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Pulmonary Hypertension In Well-Transfused Thalassemia Major Patients

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Abstract

The risk for pulmonary hypertension (PH) in thalassemia major (TM) patients remains controversial. We report echocardiography results from 60 TM patients: we evaluated the association between tricuspid regurgitation velocities (TRV), iron stores, and serologic markers of hemolysis and arginine dysregulation.

Patients were enrolled from August 2004 until May 2009. All parameters were inversely weighted by the number of exams.

TRV was comparable between sexes and it was uncorrelated with age. At the first exam, TR velocities at the upper limits of normal (2.5–2.7 m/s) were observed in 8 patients. An abnormal TRV (2.9 m/s) was found in 1 patient.

Borderline increases in TRV were associated with a reduced global arginine bioavailability ($R = -0.399$ $P = 0.005$), increased anemia (haemoglobin: $R = -0.219$ $P = 0.0461$), cardiac index ($R = 0.223$ $P = 0.0481$), and diastolic dysfunction ($E/A: R = 0.289$ $P = 0.0088$; $E/E': R = 0.223$ $P = 0.0453$), but not hemolysis, iron overload and systolic function evaluated by Magnetic Resonance Imaging, and splenectomy.

Well-transfused TM patients have a lower risk for PH than thalassemia intermedia patients.

However, they do have vascular stressors that raise their lifetime PH risk to levels higher than for

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CONFLICTS OF INTEREST

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the general population. Consequently, we support recommendations for annual echocardiographic screening and cardiac catheterization for persistent TRV above 3 m/s.

Keywords

thalassemia major; tricuspid regurgitant jet velocity; risk for pulmonary hypertension; echocardiography

INTRODUCTION

Pulmonary hypertension (PH) is a disease of the pulmonary vasculature characterized by elevated pulmonary artery pressure and pulmonary vascular resistance. It is typically progressive, leading to right ventricular overload and eventually to right ventricular failure and premature death [1]. The increase in pulmonary vascular resistance is related to a number of progressive changes in the pulmonary arterioles, including: vasoconstriction, inflammation, in-situ thrombosis and proliferative remodelling of the pulmonary vessel wall. The gold standard for diagnosing pulmonary hypertension is a right heart catheterization (RHC) and measurement of blood pressure in the pulmonary arteries. Although RHC is now a relatively safe procedure, it is invasive and impractical to perform in patients for whom it is not clearly indicated. Because it is non-invasive, widely available and relatively inexpensive, echocardiography is frequently used to screen for PH, monitor progression over time and allow identification of patients for whom diagnostic RHC is warranted [2]. Doppler echocardiography uses the peak tricuspid regurgitant (TR) jet velocity, which estimates the pressure difference between the right ventricle and right atrium using the modified Bernoulli equation [3].

Hemoglobinopathies, including thalassemias, have been recognized as one of the most common causes of PH worldwide [4]. The pathophysiology is multifactorial, characterized by chronic hemolysis, loss of splenic function (or splenectomy), hypercoagulability, vascular inflammation, liver dysfunction, hypoxemia, iron overload, left ventricular dysfunction, and increased cardiac output [5], [6], [7] and [8]. As a result, most studies clearly suggest a high prevalence of pulmonary hypertension in thalassemia intermedia patients [9] and [5].

However, regular transfusion therapy modifies pulmonary hypertension risk by preventing chronic tissue hypoxia, reduces chronic hemolysis and its negative effects on nitric oxide availability, and lowers circulating proinflammatory hormones such as placenta growth factor (PIGF) [10]. Aggressive chelation therapy is necessary to reduce cardiac and vascular iron deposition due to transfusions in order to lower cardiovascular oxidative stress and improve vascular reactivity [11] and [12]. As a result, some studies suggest that pulmonary hypertension is quite rare in well-treated thalassemia major (TM) patients [13] and [14], unlike thalassemia intermedia patients [15]. In contrast, Hagar et al reported that 57% of their hypertransfused thalassaemia patients had tricuspid regurgitation velocities (TRV) greater than 2.5 m/s and concluded that chronic transfusions were not protective against PH [16]. Similarly, a multicenter study from the Thalassemia Clinical Research Network

reported TRV > 2.5 m/s in one third of chronically transfused patients and TRV > 3 m/s in 8/148 patients [17].

In the present study we report echocardiography results from 60 thalassemia major patients. In particular, we evaluated the association between TRV, iron stores, and serologic markers of hemolysis and arginine dysregulation.

MATERIAL AND METHODS

Patient selection

From August 2004 to May 2009 patients with a diagnosis of TM were enrolled in a combined cross-sectional and longitudinal trial probing for early predictors of cardiac dysfunction (EDICT study).

All patients had received >8 transfusions per year for at least 7 years. All had pulmonary function tests, magnetic resonance imaging (MRI) scans of the heart and liver, and blood tests performed within 10 days of a regularly-schedule transfusion to limit the confounding influence of anemia.

Results of exercise performance, pulmonary function, and electrocardiogram analysis have already been published [18], [19] and [20].

The protocol for the conduct of this study was approved by The Children's Hospital of Los Angeles' Committee for the Protection of Human Subjects.

We reviewed all digitally stored medical records from that study and selected the patients with one or more accurate measurements of tricuspid regurgitation jet by echocardiography. Sixty patients suitable for analysis were identified, for a total of 83 exams. The visits were scheduled within one week of transfusion and all patients underwent a MRI exam within 3 months. Average time delay between the two studies was 7.1 ± 14.2 days.

The medical records were reviewed also for demographic data and for hematologic and biochemical parameters, determined by routine laboratory methods, all measured within three months from the MRI. Blood parameters included complete blood count, plasma amino acids, LDH, cell free hemoglobin, and haptoglobin.

Echocardiography

Two dimensional and M-mode echocardiography, conventional Doppler echocardiography and tissue Doppler imaging (TDI) were performed at rest using an Philips IE33 echocardiography machine (Best, The Netherlands) using 2.5 and 5 MHz curved array transducers. Standard 2D echocardiographic parameters of cardiac structure and function, Doppler assessment of pulmonary veins, mitral inflow, tricuspid inflow, left ventricular outflow tract, as well as tissue Doppler imaging at the mitral and tricuspid annulus were acquired by experienced echocardiography technicians with the patient in the left lateral position. Three to five beat averages were used for improved overall measurement accuracy. Continuous wave Doppler was used to measure the peak velocity of the TR jet at end-

expiration. TR jet was only reported if a full envelope was recognized. At our institution, the upper limit of normal for TR jet is considered to be 2.7 m/s.

Left ventricular diastolic function was assessed according to the Nagueh et al guidelines [21]: early (E) and late (A) transmitral peak flow velocities were recorded. In patients with severe diastolic dysfunction, left ventricular filling occurs primarily in early diastole, creating an E/A ratio greater than 2.0. Early diastolic mitral annular velocity (E') was assessed by TDI and the ratio of mitral inflow E velocity to tissue Doppler E' velocity was used to estimate LV filling pressure. Increased E/E' is a marker of abnormal LV filling and correlates with high left atrial pressure [22]. All echocardiograms were analyzed by the principal investigator (JCW).

The corrected pulmonary vascular resistance PVR(c) was also estimated [23]. PVR(c) was shown to identify the severity of PH and may be used to assess treatment response.

MRI

All MRI examinations were performed using a 4-element torso phased array coil and a 1.5 Tesla General Electric CVi scanner running system 9.1.

For cardiac and hepatic iron overload assessment, validated multiecho gradient-echo sequences were acquired. All images were processed using custom MATLAB routines (The Mathworks, Natick, MA) [24]. Region of interest (ROI) in the heart was restricted to the interventricular septum [25] while in the liver it encompassed the entire visible tissue [26]. The signal in each pixel within a ROI was fit to an exponential plus a constant model and the median of the distribution of R2* values was calculated. As recommended [27], liver R2 values [28] and R2* values [26] were converted to predicted liver iron concentration (LIC) using appropriate calibration curves.

For the evaluation of biventricular volumes and ejection fractions, fifteen serial short-axis steady-state free precession cine images were acquired during 8-second breath holds with slice thickness adjusted to span the heart. Images analysis was performed in a standard way using MASS 4.0 (Medis, The Netherlands), as previously described [29].

Statistical analysis

All analyses were performed using SAS JMP v. 5.1 statistical software (SAS Institute, Cary, NC).

All parameters were inversely weighted by the number of exams such that each patient contributed equally to correlation and group-wise analyses. All continuous variables were expressed as the mean \pm standard deviation (SD). The Shapiro-Wilks W test was applied to check the normality of the residual distributions.

For continuous values with normal distribution, comparisons between two groups were made by independent-samples t-test. First, Levene's test was applied to verify the homogeneity of variances (homoscedasticity). When the significance level of Levene's test was < 0.05 and homoscedasticity could not be assumed, the Welch statistic was used. The

Wilcoxon signed-rank test was applied for continuous values with non normal distribution. Correlation analysis was performed using Pearson's test or Spearman's where appropriated. In all tests, a 2-tailed probability value of 0.05 was considered statistically significant.

RESULTS

Patients were balanced with respect to sex (30 males and 30 females) and the mean age at the first exam was 25.2 ± 9.4 years (range: 9–47 years). The cohort was 30.5% Chinese, 11.9% other Southeast Asia (Vietnamese, Laotian, Filipino), 18.6% Indian Subcontinent (Indian, Pakistani), 23.7% Mediterranean (Italian, Greek, Cypriot), 8.5% Middle Eastern (Iranian, Lebanese, Iraqi, Saudi). Seventeen patients had evidence of inactive hepatitis C and two patients smoked.

TRV was comparable between males and females (2.12 ± 0.29 m/s vs 2.13 ± 0.21 m/s; $P=0.8032$) and it was not correlated with age ($r=0.002$, $P=0.9828$). At the first exam, TR velocities at the upper limits of normal (2.5–2.7 m/s) were observed in eight patients. An abnormal TR jet (2.9 m/s) was found in only 1 patient.

Table 1 shows the association between the TR jet and the variables used as markers of different pathological states representing risk factors in the development of PH. No correlation was detected between TR jet and markers of increased hemolytic rate: decreased haptoglobin and increased concentration of lactate dehydrogenase (LDH) in the blood. An haptoglobin lower than 25 mg/dL was found in the 40% of the cases.

The global arginine bioavailability (GAB), calculated as arginine divided by the sum of ornithine plus citrulline and serving as a more comprehensive concept of reduced NO synthetic capacity compared with systemic arginine levels, showed a significant negative correlation with TR jet ($R=-0.399$; $P=0.0050$) (Figure 1). The GAB was also significantly correlated to the PVR(c) ($R=-0.4056$; $P=0.0063$).

Hemoglobin and hematocrit were used as markers for anemia and only the hemoglobin showed a significant negative correlation with TRV ($R=-0.219$; $P=0.0461$) (Figure 2, up). Because the patient visits were timed early in the transfusion cycle, patients were generally not anemic. TRV showed a weak positive correlation with the cardiac index ($R=0.223$; $P=0.0481$) (Figure 2, bottom). Note that 44.3% of all examinations demonstrated cardiac index values above the upper limit of normal (dashed vertical line), including all of the patients with intermediate increases of TR velocity (>2.5 ms and ≤ 2.7 m/s).

There was no correlation between TRV, total lung capacity (TLC), resting O₂ saturation, diffusion capacity of the lung for carbon monoxide (DLCO) adjusted for hemoglobin and lung volumes, or lowest saturation during exercise testing.

TRV was not correlated with systolic function (volumes and EF evaluated by MRI) while it was significantly correlated with both the markers of diastolic function: E/A ratio ($R=0.289$; $P=0.0088$) and E/E' ratio ($R=0.223$; $P=0.0453$) (Figure 3). The patients with flow related increases in TR velocity had high E/A ratios but normal or low E/E' ratios. In contrast, the patient with iron cardiomyopathy and pulmonary hypertension had a high E/E' ratio. Overt

left ventricular dysfunction (LV EF < 56%) was present in the 9.8% of cases, but only in those patients whose T2* was less than 10 ms. TRV was not associated with serum ferritin levels, LIC or cardiac R2*.

Twenty-four (40%) patients were splenectomized. Splenectomized patients were significantly older than non-splenectomized patients (31.2 ± 7.5 years vs 21.8 ± 6.8 ; $P < 0.0001$) but the two groups had comparable TRV (2.10 ± 0.23 m/s vs 2.15 ± 0.19 ; $P = 0.3855$).

HCV patients were significantly older (33.5 ± 7.1 years vs 22.5 ± 6.8 ; $P < 0.0001$) but the two groups had comparable TRV (2.11 ± 0.24 m/s vs 2.13 ± 0.19 ; $P = 0.9241$). There were not enough smokers to assess smoking's impact on TRV.

In the single patient having a clearly abnormal TRV (2.9 m/s), closer review of the clinical characteristics were instructive. She was a 47 year old smoker of Italian descent who had undergone splenectomy in childhood. At the time of her abnormal TRV, she had mild diastolic dysfunction (E/E' Ratio=10.1, E/A ratio = 2.2), moderate systolic dysfunction (LV EF=42.7%), severe cardiac iron overload (cardiac T2* 6 ms), and moderate hepatic iron overload (LIC=8.5 mg/g dw). At a follow-up echo 21 months later, TRV was normal (2.2 m/s). Her diastolic function was significantly improved (E/A ratio=1.63 and E/E' Ratio=7.9) while the systolic dysfunction was improved but still present (LV EF=48.5%). Her iron overload was decreased, but still severe in the heart (cardiac T2* 9.2 ms) and moderate in the liver (LIC=7.0 mg/g dw). We conclude that her pulmonary hypertension was likely post-capillary, arising from iron cardiomyopathy, that was gradually improving on combined deferiprone and deferoxamine therapy.

DISCUSSION

The EDICT patient cohort suggests a low risk for pulmonary hypertension in well-transfused thalassemia major patients. Pathologic TR increase was found in one patient and reversed with improved iron chelation. Our finding is in agreement with studies involving optimally treated Italian patients with thalassemia major [15] and [30]. A recent multicenter Italian study reported TRV > 3.0 m/s in 5.7% and RHC confirmed pulmonary hypertension in 2.1% [31]. However, the prevalence of pulmonary hypertension varies significantly with genotype [32] and [33] and our study cohort was much more ethnically and genetically diverse than the Italian studies. Previous reports from a large American cohort suggested TRV > 2.5 m/s in 33% of patients and TRV > 3 m/s in 5% [17].

While we were unable to document any TRV suggestive of PH, mild elevations in TRV (2.5 – 2.7 m/s) were relatively common (13%). TRV was associated with low hemoglobin and high cardiac index, suggesting that increased pulmonary blood flow (to compensate for the anemia [13] and [29]) might account for mild elevations in TRV even if pulmonary vascular resistance is normal. The observation that TRV depends upon cardiac index is well known [34]. Elite athletes have significantly higher TRV at rest than nonathletes, presumably related to their large hearts and increased stroke volumes [35]. Increased TRV's in these athletes are not indicative of any vascular disease. Although TM patients are not typically

athletes, their resting cardiac dimensions, stroke volumes and cardiac index are all significantly larger than those found in elite athletes; almost half of the patients in this cohort had resting cardiac outputs above the upper limits of normal. As a result, resting TRV of 2.5 – 2.7 may be completely normal for TM patients. However, increased pulmonary blood flow can compromise the normal compliance of pulmonary vasculature by exposing the endothelium to elevated shear forces and mechanical strain [36]. High vessel pulsatility upregulates endothelial arginase, stimulating vascular remodelling. As a result, high pulmonary flow can promote pulmonary vascular disease over a lifetime in some conditions, such as unrepaired atrial septal defects [37] and single pulmonary artery [38]. Thus, further work will be necessary to understand the clinical relevance of these flow-mediated increases in TRV as thalassemia patients age into their fifties, sixties, and seventies.

Similarly, the association of TRV with left ventricular diastolic markers suggests that mild TRV elevations can also result from elevated downstream (post-capillary) filling pressures, rather than changes in pulmonary vascular resistance. PH is common in non-thalassemic patients with left-sided cardiac disease and has been reported in greater than 60% of patients with left ventricular systolic dysfunction, greater than 80% of patients with left ventricular diastolic dysfunction, and in 78% of patients prior to mitral valve surgery [39] and [40]. In fact, PH frequently develops in response to left-sided cardiac disease due to elevated pulmonary venous pressure and is associated with a series of lesions that range from diseases of the pulmonary veins, mechanical obstruction in the left atrium or at the mitral valve, or to elevation in the left atrial and pulmonary venous pressure due to mitral regurgitation, abnormal left atrial compliance, or to LV diastolic dysfunction [40]. In our TM population, overt ventricular dysfunction was detected in 9 exams, but was associated with a pathological TR jet in only one patient (11.1%). In fact, TRV and LV EF were uncorrelated. In contrast, both E/A and E/E' ratios were associated with TRV, suggesting that diastolic dysfunction is more important than systolic dysfunction in the development of PH in TM.

Hemolysis plays a key role in the development of PH in most hereditary and chronic haemolytic anaemias [41], [42] and [43], through nitric oxide (NO) scavenging, arginine catabolism, and endogenous nitric oxide synthesis inhibition [44] and [45]. Low NO bioavailability produces a vasculopathy characterized by endothelial dysfunction, increased vascular tone, inflammation, and hypercoagulability. Vascular remodeling and destruction of pulmonary vasculature ultimately result in hemolytic anemia-associated PH [46]. However, most red cell destruction in thalassemia major is intramedullary and toxic red cell contents are less likely to be released into the circulation. In fact, we found no significant association between TR jet and two reliable markers of haemolysis: haptoglobin [47] and LDH [48]. A haptoglobin level < 25 mg/dL was shown to have a high sensitivity and specificity (83% and 96%, respectively) in predicting hemolytic disease [49] and serum haptoglobin can be used in the diagnosis of hemolysis in patients receiving multiple units of packed red blood cells [47]. Using the upper limit of 25 mg/dL for the haptoglobin in our population, hemolysis was present in the 40% of the cases but no significant PH was detected in these patients. Our finding is in agreement with the study of Hagar et al who did not find a difference in several markers of haemolysis between TM patients with and

without PH [16]. It also suggests that significant differences exist between PH in TM and in sickle cell disease [41] and [48].

Further evidence that hemolysis is not central to PH in well transfused TM patients was the essentially normal global arginine bioavailability index. In SCD, reduced GAB was shown to be independently associated with pulmonary hypertension and increased mortality [50]. Three categories of GAB were defined in that study: *high* (> 0.6254), *medium* (*between* 0.3245 and 0.6254) and *low* (<0.3245). Mean GAB in our patients was 0.81 ± 0.29 , comparable to values reported in healthy subjects (0.82 ± 0.27) and higher than the mean value reported in 209 SCD patients (0.50 ± 0.26) [50]. So, the normal GAB found in TM may explain the significantly lower rate of PH. Paradoxically, TRV retained an association with global arginine bioavailability (GAB). However, arginase is upregulated in non-hemolytic vascular diseases, so GAB is a nonspecific marker of vascular stress [51].

Iron overload is known to impair blood vessel reactivity in thalassemia major [52], [12] and [11] and is correlated with pulmonary hypertension in thalassemia intermedia [53]. However, neither serum ferritin levels, hepatic iron, or cardiac iron were associated to TRV in our study. One possible explanation is that we had so few patients with increased TRV that we could not demonstrate a relationship with iron. Alternatively, regular chelation therapy may have protected the vascular endothelium independently of the organs studied by MRI [12].

Finally, a number of studies showed an association between splenectomy and pulmonary hypertension, both in thalassemia patients [54], [55], [31] and [17] and patients without hemoglobinopathies [56]. Splenectomy leads to increased platelet activation [57] and [44] and red cell microparticles [58], upregulating vascular adhesion molecules and promoting thrombosis. However, in our population TRV was comparable between splenectomized and non-splenectomized patients and no age-effect was observed, counter to the results reported by the Thalassemia Clinical Research Network [17]. A couple of key differences in the patient populations and study design may explain the disparity. Firstly, our patient population was younger, with only five patients older than 40 years of age. Secondly EDICT patients were receiving 2–3 units of blood every three weeks and were within 10 days of their last transfusion, effectively suppressing their ineffective erythropoiesis. In contrast, the TCRN cohort used a cutoff of eight transfusions per year (transfusion interval < 6.5 weeks), contained a sizable cohort of thalassemia intermedia patients who had initiated chronic transfusion therapy later in life, and studied patients randomly within the transfusion interval; late onset of transfusion therapy is a known risk factor for pulmonary hypertension [59]. Since both the degree of ineffective erythropoiesis [53], and hemoglobin level (the present work) impact TRV, differences in transfusion management and exam timing could explain disparities between the two studies. It should be considered that plasma hemoglobin and microparticle levels are higher in post-splenectomy beta thalassemia patients [59] and [60], but this effect might likely be masked in our well-transfused cohort.

A limitation of this study is that RHC was not performed. However a confirmatory RHC is only recommended for patients with TRV >3.4 m/s or in patients with a TRV between 2.9

and 3.4 m/s in the presence of other signs suggestive of pulmonary hypertension [61] and none of our patients fit that criteria.

CONCLUSIONS

Well-transfused thalassemia major patients have a low risk for pulmonary hypertension relative to thalassemia intermedia patients. Borderline increases in TRV were associated with a reduced global arginine bioavailability, increased anemia, cardiac index, and diastolic dysfunction but not hemolysis or splenectomy. However, thalassemia major patients do have vascular stressors that raise their potential risk for pulmonary hypertension relative to the general population. Consequently, we support recommendations for annual echocardiographic screening in these patients to assess TRV and diastolic function indices and cardiac catheterization for persistent elevations of TRV above 3 m/s.

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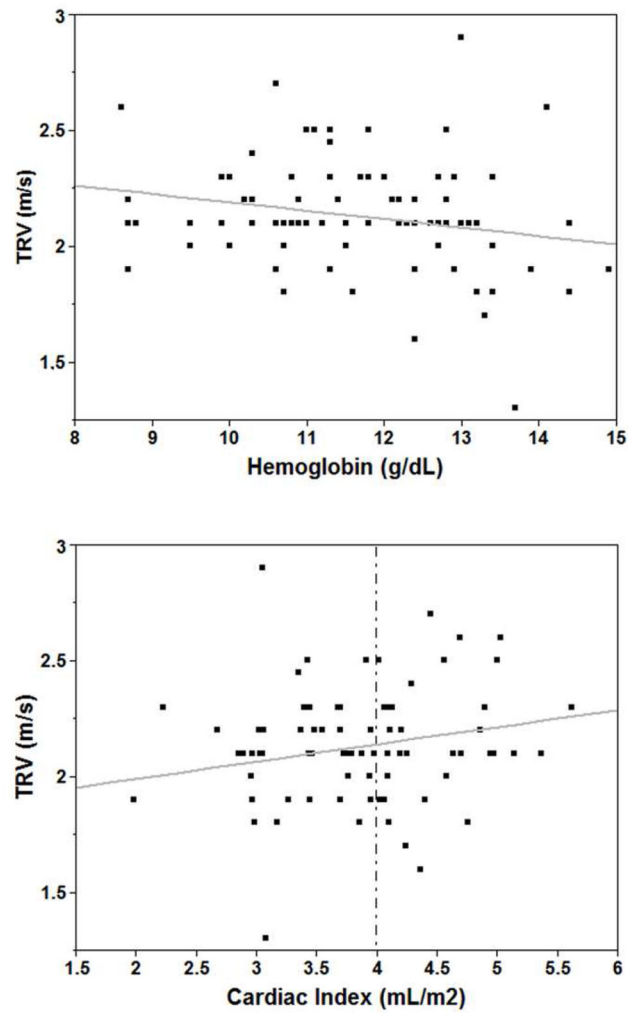


Figure 2. Scatter plot and fitted regression line showing the relationship between TRV and hemoglobin (up) and between TRV and cardiac index (bottom).

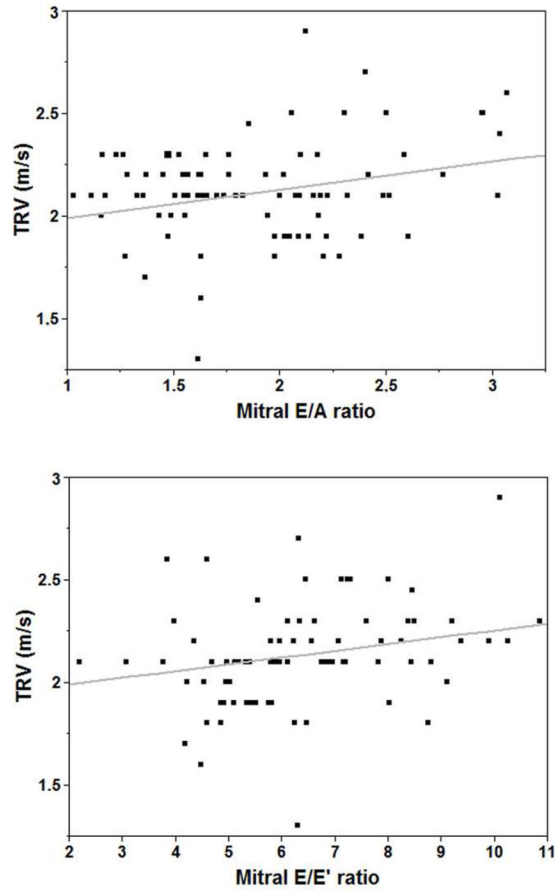


Figure 3. Scatter diagram and regression line of TRV compared to E/A ratio (up) and E/E' ratio (bottom); E/E' > 10 is considered evidence of diastolic dysfunction.

Table 1

Associations with TRV.

| Variable | Mean \pm SD | Correlation with TRV | |
|---|-----------------------|----------------------|---------|
| | | R | P-value |
| Age | 25.53 \pm 8.04 | 0.002 | 0.9828 |
| <i>Hemolysis</i> | | | |
| Haptoglobin (mg/dL) | 34.02 \pm 22.61 | -0.032 | 0.7702 |
| LDH (U/L) | 443.53 \pm 178.37 | 0.151 | 0.2341 |
| Global arginine bioavailability | 0.81 \pm 0.29 | -0.399 | 0.0050 |
| <i>Anemia</i> | | | |
| Hemoglobin (g/dL) | 11.75 \pm 1.24 | -0.219 | 0.0461 |
| Hematocrit (%) | 35.14 \pm 3.73 | -0.195 | 0.0771 |
| Cardiac Index (L/min/m ²) | 3.88 \pm 0.63 | 0.223 | 0.0481 |
| <i>Hypoxia</i> | | | |
| TLC (% of predicted) | 94.06 \pm 11.69 | 0.182 | 0.1079 |
| Resting Saturation O ₂ (%) | 96.95 \pm 1.13 | 0.068 | 0.5618 |
| O ₂ saturation during exercise (%) | 93.77 \pm 4.08 | 0.165 | 0.1759 |
| Adjusted DLCO | 5.43 \pm 0.87 | 0.126 | 0.2797 |
| <i>Systolic function</i> | | | |
| LV EDVI (mL/m ²) | 91.76 \pm 15.38 | 0.109 | 0.3268 |
| LV ESVI (mL/m ²) | 34.95 \pm 8.89 | 0.017 | 0.8815 |
| LV EF (%) | 62.34 \pm 5.18 | 0.179 | 0.1063 |
| LV mass index (g/m ²) | 69.58 \pm 11.77 | 0.036 | 0.7507 |
| <i>Diastolic function</i> | | | |
| MV E/A | 1.94 \pm 0.42 | 0.289 | 0.0088 |
| MV E/E' | 6.16 \pm 1.40 | 0.223 | 0.0453 |
| <i>Iron</i> | | | |
| Serum ferritin (ng/L) | 2789.42 \pm 2477.51 | -0.096 | 0.3852 |
| Cardiac R2* (Hz) | 97.43 \pm 73.13 | -0.166 | 0.1338 |
| LIC (mg/g dw) | 11.69 \pm 9.19 | -0.079 | 0.4796 |