

Pulmonary Hypertension in Thalassemia: Association with Platelet Activation and Hypercoagulable State

Sylvia T. Singer,^{1*} Frans A. Kuypers,² Lori Styles,¹ Elliott P. Vichinsky,¹ Drucella Foote,¹ and Howard Rosenfeld³

¹ Children's Hospital and Research Center at Oakland, Hematology/Oncology Department, Oakland, California

² Children's Hospital Oakland Research Institute, Oakland, California

³ Children's Hospital and Research Center at Oakland, Cardiology Department, Oakland, California

The pathogenesis of pulmonary hypertension (PAH), a serious complication in thalassemia, is not well understood. Thromboembolism has been postulated as one of the causative factors; however, there are currently limited specific data on its role. To examine whether increased platelet activation and hypercoagulability are linked to PAH, 25 β -thalassemia major and β -thalassemia intermedia patients were evaluated with Doppler echocardiograms for estimation of pulmonary artery pressure and with laboratory assays for indications of a prothrombotic state. The association of clinical variables and abnormal coagulation assays with PAH was determined. PAH was identified in 17 (68%) patients; mean pulmonary artery systolic pressure was 39.8 ± 5.4 mm Hg. PAH was significantly associated with prior splenectomy, older age, and evidence for chronic hemolysis, diagnosed in both transfused ($n = 10$) and nontransfused ($n = 7$) patients. Increased platelet activation, measured by P-selectin, was significantly associated with PAH ($P = 0.001$). Increased thrombin-antithrombin III level was more prevalent in the presence of PAH, but increased fibrinolysis or low protein C levels were not. This study underscores the role of platelet activation in the development of PAH and stresses its occurrence even among patients who are regularly transfused, especially those who are older and have had splenectomies. *Am. J. Hematol.* 81:670–675, 2006. © 2006 Wiley-Liss, Inc.

Key words: thalassemia; pulmonary hypertension; platelet activation

INTRODUCTION

Patients with β -thalassemia who develop pulmonary hypertension (PAH) frequently have cardiac and pulmonary deterioration, which can result in significant morbidity and mortality [1–5]. While PAH is increasingly recognized as part of the clinical spectrum for β -thalassemia, little is understood about the mechanisms and risk factors for its development. Consequently, prevention and treatment efforts are inadequate. Most patients do not undergo routine monitoring of right heart pressure to detect PAH, and reports of treatment efficacy have been anecdotal [6]. There are currently limited data on the effect of any treatment modality as data supporting a selective treatment approach have been insufficient [7]. Several studies have suggested that chronic hypoxia and lung injuries due to infections and iron depositions are common causes [1,3,8–11], whereas others suggest a hypercoagulable state caus-

ing thrombotic lesions in the lungs as a risk factor [4,5,9,12–14]. We sought to determine whether hypercoagulability and platelet activation were associated with thalassemia-related PAH. Confirming such an association and identifying specific markers predicting PAH could affect early diagnosis and treatment intervention in these patients.

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*Correspondence to: Sylvia T. Singer, Children's Hospital and Research Center at Oakland, 747 Fifty Second Street, Oakland, CA 94609-1808. E-mail: tsinger@mail.cho.org

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PATIENTS AND METHODS

Study Population

The study population consisted of 25 patients with β -thalassemia who were 10 years of age and older and seen regularly at the Comprehensive Thalassemia Center in Oakland, CA. Patients receiving treatment with an anticoagulant or platelet inhibitor were excluded. Over a period of 8 months, all eligible patients who had a complete echocardiogram performed within the 6 months prior to study initiation, or agreed to have one done, were approached for enrollment in the study. Eighteen patients had β -thalassemia major (TM) and received regular transfusions; 7 had β -thalassemia intermedia (TI) and received only occasional or no transfusions. Age, spleen status, transfusion state, and history of a thrombotic event were recorded. Average ferritin levels were calculated over a year prior to study enrollment and most recent liver iron concentration was obtained by biopsy or by spectroferritometry (SQUID). Approval for the study was obtained from the institutional review board and all patients or their legal guardians signed an informed consent.

Measurement of Pulmonary Artery Pressure

Evaluation of pulmonary artery (PA) pressure was based on continuous-wave Doppler measurement of the tricuspid regurgitant jet velocity (TRV), which estimates right ventricular to right atrium systolic pressure and approximates PA systolic pressure. PAH was defined as a TRV ≥ 2.5 m/s or PA systolic pressure greater than 25 mm Hg. The PA systolic pressure was determined based on the modified Bernoulli equation [$4(\text{TRV})^2 + \text{CVP estimate}$] [1,15–17].

Laboratory Assays

Laboratory analysis for platelet activation included platelet count, P-selectin level by enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN), and von Willebrand factor:Antigen (vWF:Ag) by latex immunoassay. The presence of a hypercoagulable state was evaluated by thrombin-anti-thrombin III (TAT), protein C, protein S, and fibrinogen levels, plasminogen activator inhibitor-1 (PAI-1), and D-dimers assays (performed with commercially available assays). Soluble vascular cell adhesion molecule (sVCAM), a measure of endothelial cell activation, was performed by ELISA (BioSource International, Camarillo, CA). Mean hemoglobin (Hb) level and reticulocyte count (pretransfusion Hb in TM patients) were determined by averaging the results of tests during the 6 months before the study began.

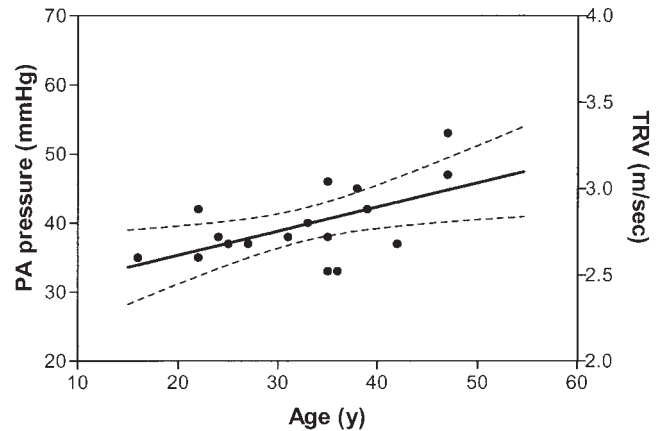


Fig. 1. Correlation of estimated mean systolic pulmonary artery (PA) pressure with age. Data shown for patients with increased pressure on Doppler echocardiogram ($n = 18$, $r = 0.64$, $P = 0.005$).

Statistical Analysis

Patients were categorized into two groups based on the presence or absence of increased TRV (>2.5 m/s), indicating PAH. The unpaired t test was used to compare each of the laboratory variables obtained from patients with or without increased TRV and Fisher exact test was applied for effect of spleen status and transfusion status. Pearson correlation coefficient (r) was calculated between patients' age and estimated level of PA pressure. Negative and positive predictive values were calculated for elevated P-selectin level for an estimated elevated PA pressure. Two-tailed P values ≤ 0.05 were considered significant. Data are reported as means \pm standard deviation (SD) unless otherwise indicated.

RESULTS

Twenty-five patients, ages 10 to 47 years (26 ± 10 years) were screened by echocardiogram, 17 (68%) had increased TRV, mean 2.7 m/s (range 2.5–3.3 m/s), and a mean systolic PA pressure of 39.8 ± 5.4 mm Hg (range 33–53 mm Hg) or mean TRV. Ten of 17 were regularly transfused TM patients and 7 had TI. The 8 remaining patients (all with TM) had trace or no tricuspid regurgitation and a normal pulmonary pressure was assumed. Two of the patients with PAH had a prior thrombotic event.

The presence and severity of PAH correlated with age ($P = 0.005$, $r = 0.64$); Six of seven patients with a PA pressure ≥ 40 mm Hg were over 30 years old (Figure 1). There was no evidence of abnormal TRV in six of seven patients under age 20.

The prevalence of PAH was high in the nontransfused TI patients (7/7); nevertheless, 10/18 transfused

TABLE I. Correlation of Clinical and Laboratory Findings with Presence or Absence of PAH

	PAH (n = 17)	No PAH (n = 8)	P value
Spleen removed	16	2	0.001
TM on transfusions	10	8	ns
TI not on regular transfusions	7	0	
Age (years)	33 ± 9	17 ± 5	0.005
Liver iron ^a μ/g dry wt	13 ± 8 (n = 13)	15 ± 7 (n = 6)	ns
Ferritin level (nl 30–300 ng/ml)	2272 ± 1600 (n = 15)	1850 ± 1200 (n = 8)	ns
Platelet count th/×10 ³	566 ± 200	369 ± 143	0.028
Hb (gr/dL)	9.5 ± 1	9.8 ± 1	ns
Reticulocytes (%)	5.4 ± 4	1.7 ± 1	0.02
P selectin (nl 9–48 ng/ml)	64 ± 11	32 ± 10	0.0001
TAT (nl < 4 μg/L)	16 ± 33	13 ± 6	0.35
vWF: Ag (nl 50–150%)	128 ± 33	105 ± 50	ns

^aSafe level < 3 μ/g dry wt.

TM, Thalassemia major; TI, Thalassemia intermedia; TAT, Thrombin-anti thrombin III; vWF:Ag, von Willebrand factor:Antigen; sVCAM, Soluble vascular cell adhesion molecule; ns, not significant.

Results are reported as mean ± standard deviation.

patients (55.5%) had evidence of PAH. Estimated systolic pulmonary artery pressure did not differ between the transfused and nontransfused groups (40 ± 5.6 mm Hg versus 39.6 ± 5.8 mm Hg).

Although mean hemoglobin levels were similar in patients with or without PAH (9.5 ± 1.2 g/dL versus 9.8 ± 1.0 g/dL), the percentage of reticulocytes was higher in patients with PAH than in those without ($5.4 \pm 4\%$ versus $1.7 \pm 1\%$, $P < 0.02$). In nontransfused TI patients hemoglobin levels were lower compared to the transfused patients (8.4 ± 1.7 versus 10 ± 0.9 g/dL) and percentage reticulocytes was higher ($8.3 \pm 3\%$ versus $2.7 \pm 2\%$). Percentage reticulocytes did not correlate with the extent of TRV.

Iron levels as measured by ferritin were not correlated with presence of PAH; however, data on liver iron concentration for better determination of such association were unavailable in most TI patients (Table I).

Effect of Splenectomy

The presence of PAH was associated with prior splenectomy and noted in 16 of the 18 splenectomized patients ($P < 0.001$). Postsplenectomy thrombocytosis correlated with evidence of PAH; platelet count was highest (mean $765 \times 1000/\text{mm}^3$) in splenectomized nontransfused TI patients ($n = 6$), all 6 of whom had increased TRV, followed by the transfused splenectomized patients (mean $515 \times 1000/\text{mm}^3$), 10/12 of whom had increased TRV. Platelet count was normal (mean $256 \times 1000/\text{mm}^3$) in the 7 patients who had not had splenectomies, only 1 of whom had an increased TRV.

Platelet Activation

P-selectin was elevated in 14/17 (87.5%) patients with PAH and in none of those without PAH ($P =$ American Journal of Hematology DOI 10.1002/ajh

0.001, positive predictive value 1.0, negative predictive value 0.72) (Figure 2). The level of P-selectin did not show a significant correlation with the extent of TRV. Among patients who had splenectomies, P-selectin remained elevated despite regular transfusions (67.8 ± 13 ng/ml versus 63 ± 11 ng/ml for nontransfused patients), suggesting that transfusions do not eliminate ongoing platelet activation. All 7 patients who had not had splenectomies had normal P-selectin levels (31 ± 9 ng/ml). When we further examined for markers of interaction of activated platelets and damaged red blood cells within the pulmonary vasculature by measuring sVCAM and vWF:Ag, we found no significant association with PAH, although the ranges for both measurements were higher in those with PAH (Table I).

Increased Coagulation

When coagulation and fibrinolysis markers were studied to determine whether activated platelets and damaged RBCs had a procoagulant effect, we found that TAT levels were elevated in some patients but did not correlate with the presence of PAH; 13/17 (81%) patients with and 50% of the 8 without PAH had elevated levels ($P = 0.35$). TAT levels were increased mostly in older, nontransfused TI patients who had splenectomies. There was no evidence of increased fibrinolysis: fibrinogen level, PAI-1 activity, and D-dimers concentration were normal in all patients except 2 who had increased D-dimers concentration at a 250 to 500 ng/ml range.

Protein C levels were below normal range in the majority of the 25 patients (mean $51 \pm 12\%$, normal 66–154%) but there was no correlation with presence of PAH. Still, the nontransfused patients who had splenectomies had the lowest levels ($39 \pm$

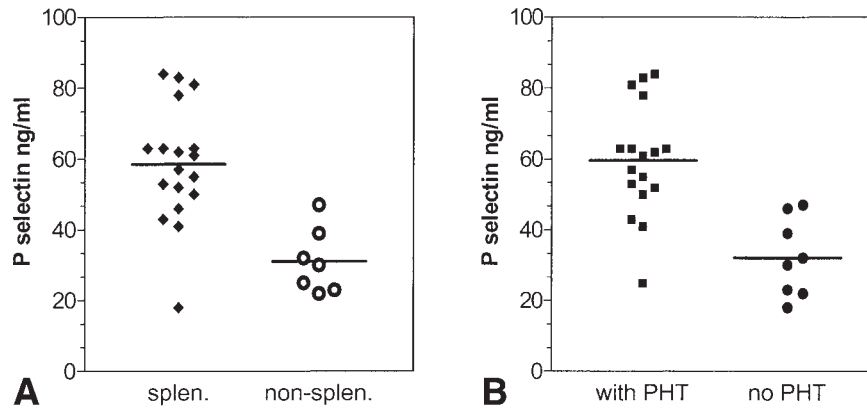


Fig. 2. Association of spleen status (A) and PAH (B) with P-selectin. Patients with PAH had higher P-selectin levels (normal: 9–48 ng/ml) compared to those without PAH and most had a prior splenectomy. splen. = undergone splenectomy; non-splen. = have not undergone splenectomy.

7%). Protein S levels were in the low normal range ($68 \pm 14\%$, normal 67–167%).

DISCUSSION

PAH poses a serious complication for thalassemia patients who frequently already have compromised cardiac function. However, the prevalence of PAH in thalassemia, age of onset, and rate of progression have not been well studied. In this study, we explored the characteristics of thalassemia patients with PAH and examined whether specific abnormalities in the coagulation system are associated with its development. Echocardiography has been used extensively for determination of PAH and reported to correlate with catheter measurements at 0.89–0.97, yet determination by echocardiography may not be sensitive enough in cases of lower PA systolic pressure [18]. This must be taken into consideration for estimations of frequency of PAH in the present study.

The frequency of PAH ranges from 10 to 74% in previous studies, and an overall higher rate is noted in TI patients [1,3,19,20]. A frequency of 59% was reported in one study of TI patients who were either nontransfused or were “late starters” in receiving regular transfusions [1]. The 68% frequency of PAH in our study population falls within this range, but probably does not truly represent the prevalence of PAH in the general population of thalassemia patients, since we screened a small group of patients seen at our clinic over a limited time period. Nonetheless, our findings indicate a significant correlation of older age with presence and severity of PAH, suggesting a need for closer monitoring of right heart pressure, particularly in those over 15–20 years old.

The mechanisms leading to PAH in thalassemia patients are poorly understood and are likely multi-

factorial. Chronic hypoxia, a combination of anemia and left ventricular dysfunction causing increased ventricular pressure, and pulmonary vascular remodeling have all been postulated [1,3,19–21]. Lung injuries due to infections and iron depositions resulting in interstitial fibrosis have also been suggested [10,11]. More recently, chronic hemolysis in patients with sickle cell disease and other hemolytic anemias was suggested as an important factor in the pathogenesis of PAH [16], as also suggested by our finding. PAH has also been attributed to the presence of a pulmonary thromboembolism. Direct evidence comes from autopsy findings of pulmonary thrombotic lesions in patients with biventricular heart failure [4,5]. Thromboembolic events are known to occur in thalassemia patients, mostly in TI patients [13,14,22,23]. We found a significant association of PAH with prior splenectomy, findings that concur with previous observations linking cardiac disease and PAH to splenectomy in hemolytic disorders including thalassemia [12,24,25]. A proposed mechanism involves abnormal red cell membrane phosphatidylserine exposure, which can trigger low-grade hypercoagulability, which is enhanced in the absence of spleen [26,27]. Alternatively, growing evidence suggests hemolysis-induced nitric oxide scavenging, causing platelet activation, thrombosis, and endothelial dysfunction [28]. Splenectomy was proposed as a risk factor for chronic thromboembolic pulmonary hypertension even in healthy splenectomized individuals [29,30], suggesting a general increased risk, possibly enhanced in chronic hemolytic disorders.

Thrombocytosis is a known postsplenectomy consequence [31]; however, the effect of long-standing absence of spleen and thrombocytosis on increased platelet activation and development of PAH in thalassemia has not been explored. We found a signifi-

cant association of P-selectin, a marker for in vivo platelet activation and an essential component in thrombus formation [32], with PAH. P-selectin may serve as an important marker for diagnosis of PAH in thalassemia. If it proves significant in larger scale studies, P-selectin inhibitors could have a role in treatment of these patients [33,34]. Activated platelets can affect vascular endothelial disruption, as noted by studies showing increased levels of vascular adhesion molecules and vasoactive substances in PAH [35,36]. More sensitive studies for detecting endothelial activation and proliferation are needed to examine this platelet-endothelial interaction in thalassemia.

The number of patients examined in this study was insufficient for reaching conclusions relating to the presence of a low-grade hypercoagulable state as indicated by increased TAT in some patients. Low protein C levels have been described in thalassemia patients, resulting from decreased liver synthesis or down-regulation by inflammatory mediators [37] and may contribute to a hypercoagulable state in thalassemia, but its role to development PAH is unknown.

In this study, PAH was found among transfused TM patients, most of whom had splenectomies, implying that regular transfusions do not prevent but rather attenuate the progression of increased pulmonary pressure. Although recently reported to decrease TAT levels and lessen PAH [38], platelet-activated endothelial pulmonary vascular changes and chronic hemolysis may not be completely turned off by transfusions. Moreover, regular transfusions result in iron overload, as also noted in these study patients, a process that may cause pulmonary fibrosis and vascular resistance, thereby contributing to the development of PAH. We found that PAH was associated with chronic hemolysis, but not with the presence of anemia. Hemolysis is thought to result in reduced bioavailability of arginine and nitric oxide in sickle cell disease, causing endothelial dysfunction and PAH [39]; a similar mechanism may affect thalassemia patients.

Taken together, our findings highlight the high prevalence of mild to moderate PAH in TI patients as well as in transfused TM patients and suggest that platelet activation may be a potential contributing mechanism of its development. Platelet activation likely contributes to a procoagulant state, endothelial cell stimulation, and increased pulmonary in situ thrombosis. We identified P-selectin as a potential marker for the presence of PAH that could be used diagnostically as well as for future, targeted, effective treatments for PAH in thalassemia. Further understanding of the pathogenesis of PAH in these patients and therapeutic trials of antiplatelet, anticoagulation, and pulmonary vascular remodeling agents are needed.

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