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Pulmonary Arterial Hypertension in Previously Splenectomized Patients with β-Thalassemic Disorders

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Abstract

Our aim was to study the cause and describe the clinical features of pulmonary arterial hypertension (PHT) in splenectomized β-thalassemia (β-Thal) patients. Ten splenectomized β-Thal patients with systolic pulmonary artery (PA) pressure >30 mm Hg were evaluated by echocardiography, right-heart catheterization, and pulmonary angiography. Five of these patients later underwent hemodynamic studies. Echocardiography and pulmonary angiography on the 10 patients showed normal values of left ventricular systolic function and no findings of acute or chronic pulmonary embolism. Hemodynamic evaluation showed very high PA pressures associated with markedly increased pulmonary vascular resistance indices (PVRIs). Hematological evaluation of the 10 patients showed marked anemia, markedly increased numbers of nucleated red blood cells (nRBCs), and serum ferritin. Mean platelet count, plasma β_2 thromboglobulin, and thrombin–antithrombin III complex levels were significantly increased. It was concluded that PHT can be found in splenectomized β-Thal patients. Features associated with PHT were female sex, hemoglobin E/β-Thal, status many years postsplenectomy, marked anemia, markedly increased nRBC count, thrombocytosis, and very high serum ferritin levels. PHT was not due to pulmonary emboli. Our findings suggested that severe PHT was due to increased PVRI from thrombotic pulmonary arteriopathy, likely from chronic low-grade hypercoagulability and platelet activation after splenectomy. *Int J Hematol.* 2003;78:139-145.

Key words: Thalassemia; Splenectomy; Pulmonary hypertension

1. Introduction

Compared with systemic arterial hypertension, pulmonary arterial hypertension (PHT) is less common, is more difficult to manage, and has a worse prognosis. It results from reductions in the caliber of the pulmonary arteries and/or increases in pulmonary blood flow [1]. In thalassemia (Thal), a hereditary hemolytic anemia, PHT could simply be due to an increased pulmonary blood flow from chronic anemia. Decreased caliber of the pulmonary vessels had never been suspected until Sonakul et al [2,3], in reviewing autopsy materials of Thal patients, found many old and new irregularly distributed thrombi mostly in 100- to 800-μm arteries and vascular changes indistinguishable from those of primary arteriosclerosis in larger arteries. Evidence of vasculitis and of infarction was lacking. Most of those patients had undergone splenectomy and had hemoglobin E/β-thalassemia (E/β-Thal). The nature of the studies did not provide evidence of the cause of pulmonary thrombi, but the authors favored the diagnosis of thromboembolism. Although deep vein thrombosis (DVT) has been reported to be increased in β-Thal [4,5], our experience does not support this conclusion. Instead of acute pulmonary embolism (PE) from DVT, we not infrequently see cases of suspected acute PE that lack both clinical evidence of acute and/or chronic DVT and evidence of thrombi in the pulmonary artery (PA) on ultrafast computed tomographic (CT) scans of the lungs. Lack of knowledge of the true nature of pulmonary thrombi in Thal patients has led physicians to treat such patients for PE, a therapy that may not be appropriate. We therefore performed this study to determine the nature of pulmonary thrombi and the cause of PHT in these patients. Because the pathology of pulmonary vasculature in Thal patients as

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reported by Sonakul et al [2,3] is quite similar to that of primary PHT, in which thrombi can form in situ in small PAs or arterioles [1], studies for evidence of coagulation and platelet activation were performed to understand the pathogenesis. Clinical features, if any, are described.

2. Materials and Methods

During July 1998 to June 1999, 100 patients with Thal disease in our clinic were screened for clinical evidence of PHT (eg, loud P_2, right ventricular heave, and elevated jugular venous pressure) and acute and chronic DVT (eg, unilateral leg swelling, dilated superficial veins, tender venous cord, chronic ankle ulcers). No patient was found to have clinical evidence of DVT, but 17 had clinical evidence of PHT. All had undergone splenectomy in the past. None had clinical evidence of chronic liver disease or portal hypertension. After being informed of the objectives of the study, 16 patients consented to participate. To confirm the diagnosis, establish disease severity, and find causes of PHT, we used echocardiography and right-heart catheterization. Of the 16 patients enrolled, 10 had findings compatible with PHT at echocardiography (systolic PA pressure >30 mm Hg) [1] and underwent right-heart catheterization for additional measurement of PA pressure and definitive assessment of PE by pulmonary angiography. Hemodynamic studies were performed later on 5 of these patients.

Cardiac evaluation consisted of chest roentgenogram, 12-lead electrocardiography (ECG), echocardiography, and right-heart catheterization with pulmonary angiography.

Echocardiography was used to obtain systolic PA pressure and to evaluate left ventricular (LV) systolic function. PA pressure was estimated by adding the peak pressure gradient of tricuspid regurgitation as recorded on the Doppler flow chart to the height (centimeters) of jugular venous pulse obtained by physical examination [6]. This method of measurement was not applicable in the absence of tricuspid regurgitation. The person performing echocardiography was blinded to cardiac catheterization data. LV systolic function was evaluated from LV ejection fraction, which was obtained from M-mode measurement. We followed the methods recommended by the American Society of Echocardiography [7].

Right-heart catheterization was done after patients were premedicated with antihistamine and a mild sedative. Venous access was through the femoral vein with a 7F Cournand catheter inserted retrograde to the PA. PHT was considered present when PA systolic and mean pressures exceed 30 and 20 mm Hg, respectively [1].

After PA pressure was measured, pulmonary angiography was performed selectively and serially into the right and left PAs via a 7F pigtail catheter. An injection of nonionic contrast medium (30 mL) was delivered into each PA with a power injector at the rate of 15 mL/s. Image acquisition was done simultaneously at the rate of 30 frames/s for 15 seconds with a 512 × 512 format (262,144 pixels). Image interpretation was done independently by a radiologist and 2 cardiologists; disagreement was settled by consensus. PE was diagnosed if 1 or more of the following 3 findings was present: intraluminal filling defect, abrupt vessel cut off, or a "railroad sign" caused by squeezing of contrast medium through the space between the obstructive area and the vascular wall. Changes consistent with previous thromboembolism, such as PA web [8] and irregular walls of the vascular trees were also sought.

Negative findings at pulmonary angiography in all 10 patients suggested that PE was not the cause of PHT. To understand the mechanism of PHT, we later performed hemodynamic studies using thermodilution technique to assess cardiac output, which allowed calculation of cardiac index and pulmonary vascular resistance index (PVRI), as previously described [9]. Pulmonary capillary wedge pressure (PCWP) also was measured. Five of the 10 patients agreed to the study.

Hematologic evaluation for evidence of hemostatic/thrombotic activation consisted of measurement of plasma fibrinogen, plasma thrombin–antithrombin III complex (TAT), and prothrombin fragment 1.2 (F 1.2) for evidence of thrombin generation or coagulation activation, and plasma β_2-thromboglobulin (β TG) as a reflection of platelet activation. Other blood tests were complete blood cell count, hemoglobin (Hb) analysis, serum ferritin, serum antibodies to human immunodeficiency virus (HIV) 1 and 2 and hepatitis C virus (HCV), and serum hepatitis B surface antigen (HBsAg).

Blood was drawn from the antecubital vein after the patient had fasted overnight. A tourniquet was applied briefly for easy venous access, after which it was released. Venous blood was obtained through a 20-gauge scalp vein needle. After the first 2 mL was discarded, blood was drawn into a new plastic syringe for biochemical, hematological, and infectious markers assays. Blood for plasma TAT, F 1.2, and β TG assays was obtained from a new plastic syringe. Blood anticoagulated with 3.2% sodium citrate (9:1 ratio) was centrifuged at 2000*g* for 20 minutes to obtain plasma, which was stored at –80°C for later assay of TAT [10] and F 1.2 [11]. Blood anticoagulated in a Diatube H tube (Becton Dickinson, Franklin Lakes, NJ, USA) was processed strictly according to the insert of the β TG enzyme-linked immunosorbent assay (ELISA) kit to prevent in vitro platelet activation. The separated plasma was stored at –80°C for later assay of β TG [12].

Plasma TAT and F 1.2 levels were measured with the Enzygnost TAT and Enzygnost F 1 + 2 micro ELISA kits (Behring Diagnostics, San Jose, CA, USA), respectively. Plasma β TG level was measured with the Asserachrom ELISA kit (Diagnostica Stago, Asnieres-sur-Seine, France). Plasma fibrinogen was measured by the method of Ellis and Stransky [13]. Complete blood cell count was performed by Coulter counter JT3 (Coulter Electronics, Luton, England), Hb analysis by high performance liquid chromatography [14] (Bio-Rad, Hercules, CA, USA), and serum ferritin, anti–HIV-1/HIV-2 antibodies, and HBsAg by microparticle enzyme immunoassay [15] with AxSYM ferritin, AxSYM HIV-1/HIV-2, and AxSYM HB_S Ag (V2), respectively (Abbott, Abbott Park, IL, USA). Serum anti-HCV was measured by immunometric technique with a Vitros Anti-HCV assay kit (Ortho-Clinical, Amersham, UK).

3. Results

Because of the small number of patients, results are shown in median and range when appropriate. Patient characteristics are shown in Table 1. The patients were underweight for height [16], as is usual in Thal major [17].

Table 1.
Patient Characteristics (n = 10)*

Diseases	E/β-Thal (n = 9), β-Thal (n = 1)†
Age, y	26 (21-39)
Sex, M:F	2:8
Height, cm	160 (135-166)
Weight, kg	44.5 (27.3-52)
Duration postsplenectomy, y	16 (5-21)

*Results are shown in median and range where applicable. Numbers in parentheses are number of patients or range. E/β-Thal indicates hemoglobin E/β-thalassemia; β-Thal, homozygous β-thalassemia.

†M:F of E/β-Thal was 2:7; the only β-Thal patient was a woman.

Results of cardiac evaluations to confirm and to determine the cause of PHT are shown in Table 2. All patients except 1 had cardiomegaly and an enlarged PA trunk consistent with PHT. Six patients had radiographic changes of extramedullary hematopoiesis indicating intense compensatory erythropoietic activity, a common finding in Thal [17]. ECG results showed that right-axis deviation and right ventricular hypertrophy were present in approximately one half of the patients but were present in all patients with systolic PA pressure more than 45 mm Hg. PA pressure determined by echocardiography and by right-heart catheterization correlated well (correlation coefficient = 0.7). Right-heart catheterization results confirmed elevated PA pressure and no intracardiac shunt. Pulmonary angiographic findings were negative for evidence of acute and chronic PE, suggesting PE as an uncommon cause of PHT.

Cardiac output assessment of the 5 patients is shown in Table 3. Note the markedly increased values of both PVRI and PA pressure in cases 4 and 5 and the borderline PHT in case 1.

Table 2.
Cardiac Evaluation (n = 10)*

Investigative Tool	
Chest roentgenogram	
Cardiomegaly	9 patients
Enlarged pulmonary trunk	9 patients
Osteopenia	10 patients
Extramedullary hematopoiesis	6 patients
Lung findings	All within normal limits
Electrocardiography	
Rhythm	All normal sinus
Axis	
Normal	5 patients
Right-axis deviation	5 patients
Chamber hypertrophy	
Right ventricular hypertrophy	6 patients
Echocardiography	
Left ventricular ejection fraction (normal value, ≥0.6)	0.74 (0.6-0.86)
Right-heart catheterization	
Systolic/diastolic pressure, mm Hg	
Right ventricle	45 (30-92)/10 (7-13)
Pulmonary artery	46 (33-86)/22 (13-40)
Intracardiac shunt	None
Pulmonary angiogram	No emboli

*Results are shown in median and range (in parenthesis) where applicable.

Results of testing with selected serum infectious markers and hematologic evaluations for evidence of hemostatic/thrombotic activation are shown in Table 4. Although both TAT and F 1.2 are markers of thrombin generation, only the former showed a statistically significant difference from normal, indicating that thrombin generation had occurred. All but 1 patient had an elevated plasma β TG level, indicating platelet activation, and all but 2 patients had a low normal plasma fibrinogen concentration consistent with low-grade consumption coagulopathy. Values of all other hematologic evaluations, such as peripheral blood Hb, percentage neutrophils, platelet count, percentage nucleated red blood cells (nRBC), and serum ferritin, were statistically significantly different from normal. Chronic anemia, thrombocytosis, neutropenia, and lymphocytosis are consistently found in splenectomized β-Thal patients [17,18]. Markedly increased nRBC count indicated intense compensatory erythropoietic activity. Markedly increased serum ferritin level consistent with iron overload was related to multiple previous blood transfusions and the increased intestinal iron absorption associated with Thal [19].

4. Discussion

Our studies using echocardiography and right-heart catheterization confirmed that PHT can be found in splenectomized β-Thal patients. In contrast to the suggestion of Sonakul et al [2], our pulmonary angiographic findings did not support the diagnosis of old or recent PE [8]. In support of our conclusion, results of animal studies have suggested that more than 22 million thromboemboli to the pulmonary arterioles would be required to raise mean PA pressure 5 mm Hg [20], and none of our patients ever had clinical evidence of acute or chronic DVT on physical examination or positive findings on ultrafast CT scan of the lungs during clinically suggestive acute PE.

To understand the mechanism of PHT, we performed hemodynamic studies on 5 of the 10 patients who had systolic PA pressure >30 mm Hg, all of whom had E/β-Thal, to determine their cardiac indices, PCWP, and PVRI, because PA pressure is governed by these 3 factors. Cardiac index values were increased in all except patient 5, who had a very high PVRI. Increased cardiac index was expected because of chronic anemia [21]. Resultant PHT was nonspecific or reactive [1].

Increased PCWP values were found in all except patient 1, who had borderline PHT in this study. Increased PCWP also could have caused PHT. However, increases in both cardiac indices and PCWP led only to a slight increase in PA pressure (cases 2 and 3 versus cases 4 and 5 in Table 3). Our finding of normal LV systolic function on echocardiogram suggested that increased PCWP was likely due to LV diastolic dysfunction, possibly secondary to chronic anemia [21] and chronic iron overload [22]. LV diastolic dysfunction is an early sign of LV dysfunction in β-Thal major [23].

Increased PVRI was found only in cases of markedly increased PA pressure (cases 4 and 5). With a mean PA pressure >25 mm Hg at rest as a definition [24], only these 2 cases were PHT. This finding further stressed the importance of increased PVRI in the pathogenesis of disease. Pathologic

Table 3.
Cardiac Output Assessment of 5 Splenectomized Female Hemoglobin E/β-Thalassemia Patients*

Case No.	Age/Post-splenectomy Period, y	Body Weight, kg/ Height, cm	LVEF (N, ≥0.6)	Hemoglobin, g/dL	Cardiac Catheterization				
					Cardiac Index, L/min per m^2 (N, 2.6-4.2)	PCWP, mm Hg (N, 2-10)	PVRI, dyne·sec·cm^{-5}·m^2 (N, 123 ± 54)	Systolic/Diastolic Pressure (*mean*), mm Hg	
								RV (N, 15-30/2-8)	PA (N, 15-30/4-12)
1	30/7	45/160	0.6	6.5	5.89	9	67.91	32/11	31/10 (*20*)
2	22/10	57/155	0.78	7.7	4.48	13	89.28	37/13	32/15 (*22*)
3	28/19	45/160	0.76	4.2	7.37	15	97.69	37/14	32/14 (*24*)
4	21/7	37/145	0.7	6.5	6.89	17	406.39	80/8	82/32 (*52*)
5	28/23	50/165	0.86	6.1	4.17	12	613.91	66/7	63/28 (*44*)

*LVEF indicates left ventricular ejection fraction; N, normal value; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; RV, right ventricle; PA, pulmonary artery.

changes in small PAs on postmortem examination of a patient who died a few months after right-heart catheterization explained the cause of increased PVRI. She was a 24-year-old E/β-Thal patient who was 21 years postsplenectomy. PA pressure was 75/32 mm Hg, and the PAs (size, 50-400 μm) showed changes similar to those of primary PHT [1] or thrombotic pulmonary arteriopathy (Figure 1). There was no vasculitis, pulmonary infarction, or PA web, only minimal iron deposition and unremarkable pulmonary venules. These findings supported those previously described by Sonakul et al [2] and were more in keeping with in situ thrombus formation than embolism [25]. The elevated plasma TAT levels were supportive of hypercoagulability contributing to the vascular abnormalities.

This systemic hypercoagulability [26] is likely related to the presence of phosphatidylserine (PS) [27] on the surface of β-Thal RBC membranes [28,29]. These highly thrombogenic RBCs normally would have been cleared by macrophages [30], but splenectomy allows an increased number of such PS-exposed RBCs to circulate [31] and enhance hypercoagulability. Intense compensatory erythropoiesis would further increase the number of these cells. Splenectomy also results in slower clearance of activated clotting factors [32,33]. Decreased plasma levels of protein C, protein S, antithrombin III [5,26,34], and heparin cofactor II [35] also facilitate thrombin formation. Thrombin from the coagulation process can activate platelets [36], and the platelet activation can facilitate the process further [37]. Increased plasma β TG levels confirmed that there was platelet activation, and subsequent release of growth factors by activated platelets may lead to vascular changes [1]. Thrombocytosis, which is consistently found in splenectomized E/β-Thal patients, likely augments thrombosis and vascular changes. Previous studies [38-40] have shown the importance of splenic absence, coagulation, and platelet activation in the pathogenesis of pulmonary microthrombi.

PS-exposed RBCs adhere selectively to vascular endothelial cells (VEC) [41,42], especially during infection [43], the frequency of which has been reported to be increased in splenectomized Thal patients [44,45]. Furthermore, markedly

Table 4.
Hematologic Evaluation (n = 10)*

Feature	Result	Normal Value, mean ± 2 SD	*P*†
Hemoglobin typing	E/β-Thal, 9; β-Thal, 1	AA_2	—
Hemoglobin, g/L	M, 59 (56-62)	M, 155 ± 25	.020
	F, 64.5 (55-75)	F, 140 ± 20	<.001
WBC count, $\times 10^9$/L	10.5 (5.2-17.5)	7.5 ± 2.5	.021
Neutrophils, %	37 (25-50)	60 ± 15.0	<.001
Platelet count, $\times 10^9$/L	513 (310-885)	270 ± 130	<.001
nRBC count, /100 WBCs	1062 (180-2567)	0	—
TAT, μg/L	9.63 (6.04-16.14)	3.0 ± 2.4	.002
F 1.2, nmol/L	1.35 (0.75-1.92)	1.03 ± 0.48	.067
β TG, IU/mL	87 (26-196)	25.3 ± 12.8	.019
Fibrinogen, mg/dL	203 (132-238)	289 ± 114	.001
Ferritin, ng/mL	M, 2706 (2318-3094)	M, 163.5 ± 136.5	—
	F, 3858 (987-6778)	F, 70 ± 60	.005
Anti-HIV antibody	Negative	Negative	—
HB_sAg	Negative	Negative	—
Anti-HCV antibody	Positive, 5 patients	Negative	—

*Results are shown in median and range where applicable. Numbers in parentheses are range. E/β-Thal indicates hemoglobin E/β-thalassemia; β-Thal, homozygous β-thalassemia; WBC, white blood cell; nRBC, nucleated red blood cell; TAT, thrombin antithrombin III complex; F 1.2, prothrombin fragment 1.2; β TG, β_2-thromboglobulin; HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.
†*t* test.

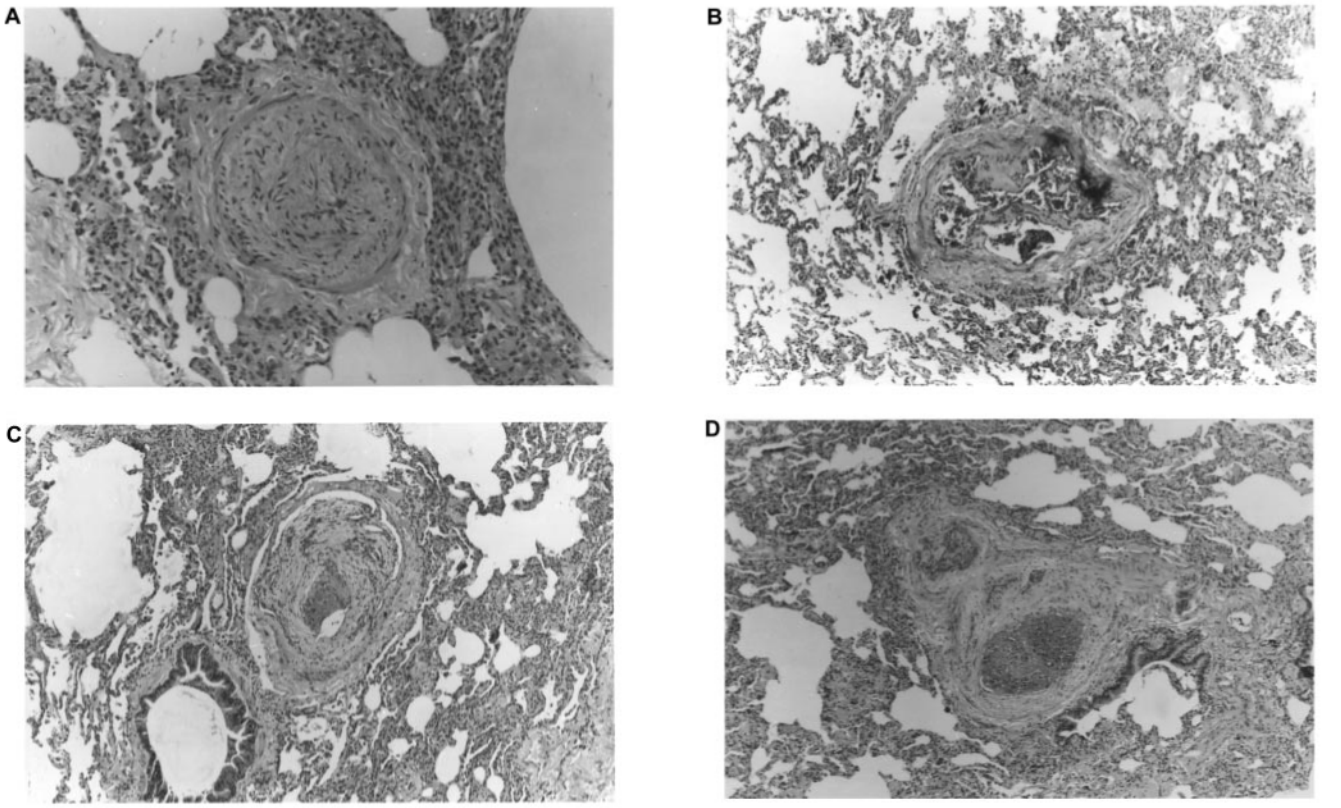

Figure 1. A, Prominent medial hypertrophy with intimal fibrosis resulted in total luminal occlusion of this pulmonary artery (hematoxylin and eosin, original magnification ×400). B, Pulmonary hypertension, grade IV. In addition to medial hypertrophy and intimal fibrosis, numerous endothelial-lined vascular channels with focal fibrinoid necrosis were noted (hematoxylin and eosin, original magnification ×200). C, Severe intimal fibrosis, muscular hypertrophy with focal recanalization of the lumen, and fibrin thrombi were noted (hematoxylin and eosin, original magnification ×200). D, Plexiform lesion of this pulmonary artery was characterized by aneurysmal expansion of the vascular wall with fibrin thrombi occluding the lumen (hematoxylin and eosin, original magnification ×200).

increased numbers of nRBCs, which deform poorly, may facilitate adherence by impeding flow in the microcirculation. Once adherent, these cells would promote local clot formation, particularly in the presence of systemic hypercoagulability. In addition, pulmonary endothelial damage from increased pulmonary blood flow [46,47] and lung injury from recurrent respiratory tract infections may explain why thrombi are limited to the PA in this systemic hypercoagulable state.

Pathogenesis of thrombotic pulmonary arteriopathy is not yet completely understood [1]. In primary PHT [1], VEC injury or dysfunction is believed to be the cause. We propose, on the basis of our findings and those of others [38-43], one possible pathophysiologic change that occurs in postsplenectomy β-Thal and leads to PHT (Figure 2).

Aessopos et al [48,49] also found increased cardiac output and PVRI in splenectomized β-Thal intermedia patients, an effect originally thought to be due to hypoxia and cirrhosis. We did not find consistent clinical evidence of these changes. We found hypoxia only in cases of severe PHT, which likely was the effect of rather than the major cause of increased PVRI. Iron excess resulting in LV, lung, and liver dysfunction also has been thought to contribute to PHT [50], but previous studies from Thailand [2,51] failed to show iron excess in heart or lungs.

All our patients were relatively young, and the time of their PHT diagnosis was distant from splenectomy. Almost all patients were female, as were those who had primary PHT [1]. The greater prevalence of E/β-Thal cases in our series and in a previous autopsy report [3] (ratio of E/β-Thal to homozygous β-Thal, approximately 3.3:1) might have been due to a higher incidence of the disease among Thais [52]. However, there could have been differences between homozygous β-Thal and E/β-Thal RBCs, as has previously been shown in α- and β-Thal RBCs [53]. That only 3 of our 10 PHT patients had the disease sooner than 15 years postsplenectomy indicates the process is slow and progressive (Table 3), in agreement with previous autopsy findings [2]. However, absence of PHT in many of our patients who were many years postsplenectomy and who had evidence of in vivo coagulation activation [31] suggested that other modifying factors, such as genetic predisposition [54], shear stress, and inflammation [55], may be operative. The finding of severe PHT 7 years postsplenectomy in case 4 (Table 3) is supportive. A markedly increased nRBC count, which is compensatory to severe anemia, also might indicate the presence of more severe disease

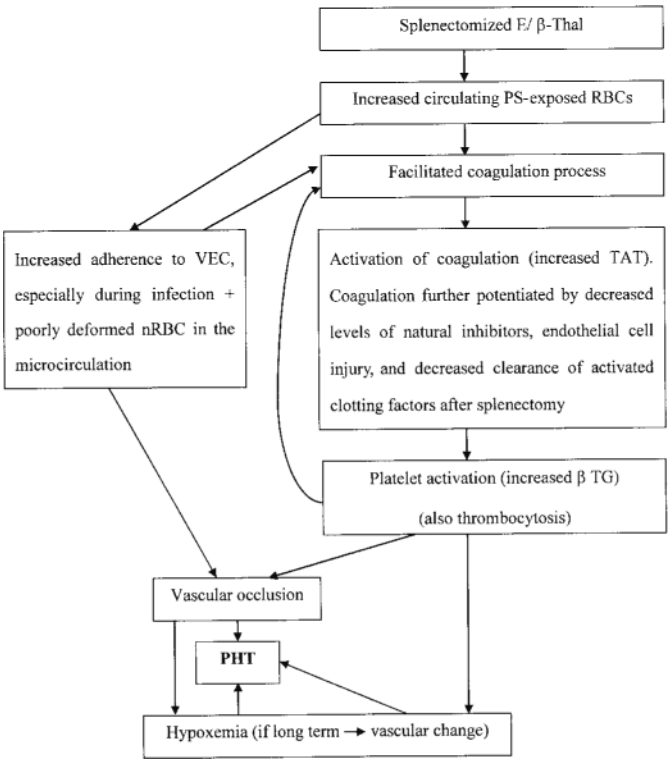

Figure 2. Proposed pathogenesis of vasculopathy and microthrombi leading to increased pulmonary vascular resistance index in splenectomized hemoglobin E/β-thalassemia (E/β-Thal) patients with severe pulmonary hypertension (PHT). PS indicates phosphatidylserine; nRBC, nucleated red blood cell; VEC, vascular endothelial cell; TAT, plasma thrombin antithrombin III complex; β TG, plasma β_2-thromboglobulin.

in this group of patients. More studies are required to define the determinants of this serious complication.

In conclusion, our studies on PHT in postsplenectomy β-Thal do not support PE as the likely cause. Increased cardiac index from chronic anemia and increased PCWP possibly from chronic anemia and chronic iron overload could cause a minor rise in PA pressure. A further rise in PA pressure requires an increased PVRI due at least to thrombotic pulmonary arteriopathy from chronic hypercoagulation. Systemic hypercoagulability and platelet activation are in turn facilitated by the presence of increased numbers of circulating PS expressing RBCs and nRBCs that results in part from splenectomy. The results of this study suggest that splenectomy in β-Thal creates long-term risk of low-grade hypercoagulability and PHT. It also provides a model for testing whether appropriate therapeutic intervention can prevent or ameliorate the severity of PHT found in splenectomized β-Thal patients.

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