

Chronic sickle cell lung disease: new insights into the diagnosis, pathogenesis and treatment of pulmonary hypertension

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Summary

Pulmonary hypertension is a common complication of sickle cell disease (SCD). In spite of the mild elevations in pulmonary artery pressures in these patients, the associated morbidity and mortality is high. In fact, in adult patients with SCD, pulmonary hypertension is emerging as the major independent risk factor for death. The aetiology of pulmonary hypertension is probably multifactorial, including haemolysis, impaired nitric oxide bioavailability, chronic hypoxaemia, thromboembolism, parenchymal and vascular injury because of sequestration of sickle erythrocytes, chronic liver disease and asplenia. Interestingly, pulmonary hypertension is emerging as a common, and probably, invariant sequella of lifelong haemolytic anaemia in other hereditary and acquired haemolytic diseases, such as thalassaemia, stomatocytosis and spherocytosis. There are currently limited specific data on the effects of any treatment modality for pulmonary hypertension in patients with SCD. It is likely that maximization of SCD therapy, in all patients, and treatment with selective pulmonary vasodilators and antiproliferative agents, in patients with severe disease, would be beneficial. A large trial evaluating the effects of therapy for pulmonary hypertension in the SCD population is clearly indicated.

Keywords: sickle cell disease, haemolysis, pulmonary hypertension, nitric oxide.

Sickle cell disease (SCD) occurs in individuals who are homozygous for a single nucleotide substitution in the β -globin gene that ultimately renders their haemoglobin (HbS) much less soluble than normal haemoglobin (HbA) when deoxygenated. This insolubility causes polymerization and aggregation of HbS inside sickle erythrocytes as they traverse the microcirculation. Rigid, dense and sickled cells become entrapped in the microcirculation producing ischaemia and reperfusion injury, propagating inflammatory,

thrombotic and oxidant stress. Intracellular polymerization ultimately damages the membrane and depletes erythrocyte energy stores, leading to chronic and episodic extravascular and intravascular haemolytic anaemia. Intravascular haemolysis produces a state of endothelial dysfunction, vascular proliferation and pro-oxidant and proinflammatory stress. These disease mechanisms could ultimately produce a proliferative vasculopathy affecting the brain, kidney and lung vasculature, a hallmark of which is the development of pulmonary hypertension in adulthood.

It is estimated that around 250 000 children worldwide are born with homozygous sickle cell anaemia every year (Serjeant, 1997). Approximately 0.15% of African-Americans are homozygous for SCD, and 8% have sickle cell trait. Mortality rates for children with SCD have declined over the last three decades, owing to widespread implementation of newborn screening programmes for the early detection of SCD, improvements in parental education, implementation of nationwide penicillin prophylaxis, effective immunization against *Haemophilus influenzae* and *Streptococcus pneumoniae*, advances in the safety of and access to transfusion medicine, and hydroxyurea therapy (Gaston *et al*, 1986; Platt, 1994; Centers for Disease Control and Prevention, 1998; Olney, 1999; Ashley-Koch *et al*, 2000; Steinberg *et al*, 2003). Despite significant improvements in the life-expectancy of patients with SCD, the median age at death is 42 years for men and 48 years for women (Platt *et al*, 1994). Pulmonary complications account for a large proportion of deaths among adults with SCD (Thomas *et al*, 1982; Powars *et al*, 1988; Gray *et al*, 1991; Platt *et al*, 1994). According to the Cooperative Study of Sickle Cell Disease (CSSCD), a prospective multicentre study of 3764 patients, over 20% of adults probably had fatal pulmonary complications of SCD (Platt *et al*, 1994). Amongst the 299 patients enrolled in the long-term follow-up study of patients who participated in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia, pulmonary disease was the most common cause of mortality, accounting for 28% of all deaths (Steinberg *et al*, 2003).

Acute and chronic pulmonary complications of sickle cell disease are common but often under-appreciated by health care providers. Acute complications include asthma and the acute

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chest syndrome and chronic complications include pulmonary fibrosis, pulmonary hypertension and cor pulmonale (recently reviewed by Minter & Gladwin, 2001; Siddiqui & Ahmed, 2003). Amongst the chronic cardiopulmonary complications of SCD, pulmonary hypertension has emerged as the major threat to the well-being and longevity of patients with SCD.

Epidemiology

Pulmonary hypertension, a disorder characterized by an elevated pulmonary artery pressure (PAP) and pulmonary vascular resistance, is an increasingly recognized complication of SCD (Collins & Orringer, 1982; Simmons *et al*, 1988; Verresen *et al*, 1990; Norris *et al*, 1992; Sutton *et al*, 1994; Castro, 1996). Pulmonary hypertension has been defined by the 1981 National Heart Lung and Blood Institute/National Institutes of Health (NHLBI/NIH) national registry as a mean pulmonary artery pressure (MPAP) ≥ 25 mmHg at rest or ≥ 30 mmHg with exercise (Rich *et al*, 1987). Echocardiographic studies performed at tertiary care sickle cell centres have reported that 20–30% of screened patients have pulmonary hypertension (MPAP: ≥ 25 mmHg; Sutton *et al*, 1994; Castro, 1996). Recent autopsy studies suggest that up to 75% of sickle cell patients have histological evidence of pulmonary arterial hypertension at the time of death (Haque *et al*, 2002). Furthermore, sickle cell patients with pulmonary hypertension have a significantly increased mortality rate compared to patients without pulmonary hypertension. Sutton *et al* (1994) reported a 40% mortality rate at 22 months with an odds ratio for death of 7.86 (2.63–23.4). Powars *et al* (1988) reported a mean 2.5 years survival in sickle cell patients with chronic lung disease with pulmonary hypertension. Castro *et al* (2003) similarly reported a 50% 2-year mortality rate in patients with SCD with pulmonary hypertension confirmed by right heart catheterization.

These data are consistent with the results of our pulmonary hypertension screening study (Gladwin *et al*, 2004a). We enrolled 195 adult patients with SCD that were screened with transthoracic Doppler-echocardiograms and the tricuspid regurgitant jet velocity (TRV) was used to estimate the pulmonary artery systolic pressure. The Doppler-echocardiogram can be used to measure the velocity of regurgitant blood across the tricuspid valve using the modified Bernoulli's equation ($4 \times \text{TRV}^2$ plus central venous pressure estimate) to calculate the pulmonary artery systolic pressure (method described in detail in Gladwin *et al*, 2004a). In our study, to avoid the more subjective estimation of central venous pressure, pulmonary hypertension was prospectively defined by a specific Doppler TRV value ≥ 2.5 m/s and moderate-to-severe pulmonary hypertension defined by a TRV ≥ 3.0 m/s. Right heart catheterization was performed in consenting patients with TRV ≥ 2.8 m/s. It was found that 32% of patients with SCD had elevated pulmonary artery systolic pressures (defined as TRV ≥ 2.5 m/s) and 9% had moderately to severely elevated pressures (defined as TRV ≥ 3.0 m/s). On

univariate statistical analysis, all markers of haemolytic anaemia, including low Hb and haematocrit, high aspartate aminotransferase but not alanine aminotransferase and high lactate dehydrogenase (LDH) levels were associated with elevated pulmonary pressures. Increasing age was also a univariate predictor of a high TRV and patients with pulmonary hypertension were significantly older than patients without pulmonary hypertension (38 ± 19 years for patients with TRV ≥ 3.0 m/s, 39 ± 12 years for patients with TRV = 2.5–2.9 m/s and 34 ± 10 years for patients with TRV < 2.5 m/s, $P = 0.02$). Multiple logistic regression analysis identified a history of renal or cardiovascular complications, increased systemic systolic blood pressure, the marker of haemolysis LDH, elevated alkaline phosphatase and low transferrin levels as independent predictors of pulmonary hypertension. In men, a history of priapism was an independent factor associated with pulmonary hypertension. These associated risk factors for pulmonary hypertension suggest that pulmonary hypertension represents a component of the systemic vasculopathy of SCD (systemic hypertension, renal failure and priapism) and is mechanistically linked to haemolytic rate, iron overload and cholestatic hepatic dysfunction. Interestingly, the development of pulmonary hypertension was not associated with markers of inflammation, fetal Hb levels or platelet counts. In another recent prospective study of 60 patients systematically sampled at a comprehensive sickle cell treatment centre, the prevalence of pulmonary hypertension (defined by an age and body mass index-adjusted nomogram) was 30% (Ataga *et al*, 2004a).

Consistent with retrospective studies indicating that pulmonary hypertension is associated with a higher mortality, a measured TRV of at least 2.5 m/s, when compared with a velocity of < 2.5 m/s, was associated with a marked increased risk of death [relative risk (RR): 10.1; 95% confidence interval (CI): 2.2–47; $P < 0.001$] and remained so after adjustment for other possible risk factors in proportional hazards regression analysis. The 18-month mortality was 16% for patients with a TRV ≥ 2.5 m/s and was $< 2\%$ in patients without pulmonary hypertension. A study by De Castro *et al* (2004) presented at the most recent meeting of the American Society of Hematology reported a similar prevalence of pulmonary hypertension and a remarkably similar 17% mortality rate for patients with pulmonary hypertension over 2 years compared with approximately 2% for subjects without pulmonary hypertension. Taken together, the retrospective (Powars *et al*, 1988; Sutton *et al*, 1994; Castro *et al*, 2003) and prospective studies (Ataga *et al*, 2004b; De Castro *et al*, 2004; Gladwin *et al*, 2004a) strongly support the contention that pulmonary hypertension is the greatest risk factor facing the ageing population of patients with SCD.

It is clear that this disease, similar to idiopathic pulmonary arterial hypertension (formerly primary pulmonary hypertension) and pulmonary hypertension associated with other conditions [e.g. scleroderma and human immunodeficiency virus (HIV)] carries an unacceptably high morbidity and

mortality rate. In fact, we continue to follow patients in our original cohort of 195 individuals and pulmonary hypertension continues to be a strong independent risk factor for mortality (RR: 7.4, 95% CI: 2.4–22.6, $P < 0.001$) with a 40-month mortality rate of approximately 40% (Fig 1).

Clinical manifestations

The diagnosis of pulmonary hypertension in patients with SCD can be challenging. Exertional dyspnoea, the most typical presentation of pulmonary hypertension, is also a cardinal symptom of chronic anaemia, and therefore a high index of suspicion for the disease is necessary. More specific symptoms, such as angina, syncope and lower extremity oedema, are uncommon and usually associated with severe and advanced pulmonary hypertension. Physical findings suggestive of right ventricular dysfunction, such as jugular venous distension, right ventricular S3 gallop, accentuated pulmonary component of S2, ascites and peripheral oedema, are also associated with more advanced pulmonary hypertension. Other conditions commonly present in patients with SCD, such as left ventricular dysfunction, pulmonary fibrosis and liver cirrhosis, could also present in a similar fashion and could also result in pulmonary hypertension. Patients with pulmonary hypertension tend to be older, have higher systolic arterial blood pressure, lower Hb levels, higher indices of haemolysis (such as high bilirubin or LDH values), lower Hb-oxygen saturation, greater degree of renal and liver dysfunction and a higher number of lifetime red blood cell transfusions (Gladwin *et al*, 2004a), suggesting a greater burden of SCD and perhaps a generalized vasculopathy associated with chronic intravascular haemolysis.

In contrast to patients with traditional forms of pulmonary arterial hypertension (e.g. idiopathic, scleroderma-associated), who are usually symptomatic with MPAP in the range of 50–60 mmHg, in patients with SCD the degree of elevation in

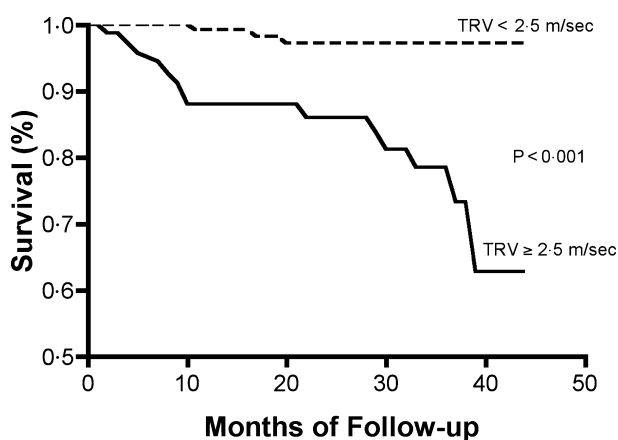


Fig 1. Kaplan–Meier survival curves according to the tricuspid regurgitant jet velocity (TRV). The survival rate is significantly higher among patients with a TRV of <2.5 m/s than among those with a TRV of at least 2.5 m/s ($P < 0.001$).

mean pulmonary pressures is mild-to-moderate, in the range of 30–40 mmHg, with mild elevations in pulmonary vascular resistance (Table I). Another peculiar finding seen in patients with SCD and pulmonary hypertension is mild elevations in pulmonary capillary wedge pressure, a finding not seen in other forms of pulmonary arterial hypertension, where the pulmonary capillary wedge pressure is normal (≤ 15 mmHg). This raises the question of a potential contribution of left-sided heart disease, in particular diastolic dysfunction and high output biventricular failure, to the development of pulmonary arterial hypertension. While we previously observed that measures of left ventricular systolic and diastolic dysfunction were not associated with elevated pulmonary artery systolic pressures or mortality in patients with SCD and pulmonary hypertension (Gladwin *et al*, 2004a), it remains possible that there may also be a comorbid component of left-sided disease in subgroups of patients. We have also recently observed increases in PAPs in these patients associated with mild exercise and vaso-occlusive painful crisis (Kato *et al*, 2004). As such, it is important to recognize that patients with normal resting pulmonary pressures could still have abnormal elevations in their pressures during exercise. For this reason, we recommend the use of exercise echocardiogram with assessment of pulmonary pressures to evaluate patients with significant exertional dyspnoea and normal resting TRVs. Although the criteria for an abnormal response to exercise are not well established, in healthy non-trained men TRV increases from an average baseline of 1.72 m/s to an average of 2.46 m/s at mid-level exercise and to 2.27 at peak exercise (240 W; Bossone *et al*, 1999).

It is important to emphasize that pulmonary hypertension in patients with SCD is clearly a different disorder than other forms of pulmonary arterial hypertension, given the presence of chronic anaemia, which requires a resting high cardiac output (usually around 10 l/min) to compensate for a decrease in oxygen carrying capacity. It is likely that in patients with critical anaemia, any degree of pulmonary hypertension would be poorly tolerated and would result in significant morbidity and possible mortality. Consistent with this hypothesis are the

Table I. Haemodynamic profiles in patients with sickle cell disease.

| | Without PHT | With PHT |
|-------------------------------|-------------|----------|
| PA systolic (mmHg) | 31 ± 1 | 54 ± 2 |
| PA diastolic (mmHg) | 12 ± 1 | 26 ± 1 |
| PA mean (mmHg) | 19 ± 1 | 36 ± 1 |
| RAP (mmHg)* | 8 ± 2 | 12 ± 1 |
| PCWP (mmHg) | 10 ± 1 | 17 ± 0.9 |
| CO (l/min) | 9 ± 0.8 | 9 ± 0.4 |
| PVR (dyn/s/cm ⁵)* | 93 ± 19 | 184 ± 26 |

Data from Castro *et al* (2003) and Gladwin *et al* (2004b).

PHT, pulmonary hypertension; PA, pulmonary artery; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; PVR, pulmonary vascular resistance.

*Not reported by Castro *et al* (2003).

results of a recently presented study evaluating the cardiopulmonary function of patients with SCD (Machado *et al*, 2004). When compared with age-, gender- and Hb-matched patients with SCD without pulmonary hypertension, individuals with pulmonary hypertension and MPAP of 36 mmHg had a significantly shorter mean 6-min walk distance (427 m vs. 308 m, respectively; $P = 0.03$) and a trend towards lower percentage predicted peak oxygen consumption during cardiopulmonary exercise testing (55% vs. 44%, respectively; $P = 0.41$). In comparison, in a recent randomized trial evaluating the effects of the selective endothelin (ET) antagonist sitaxentan in patients with idiopathic, collagen vascular disease or intracardiac shunt-related pulmonary arterial hypertension with MPAP of 54 mmHg, the mean baseline 6-min walk distance was 398 m and the mean peak oxygen consumption was 46% of that predicted (Barst *et al*, 2004a). Taken together these data suggest that, in patients with SCD and chronic anaemia, mild-to-moderate pulmonary hypertension has a severe adverse impact on functional and aerobic exercise capacity.

The 6-min walk test measures the distance an individual can walk at an unhurried pace. The test is fairly easy to perform, can be administered in most ambulatory settings and has been validated in patients with idiopathic, scleroderma and cardiac shunt-associated pulmonary arterial hypertension (for details of test administration see American Thoracic Society (ATS) Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002). The 6-min walk distance correlates with functional status, peak oxygen consumption and with survival in patients with pulmonary arterial hypertension (Key *et al*, 1998; Miyamoto *et al*, 2000; Paciocco *et al*, 2001). It is currently the most commonly utilized outcome measure in treatment trials of pulmonary hypertension, and can be used to follow response to therapy.

Diagnostic evaluation

The diagnostic evaluation of patients with SCD suspected of having pulmonary hypertension should follow the same guidelines established for other causes of pulmonary hypertension (Barst *et al*, 2004b; McGoon *et al*, 2004). The diagnosis of pulmonary hypertension usually involves two stages: detection (during the evaluation of a symptomatic patient or

as a result of generalized screening) and cardiopulmonary characterization, where selected diagnostic studies are utilized to characterize associated diseases, haemodynamic perturbations and their aetiology, and the degree of functional impairment. A unique feature of SCD, shared by patients with scleroderma, is that the extremely high prevalence of pulmonary hypertension in the population (one-third of all patients with both scleroderma and SCD) and the high associated mortality, demands universal non-invasive screening of all adults with Doppler echocardiography. While no data on prevalence and risk are available for children, we currently recommend that children with high haemolytic rates (Hb values <7 g/dl with high LDH values) and recurrent acute chest syndrome be screened. It is important that such screening be performed in steady-state, as pressures rise during vaso-occlusive painful crisis (Kato *et al*, 2004).

Symptom evaluation and functional assessment

Quantification of the degree of symptomatic exercise limitation should be evaluated with the World Health Organization classification and could be used in evaluating response to therapy (Table II). The most commonly utilized exercise test in patients with pulmonary hypertension is the 6-min walk test. While the 6-min walk test has not been extensively validated in patients with SCD, our preliminary data in patients with SCD demonstrated that the 6-min walk distance directly correlated with peak oxygen consumption and inversely with the degree of pulmonary hypertension, and that the 6-min walk distance improved with therapy, suggesting that the test could be used in this patient population (Machado *et al*, 2004).

Laboratory tests

Minimally required tests to exclude other associated conditions would include serological testing to screen for collagen vascular disorders, HIV and liver function testing. The severity of iron overload and haemolytic anaemia should be assessed.

Transthoracic Doppler echocardiography

Doppler echocardiography provides essential information, such as non-invasive estimation of pulmonary artery systolic

Table II. World Health Organization classification of functional status of patients with pulmonary hypertension.

| | |
|-----------|---|
| Class I | Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope |
| Class II | Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope |
| Class III | Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope |
| Class IV | Patients with pulmonary hypertension resulting in the inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may be present at rest, and discomfort is increased by any physical activity |

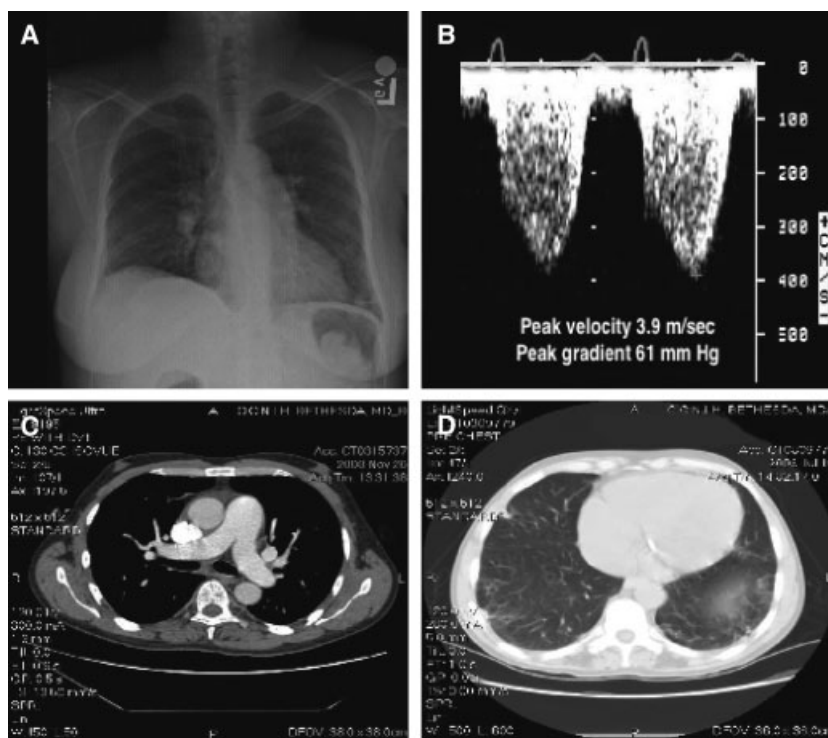


Fig 2. Imaging studies in sickle cell disease and pulmonary hypertension. (A) Chest radiograph demonstrating enlargement of main pulmonary arteries and mild basilar pulmonary fibrosis. (B) Tricuspid regurgitant jet velocity (TRV) estimation by Doppler echocardiogram. (C) Enlargement of pulmonary arteries in severe pulmonary hypertension. (D) Mild pulmonary fibrosis typical of patients with sickle cell disease and pulmonary hypertension.

pressure (via calculation of the TRV; Fig 2), valvular function and right and left ventricular function. The use of echocardiography to estimate pulmonary artery systolic pressures has been validated in patients with SCD, and non-invasive assessment correlates well with the measurement of pulmonary arterial pressures by right heart catheterization (Gladwin *et al*, 2004a).

As previously mentioned, the presence of pulmonary hypertension assessed by echocardiography is an independent risk factor for mortality in patients with SCD. Based on these data we recommend that adults with SCD undergo echocardiographic screening for pulmonary hypertension.

Because an elevated TRV is also seen with left heart failure, the echocardiogram also provides important information about the presence of left ventricular systolic dysfunction (observed in 2% of patients in our cohort) and diastolic dysfunction (observed in 15% of patients in our cohort).

Pulmonary function testing

Pulmonary function testing (including spirometry, measurement of lung volumes and diffusing capacity) will exclude or identify the presence of airflow obstruction or pulmonary parenchymal disease that could potentially exacerbate pulmonary hypertension associated with hypoxaemia. Most patients with SCD develop abnormal pulmonary function characterized by airflow obstruction, restrictive lung disease,

abnormal diffusing capacity and hypoxaemia (Koumbourlis *et al*, 1997, 2001; Lonsdorfer *et al*, 1983; Powars *et al*, 1988; Young *et al*, 1988; Santoli *et al*, 1998).

Ventilation-perfusion lung scintigraphy

The ventilation-perfusion (V/Q) scan is an indispensable component of the evaluation because chronic thromboembolic pulmonary hypertension is a potentially curable cause of pulmonary hypertension. Patients with chronic thromboembolic pulmonary embolism usually have at least one large perfusion defect on V/Q scans. This issue is particularly important in patients with SCD in which thromboembolism is a documented cause of mortality. Chronic thromboembolic pulmonary hypertension can occur in patients with SCD and has been treated surgically with success (Yung *et al*, 1998). As such, patients with SCD and pulmonary hypertension should undergo imaging studies and, if those are suggestive of chronic thromboembolic pulmonary hypertension, they should undergo more invasive studies (i.e. angiography) to exclude this potentially surgically treatable condition.

Radiographic studies

Chest X-ray has a low sensitivity for the diagnosis of pulmonary hypertension but findings of central pulmonary arterial or right ventricular enlargement suggests the presence

of the disease (Fig 2). Additional clues to associated conditions (e.g. pulmonary fibrosis) could be also seen in the chest film. High-resolution chest computerized tomography (CT) can provide a more detailed view of the pulmonary parenchyma and could aid in the diagnosis of pulmonary fibrosis, a finding commonly seen in patients with SCD (Fig 2). Contrast-enhanced spiral CT can demonstrate the presence of central chronic pulmonary thromboemboli with 85–90% sensitivity (Pruszczyk *et al*, 1997). However, pulmonary angiography is always required to confirm the diagnosis and assess operability.

Screening overnight oximetry

Severe sleep apnoea syndrome, causing frequent episodes of night-time desaturation, can lead to the development of pulmonary hypertension and overnight oximetry can provide the first clues to the diagnosis. More importantly, night-time oxygen desaturation is a well documented entity in children and adolescents with SCD (Castele *et al*, 1986; Needleman *et al*, 1999; Hargrave *et al*, 2003). Several lines of evidence suggest that nocturnal hypoxaemia could contribute to the development of neurological events and vaso-occlusive crisis via mechanisms that could involve upregulation of several cell-adhesion mediators (Kirkham *et al*, 2001; Hargrave *et al*, 2003; Setty *et al*, 2003). These effects could also play a role in the development of the vasculopathy associated with pulmonary hypertension.

Right heart catheterization

Pulmonary arterial hypertension is traditionally defined as a MPAP ≥ 25 mmHg at rest or ≥ 30 mmHg with exercise, and pulmonary capillary wedge pressure ≤ 15 mmHg and pulmonary vascular resistance > 3 units. Thus, right heart catheterization is essential to confirm the diagnosis and assess the severity of pulmonary hypertension, while excluding other contributors, such as significant left ventricular dysfunction. In our practice we routinely catheterize patients with TRVs of 2.9 m/s or greater. Diastolic or systolic left ventricular dysfunction as an independent cause of pulmonary hypertension or as a comorbidity is important to exclude by right heart catheterization. While the pulmonary capillary wedge pressure is high in patients with SCD, even in the absence of left ventricular dysfunction, an increased gradient between the pulmonary capillary wedge pressure and the pulmonary artery diastolic pressure (≥ 8 mmHg) with an absolute pulmonary capillary wedge pressure ≤ 18 mmHg supports the diagnosis of intrinsic pulmonary vascular disease.

Uncontrolled studies in patients with idiopathic pulmonary hypertension suggest that chronic treatment with calcium-channel blockers prolong survival in a subgroup of patients who acutely respond to inhaled nitric oxide (NO), adenosine or prostacyclin. The current definition of a positive acute response includes a 10 mmHg decrease in MPAP to reach a MPAP of ≤ 40 mmHg. The role of acute vasodilator challenge, however, is not established in patients with SCD and, although we perform

acute vasodilator challenge testing for prognostic information, we rarely use calcium-channel blockers in this population.

Pathogenesis

The aetiology of the pulmonary arterial hypertension in individuals with SCD is probably multifactorial, and could include haemolysis producing endothelial dysfunction and oxidant and inflammatory stress, chronic hypoxaemia with activation of proliferative mediators, chronic thromboembolism and *in situ* thrombosis, parenchymal and vascular injury because of sequestration of sickle erythrocytes, chronic liver disease, iron overload and asplenia (Fig 3).

Haemolysis

An apparent central process in the development of pulmonary hypertension is chronic intravascular haemolysis (Reiter *et al*, 2002; Jison & Gladwin, 2003; Gladwin *et al*, 2004a). Cell-free plasma Hb destroys NO at a rate 1000-fold faster than intraerythrocytic Hb (Gladwin *et al*, 2003a, 2004a; Schechter & Gladwin, 2003). As a result of haemolysis, Hb is released into plasma where it reacts with and destroys NO, resulting in abnormally high rates of NO consumption and a state of resistance to NO activity. Consequently, smooth muscle guanylyl cyclase is not activated and vasodilation is inhibited. We have previously demonstrated that plasma from patients with SCD contains cell-free ferrous Hb, which stoichiometrically consumes micromolar quantities of NO and abrogates forearm blood flow responses to NO donor infusions, and that Hb oxidation by NO inhalation restores NO bioavailability (Gladwin *et al*, 1999; Reiter *et al*, 2002; Fig 4). As such, plasma Hb- and oxygen-free radical-mediated consumption of NO produces a state of resistance to NO in patients with SCD (Aslan *et al*, 2001, 2003; Reiter *et al*, 2002; Eberhardt *et al*, 2003; Gladwin *et al*, 2003b; Reiter & Gladwin, 2003; Kaul *et al*, 2004; Nath *et al*, 2004).

Downstream effects of haemolytic anaemia include increased endothelin-1 (ET-1) expression, haem and free iron-mediated oxygen radical generation, platelet activation and increased endothelial adhesion molecule expression (Hebbel, 1985; Reiter *et al*, 2002; Gladwin *et al*, 2003b; Setty *et al*, 2003). In patients with SCD, plasma ET-1 levels are increased in steady-state and during crisis (Graido-Gonzalez *et al*, 1998; Ergul *et al*, 2004). *In vitro*, sickle erythrocytes increase ET-1 production by cultured human endothelial cells (Phelan *et al*, 1995; Shiu *et al*, 2002), and ET receptor A antagonism abrogates the vasoconstrictive effects of conditioned media from pulmonary endothelial cells exposed to sickled erythrocytes on aortic rings (Ergul *et al*, 2004). In addition, ET-1 activates Gardos channels in human sickle erythrocytes, an effect that may promote sickle cell dehydration and facilitate red blood cell sickling and adhesion (Rivera *et al*, 2002).

Intravascular haemolysis has the potential to drive a procoagulant state. Platelet activation is profoundly inhibited

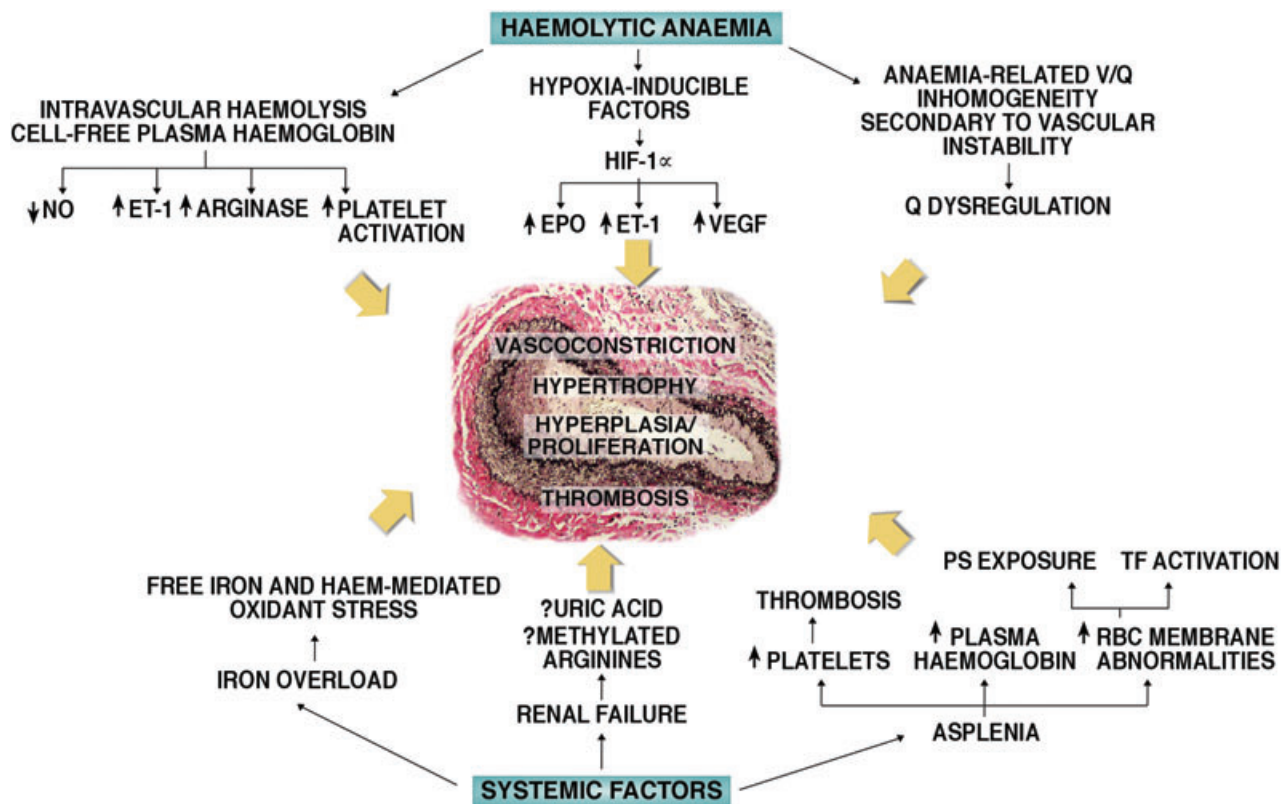


Fig 3. Pathogenesis of pulmonary hypertension in patients with sickle cell disease. NO, nitric oxide; ET-1, endothelin-1; HIF, hypoxia-inducible factor; EPO, erythropoietin; VEGF, vascular endothelial growth factor; PS, phosphatidyl serine; TF, tissue factor.

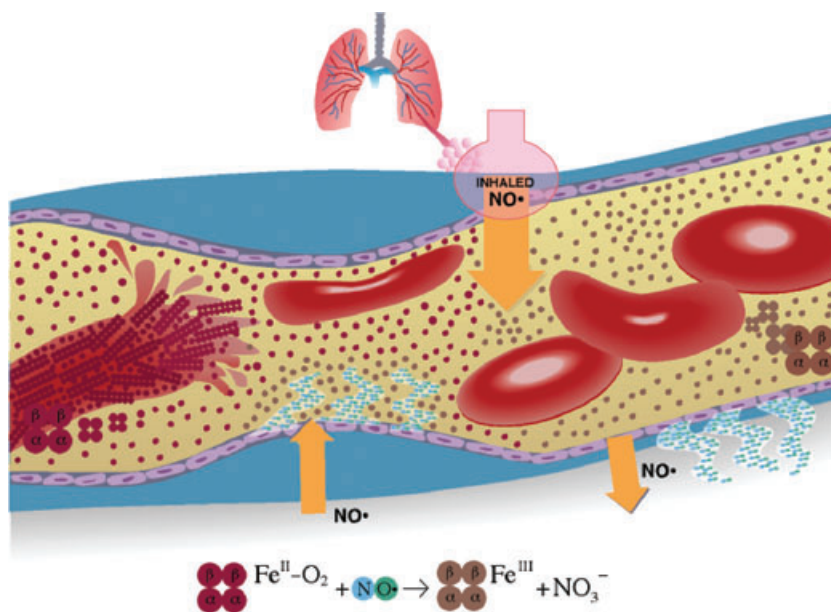


Fig 4. Cell-free haemoglobin limits nitric oxide bioavailability in sickle cell disease. Cell-free ferrous haemoglobin (red) consumes nitric oxide (NO) produced by endothelial cells, diverting NO from smooth muscle cells. The major products of this reaction are methaemoglobin (brown) and nitrate; iron-nitrosylhaemoglobin is formed as a minor product. Inhaled NO therapy oxidizes cell-free oxyhaemoglobin in the pulmonary vasculature to methaemoglobin; therefore, endogenously produced NO is free to diffuse to the smooth muscle and regulate vessel tone and regulate adhesion molecule expression (Reiter *et al*, 2002). Reproduced with permission from Nature Publishing Group (<http://www.nature.com/>).

by NO and such NO-dependent inhibition may in turn be blocked by Hb-mediated NO scavenging (Radomski *et al*, 1987, 1993; Keh *et al*, 1996; Michelson *et al*, 1996). Additionally, haemolytic anaemia is associated with Hb desaturation and ventilation/perfusion inhomogeneity (Gladwin & Rodgers, 2000; Setty *et al*, 2003); it is possible that such a hypoxic state can induce hypoxia-inducible factor-1 (HIF-1)-dependent factors, such as erythropoietin, vascular endothelial growth factor and ET. These mediators may produce a proliferative vasculopathy in the lung and other organs, such as the kidney.

In addition to Hb decompartmentalization, haemolysis releases erythrocyte arginase, which converts L-arginine, the substrate for NO synthesis, to ornithine (Belfiore, 1964; Azizi *et al*, 1970; Morris *et al*, 2003; Schnog *et al*, 2004; unpublished observations). Consistent with this observation, in patients with SCD, the arginine-to-ornithine ratio decreases significantly as pulmonary pressures increase (Gladwin *et al*, 2004a).

In support of the role of haemolysis as an important contributing mechanism in this disorder, pulmonary arterial hypertension is an increasingly recognized complication of other chronic hereditary and acquired haemolytic anaemias including thalassaemia intermedia and major (Aessopos *et al*, 1995, 2001; Koren *et al*, 1987; Grisaru *et al*, 1990; Jootar & Fucharoen, 1990; Du *et al*, 1997; Finazzo *et al*, 1998; Derchi *et al*, 1999; Merault *et al*, 2000; Zakyntinos *et al*, 2001; Hahalis *et al*, 2002; Littera *et al*, 2002; Taher *et al*, 2002; Atichartakarn *et al*, 2003), paroxysmal nocturnal haemoglobinuria (Heller *et al*, 1992; Uchida *et al*, 1998), hereditary spherocytosis and stomatocytosis (Verresen *et al*, 1991; Stewart *et al*, 1996; Hayag-Barin *et al*, 1998; Jais *et al*, 2003; Murali *et al*, 2003; Jardine & Laing, 2004), microangiopathic haemolytic anaemias (Stuard *et al*, 1972; McCarthy & Staats, 1986; Jubelirer, 1991; Suzuki *et al*, 1997; Labrune *et al*, 1999; Fischer *et al*, 2000; Alvarez Navascues & Marin, 2001), pyruvate kinase deficiency (Chou & DeLoughery, 2001), and possibly malaria (Huchzermeyer, 1988; Saissy *et al*, 2003). Additionally, certain conditions are associated with both intravascular haemolysis and risk of pulmonary hypertension, such as schistosomiasis (Strauss *et al*, 1986; de Cleva *et al*, 2003), and iatrogenic haemolysis from mechanical heart valves (Kyllonen *et al*, 1976; Iwaki *et al*, 2003), left ventricular assist devices and cardiopulmonary bypass procedures (Takami *et al*, 1996; Chukwemeka *et al*, 2000; Pierangeli *et al*, 2001; Gerrah *et al*, 2003; Philippidis *et al*, 2004).

Hypoxia

Chronic lung injury as a consequence of infection, broncho-reactive lung disease, fat embolism and undetected episodes of regional pulmonary hypoxia (resulting in sickling, increased vascular adhesion and the production of vasoactive substances) may lead to a vicious cycle of chronic fibrotic pulmonary parenchymal damage, altered vascular tone, vascular proliferation, hypoxia and a consequent pulmonary vasculopathy.

Interestingly, however, the number of episodes of acute chest syndrome (a potential cause of chronic lung disease and pulmonary fibrosis) was not associated with pulmonary hypertension in our prospective prevalence study (Gladwin *et al*, 2004a). A similar prevalence of pulmonary hypertension in patients with thalassaemia intermedia, who do not develop the acute chest syndrome, suggests that acute lung injury may worsen pulmonary hypertension but certainly is not aetiological. Interestingly, in our cohort, individuals with pulmonary hypertension have a higher incidence of restrictive lung disease and pulmonary fibrosis on high-resolution chest CT than age- and Hb-matched patients with SCD without pulmonary hypertension (unpublished observations). Further, in patients with thalassaemia restrictive ventilatory defects and pulmonary fibrosis have also been documented (Tai *et al*, 1996; Carnelli *et al*, 2003). Taken together these data suggest that similar pathogenic mechanisms that lead to pulmonary hypertension could also be involved in the genesis of pulmonary fibrosis in these patients.

Thrombosis

A hypercoagulable state, including low levels of protein C and S, elevated levels of thrombin-antithrombin complexes and D-dimers and increased activation of tissue factor, is seen in patients with SCD in steady-state (Berney *et al*, 1992; el-Hazmi *et al*, 1993; Kurantsin-Mills *et al*, 1992; Marfaing-Koka *et al*, 1993; Peters *et al*, 1994; Hagger *et al*, 1995; Shet *et al*, 2003). This hypercoagulable state could potentially promote vascular obstruction. *In situ* thrombosis is observed in both idiopathic pulmonary arterial hypertension and in patients with SCD at autopsy (Adedeji *et al*, 2001; Manci *et al*, 2003; Vichinsky, 2004). Thromboembolism is a reported cause of death in the sickle cell population but most of these data derive from autopsy studies (Manci *et al*, 2003), and a discrimination between *in situ* versus embolic aetiology of vascular thrombosis was rarely considered. Recent autopsy studies suggest that much of the thrombosis is *in situ*, similar to what occurs in other forms of pulmonary arterial hypertension (Haque *et al*, 2002).

Asplenia

Functional asplenia could also contribute to the development of pulmonary hypertension in patients with SCD. Splenectomy has been reported to be a risk factor for the development of pulmonary hypertension (Hoepfer *et al*, 1999), particularly in patients with haemolytic disorders postsplenectomy (Hayag-Barin *et al*, 1998; Chou & DeLoughery, 2001; Atichartakarn *et al*, 2003). It has been speculated that the loss of splenic function increases the circulation of platelet-derived mediators and that senescent and abnormal erythrocytes in the circulation trigger platelet activation, promoting pulmonary microthrombosis and red cell adhesion to the endothelium. Intravenous injection of haemolysate promotes the formation of platelet-rich thrombi in the pulmonary vascular bed of

rabbits after ligation of the splenic artery, without any thrombus formation in the animals without splenic artery ligation (Kisanuki *et al*, 1997). A role for intensification of haemolysis by splenectomy has also been suggested by the demonstration of significantly higher plasma Hb and erythrocyte-derived microvesicles levels in postsplenectomy patients with thalassaemia intermedia when compared to non-splenectomized patients with the disease (Westerman *et al*, 2004).

Treatment

There are limited data on the specific management of patients with SCD and pulmonary hypertension. Most of the recommendations are based on expert opinion or extrapolated from data derived from other forms of pulmonary hypertension. Our general approach usually includes maximization of SCD-specific therapy (i.e. treatment of primary haemoglobinopathy), treatment of associated cardiopulmonary conditions and targeted therapy with pulmonary vasodilator/antiremodelling agents (Fig 5). These steps are now reviewed in detail.

Intensification of sickle cell disease therapy

In SCD, a role for chronic intravascular haemolysis as a central mechanism in the development of pulmonary hypertension is supported by a correlation between markers of increased

haemolytic rate and severity of pulmonary hypertension. Based on this observation it is likely that maximization of SCD therapy would be beneficial by ameliorating the principal mechanism involved in the pathogenesis of pulmonary hypertension. We have also observed a significant worsening of pulmonary hypertension during acute episodes of vaso-occlusive crisis and the acute chest syndrome (Kato *et al*, 2004). As such, we recommend that all patients with SCD and pulmonary hypertension undergo maximization of therapy with hydroxyurea or simple/exchange transfusions.

Hydroxyurea has been shown to decrease pain, incidence of acute chest syndrome and overall mortality (Charache *et al*, 1995; Steinberg *et al*, 2003). It is possible that some of the benefits seen in pulmonary and cardiovascular deaths could be related to an improvement in pulmonary hypertension. Long-term transfusion therapy in patients with SCD reduces the synthesis of sickle cells and its pathological effects. The risks of most complications of the disease are reduced, including the risks of pulmonary events and central nervous system vasculopathy (Koshy *et al*, 1988; Pegelow *et al*, 1995; Adams *et al*, 1998). It is also possible that exchange transfusion, targeted to a Hb level of 8–10 g/dl and a HbS level of <40%, might improve cardiopulmonary function and prevent the progression of pulmonary hypertension. This thesis is supported by a recent report by Aessopos *et al* (2004) that in well-transfused, iron-chelated patients with thalassaemia major, pulmonary

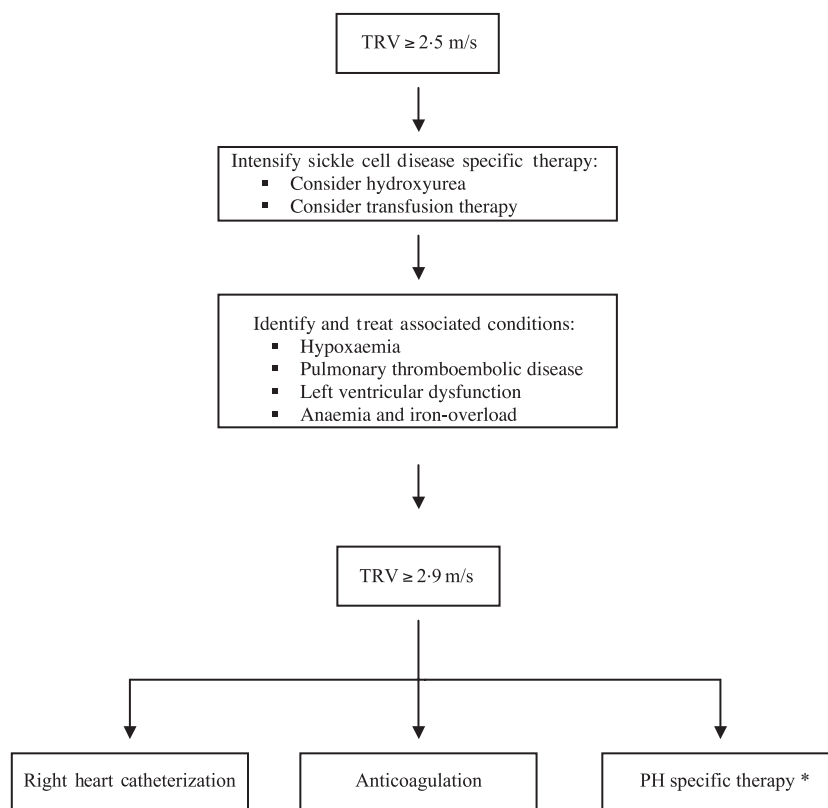


Fig 5. Treatment algorithm. *Studies are also evaluating role in patients with tricuspid regurgitant jet velocity (TRV) of 2.5–2.9 m/s; available drugs listed in Table III.

hypertension was completely prevented. These data have to be balanced with the lack of association between fetal Hb levels and the use of hydroxyurea and protection against the development of pulmonary hypertension in our cohort study (Gladwin *et al*, 2004a). Even if transfusion therapy does not lower haemolytic rates sufficiently to inhibit the development of vasculopathy, a higher Hb level and higher oxygen-carrying capacity are likely to reduce morbidity and possibly mortality by prevention of comorbid events.

Treatment of associated conditions

An aggressive search for associated conditions, such as iron overload, chronic liver disease, HIV infection, nocturnal hypoxaemia and thromboembolic disorders, should always be undertaken given the availability of specific therapies. Oxygen desaturation, especially unrecognized nocturnal hypoxaemia should be pursued (Kirkham *et al*, 2001; Hargrave *et al*, 2003; Setty *et al*, 2003).

There is evidence of a beneficial mortality effect of warfarin anticoagulation in patients with idiopathic pulmonary arterial hypertension, based on retrospective analysis of single centre

studies (Fuster *et al*, 1984; Rich *et al*, 1992; Frank *et al*, 1997). The potential benefits of warfarin therapy observed in patients with idiopathic pulmonary arterial hypertension have to be weighed against the risk of haemorrhagic stroke in adults with SCD. We believe that the relatively low risk of haemorrhagic stroke (0.21 events per 100 patient years; Ohene-Frempong *et al*, 1998) compared with the high risk of death in patients with TRVs ≥ 3.0 m/s (16–50% 2-year mortality; Castro *et al*, 2003; Jison & Gladwin, 2003; Gladwin *et al*, 2004a) supports anticoagulation in patients without a specific contraindication.

Specific therapy for pulmonary hypertension

There are limited data on the specific efficacy of selective pulmonary vasodilator/remodelling pharmacological agents in patients with SCD. Considering the fact that there are probably 15–20 000 patients in the United States with SCD-associated pulmonary hypertension, compared with approximately 15 000 patients with all other forms of pulmonary hypertension, a concerted effort to evaluate these drugs in this population is indicated. We will now briefly review the available drugs.

Table III. Pharmacological agents for the treatment of pulmonary arterial hypertension.

| Drug | Usual dose | Indication* | Level of evidence† | Common adverse effects | Caveats in sickle cell disease |
|----------------------------------|--|----------------------|--------------------|---|---|
| Prostanoids | | | | | |
| Epoprostenol | 1–20 ng/kg/min continuous IV infusion | PAH WHO class III/VI | A | Flushing, headache, jaw pain, diarrhoea, rash, line sepsis/thrombosis | Line sepsis/thrombosis, hyperdynamic state |
| Treprostinil | 1.25–20 ng/kg/min continuous SQ or IV infusion | PAH WHO class III/VI | B | Flushing, headache, jaw pain, rash, site pain, line sepsis/thrombosis | Line sepsis/thrombosis, hyperdynamic state, site pain |
| Iloprost | 2.5–5.0 µg, six to nine inhalations per day | PAH WHO class III/VI | B | Cough, headache, flushing | ? Hyperdynamic state |
| Beraprost | 80 µg orally four times daily | PAH WHO class III/VI | B | Flushing, headache, jaw pain, diarrhoea | ? Hyperdynamic state |
| ET-1 receptor antagonists | | | | | |
| Bosentan | 125 mg orally twice daily | PAH WHO class III/VI | A | Hepatotoxicity, decrease in haemoglobin, headache, flushing | Hepatotoxicity, decrease in haemoglobin |
| Sitaxentan | 100 mg orally once daily | PAH WHO class III/VI | B | Hepatotoxicity, decrease in haemoglobin, headache, flushing | Hepatotoxicity, decrease in haemoglobin |
| PDE-5 inhibitors | | | | | |
| Sildenafil | 25–100 mg orally three times daily | PAH WHO class III/VI | B‡ | Headache, nasal congestion, visual disturbances | Priapism |
| L-Arginine | 0.1 g/kg orally three times daily | | C | Hypotension, methaemoglobinaemia | |

ET-1, endothelin-1; PDE-5, phosphodiesterase-5; PAH, pulmonary arterial hypertension; IV, intravenous; WHO, World Health Organization; SQ, subcutaneous.

*Indication based on studies in patients with traditional forms of pulmonary hypertension. There is no specific data for patients with sickle cell disease and pulmonary hypertension.

†Grading of evidence for efficacy: A, data derived from multiple randomized clinical trials or meta-analyses; B, data derived from single randomized clinical trial or from multiple randomized clinical trials with heterogeneous results; C, data derived from small non-randomized studies and/or consensus opinions of experts.

‡Phase III randomized clinical trial recently completed, level of evidence could increase to A.

There is well documented evidence for the beneficial effects of therapy with prostanoids (epoprostenol, treprostinil, iloprost, beraprost; Badesch *et al*, 2004), ET antagonists (bosentan and sitaxsentan; Channick *et al*, 2004), and possibly phosphodiesterase-5 inhibitors (sildenafil; Ghofrani *et al*, 2004) in patients with traditional forms of pulmonary arterial hypertension. There are no long-term data on the specific treatment of pulmonary hypertension in SCD and the choice of agents at this juncture is largely empirical and based on the safety profile of the drugs and doctor preference (Table III).

The systemic use of prostanoids produce significant systemic vasodilation and increases in cardiac output, raising the concern for the potential development of high output heart failure in anaemic patients. In addition, the risk of chronic intravenous line-related complications, such as thrombosis and sepsis, is probably higher in patients with SCD. The main toxicity of ET-1 receptor antagonists is hepatocellular injury, which could limit their applicability in patients with SCD at risk for liver dysfunction (e.g. iron overload, hepatitis C). Another class effect of these agents is a dose-related decrease in Hb levels, usually in the range of 1 g/dl (Barst *et al*, 2004a). The main concern related to the use of sildenafil is the potential for the development of priapism in men with SCD.

Because alterations in NO bioavailability are likely to be involved in the pathogenesis of the pulmonary hypertension associated with SCD and possibly other chronic haemolytic disorders, therapeutic interventions that enhance NO effects, such as inhaled NO, L-arginine and sildenafil, may be of potential benefit. Chronic-inhaled NO could potentially be beneficial because of its ability to selectively dilate the pulmonary vasculature as well as oxidatively inactivate circulating plasma Hb (Reiter *et al*, 2002). However, the use of chronic-inhaled NO is investigational, potentially expensive and requires relatively complicated delivery systems (Channick *et al*, 1996). L-Arginine is the nitrogen donor for the synthesis of NO by NO synthase. When given for 5 d to 10 patients with SCD 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 SCD (Morris *et al*, 2003). Sildenafil use resulted in symptomatic improvement and near normalization of pulmonary pressures in one patient with thalassaemia intermedia (Littera *et al*, 2002). We have recently presented our experience with sildenafil in patients with SCD and pulmonary hypertension (Machado *et al*, 2004). We treated 12 patients (nine females and three males) with mean estimated pulmonary artery systolic pressure of 51 mmHg (mean TRV of 3.1 m/s) for a mean of 6 months. Sildenafil therapy was associated with a 10 mmHg decrease in estimated pulmonary artery systolic pressure and a 78 m improvement in the 6-min walk distance, an effect similar to the one seen in case series of sildenafil in other forms of pulmonary hypertension. Transient headaches occurred in two patients and

transient periorbital oedema occurred in four individuals. No episodes of priapism occurred in the three men enrolled in the study, but one male had prior erectile dysfunction and two males were on chronic exchange transfusion therapy.

We currently recommend specific pulmonary vasodilator/remodelling therapy for symptomatic patients with moderate-to-severe pulmonary hypertension (TRV of 2.9 m/s or greater). Based on the lack of data from any long-term study we cannot recommend any specific agent and the choice of regimen should be individualized to each patient. Additional studies are planned to evaluate the efficacy of these agents in patients with both mild and severe pulmonary hypertension.

Conclusions and future directions

Pulmonary hypertension is a common complication of adults with SCD (and other chronic haemolytic disorders) that is associated with high morbidity and mortality. Based on this evidence, echocardiographic screening for the presence of pulmonary hypertension should be strongly considered in the adult patient population. Given the probable relationship between pulmonary hypertension and haemolysis it is likely that intensification of SCD-specific therapy can limit the progression of the disease at its earliest stages and potentially reduce the associated morbidity and mortality at later stages. In patients with more severe pulmonary hypertension, specific therapy with vasodilators/antiremodelling agents should be strongly considered.

Further studies are necessary to fully understand the biology of pulmonary hypertension in patients with haemolytic disorders, such as the effects of mild elevations in pulmonary pressures on the cardiopulmonary function, mechanisms of increased mortality and the contribution of the left ventricle to the elevations in pulmonary arterial pressures. Finally, large randomized trials evaluating the effects of specific therapy for pulmonary hypertension in patients with SCD are clearly indicated.

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