Pulmonary hypertension in patients with hemoglobinopathies: could a mechanism for dysfunction provide an avenue for novel therapeutics?

Advances in the care of patients with thalassemia, sickle cell disease, and other hemolytic disorders through early detection, education, improvements in red cell transfusion and iron chelation therapy, penicillin prophylaxis, vaccination, and hydroxyurea therapy have led to a significant prolongation of the life expectancy of such patients. As this patient population ages, new chronic complications of these hemoglobinopathies develop. In this context, pulmonary hypertension is emerging as one of the leading causes of morbidity and mortality in adult sickle cell and thalassemia patients.

Retrospective and prospective studies estimate a pulmonary hypertension prevalence of 20-40% in patients with sickle cell disease,[1-3] 40-50% in patients with thalassemia intermedia,[4] and 10-75% in patients with thalassemia major.[5-7] In addition, post-mortem studies in patients with sickle cell disease have revealed obliterative pulmonary vasculopathy with medial and intimal hyperplasia and plexogenic dilations of pulmonary vessels in one-third of patients.[8]

A study by Derchi and colleagues, published in this issue of the journal (see page 450) adds important new data on the use of sildenafil in this at risk population. In Derchi's case series, seven patients with thalassemia intermedia (n=4), thalassemia major (n=2), and sickle thalassemia (n=1) and severe pulmonary hypertension were treated with 50 mg of sildenafil twice daily for a period ranging from 4 weeks to 48 months. Pulmonary pressures decreased in all patients and functional status and six-minute walked distance improved. We will now attempt to frame these therapeutically provocative findings in the context of emerging data on the prognosis, pathophysiology, and therapy of pulmonary hypertension in patients with chronic hemolytic anemias.

Pulmonary hypertension: a harbinger of mortality

Pulmonary arterial hypertension, either idiopathic or associated with conditions such as scleroderma, cirrhosis or human immunodeficiency virus infection, is traditionally defined as a mean pulmonary artery pressure (MPAP) ≥25 mmHg at rest or ≥30 mmHg with exercise, pulmonary capillary wedge pressure ≤15 mmHg, and pulmonary vascular resistance > 3 units. Patients with idiopathic primary pulmonary hypertension tend to be young, have few comorbid conditions, and are usually symptomatic with mean pulmonary arterial pressures in the range of 50-60 mmHg. In contrast, patients with hemolytic anemias tend to have mild-to-moderate elevations in mean pulmonary pressures in the range of 30-40 mmHg, with mild elevations in pulmonary vascular resistance.[9,10]

The results of our pulmonary hypertension screening study reflect this observation. We studied 195 adult patients with sickle cell disease who were screened by transthoracic Doppler-echocardiograms using tricuspid regurgitant jet velocity (TRV) to estimate the pulmonary artery systolic pressure. Pulmonary hypertension was prospectively defined as a tricuspid regurgitant jet velocity (TRV) ≥2.5 m/sec. Thirty-two percent of patients had elevated pulmonary artery systolic pressures (TRV ≥2.5 m/sec) and 9% had moderately-to-severely elevated pressures (TRV ≥3.0 m/sec). Right heart catheterization was performed in consenting patients with TRV ≥2.5 m/sec. The mean pulmonary artery pressure was 34.5 mmHg and pulmonary vascular resistance was 148.5 dyn·sec·cm^{-5}.[10]

Despite this mild-to-moderate elevation in pulmonary artery pressures, 2-year mortality rates of up to 50% have been demonstrated in patients with sickle cell disease and pulmonary hypertension.[9] Furthermore, in our prospective study, a measured TRV of at least 2.5 m/sec, as compared to a velocity of less than 2.5 m/sec, was an independent predictor of mortality associated with a markedly increased risk of death (RR 10.1; 95% CI, 2.2-47; p<0.001), with an 18-month mortality of 16% for patients with a TRV ≥2.5 m/sec and less than 2% in patients without pulmonary hypertension. Taken together, these data suggest that pulmonary hypertension in patients with hemolytic diseases is likely to be a different disorder than other forms of pulmonary arterial hypertension, due to the presence of chronic anemia. Chronically anemic patients require a high resting cardiac output to compensate for a decrease in oxygen carrying capacity. It is likely that any degree of pulmonary hypertension would be poorly tolerated in these patients and would result in significant morbidity and possibly mortality. Another distinctive finding seen in patients with sickle cell disease and thalassemia and pulmonary hypertension is mild elevation in pulmonary capillary wedge pressure, a finding not seen in other forms of pulmonary arterial hypertension, raising the question of a potential contribution of left-sided heart disease to the development of pulmonary hypertension.
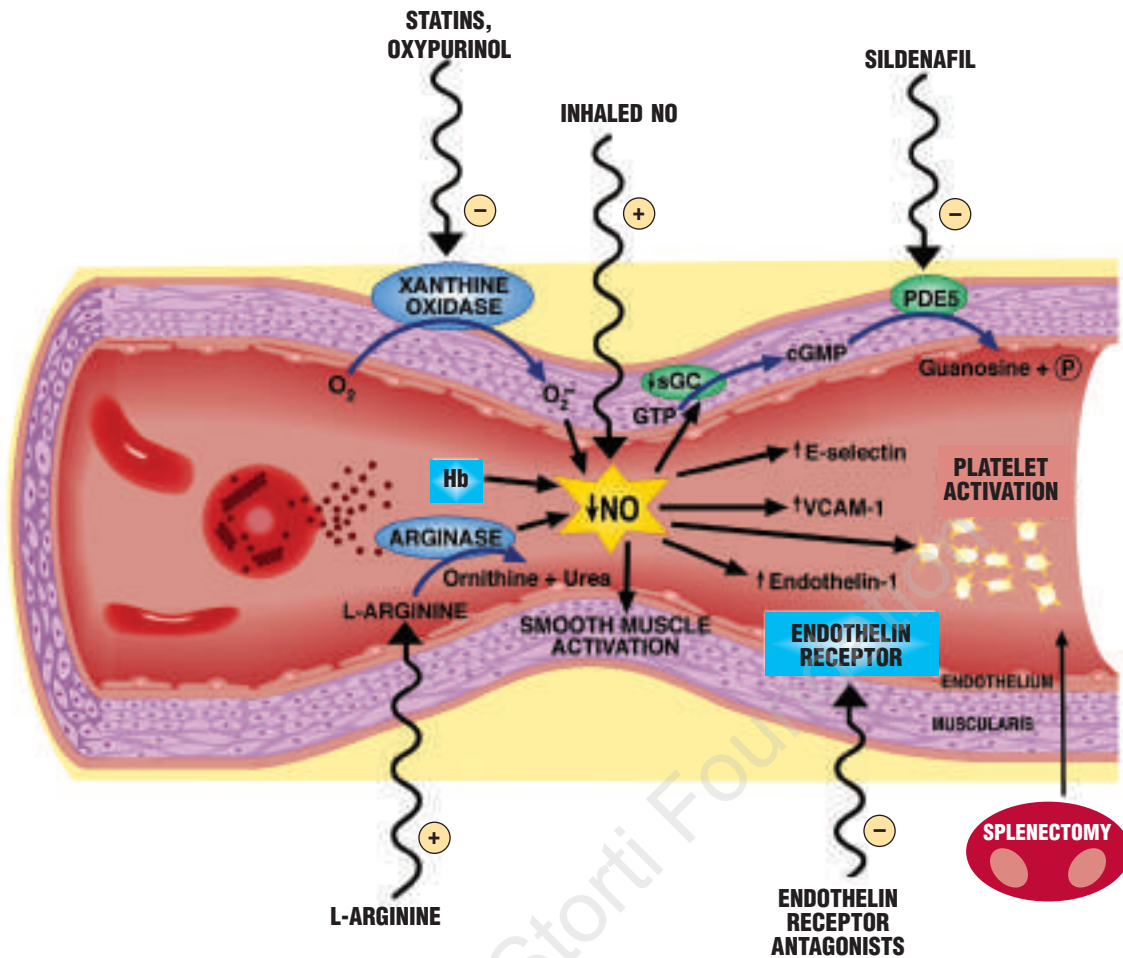
Hemolysis-associated pulmonary hypertension

Pulmonary hypertension develops in most forms of hereditary and chronic hemolytic anemia including sickle cell disease,[2,8-11] thalassemia intermedia and major,[4,6,12-14] hereditary spherocytosis,[15-17] stomatocytosis,[18-20] paroxysmal nocturnal hemoglobinuria,[21,22] microangiopathic hemolytic anemia,[23,24] pyruvate kinase deficiency,[25] and possibly malaria, suggesting that a common mechanism(s) could be responsible for the pathogenesis of the disease.

In sickle cell disease, a role for chronic intravascular hemolysis as a central mechanism in the development of secondary pulmonary hypertension is supported by a correlation between markers of increased hemolytic rate (such as low hemoglobin and hematocrit, high lactate dehydrogenase and aspartate aminotransferase, high bilirubin, high iron and ferritin, low transferrin, and a history of greater than 10 transfusions) and severity of the pulmonary hypertension.[10] These observational data suggest that pulmonary hypertension in patients with hemoglobinopathies is linked to chronic hemolysis and provide evidence for a novel mechanism of disease: hemolysis-associated pulmonary hypertension (HAPHT).

Pathophysiology

In chronic hemolytic diseases, hemoglobin is released from the erythrocytes into plasma. For example, in sickle cell disease, polymerization of hemoglobin S can lead to premature destruction of 10% of the erythrocytes every 24 hours, which is equivalent to the daily release of up to 30 grams of hemoglobin from the erythrocytes.[26] Nitric oxide is a free radical that is produced by endothelial cells and, through activation of guanylyl cyclase,

Figure 1. Pathophysiology of hemolysis-associated pulmonary hypertension and mechanism of action of pulmonary vasodilator/remodeling pharmacological agents.

causes vasodilatation of the vascular smooth muscle. However, hemoglobin oxidizes nitric oxide to nitrate, effectively scavenging it. Nitric oxide reacts with free hemoglobin at least 1000 times more rapidly than it does with erythrocytic hemoglobin, due to the loss of critical nitric oxide diffusional barriers created by the erythrocytic membrane.[27,28] Consequently, smooth muscle guanylyl cyclase is not activated and vasodilatation is inhibited. Supporting this hypothesis, plasma from patients with sickle-cell disease contains cell-free ferrous hemoglobin, which stoichiometrically consumes micromolar quantities of nitric oxide and abrogates forearm blood flow responses to infusions of nitric oxide donor, and hemoglobin oxidation by nitric oxide inhalation restores nitric oxide bioavailability.[29] As such, plasma hemoglobin and oxygen free radical-mediated consumption of nitric oxide produces a state of resistance to nitric oxide in patients with hemolytic disorders.[26,30] The scavenging of nitric oxide also upregulates adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and E-selectin, induces the expression of endothelin-1, and could influence platelet activation.[26,31-34]

Furthermore, hemolysis releases arginase from the erythrocytes. L-arginine is a precursor of nitric oxide. Arginase hydrolyzes L-arginine to ornithine and urea, thereby decreasing production and bioavailability of nitric oxide. Morris and colleagues recently determined that arginase activity tended to be higher in patients with sickle cell disease and pulmonary hypertension (0.82 ± 0.6 vs. 0.43 ± 0.2 µmol/mL/hour, $p=0.07$)[35] and arginase activity was independently associated with mortality in this population (risk ratio 2.3; CI 1.1-4.9, $p=0.03$, Morris *et al., submitted for publication*).

In addition to hemolysis, other factors are likely to contribute to the development of pulmonary hypertension in patients with hemolytic disorders. Functional asplenia or surgical splenectomy has been reported to be a risk factor for the development of pulmonary hypertension,[36] particularly in patients with hemolytic disorders post-splenectomy.[16,25,37] It has been speculated that the loss of splenic function increases the circulation of platelet-derived mediators because senescent and abnormal erythrocytes in the circulation trigger platelet activation promoting pulmonary microthrombosis and red cell adhesion to the endothelium. Westerman and colleagues have also shown that splenectomized patients with thalassemia intermedia

have a higher plasma hemoglobin concentration than do non-splenectomized patients with thlassemia intermedia (48.5±3.6 mg/dL vs. 17.18±5.58 mg/dL, *p*=0.014) and higher circulating hemoglobin containing vesicles (24.19±12.2 mg/dL vs. 4.36±0.81 mg/dL, *p*=0.008),[38] suggesting that worsening of pulmonary hypertension after splenectomy may also be due to increased cell-free plasma hemoglobin. *In situ* thrombosis, pulmonary thromboembolism, chronic liver dysfunction due to viral hepatitis or iron overload are also potential pathogenetic contributors and should be actively searched for and treated in patients presenting with pulmonary hypertension.

Diagnosis

The presence of pulmonary hypertension in patients with hemolytic disorders is likely to be unappreciated by both the patient and the care provider since its major symptom, dyspnea, is often ascribed to anemia. Doppler echocardiography provides information such as non-invasive estimation of pulmonary artery systolic pressure, valvular, right and left ventricular function. More importantly, since the presence of pulmonary hypertension assessed by echocardiography is an independent risk factor for mortality in patients with sickle cell disease, we recommend that adults with chronic hemolytic disorders undergo echocardiographic screening for pulmonary hypertension. The measurement of TRV is of central diagnostic and prognostic importance, with an absolute value greater than 2.5 m/sec identifying high-risk patients. Using this threshold, 30% of sickle cell patients[10] and 59% of thalassemia patients[4] have pulmonary hypertension. Right heart catheterization is essential to confirm the diagnosis in patients with higher TRV values (greater than 2.9 m/sec) and assess the severity of pulmonary hypertension, while excluding other contributors such as significant left ventricular dysfunction.

Treatment

There are very limited data on the specific management of patients with hemolytic disorders and pulmonary hypertension. Standard therapies to intensify the treatment of the underlying hemoglobinopathy such as hydroxyurea and simple or exchange transfusion should be optimized. Based on the role of hemolysis in the pathogenesis of pulmonary hypertension, it is likely that measures decreasing hemolytic rate would be beneficial. For example, a recent report by Aessopos and colleagues demonstrated that in well-transfused, iron-chelated patients with thalassemia major, pulmonary hypertension was completely prevented.[39] The use of selective pulmonary vasodilator/remodeling pharmacological agents such as prostacyclins, endothelin receptor antagonists, and phosphodiasterase-5 inhibitors should be considered in symptomatic patients with severe pulmonary hypertension. However, there are specific treatment concerns in patients with sickle cell disease and thalassemia. The use of prostacyclins, in addition to the risk of line sepsis or thrombosis, raises the potential concern of exacerbating a hyperdynamic state. Hepatotoxicity is a significant adverse effect of endothelin receptor antagonists, thus these agents must be used with caution in this population of patients predisposed to the development of cirrhosis secondary to hepatitis C and iron overload from repeated transfusions. Sildenafil has a theoretical risk of increasing priapism in men with sickle cell disease.

Since alterations in nitric oxide bioavailability play an important role in the pathogenesis of the pulmonary hypertension associated with chronic hemolytic disorders, therapeutic interventions that enhance nitric oxide effects such as inhaled nitric oxide, L-arginine and sildenafil may be of value. These agents target discrete components of the nitric oxide signaling pathway (Figure 1). Nitric oxide gas diffuses directly to smooth muscle guanylate cyclase, binds to its heme group and activates the enzyme, which converts GTP into the second messenger cGMP. This activates cGMP-dependent protein kinases and triggers the sequestration of intracellular calcium and vascular smooth muscle relaxation. L-arginine is the amino acid substrate for the nitric oxide synthase enzyme system, which converts L-arginine to citrulline and nitric oxide. Finally, the second messenger cGMP is degraded by phosphodiesterase 5, which is selectively inhibited by sildenafil. Inhaled nitric oxide could potentially be beneficial due to its ability to selectively dilate the pulmonary vasculature as well as oxidatively inactivate circulating plasma hemoglobin.[26] The use of chronic inhaled nitric oxide is investigational, potentially expensive and requires relatively complicated delivery systems.[40] When given for 5 days to 10 patients with sickle cell disease and moderate to severe pulmonary hypertension, L-arginine (0.1 g/kg three times daily) decreased estimated pulmonary artery systolic pressure by a mean of 15.2%, suggesting that it may have a role in the chronic treatment of pulmonary hypertension in these patients.[35] Sildenafil use resulted in symptomatic improvement and near normalization of pulmonary pressures in one patient with thalassemia intermedia.[41] The study by Derchi *et al*.[42] adds important new data on the use of sildenafil in this population. In an observational study, seven patients with either thalassemia intermedia (n=4), thalassemia major (n=2), or sickle thalassemia (n=1) and severe pulmonary hypertension with mean tricuspid regurgitant gradients of ≥45 mmHg at rest were treated with sildenafil 50 mg twice daily from 4 weeks to 48 months. Tricuspid gradient decreased in all patients and functional status, as defined by NYHA class and the six-minute walking test, improved. Sildenafil was well-tolerated without any significant hemodynamic adverse effects. There was no priapism observed in the one sickle cell patient, a serious potential concern with this agent. However, this study must now be followed by carefully designed phase I evaluations of the risk of priapism in male patients with sickle cell disease and phase II/III placebo-controlled trials evaluating efficacy.

Conclusions

Pulmonary hypertension is a common complication of chronic hemolytic disorders and is associated with high morbidity and mortality. Studies such as the one by Derchi and colleagues show promising efficacy and safety data and provide *proof of concept* for designing larger, multi-center, randomized, double-blind, placebo-controlled trials evaluating the safety and efficacy of selective pulmonary vasodilator/remodeling pharmacological agents in this population of patients.

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References

1. Simmons BE, Santhanam V, Castaner A, Rao KR, Sachdev N, Cooper R. Sickle cell heart disease. Two-dimensional echo and Doppler ultrasonographic findings in the hearts of adult patients with sickle cell anemia. Arch Intern Med 1988;148: 1526-8.
2. Sutton LL, Castro O, Cross DJ, Spencer JE, Lewis JF. Pulmonary hypertension in sickle cell disease. Am J Cardiol 1994; 74:626-8.
3. Castro O. Systemic fat embolism and pulmonary hypertension in sickle cell disease. Hematol Oncol Clin North Am 1996;10:1289-303.
4. Aessopos A, Farmakis D, Karagiorga M, Voskaridou E, Loutradi A, Hatziliami A, et al. Cardiac involvement in thalassemia intermedia: a multicenter study. Blood 2001; 97: 3411-6.
5. Derchi G, Fonti A, Forni GL, Galliera EO, Cappellini MD, Turati F, et al. Pulmonary hypertension in patients with thalassemia major. Am Heart J 1999;138:384.
6. Du ZD, Roguin N, Milgram E, Saab K, Koren A. Pulmonary hypertension in patients with thalassemia major. Am Heart J 1997;134:532-7.
7. Grisaru D, Rachmilewitz EA, Mosseri M, Gotsman M, Lafair JS, Okon E, et al. Cardiopulmonary assessment in b-thalassemia major. Chest 1990;98:1138-42.
8. Haque AK, Gokhale S, Rampy BA, Adegboyega P, Duarte A, Saldana MJ. Pulmonary hypertension in sickle cell hemoglobinopathy: a clinicopathologic study of 20 cases. Hum Pathol 2002;33:1037-43.
9. Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. Blood 2003;101:1257-61.
10. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 2004; 350:886-95.
11. Collins FS, Orringer EP. Pulmonary hypertension and cor pulmonale in the sickle hemoglobinopathies. Am J Med 1982;73:814-21.
12. Koren A, Garty I, Antonelli D, Katzuni E. Right ventricular cardiac dysfunction in b-thalassemia major. Am J Dis Child 1987;141:93-6.
13. Aessopos A, Stamatelos G, Skoumas V, Vassilopoulos G, Mantzourani M, Loukopoulos D. Pulmonary hypertension and right heart failure in patients with beta-thalassemia intermedia. Chest 1995;107:50-3.
14. Zakynthinos E, Vassilakopoulos T, Kaltsas P, Malagari E, Daniil Z, Roussos C, et al. Pulmonary hypertension, interstitial lung fibrosis, and lung iron deposition in thalassaemia major. Thorax 2001;56:737-9.
15. Verresen D, De Backer W, Van Meerbeeck J, Neetens I, Van Marck E, Vermeire P. Spherocytosis and pulmonary hypertension: coincidental occurrence or causal relationship? Eur Respir J 1991;4:629-31.
16. Hayag-Barin JE, Smith RE, Tucker FC Jr. Hereditary spherocytosis, thrombocytosis, and chronic pulmonary emboli: a case report and review of the literature. Am J Hematol 1998; 57:82-4.
17. Jardine DL, Laing AD. Delayed pulmonary hypertension following splenectomy for congenital spherocytosis. Intern Med J 2004;34:214-6.
18. Stewart GW, Amess JA, Eber SW, Kingswood C, Lane PA, Smith BD, et al. Thrombo-embolic disease after splenectomy for hereditary stomatocytosis. Br J Haematol 1996; 93: 303-10.
19. Jais X, Till SJ, Cynober T, Ioos V, Garcia Q, Tchernia G, et al. An extreme consequence of splenectomy in dehydrated hereditary stomatocytosis: gradual thrombo-embolic pulmonary hypertension and lung-heart transplantation. Hemoglobin 2003;27:139-47.
20. Murali B, Drain A, Seller D, Dunning J, Vuylsteke A. Pulmonary thromboendarterectomy in a case of hereditary stomatocytosis. Br J Anaesth 2003;91:739-41.
21. Heller PG, Grinberg AR, Lencioni M, Molina MM, Roncoroni AJ. Pulmonary hypertension in paroxysmal nocturnal hemoglobinuria. Chest 1992;102:642-3.
22. Uchida T, Miyake T, Matsuno M, Nishihara T, Ide M, Kawachi Y, et al. [Fatal pulmonary thromboembolism in a patient with paroxysmal nocturnal hemoglobinuria]. Rinsho Ketsueki 1998;39:150-2.
23. Stuard ID, Heusinkveld RS, Moss AJ. Microangiopathic hemolytic anemia and thrombocytopenia in primary pulmonary hypertension. N Engl J Med 1972;287:869-70.
24. Jubelirer SJ. Primary pulmonary hypertension. Its association with microangiopathic hemolytic anemia and thrombocytopenia. Arch Intern Med 1991;151:1221-3.
25. Chou R, DeLoughery TG. Recurrent thromboembolic disease following splenectomy for pyruvate kinase deficiency. Am J Hematol 2001;67:197-9.
26. Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO 3rd, Schechter AN, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nat Med 2002; 8: 1383-9.
27. Liu X, Miller MJ, Joshi MS, Sadowska-Krowicka H, Clark DA, Lancaster JR Jr. Diffusion-limited reaction of free nitric oxide with erythrocytes. J Biol Chem 1998;273:18709-13.
28. Carlsen E, Comroe JH Jr. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. J Gen Physiol 1958;42: 83-107.
29. Reiter CD, Gladwin MT. An emerging role for nitric oxide in sickle cell disease vascular homeostasis and therapy. Curr Opin Hematol 2003;10:99-107.
30. Aslan M, Ryan TM, Adler B, Townes TM, Parks DA, Thompson JA, et al. Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. Proc Natl Acad Sci USA 2001;98:15215-20.
31. Ergul S, Brunson CY, Hutchinson J, Tawfik A, Kutlar A, Webb RC, et al. Vasoactive factors in sickle cell disease: in vitro evidence for endothelin-1-mediated vasoconstriction. Am J Hematol 2004;76:245-51.
32. Gladwin MT, Schechter AN, Ognibene FP, Coles WA, Reiter CD, Schenke WH, et al. Divergent nitric oxide bioavailability in men and women with sickle cell disease. Circulation 2003;107:271-8.
33. Graido-Gonzalez E, Doherty JC, Bergreen EW, Organ G, Telfer M, McMillen MA. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. Blood 1998;92:2551-5.
34. Setty BN, Stuart MJ, Dampier C, Brodecki D, Allen JL. Hypoxaemia in sickle cell disease: biomarker modulation and relevance to pathophysiology. Lancet 2003;362:1450-5.
35. Morris CR, Morris SM Jr, Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, et al. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? Am J Respir Crit Care Med 2003;168:63-9.
36. Hoeper MM, Niedermeyer J, Hoffmeyer F, Flemming P, Fabel H. Pulmonary hypertension after splenectomy? Ann Intern Med 1999;130:506-9.
37. Atichartakarn V, Likittanasombat K, Chuncharunee S, Chandanamattha P, Worapongpaiboon S, Angchaisuksiri P, et al. Pulmonary arterial hypertension in previously splenectomized patients with b-thalassemic disorders. Int J Hematol 2003;78:139-45.
38. Westerman M, Pizzey A, Hirschman J, Cerino M, Eze A, Ramonton P, et al. Plasma 'free' HB is related to red cell derived vesicle numbers in sickle cell anemia and thalassemia intermedia: implications for nitric oxide (NO) scavenging and pulmonary hypertension. Blood 2004;104:465A.
39. Aessopos A, Farmakis D, Hatziliami A, Fragodimitri C, Karabatsos F, Joussef J, et al. Cardiac status in well-treated patients with thalassemia major. Eur J Haematol 2004; 73:359-66.
40. Channick RN, Newhart JW, Johnson FW, Williams PJ, Auger WR, Fedullo PF, et al. Pulsed delivery of inhaled nitric oxide to patients with primary pulmonary hypertension: an ambulatory delivery system and initial clinical tests. Chest 1996;109: 1545-9.
41. Littera R, La Nasa G, Derchi G, Cappellini MD, Chang CY, Contu L. Long-term treatment with sildenafil in a thalassemic patient with pulmonary hypertension. Blood 2002; 100:1516-7.
42. Derchi G, Forni GL, Formisano F, Cappellini MD, Galanello R, D'Ascola G, e al. Efficacy and safety of sildenafil in the treatment of severe pulmonary hypertension in patients with hemoglobinopathies. Haematologica 2005; 90:452-8.