Sickle cell disease, pathophysiology and clinical complications
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Large and medium sized pulmonary artery obstruction does not play a role of primary importance in the etiology of sickle cell disease associated pulmonary hypertension

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Abstract

Pulmonary hypertension (PHT) occurs in approximately 30% of adult sickle cell patients and is a risk factor for early death. The potential role of pulmonary artery obstruction, whether due to emboli or in situ thrombosis, in the etiology of sickle cell disease (SCD) related PHT is unknown. Consecutive sickle cell patients were screened for PHT (defined as a tricuspid regurgitant jet flow velocity ≥2.5m/s) employing echocardiography and were evaluated for pulmonary artery obstruction with ventilation-perfusion (VQ) scintigraphy. Fifty-three HbSS, 6 HbSβ0-thalassemia, 20 HbSC and 6 HbSβ+-thalassemia patients were included. The overall prevalence of PHT was 41% in HbSS/HbSβ0-thal patients and 13% in HbSC/HbSβ+-thal patients. High probability VQ-defects (PIOPED criteria) were detected in 2 patients, one of whom had PHT. In HbSS/HbSβ0-thal patients with PHT, 19 (86%), 2 (9%) and 1 (5%) had low, intermediate or high-probability scans as compared to 30 (97%), 1 (3%) and 0(0%) in HbSS/HbSβ0-thal patients without PHT (p=0.31). In HbSC/HbSβ+-thal patients with PHT, 3(100%), 0(0%) and 0(0%) had low, intermediate and a high-probability scans as compared to 19(90%), 1(5%), and 1 (5%) in HbSC/HbSβ+-thal patients without PHT (p=0.86). There were no statistical differences in irregular distribution of the radiopharmaceutical or non-specific signs associated with PHT between patients with and without PHT.

Although small pulmonary artery obstruction cannot be excluded, large to medium sized pulmonary artery obstruction is an unlikely primary causative factor in SCD-related PHT.
Introduction

Pulmonary hypertension (PHT) is a recognized complication of sickle cell disease (SCD) occurring in approximately 30% of adult patients with sickle cell anemia. Once manifest, it is associated with an increased risk of early death. The pathophysiology of SCD related PHT is not completely elucidated, but reduced nitric oxide availability due to chronic hemolysis is considered to be of major importance. However, other factors, such as in situ thrombosis and/or pulmonary embolism (referred to as pulmonary artery obstruction from here on) may also be at play. SCD, as well as other forms of hemolytic anemia (such as thalassemia), are characterized by a hypercoagulable state, and it is increasingly recognized that PHT occurs in chronic hemolytic anemias other than SCD. Several small autopsy studies have shown pulmonary artery obstruction to occur in sickle cell patients, with a large landmark autopsy study demonstrating pulmonary artery obstruction in 16% of HbSS patients. Furthermore, large pulmonary artery obstruction has been reported as the cause of PHT in two sickle cell patients. Also, in a retrospective study, Stein and colleagues have recently shown that pulmonary embolism and/or in situ thrombosis seems to occur more frequently in sickle cell patients as compared to matched controls without SCD. Pulmonary artery obstruction could therefore play a role of importance in the development SCD-related PHT as described in other forms of PHT. If pulmonary artery obstruction plays a role in the pathophysiology of SCD related PHT, early recognition would be of cardinal importance to institute potential therapies such as anticoagulation as early as possible. Ventilation and perfusion (VQ) scintigraphy is an accurate and recommended method to screen for pulmonary artery obstruction in the diagnostic work-up of PHT. By employing VQ-scintigraphy, we set out to investigate whether large to medium sized pulmonary artery obstruction occurs in consecutive ambulatory adult sickle cell patients, and if so, whether it is associated with PHT.
Methods

Patients
Consecutive sickle cell patients visiting the outpatient hematology/internal medicine clinics of the Academic Medical Center (Amsterdam, the Netherlands), the Slotervaart Hospital (Amsterdam, the Netherlands) and the Erasmus Medical Center (Rotterdam, the Netherlands) were eligible for this study. Inclusion criteria were: high performance liquid chromatography confirmed diagnosis of SCD, age 18 years and older and written informed consent. Exclusion criteria were: known congestive heart failure, known chronic obstructive pulmonary disease or poorly controlled asthma, pregnancy and a painful crisis in the preceding 2 weeks (as pulmonary artery pressure increases during a painful crisis) and/or an acute chest syndrome in the preceding three months before study entry. The patients’ medical histories were studied by chart review. Acute chest syndrome was defined as the finding of a new pulmonary infiltrate on chest radiography in combination with fever, respiratory symptoms, or chest pain for which medical treatment was needed. The protocol was reviewed by a central medical ethical committee (Slotervaart Hospital) and subsequently reviewed and approved in the Academic Medical Center and Erasmus Medical Center. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Trans-thoracic echocardiography
Trans-thoracic echocardiography was performed using conventional clinical echocardiographic equipment with 2.5- or 3.5-MHz transducers. Trans-thoracic M-Mode, Doppler and two-dimensional images were obtained from para-sternal long- and short-axis, apical four- and two chamber, and subcostal four-chamber views. Echocardiograms were reviewed to assess the pericardium, valvular anatomy and function, left- and right-sided chamber size, cardiac function and the presence of shunts. Left ventricular diastolic function was assessed by transmitral inflow and pulmonary vein flow signal analysis. Tricuspid regurgitant jet flow velocity (TRV) was identified by color flow Doppler techniques and maximum jet velocity was measured and recorded. Right ventricular systolic pressure was estimated based on the modified Bernoulli equation and considered to be equal to the systolic pulmonary artery pressure (sPAP) in the absence of right ventricular outflow obstruction. This has been validated to correlate with pulmonary artery systolic pressure in the absence of right ventricular outflow obstruction and pulmonary stenosis in patients with SCD. PHT was defined as a TRV ≥ 2.5 m/s. Pulmonary-artery pressures were considered normal in patients with trace or no tricuspid regurgitation (with the TRV assigned a value of 1.3 m/s in the latter group). Diastolic dysfunction was defined as an E/A < 1.0.
VQ-scintigraphy
VQ-scintigraphy was performed with 100-200 MBq of technetium-99m macroaggregated albumin particles (Mallineckrodt, Petten, The Netherlands) in all participating centers. Images were acquired in six views (500 kilocounts per view, 256 matrix). Krypton-81m gas was used for ventilation scintigraphy acquiring the same six views as in the perfusion scintigraphy (500 kilocounts per view, 256 matrix). VQ-scans were anonymously analyzed in PAX-2000 by two trained observers who were blinded to genotype and echocardiographic findings. The VQ-scans were analyzed according to the Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED) criteria for the diagnosis of pulmonary embolism.17 Perfusion abnormalities with an evident extrapulmonary cause, e.g. cardiomegaly or lung lobectomy were not scored as being perfusion defects. The probability scores normal, low and very low were combined in the category low probability.14 Next, a more detailed analysis was performed in which scintigraphic abnormalities were analyzed for each segment separately. Segments could be either normal, have an irregular distribution of the radiopharmaceutical (without perfusion defects) or could have a sub-segmental or segmental perfusion defect. In this analysis results on ventilation were disregarded. Lastly, all VQ-scans were analyzed in order to detect non-specific signs such as patchiness and diffuse left to right differences, which have been described in PHT patients.18 The medial basal segment was not scored because it is not recognizable on planar VQ-scintigraphy. Disagreement was solved by consensus between the two observers upon second review. To graph data, detailed scintigraphic abnormalities were summarized with a quantitative score: completely normal segments scored 0, segments with irregular distribution of the radiopharmaceutical (without defects) scored 1, sub-segmental defects scored 2, and segmental defects scored 4 points. Per patient all scored points were added up and divided by four to quantify the total lung area with perfusion defects. This score expresses the total abnormally perfused lung area in units equivalent to lung segments (segmental equivalents).

Statistics
All numbers are medians with corresponding inter-quartile range (IQR) unless stated otherwise. Difference in continuous data between groups was tested with the Mann-Whitney-U test. Difference in categorical data between groups was tested with the Chi-square test or the Fisher's exact test when appropriate. For the analysis of perfusion defects as segmental equivalents ordinal categories were employed. For graphical summary of the data the difference between groups was calculated as difference in segmental equivalents. For correlation studies the Spearman rank correlation coefficient ($r_s$) was calculated. P-values ≤0.05 were considered statistically significant. Statistical analysis was performed by using SPSS 12.0.2 (SPSS Inc, Chicago, IL).
Results

Patients
Ninety consecutive patients were eligible for the study, 5 of whom declined participation. Eighty-five patients were included in the study. For data analysis, the more severe SCD genotypes, HbSS and HbSβ0-thalassemia, were grouped together, as were the relatively milder SCD genotypes HbSβ+-thalassemia and HbSC.19,20 For patient demographics see Table 1.

Trans-thoracic echocardiography
Trans-thoracic echocardiography was performed in 78 of the 85 included patients as 7 patients failed to meet their appointment on several occasions. The overall prevalence of PHT was 32% (23 patients (41%) in the HbSS/HbSβ0-thal group and 3 patients (13%) in the HbSC/HbSβ+-thal group (P=0.018). The TRV in sickle cell patients with PHT was: median 2.7m/s (IQR 2.6-2.8m/s), mean 2.7±0.2m/s, range 2.5-3.5m/s. The median sPAP was higher in the HbSS/HbSβ0-thal group as compared to the HbSC/HbSβ+-thal group. No difference in the prevalence of PHT was detected between male and female patients. No intra-cardiac shunts or right ventricular outflow obstruction were detected.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics.</th>
<th>HbSS (n=53)/HbSβ+-thal (n=6)</th>
<th>HbSC (n=20)/HbSβ+-thal (n=6)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>31 (21-47)</td>
<td>30 (24-39)</td>
<td>0.89</td>
</tr>
<tr>
<td>Sex (Males:female)</td>
<td>15:44</td>
<td>14:12</td>
<td>0.01*</td>
</tr>
<tr>
<td>History of ACS (%)</td>
<td>41.7%</td>
<td>8.3%</td>
<td>0.004*</td>
</tr>
<tr>
<td>Tricuspid regurgitant flow (m/s)</td>
<td>2.4 (1.6-2.6)</td>
<td>2.1 (1.3-2.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>sPAP (mmHg)</td>
<td>28 (12-33)</td>
<td>23 (12-28)</td>
<td>0.90*</td>
</tr>
<tr>
<td>Diastolic dysfunction (%)</td>
<td>8.3%</td>
<td>4.8%</td>
<td>0.90*</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.5 (7.4-9.4)</td>
<td>11.5 (10.6-12.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocytes (&lt;10⁹/L)</td>
<td>8.9 (7.2-10.5)</td>
<td>7.1 (4.6-9.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fetal Hb (%)</td>
<td>8.6 (4.1-15.1)</td>
<td>1.0 (1.0-2.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>425 (344-647)</td>
<td>234 (214-299)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Numbers are medians (IQR). P-values are based on Mann-Whitney test. *P-value based on Chi-square test. ACS = acute chest syndrome, Hb = Hemoglobin, LDH = lactate dehydrogenase.

VQ scintigraphy
In total 83 patients underwent perfusion lung scanning. Two subjects repeatedly did not appear for their appointment and had no VQ-scintigraphy performed. All scans were of adequate quality for analysis.
The inter-observer overall agreement was 0.92 and the Kappa coefficient for sub-segmental and segmental defects versus none or any defect (high probability and intermediate) showed moderate agreement (Kappa = 0.45).

According to the P I O P E D-criteria 49 (92%) patients had low probability VQ scans, 3 (6%) had intermediate probability scans and 1 (2%) had a high-probability scan in the HbSS/HbSβ⁰-thal group. In the HbSC/HbSβ⁰-thal group 22 (94%) patients had low probability VQ scans, 1 (3%) had an intermediate probability VQ scan and 1 (3%) had a high-probability VQ scan. Results of VQ scans analyzed with P I O P E D criteria did not differ between the HbSS/HbSβ⁰-thal and HbSC/HbSβ⁰-thal groups (P=0.82).

Detailed analysis of scintigraphic abnormalities revealed 64 patients with no scintigraphic abnormalities, 4 patients with one or more segmental perfusion defects, 11 patients with one or more sub-segmental defects and 13 patients with one or more lung segments with irregular distribution of the radiopharmaceutical.

### Table 2. VQ scintigraphy results according to P I O P E D criteria in patients with and without PHT.

<table>
<thead>
<tr>
<th></th>
<th>HbSS/Sβ⁰-thal</th>
<th></th>
<th>HbSC/Sβ⁰-thal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHT</td>
<td>No PHT</td>
<td>P-value*</td>
<td>PHT</td>
</tr>
<tr>
<td>High</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td></td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2 (9%)</td>
<td>1 (3%)</td>
<td>0.31</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Low</td>
<td>19 (86%)</td>
<td>30 (97%)</td>
<td></td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

Numbers are counts (percentages). *= P-value based on Chi-square test.

### Figure 1. Difference in perfusion defects between patients with and without PHT.

Anterior and posterior view of the lung showing total difference in perfusion defects (segmental equivalents rounded to the nearest integer) between patients with PHT (N=25) and without PHT (N=52). The superior and posterior-basal segment (of the lower lobe) of the left lung had significantly more perfusion defects in the PHT group compared to the non-PHT group (P<0.001 and P<0.018 respectively). The difference in perfusion of the apex of the right lung was not statistically significant (P=0.68). R=right; L=left; Segments are: Ap=apex; Ant=anterior; L=Lateral; M=Medial; SL=Superior lingula; IL= Inferior Lingula; S=superior; AB=Antero-basal; LB=Latero-basal; PB=Posterolateral.

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The number of segments with irregular distribution of the radiopharmaceutical in the left lung was significantly higher than in the right lung (P=0.016). Neither a history of an acute chest syndrome nor the frequency of painful crisis was associated with high PIOPED probability score or with the detailed perfusion analysis score as described above (data not shown). The number of segments with irregular distribution of the radiopharmaceutical in the lower lobe and lingula of the left lung were significantly correlated to volume of the left ventricle end-diastolic volume (r=0.293; P=0.018). The presence of diastolic dysfunction was not related to perfusion defects (data not shown).

**Association of VQ-scintigraphy with PHT**

Six patients underwent VQ-scintigraphy but did not undergo a trans-thoracic echocardiography. All of these patients had low probability VQ scans according to the PIOPED criteria. In the remaining 77 patients, no statistical difference in pulmonary perfusion according to PIOPED criteria was observed between patients with or without PHT (see Table 2). A high probability VQ defect was detected in only 1 PHT patient. This patient had a history of an ACS complicated by a lung infarct. In the group of patients without PHT, a high probability VQ defect was detected in 1 patient with a history of pulmonary embolism. Two sickle cell patients with PHT had an intermediate probability VQ defect.

![Figure 2. Representative VQ-scans showing irregular distribution of the radiopharmaceutical.](image)

A. Anterior (ant), posterior (post), right posterior oblique (rpo) and left posterior oblique (lpo) view of a patient with a normal perfusion-scan. B. Anterior (ant), posterior (post), right posterior oblique (rpo) and left posterior oblique (lpo) view of a representative patient with irregular distribution of the radiopharmaceutical in the lower lobe of the left lung and evident cardiomegaly.
With detailed analysis, significantly more scintigraphic abnormalities were demonstrated in the superior and postero-basal segment of the left lower lobe in patients with PHT as compared to patients without PHT (P=0.001 and P=0.018 respectively) (see Figure 1). A separate analysis of the HbSS/HbSβ+-thal patients demonstrated significantly more scintigraphic abnormalities in the superior (P=0.01) and postero-basal segment (P=0.047) of the left lower lobe in patients with PHT as compared to patients without PHT. In addition, more segments with irregular distribution of the radiopharmaceutical were seen in the lung segments of the left lung of the HbSS/HbSβ+-thal group with PHT (P=0.023) (Figure 2). This detailed analysis was not performed for the HbSC/HbSβ+-thal patients because of the low prevalence of PHT in this group. No differences were found in non-specific scintigraphic signs of PHT (such as patchiness and diffuse left to right differences) between the patients with and without PHT (data not shown). There was no statistically significant correlation between the TRV and presence of or number of perfusion defects (data not shown). Twenty-one patients had only trace or no tricuspid regurgitation on trans-thoracic echocardiography. In this group, all 21 patients had low probability VQ-scans. Three patients had sub-segmental defects and the frequency of non-specific scintigraphic signs in this group was not significantly different from that of other patients without PHT (Figure 3).

![Figure 3. Total amount of perfusion defects per patient.](image)

On the Y-axis the total amount of perfusion defects per patients is quantified in segmental equivalents (SE) as defined in the material and methods section. On the X-axis every individual patient is represented according to their respective TRV. Patients who did not undergo echocardiography are also shown. * All patients with trace or no tricuspid regurgitation with the TRV assigned 1.3 m/s. ** All patients with a TRV between 1.3 m/s and 2.5 m/s with the TRV increasing from left to right. # All patients with a TRV ≥ 2.5 m/s with the TRV increasing from left to right. There was no difference in the total amount of perfusion defects between the HbSS/HbSβ+-thal patients and the HbSC/HbSβ+-thal patients (data not shown).
Discussion

A recent landmark study demonstrated that PHT occurs in approximately 30% of adult sickle cell patients and carries a strongly increased risk of death as compared to sickle cell patients without PHT.1 Given this risk of early death, it is of paramount importance to identify treatable causes and/or aggravating factors of PHT in SCD. In this study, we investigated the potential role of pulmonary artery obstruction as a causative or contributing factor in sickle cell patients with PHT employing QV-scintigraphy. QV-scintigraphy was performed in 83 prospectively and consecutively included ambulatory adult sickle cell patients, making this the largest study to date regarding QV scans in SCD. Even though the participating centers were referral hospitals for treating sickle cell patients, most sickle cell patients in the Netherlands are either cared for at referral centers or visit a referral center at regular intervals. We therefore feel that referral bias is limited. Furthermore, patient demographics and PHT prevalence were very similar to those reported in literature.1,2 Based on PIOPED criteria, large to medium sized pulmonary artery obstruction could be excluded in 25 of 26 sickle cell patients with PHT.17 As it was recently demonstrated that a low or intermediate probability QV-scan virtually excludes medium to large pulmonary artery obstruction in idiopathic pulmonary hypertension, it is unlikely that significant pulmonary arterial obstruction was missed in these sickle cell patients.14 These data indicate that large to medium sized pulmonary artery obstruction (due to either pulmonary embolism or in situ thrombosis) is not important in the pathophysiology of SCD-related PHT in the majority of patients.

A detailed analysis of the QV scans was performed in which all segments were separately studied in order to detect subtle perfusion abnormalities. Significantly more segments with irregular distribution of the radiopharmaceutical were detected in the left lower lobe of patients with PHT compared to patients without PHT. Given the anatomic localization this is likely explained by a more pronounced cardiomegaly.21 Neither general patchiness of perfusion, nor diffuse left to right lung perfusion differences, findings known to be associated with primary PHT, differed between sickle cell patients with and without PHT.18

Previous studies have demonstrated a TRV≥2.5 m/s to be strongly indicative of PHT in SCD, and screening for SCD-related PHT with echocardiography is now generally recommended.1,2,21 In our cohort 27% of patients had only trace or no detectable TRV. Even though we cannot exclude that we have missed cases of PHT in this group, long term follow-up of sickle cell patients without a detectable TRV argues against a high prevalence of PHT in this group.24 Importantly, the QV scintigraphy results in these patients were comparable to those in patients with a measurable TRV. As right heart catheterization remains the gold standard diagnostic test for PHT, we cannot exclude the possibility that some cases of PHT may have been missed or over-diagnosed. However, given the reported correlation between pulmonary artery pressure and the TRV in SCD,1 we feel that it is
unlikely that the lack of right heart catheterization in our patients would significantly alter our results.

Few studies have concentrated on VQ scintigraphy in SCD. Walker et al. demonstrated normal VQ scintigraphy results in 8 of 16 asymptomatic adult sickle cell patients, with 2 VQ-scans indicating pulmonary embolism.25 In a retrospective study, Kaur and colleagues demonstrated 3 intermediate probability VQ scans in 10 sickle cell patients presenting with chest pain.26 In a recent case control study in 26 sickle cell patients with and 17 without PHT, a high prevalence of patchy perfusion defects typical of idiopathic PHT was demonstrated in PHT patients, with 3 patients characterized by high probability VQ defects.27 These findings may be explained by the fact that these patients had more severe PHT (mean TRV 3.4m/s, range 2.8-4.2) as compared to the patients in our cohort (mean TRV 2.7m/s, range 2.5-3.5m/s with only three patients with a TRV > 2.8m/s).27 Therefore, in SCD, as in other forms of PHT, either acute or gradual obstruction of medium to large pulmonary arteries may well contribute to the progression of SCD-related PHT.12 However, our data do indicate that in sickle cell patients with relatively mild or early stage PHT, pulmonary artery obstruction is rare and therefore unlikely to be the an important causative or contributing factor in its primary etiology. Small pulmonary artery thrombosis or emboli, which are not detected with VQ scintigraphy, cannot be excluded. Based on the above, serial VQ scintigraphy may prove to be indicated in order to detect pulmonary artery obstruction as early as possible once PHT is diagnosed in patients with SCD. Whether intervention with anticoagulants will then retard the progression of PHT should be addressed in a randomized clinical trial.

In conclusion, our data demonstrate that large to medium sized pulmonary artery obstruction is not prevalent in ambulatory patients with SCD and is not associated with PHT in these patients. Pulmonary thrombo-emboli of large to medium sized pulmonary arteries are therefore unlikely to be a causative factor in SCD-related PHT.

Acknowledgement

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Chapter 7

References

Ventilation perfusion lung scan in sickle cell disease


