30% prevalence of elevated TRV  $\geq 2.5$  m/second on screening echocardiograms [4–6]. The pathogenesis of PHT is likely multifactorial and several investigators have proposed that hemolysis induced nitric oxide depletion and vascular dysfunction play a major role [3,7–10]. More recent reports highlight the role of inflammation in the pathogenesis of PHT [11,12].

Sickle cell nephropathy (SCN) is of significant concern in adults with SCD and renal failure affects 12–21% of adults with SCD [13,14]. One of the early manifestations of SCN is proteinuria. The onset of proteinuria has been reported in early childhood, and studies in children report a 16–26% prevalence of microalbuminuria and a 6.2% prevalence of proteinuria [15–18]. Risk factors associated with albuminuria in children include older age, low hemoglobin, history of acute chest syndrome (ACS), and type of SCD. Recently a study in adults found a positive association between albuminuria and PHT [19]. However, there are no data in children on the potential association between kidney dysfunction manifested by proteinuria and elevated pulmonary artery pressures. We hence conducted a study to determine if elevated pulmonary artery systolic pressures estimated on echocardiograms was associated with proteinuria in children with SCD.

### METHODS

A detailed retrospective chart review was conducted on a cohort of SCD patients. Starting in June 2005, screening echocardiograms were incorporated at our center as part of the annual evaluation of children with Hb SS and Hb S $\beta^0$  thalassemia older than 6 years of age. Screening echocardiograms were done when patients were in steady state, at least 2 weeks after admission for

vaso-occlusive crises. TRV was measured by pulsed-wave and continuous wave Doppler echocardiography. Multiple views (apical four chamber, parasternal short axis, parasternal long axis) were obtained to record optimal tricuspid Doppler flow signals and a minimum of five sequential signals were recorded. A peak TRV of  $\geq 2.5$  m/second equating to a pulmonary artery pressure of at least 30 mmHg was considered to be elevated. Other evaluations done during the visit included a detailed history, complete physical examination and laboratory studies including complete blood count, reticulocyte count, renal and liver function tests and urinalysis. Urinalysis was sent for measurement of protein, which was quantified from 0 to 3+. Urine protein 1+ equaled 30 mg/dl, 2+ equaled 100 mg/dl, and 3+ equaled  $\geq 300$  mg/dl in our laboratory. Urine protein of 1+ to 3+ was considered positive, urine protein of 0 was considered negative.

On follow up, urinalysis was repeated at least annually. Echocardiograms were repeated annually on patients with a baseline TRV  $\geq 2.5$  m/second, and every 2 years on patients with normal baseline TRV.

For this study, charts were reviewed from June 2005 to July 2010. Patients who had a screening echocardiogram and a urinalysis within 1 year were included in the study. Baseline data collected included demographics, type of SCD, clinical history, echocardiogram results, laboratory results, and urinalysis. Longitudinal data from all subsequent echocardiograms and urinalyses for each subject were also collected. Data were analyzed using Chi square test for categorical variables and *t*-test for continuous variables. The study was approved by the institutional review board.

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TABLE I. Characteristics of Patients With and Without TRV on Baseline Screening Echocardiogram

| Patient characteristics                     | Baseline TRV              |                           | P      |
|---|---------------------------|---------------------------|--------|
|   | <2.5 m/second<br>(n = 57) | >2.5 m/second<br>(n = 28) |        |
| Age at echocardiogram, mean ± SD, y         | 11.72 ± 3.93              | 13.04 ± 4.45              | 0.17   |
| Male gender, n (%)                          | 33 (57.89)                | 16 (57.14)                | 0.86   |
| Genotype, n (%)                             |                           |                           |        |
| SS  | 45 (78.9)                 | 27 (96.43)                |        |
| Sβ <sup>0</sup>                             | 12 (21.0)                 | 1 (3.57)                  | 0.22   |
| H/O acute chest syndrome, n (%)             | 23 (40.3)                 | 16 (57.14)                | 0.15   |
| Hydroxyurea, n (%)                          | 12 (21.0)                 | 5 (17.86)                 | 0.78   |
| Blood transfusion, n (%)                    | 12 (21.0)                 | 2 (7.14)                  | 0.12   |
| Stroke/abnormal TCD, n (%)                  | 10 (7.5)                  | 1 (3.57)                  | 0.09   |
| Systolic BP, mean ± SD, mmHg                | 108.42 ± 8.10             | 110.00 ± 7.63             | 0.39   |
| Oxygen saturation, mean ± SD                | 97.28 ± 2.45              | 95.56 ± 4.46              | 0.02*  |
| Hemoglobin, mean ± SD, g/L                  | 8.45 ± 1.30               | 8.30 ± 1.09               | 0.60   |
| Hematocrit, mean ± SD                       | 24.49 ± 4.13              | 23.11 ± 3.50              | 0.13   |
| Reticulocyte count, mean ± SD               | 10.43 ± 5.39              | 13.66 ± 6.73              | 0.04*  |
| WBC count (×10 <sup>3</sup> /μl), mean ± SD | 14.23 ± 5.34              | 13.53 ± 4.76              | 0.55   |
| Platelet count, mean ± SD                   | 406.48 ± 143.08           | 505.39 ± 143.75           | 0.003* |
| LDH, mean ± SD                              | 648.21 ± 254.30           | 650.50 ± 255.18           | 0.98   |
| Total bilirubin, mean ± SD                  | 3.25 ± 2.50               | 3.90 ± 2.51               | 0.30   |
| Creatinine, mean ± SD, mg/dl                | 0.52 ± 0.16               | 0.48 ± 0.16               | 0.33   |
| Positive urine protein, n (%)               | 1 (1.75)                  | 2 (7.14)                  | 0.25   |

\*P &lt; 0.05.

## RESULTS

A total of 85 patients aged 6–21 years were included in the study. Seventy-two patients had Hb SS disease and 13 patients had Sβ<sup>0</sup> thalassemia. The male to female ratio was 1.36:1. The mean age at initial screening echocardiogram was 12.16 years with a SD 4.15 years. On initial screening echocardiograms, 28 of 85 patients (32.9%) had TRV ≥ 2.5 m/second. Table I shows the baseline characteristics of patients with and without elevated TRV. As shown in Table I, low daytime oxygen saturation, high reticulocyte count, and high platelet count were significantly associated with elevated TRV. Of the 28 patients with elevated TRV, 2 patients (7.14%) had proteinuria compared to 1 of 57 patients (1.75%) with normal TRV.

Follow up data was available for 82 patients. Average follow up time was 3.23 years (range 0.27–5.15 years). As shown in Table II, 89.29% of patients with elevated TRV had repeat echocardiograms compared to 86% of patients with normal

TRV. The number of follow up echocardiograms per patient was 2.76 in the high TRV group and 2.24 in the normal TRV group. In the 28 patients with baseline high TRV, 49.27% of the repeat echocardiograms (N = 69) showed persistent elevation of TRV ≥ 2.5 m/second. In the 57 patients with baseline TRV < 2.5 m/second, 73.6% of all repeat echocardiograms (N = 110) continued to have TRV < 2.5 m/second. Patients with a TRV ≥ 2.5 m/second on initial echocardiogram had a significantly higher chance of having repeat echocardiograms with elevated TRV compared to patients with normal baseline TRV (P = 0.003).

As shown in Table III, when patients were grouped based on their initial screening echocardiograms, follow up urinalysis data was available in 92.86% of patients with elevated TRV and in 94.73% of patients with normal TRV. The number of follow up urinalysis per patient was 6.17 in the high TRV group and 7.24 in the normal TRV group. Of the 173 repeat urine analyses in patients with elevated TRV, 19.08% were positive for proteinuria

TABLE II. Follow Up Echocardiograms in Patients With and Without Elevated TRV on Baseline Screening ECHO

| Patients characteristics   | Baseline TRV  |               | P      |
|--|---------------|---------------|--------|
|  | <2.5 (n = 57) | >2.5 (n = 28) |        |
| Patients with follow-up ECHO, n (%)  | 49 (85.96%)   | 25 (89.29%)   |        |
| Total number of echocardiograms  | 110           | 69            |        |
| Number of echocardiograms per patient  | 2.24          | 2.76          | 0.029* |
| Number of patients with repeat echocardiograms showing TRV >2.5 m/second (%) | 15 (30.6%)    | 15 (60%)      |        |
| Number of repeat echocardiograms with TRV >2.5 m/second, n (%)               | 29 (26.36%)   | 34 (49.27%)   | 0.003* |
| Number of repeat echocardiograms with TRV <2.5 m/second, n (%)               | 81 (73.6%)    | 35 (50.7%)    |        |

\*P &lt; 0.05.

TABLE III. Follow Up Urinalysis in Patients With and Without Elevated TRV on Baseline Screening ECHO

| Patients characteristics                          | Baseline TRV  |               | <i>P</i> |
|---|---------------|---------------|----------|
|   | <2.5 (n = 57) | >2.5 (n = 28) |          |
| Patients with follow-up urinalysis, n (%)         | 54 (94.73%)   | 26 (92.86%)   |          |
| Total number of urinalysis                        | 413           | 173           |          |
| Number of urinalysis per patient                  | 7.24          | 6.17          |          |
| Number of urinalysis with positive protein, n (%) | 51 (12.35%)   | 33 (19.08%)   | 0.04*    |

\**P* < 0.05.

compared to 12.35% (51 of 413 urine samples) in patients with normal baseline TRV (*P* = 0.04).

As shown in Table IV, when patients were grouped based on the presence of any echocardiogram (baseline or follow up) having TRV  $\geq$  2.5 m/second, follow up urinalysis were available in 95.35% of patients with elevated TRV and 92.86% of patients with normal TRV. Of the 302 repeat urinalysis in patients with any elevated TRV, 21.52% were positive for proteinuria compared to 6.69% (19 of 284 urine samples) in patients with every TRV < 2.5 m/second (*P* = <0.001).

## DISCUSSION

Our study shows that on initial screening echocardiogram 32.9% of patients had an elevated TRV  $\geq$  2.5 m/second. Risk factors associated with increased TRV were similar to our previous study [4] and included low daytime oxygen saturation, high reticulocyte count, and high platelet count. Other studies have shown a similar association between low daytime oxygen saturation and elevated TRV [6,20]. Urine protein was present in 2 (7.14%) patients with TRV  $\geq$  2.5 m/second compared to 1 (1.75%) patient with normal TRV. This was not statistically significant, due to the small numbers in each group. Interestingly, the patient with normal TRV who had proteinuria at baseline did have a borderline high TRV of 2.4–2.7 m/second on initial echocardiogram. All of his subsequent echocardiograms showed a high TRV > 3 m/second, hence it is likely that the pulmonary artery pressures were evolving at the time of the initial echocardiogram.

On follow up, we found that patients with normal TRV at baseline had a 73.6% likelihood of continuing to have normal TRV on subsequent echocardiograms. In patients with baseline elevated TRV, 49.27% of repeat echocardiograms showed persistent elevation, while 50.7% of repeat echocardiograms were normal with TRV < 2.5 m/second. This observation highlights that the reproducibility of TRV measurement is limited. This has been previously shown by us and other investigators [21,22]. It is hence important to repeat echocardiograms and ensure that the elevated TRV is persistent before considering any intervention.

Follow up of urinalysis data showed a significant association between proteinuria and elevated TRV, when patients were grouped either based on their initial echocardiogram or on any subsequent echocardiogram showing TRV  $\geq$  2.5 m/second. A positive association between proteinuria and PHT has been reported previously in adults [19,23]. Ours is the first study showing that proteinuria is associated with elevated TRV in children with SCD.

The underlying potential pathogenesis linking proteinuria and elevated pulmonary artery systolic pressures is unclear at present. Both complications occur in childhood with increasing prevalence with age [15–17,24]. Both complications are risk factors for death in SCD patients [3,25]. The risk factors for elevated pulmonary artery pressures are markers of hemolysis such as low hemoglobin, elevated reticulocyte count, elevated LDH, and high hemolytic index. The pathogenesis of elevated pulmonary artery pressures is thought to be multifactorial with hemolysis induced nitric oxide depletion and consequent vascular dysfunction playing a major role. Recently the role of inflammation has been described in the pathogenesis.

Albuminuria is common in SCD patients and is reported in 16–26% of children with SCD. Studies in adults with SCD report a 42% prevalence of albuminuria. Risk factors associated with albuminuria include older age, type of SCD, history of ACS, and serum creatinine [16,21]. The association of low hemoglobin with albuminuria or proteinuria has varied in different reports. Several investigators showed that albuminuria/proteinuria was associated with low hemoglobin [15,18,26,27]. Others have not found an association between low hemoglobin and albuminuria [16,19,24]. However Ataga et al. [19] did find that hemoglobin levels were lower and lactate dehydrogenase levels were higher in patients with macroalbuminuria compared to patients with microalbuminuria or normoalbuminuria, hence postulating that hemolysis may play a role in albuminuria in SCD. Interestingly, they also found that the levels of sFLT-1, soluble VCAM, and NT pro-BNP were significantly higher in patients with macroalbuminuria as compared to patients with microalbuminuria or normoalbuminuria. sFLT-1 has also been shown to be associated

TABLE IV. Follow Up Urinalysis in Patients With and Without Elevated TRV on Any ECHO (Baseline or Follow Up)

| Patients characteristics                          | Baseline TRV  |               | <i>P</i> |
|---|---------------|---------------|----------|
|   | <2.5 (n = 42) | >2.5 (n = 43) |          |
| Patients with follow-up urinalysis, n (%)         | 39 (92.86%)   | 41 (95.35%)   |          |
| Total number of urinalysis                        | 284           | 302           |          |
| Number of urinalysis per patient                  | 7.28          | 7.36          |          |
| Number of urinalysis with positive protein, n (%) | 19 (6.69%)    | 65 (21.52%)   | <0.001*  |

\**P* < 0.05.

with PHT [28]. The association between albuminuria and elevated pulmonary artery systolic pressures may be in part due to endothelial activation and endothelial dysfunction that follow increased levels of s FLT-1.

Our study has some limitations. The data were collected retrospectively and in some patients the echocardiograms and urinalysis were not performed simultaneously. There were some missing urinalysis and echocardiogram data points. We hence recommend doing a prospective study to further define this association.

In conclusion, our study shows that elevated TRV is associated with proteinuria in children with SCD. The underlying pathogenesis linking the two conditions is currently being explored and it is likely that hemolysis or inflammation induced endothelial dysfunction may play a role.

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**HIGHLIGHT**by Gregory J. Kato, MD\*<sup>†</sup>**TRV: A Physiological Biomarker in Sickle Cell Disease**

(Commentary on Forrest et al., page 937)

Clinical and translational research makes the biggest leaps in human disease when effective biomarkers are developed and put into play. Effective biomarkers are often convenient surrogate markers of clinical outcomes that dramatically speed up the development of clinically useful interventions. They also often point to new mechanistic pathways that open up opportunities for new intervention strategies. The biomarkers may be biochemical, physiological, imaging, or any other reproducible indicator. Familiar examples of effective biomarkers that guide clinical management resulting in improved clinical outcomes include blood pressure, serum cholesterol, or tumor size from radiographic imaging.

In sickle cell disease (SCD), the first truly successful biomarker was fetal hemoglobin [1]. Natural history epidemiological investigation demonstrated that patients with higher levels suffer fewer clinical complications of SCD. Bench investigations showed that fetal hemoglobin inhibited polymerization of sickle hemoglobin. Intervention with hydroxyurea raises fetal hemoglobin levels, and improves clinical outcomes. Hydroxyurea is now an approved, standard of care drug in SCD, and indications for its prescription continue to expand.

The second truly successful biomarker in SCD is a physiological biomarker: transcranial Doppler (TCD) velocity of blood flow in the cerebral arteries [2]. TCD velocity predicts risk of stroke in children with SCD. Therapeutic intervention with monthly transfusion of red blood cells lowers the TCD velocity, and markedly reduces the incidence of first stroke. Three very important lessons emerged from TCD research. Successful application of TCD: (i) occurred despite lack of consensus over the precise physiological mechanism of TCD velocity; (ii) occurred despite known limitations in its specificity; (iii) required highly standardized TCD measurement conditions and specific technician training.

In this issue of *Pediatric Blood & Cancer*, Forrest et al. at Yale University show that tricuspid regurgitant velocity (TRV) measured during Doppler echocardiography in children with SCD is a biomarker that associates with proteinuria, a sign of renal disease. In order to put this observation in perspective, it is helpful to trace the findings that establish TRV as a physiological biomarker in SCD.

Elevated TRV predicts mortality in adults with SCD more strongly than any other marker. A longstanding marker of pulmonary hypertension in the echocardiography world, TRV was first introduced as a biomarker in SCD research by Sutton et al. [3] in a retrospective study. Gladwin et al. [4] published a prospective cross-sectional screening study of adults with SCD that showed that a TRV of two standard deviations above the mean

( $\geq 2.5$  m/second) was prevalent (32%), and predicted about a 10-fold risk for early mortality [4], findings confirmed by Ataga et al. [5]. Both Gladwin and Ataga attributed the findings to pulmonary hypertension, correct in principle. However, since the consensus clinical definition of pulmonary hypertension uses a threshold for diagnosis of mean pulmonary artery pressure three standard deviations above the mean (25 mmHg) by right heart catheterization, these differences in definitions have led to confusion and debate [6]. Nevertheless, TRV is emerging as a robust physiological biomarker of clinical outcome in SCD.

TRV in SCD adults reproducibly correlates with several important clinical outcomes. Several studies confirm that elevated TRV in SCD adults is associated with important clinical outcomes: pulmonary hypertension [7–9], proteinuria [10,11], leg ulcers [4,7,12–15], decreased exercise capacity [8,16], and early death [4,5]. TRV is one of several longstanding noninvasive modalities for estimating pulmonary artery pressure in the fields of echocardiography and pulmonary hypertension. This same association has been documented by right heart catheterization studies in SCD [8]. In adults, TRV  $\geq 2.5$  m/second has a positive predictive value for pulmonary hypertension of only 25% [7], although addition of NT-proBNP to the screening criteria may help provide, respectable yields for a screening test. TRV can be a useful screening tool in adults with SCD, especially if coupled with a 6-minute walk distance  $< 500$  m [16,17].

To put these numbers in context, TCD velocity is highly useful and now constitutes standard of care despite an estimated positive predictive value of only 36% [18]. Like TRV, (i) there is lack of consensus over the precise physiological mechanism of TCD velocity [6]; (ii) there are limitations in its specificity; (iii) highly standardized measurement conditions are required to minimize variability [19].

It would be ideal to identify subjects at high risk to target preventative strategies. Elevated TRV is detectable in childhood [20], decades before the typical age at which pulmonary hypertension becomes clinically symptomatic [21]. Elevated TRV in

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children with SCD is associated with many of the same clinical features found in SCD adults with elevated TRV: higher systolic blood pressure, lower hemoglobin, higher serum levels of bilirubin, and lactate dehydrogenase [20]. SCD children with elevated TRV show a greater decline in systemic arterial oxygen saturation during exercise than children without a TRV elevation [22]. Although it is not immediately associated with decreased exercise capacity as it is in adults with SCD, elevated TRV in children with SCD predicts future loss of exercise capacity [23].

Forrest et al. in this issue add another piece to this picture, showing that abnormal TRV in children with SCD is associated with proteinuria, a form of end organ disease seen also in SCD adults with high TRV [10,11]. It appears at this point that childhood echocardiography screening might be identifying an early stage of elevated TRV, but only long-term longitudinal cohort studies will give the definitive answer. These children over years or decades may be at risk to develop loss of exercise capacity and potentially clinically significant pulmonary hypertension. More research is needed in order to provide better evidence regarding screening guidelines in children with SCD.

Children with SCD and elevated TRV may be an ideal group for randomized controlled trials to test possible strategies to prevent progression to high-risk status in adulthood. Such trials might test hydroxyurea or other disease modifying strategies for this indication. Since death with high TRV appears to occur mainly in adulthood, such trials would need to use a more immediate surrogate marker, such as exercise capacity, an endpoint accepted by the Food and Drug Administration for approval of pulmonary hypertension therapeutics. If and when an intervention in presymptomatic children with high TRV is found that prevents progression to adulthood pulmonary hypertension and death, TRV will have fully followed in the footsteps of TCD as a physiological biomarker of risk in children with SCD.

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