Review Article

Sickle cell anemia–associated pulmonary arterial hypertension*

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Abstract

Pulmonary hypertension is a common complication of sickle cell anemia. Despite the fact that the elevations in pulmonary artery pressures are slight, morbidity and mortality are high. In adult sickle cell anemia patients, pulmonary hypertension is emerging as a major risk factor for death. The pathogenesis of sickle cell anemia-related pulmonary hypertension is multifactorial, including hemolysis, impaired nitric oxide bioavailability, chronic hypoxemia, thromboembolism, chronic liver disease and asplenia. In the majority of patients, pulmonary arterial hypertension is the main cause of elevated pulmonary artery pressures. However, pulmonary venous hypertension also plays a role in a subgroup of patients. Specific data on the effects of treatment modalities for pulmonary hypertension in patients with sickle cell anemia are scarce. It is likely that all patients would benefit from maximization of sickle cell anemia therapy, and that patients with the severe form of the disease would benefit from treatment with selective pulmonary vasodilators and antiproliferative agents. Large trials evaluating the effects of treatment for pulmonary hypertension in the sickle cell anemia population are underway.

Keywords: Anemia, Sickle cell; Hemolysis; Hypertension, pulmonary; Nitric oxide.

Introduction

Advances in health care of patients with sickle cell anemia and other hemolytic anemias have led to an increase in the life expectancy of these individuals. As this population grows older, new complications of these diseases tend to develop. One of these complications, pulmonary arterial hypertension, has become one of the leading causes of morbidity and mortality in adults with sickle cell anemia, thalassemia and, possibly, other hemoglobinopathies.

Sickle cell anemia occurs in homozygotic individuals in order to enable a nucleotide substitution in the beta-globin gene, which results in the synthesis of S hemoglobin (HbS), a structural variable that is far less soluble than normal hemoglobin when deoxygenated. Deoxygenated HbS polymerizes and is accreted within circulating erythrocytes in microvasculature. Sickle cells, which are typically rigid and dense, obstruct microvasculature, a process that is intensified by their propensity to adhere to vascular endothelium, resulting in a process of ischemia and reperfusion, inflammation, and oxidative stress. These events are responsible for the most typical manifestations of the disease: frequent episodes of bone pain, and acute thoracic syndrome. In addition, the membrane of erythrocytes that contain HbS is constantly exposed to mechanical damage that results in rupture and in a state of chronic intravascular hemolytic anemia. This hemolytic process results in the release of free hemoglobin and arginase enzyme in plasma, producing a state of endothelial dysfunction, and vascular proliferation, as well as oxidative and inflammatory stress. These pathological mechanisms result in a proliferative vasculopathy that can affect cerebral, renal, and pulmonary circulations, and whose principal manifestation is the development of pulmonary hypertension in adult life.

It is estimated that approximately 250,000 children are born with sickle cell anemia worldwide every year,[1] and, according to the World Health Organization, 2500 children are born with the disease annually in Brazil. Pulmonary complications account for a significant portion of the deaths related to sickle cell anemia.[2-5] In a multicenter prospective study conducted as part of the Cooperative Study of Sickle Cell Disease and involving 3764 patients, over 20% of the adults suffered from fatal pulmonary complications of the disease.[3] In addition, pulmonary diseases were the leading causes of death (28% of all deaths) among the 299 patients monitored at the Multicenter Study of Hydroxyurea in Sickle Cell Anemia.[6] Pulmonary hypertension springs among these chronic cardiopulmonary complications as one of the leading risks to well-being and longevity of patients with sickle cell anemia.

Epidemiology

Pulmonary hypertension, a disorder characterized by increased pulmonary artery pressure and pulmonary vascular resistance, has been increasingly recognized in patients with sickle cell anemia.[7-12] For example, the prevalence of pulmonary hypertension diagnosed by echocardiogram in patients examined at referral centers ranges from 20 to 30%.[7,11] In autopsy studies, approximately 75% of patients with sickle cell anemia present histological evidence of pulmonary hypertension.[13] In addition, various retrospective studies demonstrate that patients with pulmonary hypertension associated with sickle cell anemia have a worse prognosis. One study demonstrated a 40% mortality rate in a 22-month follow-up period with an odds ratio of 7.86 and a 95% confidence interval (95% CI) of 2.63-23.4.[11] In a study by other authors, mean survival for patients with sickle cell anemia complicated by chronic pulmonary disease and pulmonary hypertension was 2.5 years.[4] Similarly, another study[14] demonstrated a 50% mortality within two years in patients with pulmonary hypertension confirmed by right heart catheterization.

These retrospective data were confirmed by a screening study of pulmonary hypertension in sickle cell anemia carried out by the National Institutes of Health.[15] In the present, prospective study, 195 adults with sickle cell anemia were assessed by echocardiogram, using tricuspid regurgitant jet (TRJ) velocity to estimate systolic pulmonary artery pressure. Pulmonary hypertension was defined as TRJ velocity ≥ 2.5 m/sec. The prevalence of pulmonary hypertension in the present study was 32%, and 9% of the patients presented moderate to severe increases of systolic pulmonary pressure (defined as TRJ velocity ≥ 3 m/sec).

Using univariate analysis, high TRJ velocity was associated with markers of hemolytic anemia such as low hemoglobin, high aspartate aminotransferase,

and high lactate dehydrogenase. Advanced age was also related to pulmonary hypertension, and the age of the patients with pulmonary hypertension was significantly higher than that of the individuals without (38 ± 19 years for patients with TRJ velocity ≥ 3 m/sec, 39 ± 12 years for patients with TRJ velocity 2.5-2.9 m/sec, and 34 ± 10 years for patients with TRJ velocity < 2.5 m/sec, p = 0.02). In a multiple logistic regression, having been diagnosed with pulmonary hypertension was associated with a history of renal or cardiovascular problems, high systolic artery pressure, lactate dehydrogenase, high alkaline phosphatase, and low serum transferrin. In men, a history of priapism was also associated with pulmonary hypertension. The presence of these risk factors in the development of pulmonary hypertension suggests that this is a component of a systemic vasculopathy associated with sickle cell anemia, characterized by systemic arterial hypertension, renal insufficiency, and priapism, which it is mechanistically connected to hemolysis, iron overload, and cholestatic liver dysfunction.

In another prospective study with 60 patients, the prevalence of pulmonary hypertension (using TRJ velocity adjusted for body mass index) was 30%.[16] Similar findings were reported in a sample of participants in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia Patients' Follow-up Study in 1996.[17]

In the National Institutes of Health cohort, a TRJ velocity ≥ 2.5 m/sec was an independent marker of significantly increased risk of mortality (RR = 10.1; 95% CI: 2.2-47; p < 0.001). Mortality rate in 18 months was 16% for patients with TRJ velocity ≥ 2.5 m/sec and less than 2% in patients without pulmonary hypertension. These patients are still being monitored and recent data still demonstrate that pulmonary hypertension remains significantly associated with the risk of mortality, with a mortality rate of 40% in 45 months. In an updated analysis, relative risk of mortality for patients with TRJ velocity ≥ 2.5-2.9 m/sec was 4.4 (95% CI: 1.6-12.2; p < 0.001) and 10.6 (95% CI: 3.3-33.6; p < 0.001) for patients with TRJ velocity > 3.0 m/sec (Figure 1). Two other studies confirm these findings: with 17% mortality rate within two years for patients with pulmonary hypertension (versus 2% in the control group) in one study,[18] and 10% in 26 months (versus 1% in the control group) in the other study.[19]
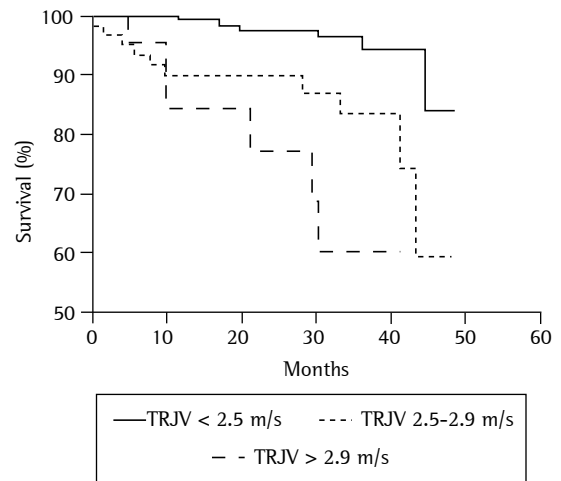

Figure 1 – Survival and severity of pulmonary hypertension in patients with sickle cell anemia. Survival is significantly higher in patients with tricuspid regurgitant jet velocity (TRJV) < 2.5 meters per second when compared with patients with TRJV > 2.5 meters per second (p < 0.001). Adapted from Gladwin et al.[15]

In summary, retrospective[4,11,14] and prospective[15,16,18,19] studies suggest that pulmonary hypertension is the leading risk factor for the well-being of adults with sickle cell anemia and, possibly, of patients with other hemolytic hemoglobinopathies. In addition, it is apparently clear that, similar to what occurs in patients with idiopathic pulmonary arterial hypertension or associated with other diseases such as scleroderma or HIV, the prognosis of pulmonary arterial hypertension associated with sickle cell anemia is poor.

Pathogenesis

Although different hemolytic anemias have specific clinical manifestations, the etiology of pulmonary hypertension in these patients seems to involve mechanisms that are common to these diseases altogether. These processes probably include hemolysis, causing endothelial dysfunction and oxidative/inflammatory stress, chronic hypoxemia, chronic thromboembolism, chronic liver disease, iron overload, and asplenia.

An important process in the development of pulmonary hypertension in patients with hemoglobinopathies is hemolytic anemia. Hemolysis results in the release of hemoglobin into plasma, where it reacts and consumes nitric oxide (NO) causing a

state of resistance to NO-dependent vasodilatory effects.[20,21] In addition, hemolysis also causes the release of arginase into plasma, which decreases the concentration of arginine, substrate for the synthesis of NO.[22] Other effects associated with hemolysis that can contribute to the pathogenesis of pulmonary hypertension are as follows: increased cellular expression of endothelin, production of free radicals, platelet activation, and increased expression of endothelial adhesion mediating molecules.[23]

Studies suggest that splenectomy (surgical or functional) is a risk factor for the development of pulmonary hypertension,[24] especially in patients with hemolytic anemias.[25-27] It is speculated that the loss of the spleen increases the circulation of platelet mediators and senescent erythrocytes that result in platelet activation (promoting endothelial adhesion and thrombosis in the pulmonary vascular bed), and possibly stimulates the increase in the intravascular hemolysis rate.[28-31]

Chronic pulmonary involvement due to repeated episodes of acute thoracic syndrome can lead to pulmonary fibrosis and chronic hypoxemia, which can eventually lead to the development of pulmonary hypertension. However, in epidemiological studies, the number of episodes of acute thoracic syndrome has not been related to pulmonary hypertension.[15,17] In addition, the prevalence of pulmonary hypertension in patients with thalassemia (who do not suffer from acute thoracic syndrome or significant pulmonary fibrosis) is quite similar to that of patients with sickle cell anemia, suggesting that chronic hypoxemia contributes to the development of pulmonary hypertension but is not a major etiological factor. Coagulation disorders, such as low levels of protein C, low levels of protein S, high levels of D-dimers and increased activity of the tissue factor, occur in patients with sickle cell anemia.[32-38] This hypercoagulable state can cause thrombosis in situ or pulmonary thromboembolism, which occurs in patients with sickle cell anemia and other hemolytic anemias.[26,27,39-43]

Clinical manifestations

The diagnosis of pulmonary hypertension in patients with sickle cell anemia is typically difficult. Dyspnea upon exertion, the symptom most typically associated with pulmonary hypertension, is also very common in anemic patients. In addition,

other disorders with similar symptomatology, such as left heart failure or pulmonary fibrosis, frequently occur in patients with sickle cell anemia. Patients with pulmonary hypertension tend to be older, present higher systemic blood pressure, more severe hemolytic anemia, lower peripheral oxygen saturation, worse renal function, impaired liver function, and a higher number of red blood cell transfusions than do patients with sickle cell anemia and normal pulmonary pressure.[15]

In contrast with patients presenting traditional forms of pulmonary arterial hypertension (idiopathic, associated with scleroderma, for example) who have symptoms such as a mean pulmonary artery pressure (mPAP) of 50-60 mmHg, patients with sickle cell anemia present a moderate increase in mPAP (30-40 mmHg), together with a mild increase in pulmonary vascular resistance (Table 1). Another characteristic finding of this group of patients is the mild increase in pulmonary capillary pressure, which does not occur in patients with pulmonary arterial hypertension. Specifically in patients submitted to hemodynamic testing in our department, 54% of the patients present pulmonary arterial hypertension, and 46% present pulmonary venous hypertension, mostly associated with diastolic dysfunction, which suggests that the etiology of pulmonary hypertension in patients with sickle cell anemia is multifactorial. Our group also using echocardiography to investigate the diastolic function of 141 patients.[44] Of this population, 18% presented echocardiographic evidence of diastolic dysfunction. In addition, diastolic dysfunction contributed to increases in TRJ velocity in only one-third of the patients with pulmonary hypertension. The most important finding, however, was that the

Table 1 – Hemodynamic profile in patients with sickle cell anemia.

	Without PH	With PH
mPAP (mm Hg)	19 ± 0.7	36 ± 1
mRAP (mm Hg)	6 ± 0.4	10 ± 1
PCP (mm Hg)	11 ± 0.5	17 ± 1
CI (L/min)	10 ± 0.5	9 ± 0.3
PVR (dyn.sec.cm^{-5})	59 ± 6	197 ± 14

Adapted from various sources;[14,15,61] PH: pulmonary hypertension; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; PCP: pulmonary capillary pressure; CI: cardiac index; and PVR: pulmonary vascular resistance.

combination of diastolic dysfunction and pulmonary hypertension increases the risk of mortality in these patients.

It should be noted that pulmonary hypertension in patients with hemoglobinopathies is quite different from classic pulmonary arterial hypertension due to chronic anemia, which results in increased cardiac index (in general, approximately 10 L/min) in an attempt to compensate for the decrease in the oxygen carrying capacity resulting from low hemoglobin. Due to this phenomenon, it is quite probable that, in these patients with severe anemia, any degree of pulmonary hypertension is poorly tolerated, which results in significant morbidity and, possibly, mortality. This hypothesis is supported by the results of a study that evaluated the cardiopulmonary function of patients with sickle cell anemia with and without pulmonary hypertension.[45] When compared with patients with normal pulmonary artery pressure, individuals with pulmonary hypertension (paired by age, gender and hemoglobin level) and mPAP of 36 ± 1.5 mmHg present lower distance covered on the 6-minute walk test (435 ± 31 versus 320 ± 20 meters; p = 0.002) and lower maximal oxygen uptake (50 ± 3% versus 41 ± 2% of normality; p = 0.02). In terms of comparison, in a study evaluating the effects of sitaxsentan, an endothelin antagonist, in patients with pulmonary arterial hypertension (idiopathic, associated with scleroderma and intracardiac shunt) with mPAP of 54 mmHg, mean distance on the 6-minute walk test was 398 meters, and mean maximal oxygen uptake was 46% of normality.[46] Therefore, we can infer that, in patients with chronic anemia, mild to moderate pulmonary hypertension has a significant deleterious impact on the aerobic functional capacity of these individuals.

Diagnostic evaluation

The diagnostic evaluation of patients with hemoglobinopathies and suspected of having pulmonary hypertension should follow the same guidelines established for the investigation of patients with other causes of pulmonary hypertension. Considering the high prevalence of pulmonary hypertension in patients with sickle cell anemia, we recommend an echocardiogram screening for all adults who suffer from the disease. It is important to note that the screening should be performed in clinically stable patients, since pulmonary pressures significantly increase during episodes of algic crises.[47] Subsequently, we will review aspects of diagnostic evaluation that are particularly relevant in patients with sickle cell anemia.

Evaluation of symptoms and functional capacity

Although the 6-minute walk test has not been validated in patients with hemoglobinopathies, preliminary data suggest that this test correlates well with maximal oxygen uptake and with the severity of pulmonary hypertension in patients with sickle cell anemia.[45] In addition, in these patients, the distance covered on the 6-minute walk test significantly improves with the treatment of pulmonary hypertension, which suggests that it can be used in this population.[48,49]

Laboratory tests

Serology for collagen-related diseases, viral hepatitis, and HIV, as well as liver function tests, should be performed in order to rule out other diseases associated with pulmonary hypertension. The degree of iron overload severity and hemolytic anemia should also be evaluated. Recently, we validated the use of serum levels of the cerebral natriuretic propeptide as a diagnostic and prognostic marker of pulmonary hypertension in patients with sickle cell anemia.[17]

Pulmonary ventilation/ perfusion scintigraphy

Ventilation/perfusion scintigraphy is an essential component of diagnostic evaluation, considering that chronic pulmonary hypertensive thromboembolism can be surgically cured. This process is particularly important in patients with sickle cell anemia, in whom pulmonary thromboembolism is a well-documented cause of mortality. In addition, chronic pulmonary hypertensive thromboembolism can occur and be successfully treated in these patients.[50]

Nocturnal pulse oximetry

Nocturnal desaturation is relatively common in children and adolescents with sickle cell anemia.[51-53] Various lines of evidence suggest that nocturnal hypoxia can contribute to the

development of neurological complications and algic crises through mechanisms that involve mediators that promote cell adhesion.[52,54,55] These effects can also be involved in the development of pulmonary arterial vasculopathy.

Treatment

There are few data on treatment evaluation of pulmonary hypertension associated with sickle cell anemia. Most recommendations are based on the opinion of specialists or evidence from treatment of other forms of pulmonary arterial hypertension. Our management typically includes intensifying the sickle cell anemia therapy, treatment of associated cardiopulmonary disorders and specific therapy for pulmonary arterial hypertension.

Intensification of sickle cell anemia therapy

Based on the observation that hemolysis plays a major role in the development of pulmonary hypertension in sickle cell anemia, it is possible that the intensification of the treatment of hemolytic anemia would be beneficial, since it modulates the principal mechanism involved in the pathogenesis of pulmonary hypertension. Therefore, we recommend the use of hydroxyurea or chronic transfusions in all patients with pulmonary hypertension. These interventions can also lead to symptomatic improvement, since they tend to increase the hemoglobin level and, consequently, the oxygen carrying capacity.

Treatment of associated disorders

Associated conditions such as iron overload, HIV, chronic liver disease, nocturnal hypoxia, and thromboembolic disorders should be specifically investigated and treated. The use of systemic anticoagulation should be considered in patients with moderate to severe pulmonary hypertension, considering the benefits demonstrated in patients with idiopathic pulmonary arterial hypertension.[56-58]

Specific therapy for pulmonary arterial hypertension

There is no high quality or long-term follow-up evidence in the evaluation of specific therapies for pulmonary arterial hypertension in patients with sickle cell anemia. The choice of drugs, at the moment, is empirical and based on the profile of adverse effects or on the preference of the health professional. However, there are specific effects of different drugs that can negatively interact with pathophysiological aspects of sickle cell anemia (Table 2).

The systemic use of prostanoids produces systemic vasodilation and increases the cardiac index, which can theoretically produce a state of heart failure with high cardiac index in patients with chronic anemia. The principal toxicity of endothelin receptor antagonists is hepatocellular lesion, which can complicate its use in patients with sickle cell anemia who frequently suffer from chronic liver disease (secondary to iron overload or viral hepatitis, for example). Another effect of this class of drugs is a drop in hemoglobin levels, which can be relevant in patients with chronic anemia.[46] The major preoccupation related to the use of sildenafil (and other phosphodiesterase-5 inhibitors) is the potential to develop priapism in men with sickle cell anemia.

Considering that alterations related to NO play an important role in the pathogenesis of pulmonary hypertension related to sickle cell anemia, therapeutic measures that increase the effect of NO can be beneficial. L-arginine is the nitrogen donor for the synthesis of NO and its use (0.1 g/kg three times a day for five days) in 10 patients with sickle cell anemia and pulmonary hypertension resulted in a 15.2% mean reduction in systolic pulmonary artery pressure.[59] In a series of recent cases, seven patients with thalassemia or sickle cell anemia complicated by severe pulmonary hypertension were treated with sildenafil for 4 weeks to 48 months. In that study, the tricuspid gradient

Table 2 – Potential adverse effects of drugs that affect pulmonary circulation and are especially relevant for patients with sickle cell anemia.

Drugs	Potential adverse effects
Prostanoids	Inconvenience Intravenous catheter-related infection risk Aggravation of hyperdynamic hemodynamic state
Phosphodiesterase-5 inhibitors	Priapism
Endothelin receptor antagonists	Liver toxicity Drop in hemoglobin levels

decreased in all patients, which was associated with an improvement in the functional class and distance covered on the 6-minute walk test.[60] We recently reported our experience with sildenafil in patients with sickle cell anemia and pulmonary hypertension, when we treated 12 patients with mean systolic pulmonary artery pressure of 51 mmHg for mean six months.[48] The therapy with sildenafil decreased systolic pulmonary artery pressure by 9 (95% CI: 0.3-17) mmHg, the distance covered on the 6-minute walk test improved by 78 (95% CI: 40-117) meters, and the plasma level of the cerebral natriuretic peptide decreased by 448 pg/ml.

Taking into account the lack of specific data from randomized long-term studies, we cannot recommend any agent as a drug of choice for pulmonary hypertension associated with hemolytic anemia. Fortunately, two multicentric randomized studies in patients with sickle cell anemia, one evaluating the effects of bosentan and another evaluating the effects of sildenafil in patients with pulmonary hypertension, are open and recruiting patients.

Final considerations

Pulmonary hypertension is a typical complication of sickle cell anemia (and, probably, of other hemolytic anemias) associated with high morbidity and mortality. Therefore, we suggest annual echocardiographic screening in this group of patients. Considering the fact that there is a relationship between the severity of hemolysis and pulmonary hypertension, it is quite probable that the intensification of specific therapy for sickle cell anemia will curb the progression of hypertensive disease in milder cases, and contribute to decrease morbidity and mortality associated with the most severe cases of the disease. In addition, we should consider the use of specific agents for pulmonary hypertension in symptomatic patients with severe pulmonary artery pressure increase.

Further studies are needed in order to evaluate the impact of pulmonary hypertension in patients with hemoglobinopathies, especially considering the high mortality rates associated with the disease and the peculiar hemodynamic profile of these patients. Randomized studies evaluating the effects of the treatment of pulmonary arterial hypertension in patients with sickle cell anemia are underway, which, we hope, will help clarify its role in this population.

References

1. Serjeant GR. Sickle-cell disease. Lancet. 1997;350(9079):725-30.
2. Gray A, Anionwu EN, Davies SC, Brozovic M. Patterns of mortality in sickle cell disease in the United Kingdom. J Clin Pathol. 1991;44(6):459-63.
3. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994;330(23):1639-44.
4. Powars D, Weidman JA, Odom-Maryon T, Niland JC, Johnson C. Sickle cell chronic lung disease: prior morbidity and the risk of pulmonary failure. Medicine (Baltimore). 1988;67(1):66-76.
5. Thomas AN, Pattison C, Serjeant GR. Causes of death in sickle-cell disease in Jamaica. Br Med J (Clin Res Ed). 1982;285(6342):633-5.
6. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA. 2003;289(13):1645-51. Erratum in: JAMA. 2003;290(6):756.
7. Castro O. Systemic fat embolism and pulmonary hypertension in sickle cell disease. Hematol Oncol Clin North Am. 1996;10(6):1289-303.
8. Collins FS, Orringer EP. Pulmonary hypertension and cor pulmonale in the sickle hemoglobinopathies. Am J Med. 1982;73(6):814-21.
9. Norris SL, Johnson C, Haywood LJ. Left ventricular filling pressure in sickle cell anemia. J Assoc Acad Minor Phys. 1992;3(1):20-3.
10. 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(7):1526-8.
11. Sutton LL, Castro O, Cross DJ, Spencer JE, Lewis JF. Pulmonary hypertension in sickle cell disease. Am J Cardiol. 1994;74(6):626-8.
12. Verresen D, De Backer W, Vermeire P. Pulmonary hypertension and sickle hemoglobinopathy. Chest. 1990;98(4):1042.
13. 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(10):1037-43.
14. Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. Blood. 2003;101(4):1257-61.
15. 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(9):886-95.
16. Ataga KI, Sood N, De Gent G, Kelly E, Henderson AG, Jones S, et al. Pulmonary hypertension in sickle cell disease. Am J Med. 2004;117(9):665-9.
17. Machado RF, Anthi A, Steinberg MH, Bonds D, Sachdev V, Kato GJ, et al. N-terminal pro-brain natriuretic peptide

levels and risk of death in sickle cell disease. JAMA. 2006;296(3):310-8.

18. De Castro LM, Jonassiant JC, Graham FL, Ashley-Koch A, Tellen MJ. Pulmonary Hypertension in SS, SC and Sβ Thalassemia: Prevalence, Associated Clinical Sydromes, and Mortality. Blood. 2004;104:462a.

19. Ataga KI, Moore CG, Jones S, Olajide O, Strayhorn D, Hinderliter A, et al. Pulmonary hypertension in patients with sickle cell disease: a longitudinal study. Br J Haematol. 2006;134(1):109-15.

20. 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(12):1383-9.

21. Kato GJ, McGowan V, Machado RF, Little JA, Taylor J 6th, Morris CR, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. Blood. 2006;107(6):2279-85.

22. Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, Sachdev V, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA. 2005;294(1):81-90.

23. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA. 2005;293(13):1653-62.

24. Hoeper MM, Niedermeyer J, Hoffmeyer F, Flemming P, Fabel H. Pulmonary hypertension after splenectomy? Ann Intern Med. 1999;130(6):506-9.

25. Atichartakarn V, Likittanasombat K, Chuncharunee S, Chandanamattha P, Worapongpaiboon S, Angchaisuksiri P, et al. Pulmonary arterial hypertension in previously splenectomized patients with beta-thalassemic disorders. Int J Hematol. 2003;78(2):139-45.

26. Chou R, DeLoughery TG. Recurrent thromboembolic disease following splenectomy for pyruvate kinase deficiency. Am J Hematol. 2001;67(3):197-9.

27. 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(1):82-4.

28. Atichartakarn V, Angchaisuksiri P, Aryurachai K, Chuncharunee S, Thakkinstian A. In vivo platelet activation and hyperaggregation in hemoglobin E/beta-thalassemia: a consequence of splenectomy. Int J Hematol. 2003;77(3):299-303.

29. Kisanuki A, Kietthubthew S, Asada Y, Marutsuka K, Funahara Y, Sumiyoshi A. Intravenous injection of sonicated blood induces pulmonary microthromboembolism in rabbits with ligation of the splenic artery. Thromb Res. 1997;85(2):95-103.

30. 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.

31. Atichartakarn V, Angchaisuksiri P, Aryurachai K, Onpun S, Chuncharunee S, Thakkinstian A, et al. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/beta-thalassaemic patients. Br J Haematol. 2002;118(3):893-8.

32. Berney SI, Ridler CD, Stephens AD, Thomas AE, Kovacs IB. Enhanced platelet reactivity and hypercoagulability in the steady state of sickle cell anaemia. Am J Hematol. 1992;40(4):290-4.

33. el-Hazmi MA, Warsy AS, Bahakim H. Blood proteins C and S in sickle cell disease. Acta Haematol. 1993;90(3):114-9.

34. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared with healthy black controls. Blood Coagul Fibrinolysis. 1995;6(2):93-9.

35. Kurantsin-Mills J, Ofosu FA, Safa TK, Siegel RS, Lessin LS. Plasma factor VII and thrombin-antithrombin III levels indicate increased tissue factor activity in sickle cell patients. Br J Haematol. 1992;81(4):539-44.

36. Marfaing-Koka A, Boyer-Neumann C, Wolf M, Leroy-Matheron C, Cynober T, Tchernia G. Decreased protein S activity in sickle cell disease. Nouv Rev Fr Hematol. 1993;35(4):425-30.

37. Peters M, Plaat BE, ten Cate H, Wolters HJ, Weening RS, Brandjes DP. Enhanced thrombin generation in children with sickle cell disease. Thromb Haemost. 1994;71(2):169-72.

38. Shet AS, Aras O, Gupta K, Hass MJ, Rausch DJ, Saba N, et al. Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. Blood. 2003;102(7):2678-83.

39. Manci EA, Culberson DE, Yang YM, Gardner TM, Powell R, Haynes J Jr, et al. Causes of death in sickle cell disease: an autopsy study. Br J Haematol. 2003;123(2):359-65..

40. Taher A, Abou-Mourad Y, Abchee A, Zalouaa P, Shamseddine A. Pulmonary thromboembolism in beta-thalassemia intermedia: are we aware of this complication? Hemoglobin. 2002;26(2):107-12.

41. 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(2):150-2. Japanese

42. Jaïs X, Till SJ, Cynober T, Ioos V, Garcia G, 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(3):139-47.

43. 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(2):303-10.

44. Sachdev V, Machado RF, Shizukuda Y, Rao YN, Sidenko S, Ernst I, et al. Diastolic dysfunction is an independent risk factor for death in patients with sickle cell disease. J Am Coll Cardiol. 2007;49(4):472-9.

45. Anthi A, Machado RF, Jison ML, Taveira-Dasilva AM, Rubin LJ, Hunter L, et al. Hemodynamic and functional assessment of patients with sickle cell disease and pulmonary hypertension. Am J Respir Crit Care Med. 2007;175(12):1272-9.

46. Barst RJ, Langleben D, Frost A, Horn EM, Oudiz R, Shapiro S, et al. Sitaxsentan therapy for pulmonary arterial hypertension. Am J Respir Crit Care Med. 2004;169(4):441-7.

47. Machado RF, Kyle Mack A, Martyr S, Barnett C, Macarthur P, Sachdev V, et al. Severity of pulmonary hypertension during vaso-occlusive pain crisis and exercise in patients with sickle cell disease. Br J Haematol. 2007;136(2):319-25.

48. Machado RF, Martyr S, Kato GJ, Barst RJ, Anthi A, Robinson MR, et al. Sildenafil therapy in patients with sickle cell

disease and pulmonary hypertension. Br J Haematol. 2005;130(3):445-53.

49. Derchi G, Forni GL, Formisano F, Cappellini MD, Galanello R, D'Ascola G, et al. Efficacy and safety of sildenafil in the treatment of severe pulmonary hypertension in patients with hemoglobinopathies. Haematologica. 2005;90(4):452-8.

50. Yung GL, Channick RN, Fedullo PF, Auger WR, Kerr KM, Jamieson SW, et al. Successful pulmonary thromboendarterectomy in two patients with sickle cell disease. Am J Respir Crit Care Med. 1998;157(5 Pt 1):1690-3.

51. Castele RJ, Strohl KP, Chester CS, Brittenham GM, Harris JW. Oxygen saturation with sleep in patients with sickle cell disease. Arch Intern Med. 1986;146(4):722-5.

52. Hargrave DR, Wade A, Evans JP, Hewes DK, Kirkham FJ. Nocturnal oxygen saturation and painful sickle cell crises in children. Blood. 2003;101(3):846-8.

53. Needleman JP, Franco ME, Varlotta L, Reber-Brodecki D, Bauer N, Dampier C, et al. Mechanisms of nocturnal oxyhemoglobin desaturation in children and adolescents with sickle cell disease. Pediatr Pulmonol. 1999;28(6):418-22.

54. Kirkham FJ, Hewes DK, Prengler M, Wade A, Lane R, Evans JP. Nocturnal hypoxaemia and central-nervous-system events in sickle-cell disease. Lancet. 20016;357(9269):1656-9.

55. Setty BN, Stuart MJ, Dampier C, Brodecki D, Allen JL. Hypoxaemia in sickle cell disease: biomarker modulation and relevance to pathophysiology. Lancet. 2003;362(9394):1450-5.

56. Frank H, Mlczoch J, Huber K, Schuster E, Gurtner HP, Kneussl M. The effect of anticoagulant therapy in primary and anorectic drug-induced pulmonary hypertension. Chest. 1997;112(3):714-21.

57. Fuster V, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary hypertension: natural history and the importance of thrombosis. Circulation. 1984;70(4):580-7.

58. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. N Engl J Med. 1992;327(2):76-81.

59. 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(1):63-9.

60. 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(4):1516-7.

61. Leight L, Snider TH, Clifford GO, Hellems HK. Hemodynamic studies in sickle cell anemia. Circulation. 1954;10(5):653-62.