Tricuspid regurgitant jet velocity is associated with hemolysis in children and young adults with sickle cell disease evaluated for pulmonary hypertension

Robert I. Liem, Luciana T. Young, Alexis A. Thompson

From the Division of Hematology, Oncology & Stem Cell Transplant (RIL, AAT); Division of Cardiology (LTY), Children's Memorial Hospital, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA.

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Correspondence:
Robert I. Liem, MD, Division of Hematology, Oncology & Stem Cell Transplant, Children's Memorial Hospital, 2300 Children's Plaza, Box 30, Chicago, 60614-3394, IL, USA.
E-mail: rliem@childrensmemorial.org

ABSTRACT

Tricuspid regurgitant jet velocity (TRJV) ≥2.5 m/sec. on echocardiography is a surrogate marker for pulmonary hypertension (PHT) in adults with sickle cell disease (SCD). We prospectively examined the relationship between TRJV and laboratory markers of hemolysis in 51 children and young adults with SCD at baseline. We found significant correlations between TRJV and lactate dehydrogenase (LDH), hemoglobin (Hb), reticulocyte count (retic) and aspartate aminotransferase (AST). LDH, retic and AST were significantly higher and Hb was lower in subjects with TRJV ≥2.5 m/sec. We conclude that hemolysis significantly contributes to TRJV elevation in children and young adults with SCD.

Key words: sickle cell disease, pulmonary hypertension, tricuspid regurgitant jet velocity, hemolysis, lactate dehydrogenase

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Recent evidence suggests that pulmonary hypertension (PHT), which may be defined as an elevated tricuspid regurgitant jet velocity (TRJV) on echocardiogram (ECHO), is a major cause of morbidity and mortality in adults with sickle cell disease (SCD).[1] Using a TRJV ≥2.5 m/sec. on Doppler ECHO to predict elevated pulmonary artery (PA) systolic pressures, PHT was identified in 32% of adults with SCD in a recent cohort and emerged as the single most important independent risk factor for early mortality.[2] By contrast, the prevalence and natural history of PHT in children and adolescents with SCD remain largely unknown despite retrospective studies that suggest a similar prevalence to adults.[3,4] Although various mechanisms have been implicated in the development of PHT in SCD, the pathophysiology remains unclear. Recently, the effect of chronic hemolysis on nitric oxide (NO) bioavailability has emerged as a potentially important contributor to the development of PHT in SCD and other chronic hemolytic disorders.[5-7] In the current study, we explored the relationship between TRJV and standard laboratory parameters for hemolysis in a pediatric cohort with SCD.

Design and Methods

Subjects

A PHT screening protocol was initiated at our institution in December 2005 as standard of care for children and young adults between 10 and 21 years old with Hb SS, SC and S-β^0 thalassemia confirmed by electrophoresis. The possibility of screening was discussed with this convenience sample and enrolment took place during routine clinical visits to our Comprehensive Sickle Cell Program. To date, of the 55 patients invited to take part in the screening program, only 4 have refused participation or have been noncompliant with screening appointments. We included subjects on hydroxyurea therapy at the time of screening but excluded subjects undergoing chronic transfusions.

Procedures

Each subject was screened after obtaining complete informed consent. A transthoracic ECHO was performed on each subject at baseline steady state and laboratory studies collected on the same day. ECHOs included 2D imaging, M-mode, spectral and color Doppler interrogation, emphasizing the

evaluation of PHT. A standard protocol was used to train a limited number of sonographers. In the absence of right ventricular (RV) outflow obstruction, TRJV was measured to estimate RV systolic pressure. The transducer was placed at the cardiac apex and a four-chamber view was obtained. The ultrasound beam was directed parallel to the tricuspid regurgitant jet to obtain the cleanest Doppler tracing of velocity. An elevated TRJV ≥2.5 m/sec. corresponded to a RV systolic pressure ≥25 mm Hg above the right atrial pressure or a PA systolic pressure ≥30-35 mm Hg calculated from the equation $4(TRJV)^2$ + an assumed right atrial pressure of 5-10 mm Hg. A single pediatric cardiologist interpreted all studies. Steady state laboratory studies performed at our institution included a complete blood count (CBC), reticulocyte count (retic), Hb electrophoresis, aspartate aminotransferase (AST), total bilirubin (Tbili) and lactate dehydrogenase (LDH). Plasma free Hb was analyzed by Mayo Medical Laboratories (Rochester, MN, USA).

Statistical analysis

Analysis was performed using SPSS version 12.0. We used univariate linear regression and Spearman's Rank Correlation Coefficient (r) to assess the relationship between TRJV and all markers. Student's *t*-test was used to compare mean values of all continuous variables. Values not normally distributed were log transformed prior to *t*-test analysis. A 2-sided *p* value of 0.05 was considered significant. Significant variables were entered into a multivariate regression model and a receiver operating characteristic curve (ROC) was generated for any unique marker identified.

Results and Discussion

We quantified TRJV in 51 patients (30 males, 21 females), 10-20 years old (mean 14.02±2.62 years), for this analysis. Of these, 41 (80%) had Hb SS disease, 6 (12%) had Hb SC, and 4 (8%) had Hb S-β^0 thalassemia. Mean TRJV was 2.35±0.39 m/sec. in this cohort (Table 1). TRJV was ≥2.5 m/sec. in 17 patients, and was not affected by age or sex. There was a significant, positive correlation between TRJV and LDH (r=0.45, *p*=0.001), with higher TRJV associated with higher LDH. Modest but significant positive and negative correlations also existed between TRJV and retic (r=0.36, *p*=0.010) and AST (r=0.37, *p*=0.007), and between TRJV and Hb (r=-0.33, *p*=0.017), respectively (Figure 1). We observed no significant relationship between TRJV and plasma Hb, Tbili, percent fetal Hb (Hb F), total white blood cell count (WBC), absolute neutrophil count (ANC) or platelet count. Subjects with TRJV ≥2.5 m/sec. had significantly higher mean LDH (488±191 vs. 344±139 U/L, *p*=0.005), retic (16.6±9.6 vs. 10.4±6.3%, *p*=0.013) and AST (50±21 vs. 38±11 U/L, p=0.021) and significantly lower mean Hb (8.6±1.2 vs. 10.0±1.7 g/dL, *p*=0.004) compared to patients with TRJV <2.5 m/sec. Mean WBC was also significantly higher (11.8±5.1 vs. 8.8±3.5×10^9/L, *p*=0.030) in patients with TRJV ≥2.5 m/sec. There was no difference in plasma Hb (3.5±1.6 vs. 3.0±1.7 μmol/L, *p*=0.345), platelet count (503±269 vs. 450±196×10^9/L, *p*=0.545), percent Hb F (11.5±11.8 vs. 10.7±10.4 %, *p*=0.421) or Tbili (66.7±35.9 vs. 51.3±39.3 μmol/L, *p*=0.072) between the 2 groups.

Table 1. Patient demographic and baseline laboratory data.

Patient characteristic	*Mean (±SD)*	*Range*
Age (years)	14.02 (±2.62)	10-20
Males (n=30)	14.03 (±2.81)	10-20
Females (n=21)	14.00 (±2.36)	10-19
Tricuspid regurgitant jet velocity (m/sec.)	2.35 (±0.39)	1.00-3.50
Lactate dehydrogenase (U/L)	381 (±168)	161-932
White blood cells (x10^9/L)	9.74 (±4.24)	3.32-22.42
Absolute neutrophils (x10^9/L)	4.80 (±2.60)	0.9-13.30
Hemoglobin (g/dL)	9.6 (±1.7)	6.7-15.2
Platelets (×10^9/L)	461 (±218)	145-1404
Reticulocytes (%)	12.4 (±8.1)	2.4-43.3
Plasma hemoglobin (μmol/L)	3.1 (±1.7)	0.5-6.4
Percent fetal Hemoglobin (%)	10.4 (±10.3)	0.4-51.50
Total bilirubin (μmol/L)	54.7 (±41.0)	15.4-177.8
Aspartate aminotransferase (U/L)	41 (±16)	20-101

Variables with a *p* value <0.05 on *t*-testing were entered into a multivariate regression model to evaluate their combined and individual effects on TRJV variability in our subjects. The combination of LDH, Hb and WBC explained approximately 30% (R^2=0.292, *p*=0.001) of the total variability in TRJV, although LDH alone was independently associated with TRJV (*p*=0.012) in the final model. This suggests that LDH was the largest contributor to TRJV variability, which was confirmed on univariate analysis (R^2=0.269, *p*=0.001). Retic count and AST were removed from the model since each was highly correlated with LDH and therefore, represented minimal additional contribution to variability in TRJV. Based on the results of our multivariate analysis, an ROC was generated for LDH only. Although the area under the curve (AUC) for LDH was significant (AUC 0.721, 95% CI 0.574-0.869, *p*=0.011), we were unable to identify a cut-off value for LDH that optimized sensitivity and specificity for predicting TRJV ≥2.5 m/sec.

The release of plasma free Hb from intravascular hemolysis causes a cascade of physiologic derangements that result from depletion of endogenous NO, a critical regulator of vasodilatation and vascular tone. Hb that is no longer compartmentalized in intact RBCs acts as a potent scavenger of NO.[8] In acute and chronic hemolytic conditions, NO depletion is implicated in clinical manifestations associated with smooth muscle and endothelial dysfunction.[9] Our data indicate that hemolysis is associated with TRJV elevation in children and young adults with SCD, a relationship in this population that

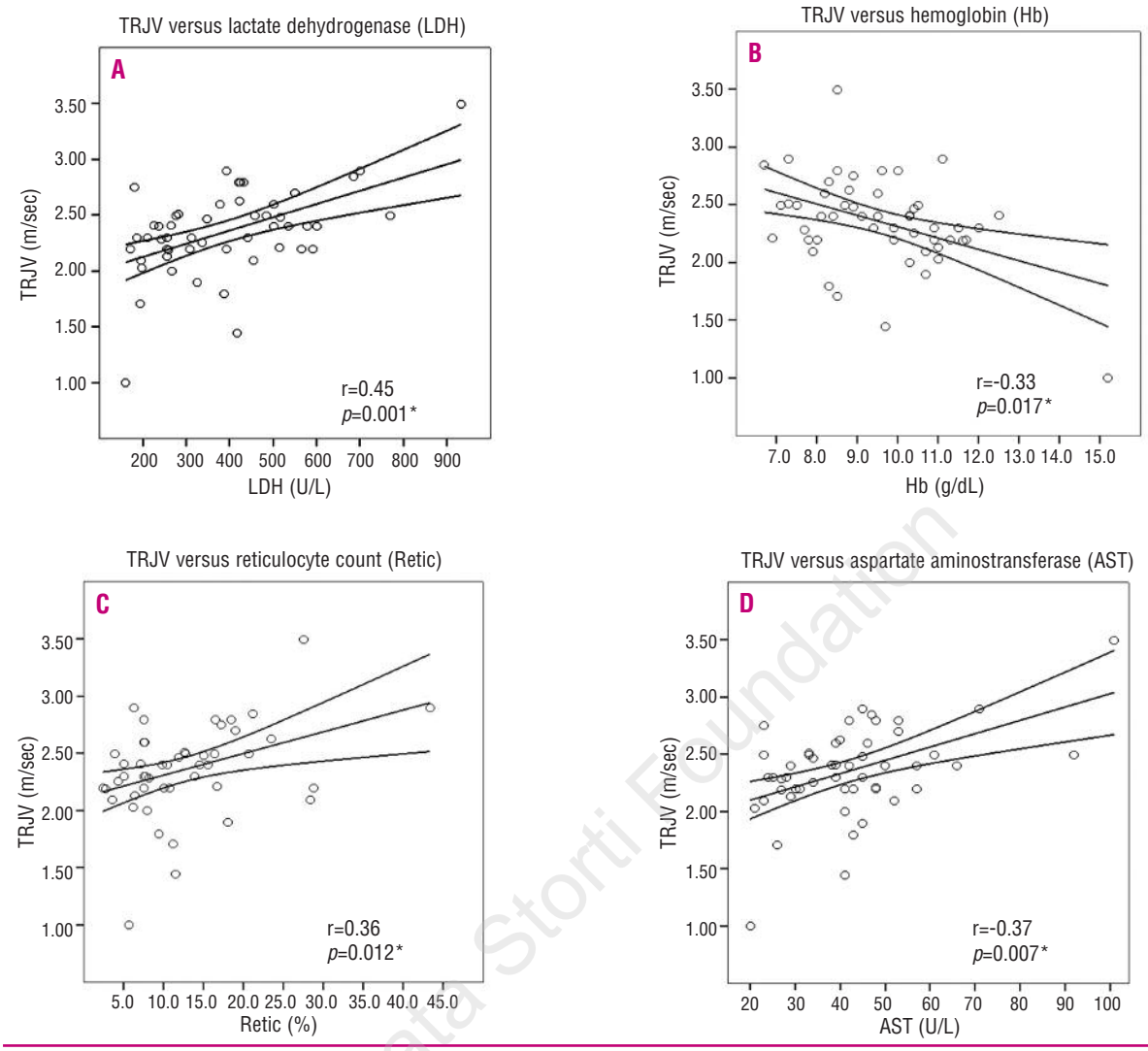

Figure 1. **Linear regression analysis between TRJV and LDH (A), Hb (B), retic (C) and AST (D) (95% CI shown). Significant positive correlations were observed between TRJV and LDH, retic and AST. A significant negative correlation was observed between TRJV and Hb. All r values were obtained using Spearman's Rank Order Correlation.**

has not been previously described in the literature. Hemolysis and its clinical implications have been the recent focus of intense investigation in SCD. Reiter *et al.*[10] demonstrated that plasma from patients with SCD inactivates NO. Growing evidence suggests that this hemolysis-related decrease in NO bioavailability is a major contributor to the development of vasculopathy related complications in adults with SCD, including PHT, priapism, leg ulcers and stroke.[5,11-13]

The relationship between TRJV and hemolysis in our cohort is observed most strongly with LDH, which is consistent with studies of adults with SCD. In their study, Kato *et al.*[5] found a significant association between steady state LDH and other markers of hemolysis. In these adults, erythrocyte LDH was the major isotype contributing to total LDH in fractionation studies. More importantly, higher LDH values were significantly associated with PHT and reduced survival after a median follow-up of 30 months. The high mortality rate associated with TRJV elevation and PHT in adults with SCD emphasizes the need for effective screening and intervention in the pediatric population, since pathophysiologic changes leading to the development of clinically relevant PHT may be reversible early in life. In our model, LDH emerged as the single most important predictor of TRJV. As an independent variable, however, LDH still explained less than 30% of the total variability. This suggests that the etiology of TRJV elevation in our young cohort is multi-factorial and only partly explained by hemolysis. Our study was not designed to evaluate the utility of LDH as a surrogate marker for elevated TRJV. Although sample size may have been a limitation, our inability to find an LDH cut-off value with optimal sensitivity and specificity for predicting a TRJV ≥2.5 m/sec. is shared by adult studies of SCD and PHT.

In our analysis, no other marker of hemolysis performed better, including plasma Hb, which requires optimal conditions for collection and measurement.[14]

Interestingly, higher total WBC was observed in our subjects with TRJV ≥2.5 m/sec. Although not directly associated with hemolysis, elevated WBC may be an important source of oxidative stress in SCD[15,16] contributing to NO dysregulation and pulmonary vascular remodeling.[17,18] To summarize, this is the first study to demonstrate that hemolysis is associated with TRJV elevation in a prospectively screened pediatric and young adult cohort. Although these findings are modest, they demonstrate the importance of hemolysis as a significant, albeit partial, contributor to TRJV elevation in our population. Larger cohort studies are needed to test the predictive value of these and other novel laboratory markers for TRJV elevation given that the small, cross-sectional, convenience sample of subjects in this study may have affected our results through selection bias. Because TRJV elevation itself is an independent predictor of early mortality in adults with SCD, therapies aimed at decreasing hemolysis in children with SCD may prove useful in preventing future clinically significant PHT.

Authors' contributions

RIL: design of study, enrollment of subjects, data analysis, manuscript preparation; AAT and LTY: design of study, interpretation of results, manuscript editing.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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