Pulmonary Hypertension in Thalassemia: Association with Hemolysis, Arginine Metabolism Dysregulation, and a Hypercoagulable State


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The thalassemia syndromes are a heterogeneous group of inherited hemoglobin disorders resulting from impaired production of either the alpha or beta globin chain subunits of the hemoglobin tetramer. The clinical spectrum is a consequence of chronic hemolytic anemia and imbalanced globin chain accumulation.[1] Depending on clinical severity, two forms of beta-thalassemia have been classified: thalassemia major (TM) and thalassemia intermedia (TI). TM is characterized by severe anemia starting during the first year of life and requiring lifelong transfusion therapy for survival, while TI has a later clinical onset with a milder anemia, permitting survival without regular transfusions, and a longer life expectancy.[2]

Heart failure is the most common cause of death in both forms of the disease.[3] Thalassemia heart disease involves mainly left ventricular (LV) dysfunction caused by transfusion-induced iron overload. However, recent studies suggest that both TM and TI patients have a unique hemodynamic pattern consistent with right ventricular (RV) cardiomyopathy, and pulmonary hypertension (PH) in addition to the LV abnormalities.[4] PH in beta-thalassemia represents a common, yet less well-explored complication in the cardiopulmonary spectrum of the disease, and is the focus of this review.

Earlier studies in both TI and TM demonstrate that adults frequently have undetected PH, with a reported prevalence of 60% to 75%,[5-9] and there is a growing body of literature that suggests asymptomatic PH is a leading factor in heart failure and death in thalassemia.[5] In a study of TM, a pulmonary artery systolic pressure (PASP) above 30 mmHg was found in all patients over 22 years.[6] More recent studies in more uniformly treated TM and TI patients have shown a lower frequency in transfused TM patients,[9] while in TI patients increased PASP was more frequently detected.. In one study 60% of TI patients had a PASP above 30 mmHg with preserved LV systolic function,[5] and another study showed a PASP above 35 mmHg in 23% of TI patients.[9]

Growing data suggest that thalassemia has many biologic and clinical risk factors responsible for the development of PH, including chronic hypoxia, long-term effect of splenectomy, red cell membrane pathology,[10-14] coagulation abnormalities,[13,15] oxidative stress,[16] iron overload,[4,8,17-20] and chronic hemolysis. Some of these risk factors occur in sickle cell disease (SCD),[21] another clinically significant hereditary anemia that is associated with a high incidence of PH.[22] In particular, analogous to SCD, hemolysis-associated PH is emerging as an important risk factor in thalassemia.[23,24] Red cell destruction, elevated free plasma hemoglobin, anemia, and abnormal nitric oxide (NO) metabolism exist in both nontransfused and transfused patients.[25]

Pulmonary Hypertension

PH is defined as a mean pulmonary artery pressure (PAP) of 25 mmHg or greater at rest or of 30 mmHg or greater during exercise and can result from a wide range of conditions.[26] Increased PAP compromises oxygenation and right-heart function, and can ultimately become life-threatening.[27] The initial injury leading to pulmonary artery hypertension (PAH) in different disease states may vary; however, there is a common pathway of vascular remodeling that results in a similar clinical and histopathologic condition. Recent investigations suggest that endothelial dysfunction is key to the PAH pathogenesis.[28] Since impaired NO bioavailability represents the central feature of endothelial dysfunction,[29] aberrant NO activity plays a foreseeable role in the PH development.[30]

NO is one of the most potent vasodilators known[31] and is essential to vascular homeostasis. It plays an important role in the maintenance of vasomotor tone, limits platelet aggregation and ischemia-reperfusion injury, modulates endothelial proliferation, and has anti-inflammatory properties. Arginine is the precursor to NO, catalyzed by a family of enzymes, the NO synthases. NO causes vasodilation through the activation of soluble guanylate cyclase to produce the intracellular messenger cyclic guanylate monophosphate (cGMP).[32] Increased consumption and decreased produc-

tion of both NO and arginine contribute to complications associated with PH.

There is growing evidence that PH is a disease process that involves altered arginine metabolism or decreased bioavailability;[33] however, the role of elevated arginase activity in PH pathogenesis has been discovered only recently.[23,33-37] Arginase is an essential enzyme in the urea cycle, responsible for the conversion of arginine to ornithine and urea.[38] Found predominantly in liver and kidneys,[39] arginase is also found in the red blood cells of humans and other primates,[40,41] making it an intriguing enzyme to study in hemolytic disorders. Plasma arginase activity is elevated in SCD as a consequence of inflammation, liver dysfunction, and most significantly through the release of erythrocyte arginase during intravascular hemolysis, as demonstrated through a strong correlation to cell-free hemoglobin levels and other markers of increased hemolytic rate.[34] Arginase activity is higher in the red cell lysate of patients with SCD compared with normal controls, and strongly correlates to plasma arginase activity.[34] Erythro-cyte arginase activity is also elevated in thalassemia patients.[42,43] In addition, arginase activity is higher in immature red blood cells and reticulocytes,[41] compared with older cells. When these early cells are destroyed in the bone marrow, a high concentration of arginase will be released, contributing to arginine dysregulation. It is likely that erythrocyte release of arginase during hemolysis limits the availability of arginine to NO synthase, resulting in a deficiency of NO and dysregulation of arginine metabolism in thalassemia patients through a similar mechanism identified in SCD.

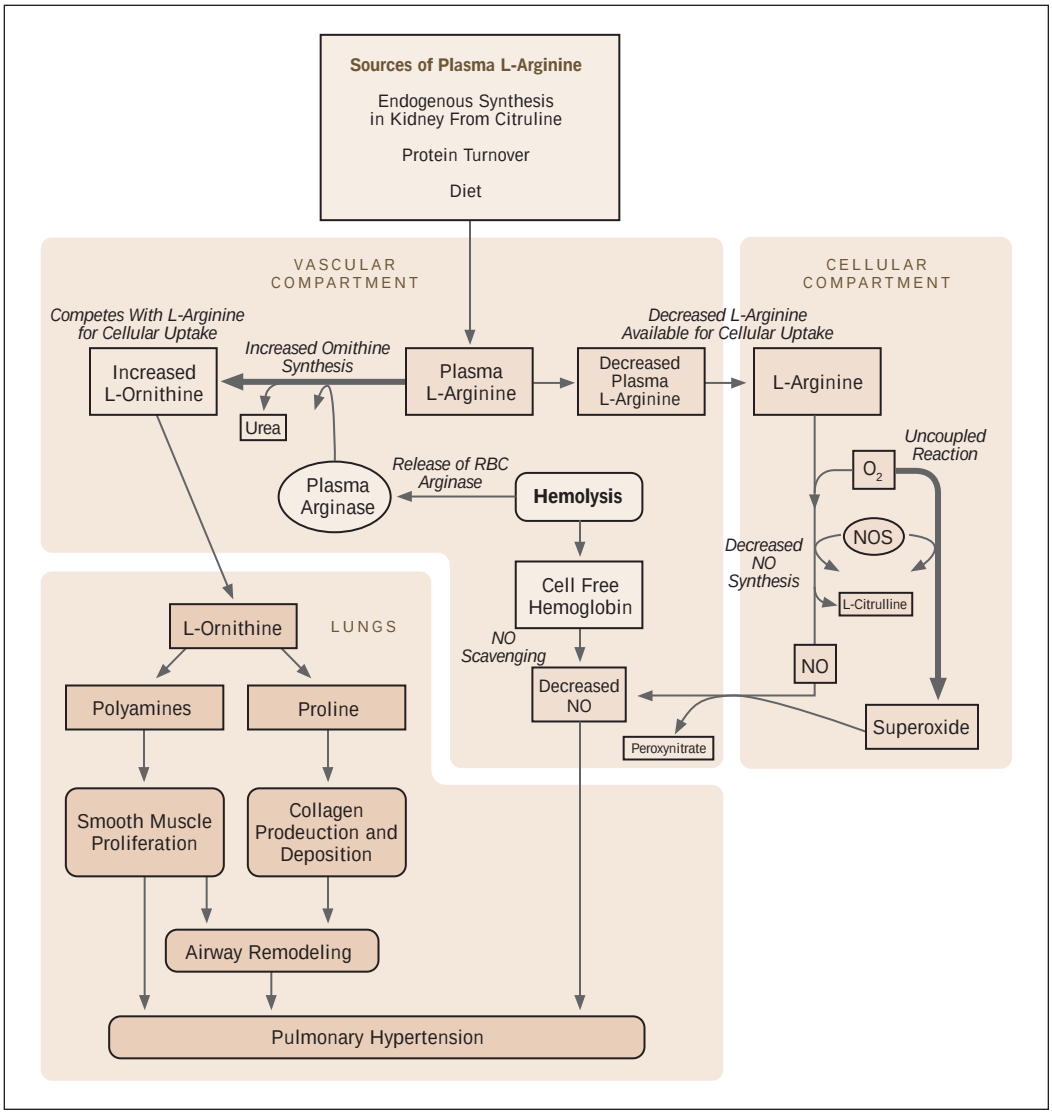

Figure 1. Altered arginine metabolism in hemolytic disorders. Arginine is synthesized endogenously from citrulline primarily via the intestinal-renal axis. Arginase and nitric oxide synthase (NOS) compete for arginine, their common substrate. In sickle cell disease, bioavailability of arginine and nitric oxide (NO) is decreased by several mechanisms linked to hemolysis, and similar mechanisms are postulated for thalassemia. The release of erythrocyte arginase during hemolysis increases plasma arginase levels and shifts arginine metabolism toward ornithine production, decreasing the amount available for NO production. The bioavailability of arginine is further decreased by increased ornithine levels because ornithine and arginine compete for the same transporter system for cellular uptake. Endogenous synthesis of arginine from citrulline may be compromised by renal dysfunction, commonly associated with thalassemia. Despite an increase in NOS in sickle cell disease, NO bioavailability is low due to low substrate availability, NO scavenging by cell-free hemoglobin released during hemolysis, and through reactions with free radicals such as superoxide. Superoxide is elevated in sickle cell disease due to low superoxide dismutase activity, high xanthine oxidase activity, and potentially as a result of uncoupled NOS in an environment of low arginine and/or tetrahydrobiopterin concentration. These mechanisms warrant exploration in the thalassemia syndromes. Endothelial dysfunction resulting from NO depletion and increased levels of the downstream products of ornithine metabolism (polyamines and proline) likely contribute to the pathogenesis of lung injury, fibrosis and pulmonary hypertension. (Reproduced from Morris et al[34] with permission from the American Medical Association.)

Since PH also develops in most hereditary and chronic hemolytic anemias in addition to SCD,[24] including thalassemia,[5-9,15,25] erythrocyte arginase release during hemolysis may contribute to endothelial dysfunction and the pathogenesis of PH through impaired production of NO.[22,23,33,34,44] These observations support a novel mechanism of disease that links oxidative stress, chronic organ damage, and hemolytic rate to endothelial dysfunction and PH.[22-24,44,45] Arginase activity and alterations in arginine metabolic pathways have also recently been implicated in the pathophysiology of PAH,[35,37] suggesting a common mechanism in the pathogenesis of otherwise distinct forms of PH.

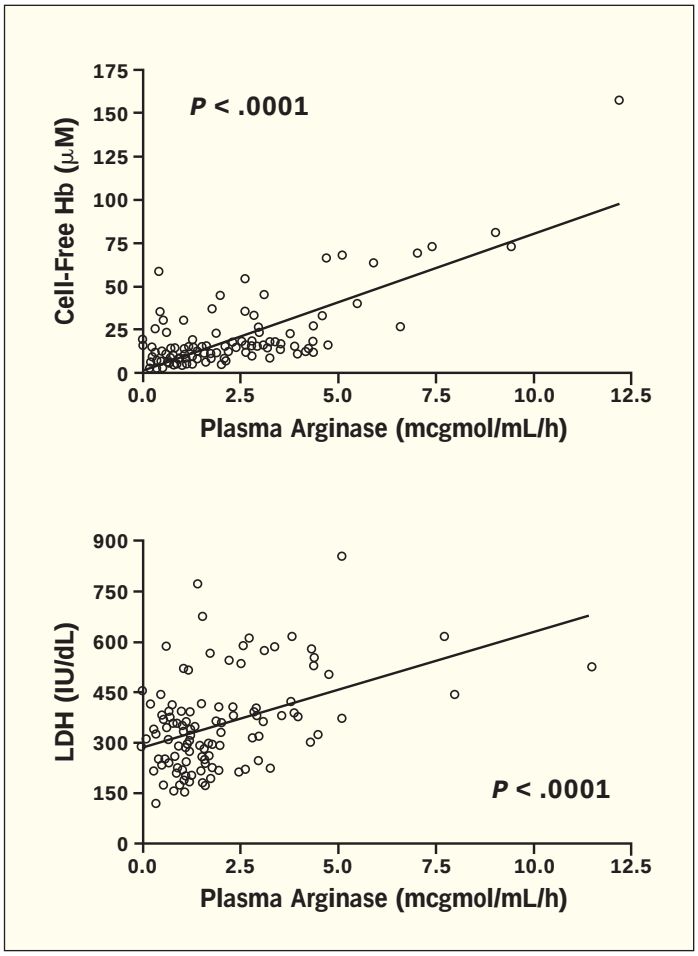

Figure 2. Association of arginase activity with hemolytic rate. Correlation of plasma arginase activity to cell-free hemoglobin (Hb, n = 138, *P* < .001) and serum lactate dehydrogenase (LDH) levels (n = 121, *P* < .001) in patients with sickle cell disease. (Reproduced from Morris et al[34] with permission from the American Medical Association.)

Pulmonary Hypertension Associated with Hemolysis

Accumulating support for the existence of a new disease paradigm involving hemolysis-associated PH[22,24] has emerged over the last few years. Although most of the studies have been done in SCD,[22,34,44] chronic hemolysis also occurs in thalassemia. Hemolysis is a condition that initiates a global attack on the arginine-NO pathway (**Figure 1**). During the hemolytic process, hemoglobin is released into plasma where it reacts with and destroys NO, resulting in abnormally high NO consumption and the formation of reactive oxygen species.[45] Consequently, smooth muscle guanylate cyclase is not activated and vasodilation is inhibited. NO destruction by hemoglobin can also cause further impairment in vascular endothelial function via transcription depression of adhesion molecules, including VCAM-1 and E-selectin, and vasoconstrictor/growth factors such as endothelin-1.[24] The simultaneous release of erythrocyte arginase during hemolysis[34] will limit the availability of arginine to NO synthase, resulting in a deficiency of NO. Once released into circulation, arginase will convert arginine to ornithine, which in turn is the precursor to proline, an amino acid involved in collagen formation,[46] lung fibrosis, airway remodeling, and vascular smooth muscle proliferation,[33,34,47] common features of pulmonary dysfunction in thalassemia.[19,48] By creating a shift toward ornithine metabolism, arginase triggers a proliferative pathway that contributes to the pathogenesis of PH. Furthermore, since arginine and ornithine compete for the same transport system for cellular uptake,[49] a decrease in the arginine-to-ornithine ratio resulting from increased arginase activity could also impair arginine bioavailability for NO production, even when plasma arginine concentration appears sufficient. Under conditions of low arginine bioavailability, NO synthase will uncouple, generating superoxide instead of NO,[50] thereby decreasing NO generating potential while further scavenging NO. This reaction leads to the formation of reactive NO species that can induce cellular damage and death, and adds to the milieu of oxidative stress. We have demonstrated an arginine deficiency in SCD associated with elevated arginase activity and a low arginine-to-ornithine ratio[34,36] that correlates to markers of hemolysis (**Figure 2**) and mortality in SCD (**Figure 3**), and severity of PH of various etiologies.[35] We have also found that arginine therapy improved PASP in SCD patients with PH by 15% within 5 days of treatment.[36] Since the arginase/proline pathway that limits arginine and therefore NO bioavailability is a hemolysis-related phenomenon, arginine and NO-based therapy may be a promising treatment for hemolysis-related PH, and is currently being explored in upcoming clinical trials through the Thalassemia Clinical Research Network. Sildenafil is another NO-enhancing therapy and PAH has recently been added to its FDA-approved indications. Standard treatment for hemolysis-associated PH has not yet been established; however, promising benefits of sildenafil therapy have been described in a small number of thalassemia patients[51,52] as well as in patients with SCD,[53] and will be an important area of future research.

Dysregulation of Arginine Metabolism in Thalassemia

As in SCD, dysregulated arginine metabolism also occurs in patients with thalassemia[23] (**Table 1**). Plasma arginine concentration tends to be lower in patients with thalassemia, with values ranging from normal to very low (19.5 to 122 mcgM, median 50 mcgM). Ornithine levels are high, the arginine-to-ornithine ratio is low, and plasma arginase activity is significantly elevated in thalassemia patients compared with control subjects.[34] Proline is also elevated, a downstream metabolite of arginase activity[46] and likely a contributor to pulmonary vascular remodeling. Citrulline, the endogenous precursor for de novo arginine synthesis, which occurs primarily in the kidney,[46] is also significantly elevated in thalassemia patients and may reflect impaired conversion of citrulline to arginine in patients with renal dysfunction. The implications of arginine dysregulation in thalassemia remain to be determined; however, given the association of low arginine bioavailability in SCD with increased hemolytic rate, severity of PH, and mortality, this relationship in patients with thalassemia is of significant clinical interest.

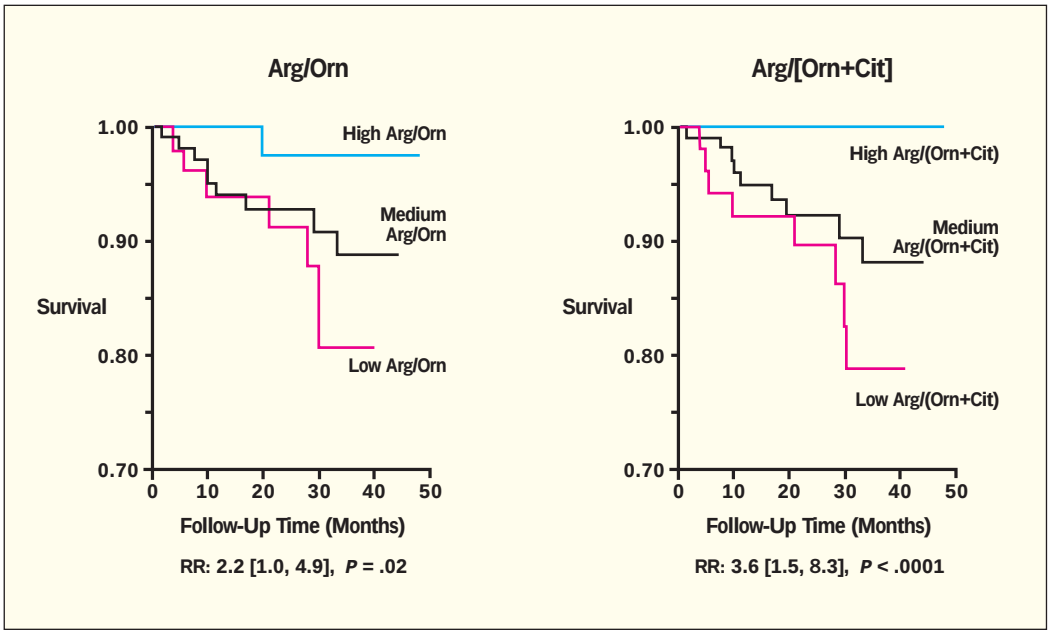

Figure 3. Association of arginine bioavailability ratios with mortality in sickle cell disease: Kaplan-Meier survival plots. Left panel shows mortality for three categories of arginine-to-ornithine ratio (Arg/Orn): high = upper quartile, > 0.8690; medium = 25th to 75th percentiles, > 0.4385 and £ 0.8690; and low = lower quartile, £ 0.4385. Right panel shows mortality for three categories of arginine-to-(ornithine + citrulline) ratio (Arg/[Orn + Cit]): high = upper quartile, > 0.6254; medium = 25th to 75th percentiles, > 0.3245 and £ 0.6254; low = lower quartile, £ 0.3245. (Reproduced from Morris et al,[34] with permission from the American Medical Association.)

Pathogenesis and Treatment of Pulmonary Hypertension in Thalassemia

Chronic hemolysis may play an important role in the pathogenesis of PH in thalassemia; yet, growing evidence suggests additional mechanisms involving a complex interaction of platelets, coagulation system, erythrocytes, and endothelial cells along with inflammatory and vascular mediators.

Hypercoagulability is a known complication in thalassemia patients,[54] ranging from 1.1% to 5.3% in TM patients and 9.6% to 29% in TI patients.[14,54,55] These, along with postsplenectomy thrombocytosis lead to a wide spectrum of clinically manifested thrombotic events, including the development of PH. Direct evidence comes from autopsy findings, which have demonstrated significant thrombotic lesions, primarily in the lungs, observed in 19 of 43 (44%) autopsies of nontransfused thalassemia patients along with striking biventricular hypertrophy with RV predominance.[10,11,18,56] Spleen removal significantly contributes to the development of PH in thalassemia as well as other disorders.[10,15,57] The resultant thrombocytosis and enhanced platelet aggregation and activation are found in 71% of splenectomized and 35% of nonsplenectomized patients as measured by increase in the urine arachnoidate metabolite levels, thromboxane A2 (TXA2) and prostacycline PGI2. An increase in CD62 (p-selectin), a platelet fraction that is expressed in hypercoagulable states, has been shown in secondary PH including thalassemia.[15,58,59] Platelet activation stimulates coagulation through upregulation of tissue factor, release of vasoactive substances like serotonin, and interaction with endothelial cells via CD40 ligand.[60] It is thought that continuously elevated vessel wall shear stress and inflammation further enhance platelet activation and adhesion, which promotes pulmonary vasculature changes.

In thalassemia erythrocytes likely contribute to clot formation, particularly in splenectomized patients. Subsequent to splenectomy, abnormal erythrocytes are not filtered out; they remain in the circulation and trigger platelet activation and thrombosis, which then affect pulmonary circulation. Abnormal phospholipid exposure in the outer leaflet of red blood cells was reported in thalassemia patients, resulting in distorted red blood cell membrane that triggers thrombosis.[13,54] In addition, platelet-red blood cell interactions via adenosine diphosphate release and adherence of erythrocytes to endothelial cells can cause changes in the microvasculature.[61] Endothelial cell dysfunction was shown in PH of various etiologies, including in patients with SCD and thalassemia, suggesting presence of inflammation, enhanced thrombosis, and arterial stiffness.[12,62,63] Endothelial dysfunction is also a result of oxidative tissue damage, which enhances hypercoagulability and in situ thrombus formation within the pulmonary artery walls, contributing to functional and structural alterations of pulmonary vessels.[64] The oxidative stress is caused by hemolysis and by the presence of iron overload and free-radical formation. Iron overload, present in both TM and TI patients, induces interstitial pulmonary fibrosis as well as left and right cardiac hemosiderosis, which results in cardiac dysfunction and affects pulmonary vascular resistance.[19] In TI patients, chronic anemia and hypoxemia lead to further vasoconstriction and increase in pulmonary vascular resistance. Hypoxia was also shown to alter the von Willebrand factor released by endothelial cells in secondary PH, affecting platelet activation and consumption.[65]

The high prevalence of PH in nontransfused TI patients has been well documented.[15,20] However, the frequency in transfused TM patients is less well defined. Mild-to-moderate PH was diagnosed in more than 50% of beta-thalassemia patients despite transfusion.[6,15,25] A study of 202 TM patients concluded that strict compliance with chronic transfusion and chelation therapy to prevent iron overload reduces the occurrence of heart failure and prevents PH.[8,9,66] Although more aggressive transfusion programs may provide greater protection from the development of PH, the occurrence of intramedullary hemolysis, thrombocytosis, iron deposits, and a resultant vasculopathy may still be able to induce PH, which likely progresses more slowly.

Nevertheless, these findings highlight the beneficial

Table 1: Distribution (mean ± SD) of amino acids linked to the L-arginine-nitric oxide pathway in thalassemia patients compared with normal controls.[34]

Variable	Control (n = 36)	Thalassemia (n = 14)	*P**
Arginine (mcgM)	67 (18)	57 (26)	.15
Ornithine (mcgM)	62 (22)	85 (68)	.05
Arg/Orn ratio	1.2 (0.5)	0.79 (0.4)	< .01
Proline (mcgM)	161 (48)	258 (116)	< .001
Citrulline (mcgM)	25 (11)	42 (17)	< .001
Arginase (mcgmol/cc/h)	0.33 (0.2)	0.71 (0.3)	< .001

* *P* values are from two-sided two-sample *t*-test.

effect of regular transfusion in either preventing or slowing the progression of PH in thalassemia.[67] Initiation of a short-term (6 to 12 month) transfusion therapy trial and assessment of its effect on PAP, or long-term transfusions in TI patients experiencing more severe PH should be strongly considered. Likewise, a more aggressive transfusion regimen could improve abnormal PAP and overall cardiac function in already transfused patients. Adequate iron chelation is critical, since iron overload leads to heart failure and cardiomyopathy[4,8,19,20,47] and will contribute to pulmonary fibrosis, lung iron deposition, and PH.[66] Preventive anticoagulation should be given to all splenectomized patients and those with PH. Currently, the choice of agent is arbitrary, as no randomized studies comparing the effect of an antiplatelet agent with that of an antithrombotic agent in thalassemia have been performed to date.

Other treatment modalities that have been successful in primary and secondary PH show promise in thalassemia. Sildenafil, though not assessed in larger randomized clinical trials in thalassemia, was effective in reducing PAP in a smaller study.[51] In a case report, treatment with epoprostenol (a prostacycline and antithrombotic agent) has proven successful.[68] Further research targeting specific pathways in the pathogenesis of PH in thalassemia could lead to novel therapeutic approaches.

Conclusion

Thalassemia patients frequently have multiorgan pathology and cardiac disease due to iron overload, chronic anemia and hypoxemia, liver disease, and a hypercoagulable state. The development of even a moderate increase in PAP can further impact on the already compromised cardiac and systemic organ function. Although multifactorial in origin, hemolysis-associated endothelial dysfunction is a newly appreciated mechanism that plays a role in the pathogenesis of PH that frequently complicates hemolytic disorders.[22,24] Intravascular hemolysis triggers a shift in arginine metabolism away from NO, toward ornithine-dependent pathways and a proliferative track that is likely contributing to the structural remodeling of the lungs seen with PH. However, the common mechanism of elevated arginase activity shared in both PAH[35,37] and hemolysis-associated PH of SCD[34] and thalassemia[23] reflects a greater pathophysiologic similarity in these conditions despite the diverse origins of disease. Although the clinical scenario of PH is the end result of complex and multifactorial mechanisms, alterations in NO and arginine bioavailability are likely to be involved in the pathogenesis of PH in thalassemia. As such, therapeutic interventions that decrease hemolytic rate, improve arginine bioavailability, enhance NO effects, or act as NO donors are of potential benefit and may alter the progression of the disorder. PH

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