

# Pulmonary hypertension in hemolytic anemias

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*F1000 Medicine Reports* 2010, **2**:10 (doi:10.3410/M2-10)

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## Abstract

Pulmonary hypertension (PH) has been reported with nearly all forms of the inherited as well as the acquired hemolytic anemias. Recent research investigating the pathophysiology of PH in sickle cell disease and thalassemia has helped elucidate the central role of hemolysis-mediated endothelial dysfunction in the development of PH in these populations. Although the most appropriate treatment of PH in patients with hemolytic anemia is not clearly defined, the associated significant increased risk of death underscores the need for randomized clinical trials in this area.

## Introduction and context

Pulmonary hypertension (PH) is a syndrome of restricted blood flow through the pulmonary arterial circulation associated with increased pulmonary vascular resistance and elevated pulmonary artery pressures. While there are case reports of this syndrome occurring in most hemolytic anemias, including hereditary spherocytosis, pyruvate kinase deficiency, unstable hemoglobin variants, paroxysmal nocturnal hemoglobinuria, and microangiopathic hemolytic anemias [1-6], data from larger cohorts have been reported only in sickle cell disease (SCD) and thalassemia and will be the focus of this report. The gold standard for diagnosis of PH is elevated mean pulmonary artery pressure (mPAP) of  $\geq 25$  mmHg measured during right heart catheterization. Echocardiography can be used as a noninvasive method to estimate pulmonary pressures by measuring the tricuspid regurgitant jet velocity (TRV). A TRV of  $\geq 2.5$  m/s has been validated as a reliable predictor of elevated pulmonary artery pressures in idiopathic PH [7] and has also been shown to correlate well with mPAP measured during catheterization in adult SCD patients [8]. Recent research has helped define the link between hemolysis and the development of pulmonary hypertension, establish the prevalence and risk factors for its development and, perhaps most importantly, identify PH as a significant marker for death.

## Recent advances

### Pathophysiology

While the initial mechanism of injury in various forms of PH may vary, the end result is a range of endothelial damage seen on histopathology, including hyperplasia, proliferation, thrombosis *in situ*, and finally irreversible plexiform arteriopathy [7]. The pathways that lead to this injury as a result of hemolysis are multifactorial and involve nitric oxide depletion and reduced bioavailability, dysregulation of arginine metabolism, oxidative stress, and a hypercoagulable state. Nitric oxide (NO), synthesized from arginine by endothelial nitric oxide synthase, plays a critical role in maintaining vascular homeostasis. Not only does NO maintain vasodilation by activating cGMP-dependent protein kinases, but it also inhibits platelet aggregation and attachment, limits ischemia-reperfusion injury and down-regulates adhesion molecules such as vascular cell adhesion molecule, selectins, and the potent vasoconstrictor endothelin-1 [9]. During hemolysis, breakdown of the erythrocyte releases hemoglobin and the enzyme arginase into circulation. Cell-free hemoglobin has been shown to be a potent scavenger of NO, effectively preventing these vasoprotective properties [10]. In addition, arginase depletes the substrate for NO synthesis by conversion of arginine to ornithine, compounding the state of reduced NO bioavailability. The downstream products

of this shift in amino acid metabolism to ornithine include proline and polyamines, metabolites known to increase vascular smooth muscle proliferation and collagen production and deposition, respectively [11]. Collectively, these changes result in vasoconstriction and vascular remodeling of the pulmonary vascular endothelium. The relationship between hemolysis, NO depletion, arginine metabolism dysregulation, and PH has been demonstrated in animal models as well as *in vitro* and *in vivo* experiments over the past few years. Multiple studies in adults as well as children with SCD have identified a strong link between the intensity of hemolysis and development of PH [8,12,13]. Mouse models of SCD as well as alloimmune hemolytic anemia with intravascular hemolysis have been shown to develop PH [14]. Canine models of intravascular hemolysis also demonstrate induction of PH that is partially reversed by administration of NO [15]. Reiter *et al.* [10] showed that plasma from SCD patients consumes NO and the inactivation of NO is directly proportional to the hemolytic rate. Arginase activity has also been shown to correlate with the hemolytic rate and a low arginine to ornithine ratio reflecting the dysregulation of arginine metabolism has been found in thalassemia and SCD. In patients with SCD, this ratio correlates significantly with PH [11].

The oxidative damage that occurs with chronic hemolysis also likely contributes to the development of PH. Reduced glutathione is a critical antioxidant and its depletion has recently been shown to correlate with hemolysis and was independently associated with PH in SCD patients [16]. Finally, the hypercoagulable state associated with SCD and thalassemia has been shown to contribute to the development of PH through a series of recent experiments. Villagra *et al.* [17] showed the correlation between platelet activation, markers of hemolysis, and severity of PH in SCD. Kuypers [18] has demonstrated the procoagulant nature of the abnormal erythrocyte membrane that occurs with oxidative damage in hemolytic anemias. Setty *et al.* [19] has reported increased tissue factor expression and thrombin generation induced by free heme. Splenectomy contributes to this hypercoagulable state and is a known risk factor for PH in thalassemia [20]. Singer *et al.* [21] have reported an association between PH and platelet activation, hypercoagulability, and splenectomy in patients with thalassemia.

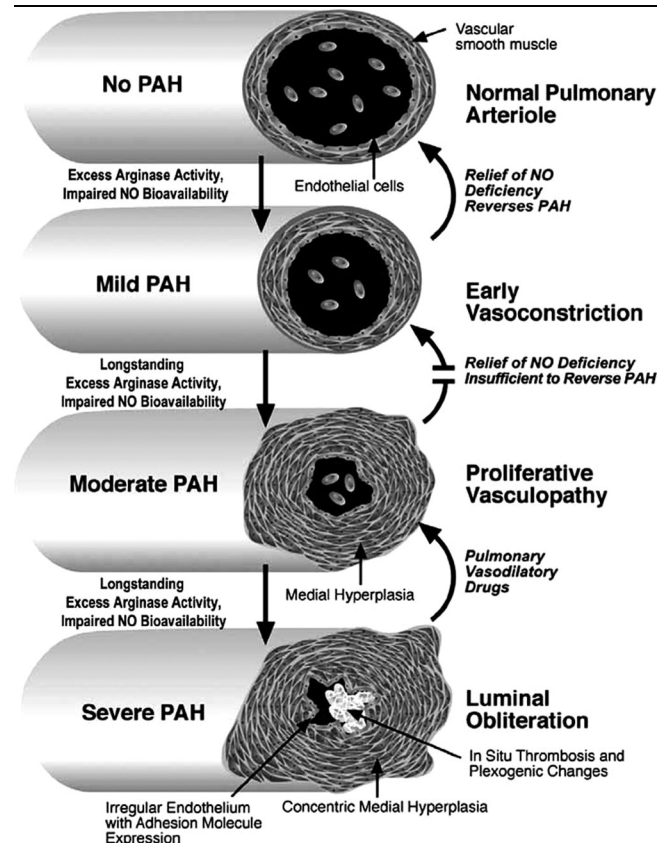
In summary, the development of PH due to hemolysis is multifactorial and involves NO deficiency, dysregulated arginine metabolism, oxidative stress, and hypercoagulability. Although the chronology of the vascular injury that leads to PH is not completely defined, there is likely

some initial vasoconstriction and proliferative vasculopathy that progresses to irreversible luminal narrowing with vascular remodeling over time (Figure 1).

**Prevalence**

Multiple prospective screening studies have found roughly a third of adult patients with SCD have PH as

**Figure 1. Progression of pulmonary hypertension in sickle cell disease and thalassemia**



In this hypothetical model, impaired nitric oxide (NO) bioavailability that results from chronic hemolysis and oxidative stress triggers chronic pulmonary vasoconstriction, mildly elevating pulmonary vascular resistance and pulmonary artery pressures. Excess arginase liberated from the erythrocyte during hemolysis consumes arginine, the obligate substrate for NO production, and creates a shift towards ornithine production that contributes to collagen deposition and vascular smooth muscle proliferation. Overabundant thrombin generation contributes to a chronic hypercoagulable state, increases arginase activity, and stimulates polyamine synthesis in vascular smooth muscle cells. As this becomes more longstanding, vascular smooth muscle hyperplasia begins to create a relatively fixed lesion, compounded in later stages by irregular, activated endothelium with expression of adhesion molecules. *In situ* thrombosis further occludes the vessel lumen, and results in plexogenic changes, further accelerating the progression of the pulmonary artery hypertension (PAH). Figure and caption reproduced with permission from [13]. Copyright © 2007 Informa Healthcare.

defined by a TRV of  $\geq 2.5$  m/s and approximately 10% have severe PH (TRV of  $>3$  m/s) [8,22-24]. Most reports of PH in pediatric SCD patients have also found a prevalence of 30%, although most of these are retrospective and therefore associated with some selection bias due to screening of symptomatic patients [25-27]. A recent study of children with SCD prospectively screened with echocardiography reported PH in 11% of 290 patients [28].

Studies of PH in thalassemia patients report less consistent results with a prevalence range of 10-75% depending on age, transfusion regimen, and degree of left ventricular dysfunction. Thalassemia major (TM) patients have a more clinically severe anemia and are typically transfusion dependent very early in life whereas thalassemia intermedia (TI) patients have a later onset of symptoms and may be less frequently transfused. Transfusions appear to have a preventative effect as PH is more frequently reported with TI compared to regularly transfused TM patients. Aessopos *et al.* [29] compared 131 transfused TM and 74 non-transfused TI patients and found PH in 23% of TI patients and no PH in the TM cohort. Singer *et al.* [21] reported PH in 10 of 18 transfused TM and 7 of 7 TI patients. Others have found PH in 44% of 36 [30] and 9.8% of 368 [31] regularly transfused TM patients.

#### **Amino-terminal pro-brain natriuretic peptide**

High levels of brain natriuretic peptide (BNP) reflect cardiac volume and pressure afterload as this hormone is released in response to cardiomyocyte stretching. The proBNP hormone is cleaved into the biologically active BNP and an easily measurable inactive amino-terminal proBNP. The amino-terminal proBNP level has been shown to correlate with severity of pulmonary artery pressure elevation and right ventricular dysfunction in primary PH [7]. It has more recently been validated as a biomarker for PH in SCD. Machado *et al.* [24] found that amino-terminal proBNP levels were higher in SCD patients with PH and correlated directly with TRV ( $R = 0.5$ ,  $P < 0.001$ ). A level of 160 pg/ml or greater had 78% positive predictive value for the diagnosis of PH in this cohort. Importantly, they also found this level was an independent predictor of mortality (relative risk for death 5.1; 95% confidence interval 2.1-12.5;  $P < 0.001$ ).

#### **Risk of mortality**

Heart failure is the most common cause of death in thalassemia [32]. While this is commonly due to left ventricular dysfunction resulting from transfusion-induced iron overload, right heart failure is now increasingly recognized [33]. Long-term longitudinal studies of thalassemia patients are needed to further

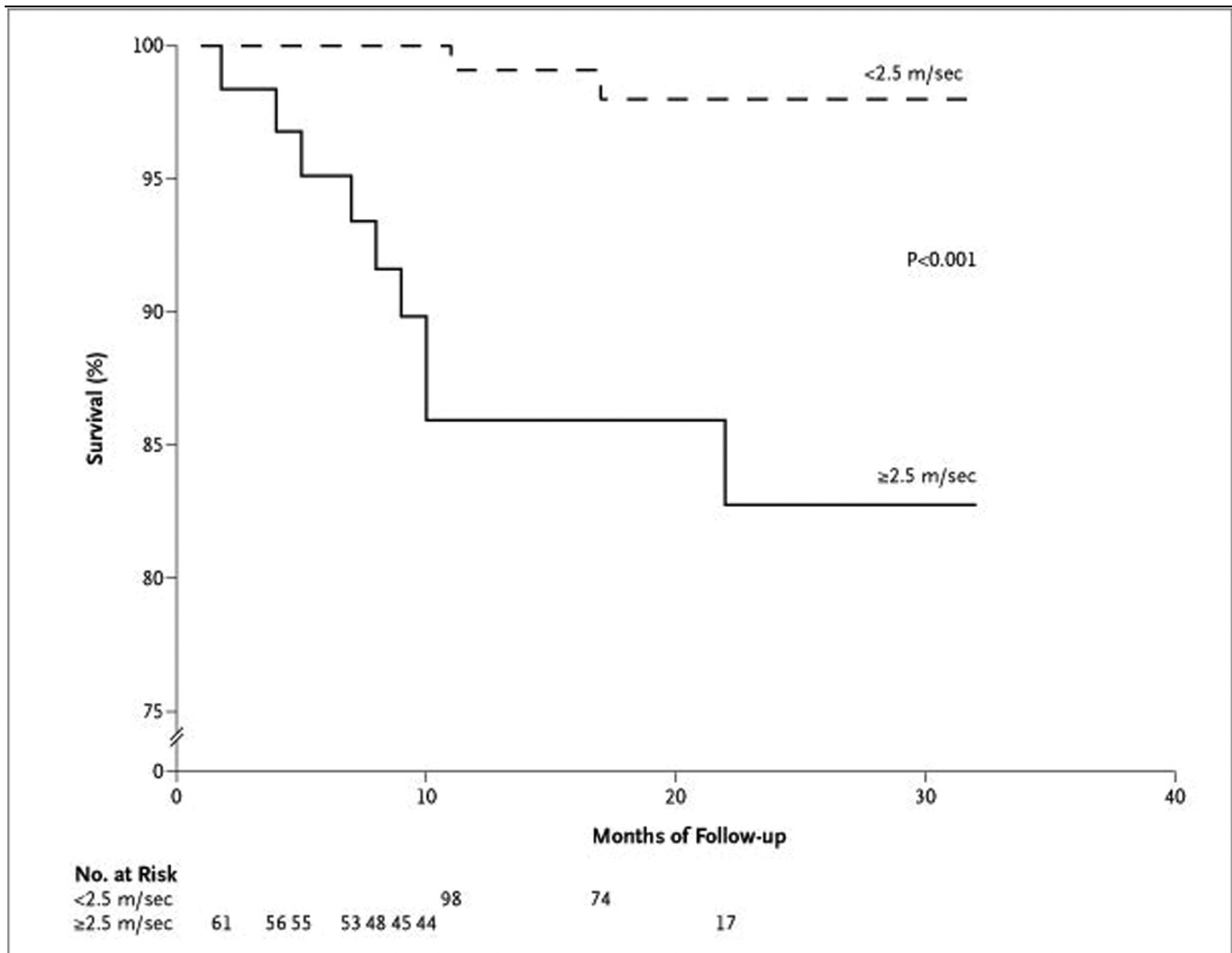
delineate the contribution of PH to mortality in this population.

Gladwin *et al.* [8] identified PH as a significant risk factor for death in adults with SCD. In a cohort of 195 patients, the rate ratio for death was 10.1 for those with TRV  $>2.5$  m/s at a mean follow up of 18 months (Figure 2). This significantly increased risk of death has been validated in multiple other studies [22-24]. In a recent report of 88 children, all 18 patients with PH were alive at a mean follow-up period of 3 years [34], suggesting there may be some progressive damage over time that ultimately leads to death in adulthood. It is important to note that this increased mortality is seen despite the fact that the mPAPs in hemolytic anemias are much lower than those reported with idiopathic PH. It remains unclear if PH is the direct cause of death or simply a marker of severe vasculopathy in SCD. PH has been reported as a frequent finding in autopsies of SCD patients with sudden death [35] and vaso-occlusive pain crisis is known to commonly precede sudden death in these patients. Recent work by Machado *et al.* [36] may shed light on the pathophysiology of sudden death with the finding that, during crisis, there is worsening of hemolysis as measured by hemoglobin and lactate dehydrogenase and also worsening of PH as measured by TRV. They have speculated that acute worsening of PH during crisis in patients who are already at the limits of cardiopulmonary compensation could potentially result in either dysrhythmia or acute right ventricular failure. More research is needed to further investigate these results and clarify the cause and effect relationship of PH and death in patients with hemolytic anemia.

#### **Implications for clinical practice**

Patients with hemolytic anemia and PH may be asymptomatic or only mildly symptomatic. Even if dyspnea on exertion or other symptoms develop, these may be misinterpreted as either deconditioning or simply due to chronic anemia. Therefore, clinicians must maintain an index of suspicion and screen patients with echocardiography. There are ongoing clinical trials evaluating the most appropriate treatment strategies for hemolysis-associated PH, although definitive treatment guidelines have not been fully established. The findings that regular transfusions may prevent or slow the progression of PH in thalassemia [37,38] suggest a trial of aggressive transfusion therapy is appropriate for these patients. The known association of PH with hypercoagulability, especially after splenectomy, in thalassemia suggests preventative anticoagulation is warranted, although there are no data to clarify if antiplatelet or antithrombotic agents are more beneficial. Because of the high prevalence of PH after splenectomy in TI, the

**Figure 2. Kaplan-Meier survival curves according to the tricuspid regurgitant jet velocity**



The survival rate was significantly higher among patients with a tricuspid regurgitant jet velocity of <2.5 m/s (indicating normal pulmonary artery pressure) than among those with a tricuspid regurgitant jet velocity of at least 2.5 m/s ( $P < 0.001$ ). Because patients were enrolled over a 20-month period, the first patients enrolled were followed for the entire time. Thus, the number of patients at risk at the time of each death is shown for both groups. (Figure and caption reproduced with permission from [8]. Copyright © 2004 Massachusetts Medical Society. All rights reserved.)

surgery should be avoided if at all possible for these patients [39].

For SCD-associated PH, most hematologists would maximize sickle cell therapy with hydroxyurea or chronic transfusions. It should be noted, however, that multiple studies did not find an association with hydroxyurea use and decreased TRV [8,24,40,41]. It is known that hydroxyurea decreases hemolysis and induces NO in endothelial cells [42] as well as improves clinical symptoms with less reported pain and fewer hospitalizations [43,44]. Treatment with hydroxyurea may therefore help prevent the acute episodes that exacerbate PH and are potentially associated with sudden death in these patients.

No prospective trials have evaluated the efficacy of transfusion therapy to decrease PH, although chronic transfusions are known to lower plasma free hemoglobin [45] and there are a few case reports of improvement of TRV with transfusion therapy [13]. One retrospective evaluation of 55 nontransfused and 20 transfused pediatric SCD patients found significantly lower TRV in the transfused population [46].

Therapies specifically targeted at PH pathology include vasodilatory agents, such as phosphodiesterase-5 inhibitors and prostanoids, as well as endothelin receptor antagonists and arginine supplementation. In a pilot study of 12 SCD patients with PH, sildenafil showed a

decrease in TRV and pulmonary artery pressures as well as functional improvement [47]. However, a large multicenter trial treating PH with sildenafil was prematurely closed as there was no functional improvement and increased vaso-occlusive pain was reported in the treatment group. Therefore, the role of sildenafil therapy is still unclear. Treatment with arginine supplementation was also shown to decrease pulmonary artery pressures in ten SCD patients [48]; however, the number of pills and dosing schedule required for an effect limit the use of arginine in clinical practice. A recent study of 14 SCD patients treated with the endothelin receptor antagonist bosentan showed promising results with improved TRV and mPAP [49].

Pulmonary hypertension is now an increasingly recognized complication of hemolysis and is associated with mortality in patients with hemolytic anemia. It remains to be seen if PH directly results in death or simply provides a window into the severity of vasculopathy for patients with SCD. Ongoing research aims to define the most effective treatment strategies for these patients and enrolment in clinical trials is therefore of paramount importance.

### Abbreviations

BNP, brain natriuretic peptide; mPAP, mean pulmonary artery pressure; NO, nitric oxide; PH, pulmonary hypertension; SCD, sickle cell disease; TI, thalassemia intermedia; TM, thalassemia major; TRV, tricuspid regurgitant jet velocity.

### Competing interests

The authors declare that they have no competing interests.

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