No association of the hypercoagulable state with sickle cell disease related pulmonary hypertension

Pulmonary hypertension (PHT) occurs in approximately 30% of adult patients with sickle cell disease (SCD) and is a risk factor for early death.[1] Hypercoagulability has been linked to PHT in general and pulmonary artery thrombosis contributes to PHT progression regardless of its cause.[2] Sickle cell patients are characterized by a hypercoagulable state[3] and both autopsy[4] and imaging studies[5] in sickle cell patients with moderate to severe PHT suggest a role of pulmonary artery thrombosis (albeit *in situ* thrombosis or pulmonary embolism) in SCD-related PHT. However, in a series of 78 consecutive adult sickle cell patients, large to medium sized pulmonary artery thrombosis was ruled out with VQ scintigraphy in 25 of 26 sickle cell patients with mostly mild PHT, suggesting that pulmonary artery thrombosis is probably a late event.[6] Nonetheless, organized thrombi in small pulmonary arteries, especially in association with obliterating vessel wall changes,[4] may remain undetected with VQ scintigraphy. To further investigate the potential role of the hypercoagulable state in SCD related PHT sensitive markers of haemostasis were determined in sickle cell patients screened for PHT.

Study design is extensively reported elsewhere.[6] Consecutive outpatients were screened for PHT with trans-thoracic echocardiography with mild and moderate-severe PHT defined as a tricuspid regurgitant jet flow velocity (TRV) of 2.5-2.9 m/s and ≥3m/s, respectively.[1] The study was carried out in accordance with the principles of the Declaration of Helsinki. Venous blood (3.2% citrate) was collected from the antecubital vein and platelet-poor plasma was prepared by two-step centrifugation (4000 rpm, 15 min followed by 10000 rpm, 5 min).

Endogenous thrombin potential (ETP) was determined with the Calibrated Automated Thrombogram (Thrombinoscope BV, Maastricht, The Netherlands) in a 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland). Thrombin generation was triggered with 1 pM recombinant relipidated tissue factor (rTF) and 4 µM phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine vesicles in HEPES-buffered saline in presence and absence of thrombomodulin (kind gift from Prof. C. Hemker, Synapse BV, Maastricht, the Netherlands) added at a concentration that upon addition to normal pool plasma would reduce the ETP with 50%).[7]

Prothrombin fragment 1+2 (F1+2), soluble tissue factor (ELISA; Behring, Marburg, Germany), D-dimers (TintElize D-Dimer assay, Trinity Biotech, Kordia Life Sciences), and plasminogen activator inhibitor-1 activity (PAI-1:act, Chromolize, Trinity Biotech) were measured according to manufacturer's procedures.

For statistical analysis, HbSS and HbSβ⁰-thalassemia patients were grouped together, as were HbSβ⁺-thalassemia and HbSC patients. Between group differences were tested with the Mann-Whitney U test. For correlation studies the Spearman rank correlation coefficient (rs) was calculated. p values <0.05 were considered statistically significant (SPSS 12.0.2, SPSS Inc, Chicago, IL, USA).

Of 25 PHT patients, 23 had mild PHT. Only 3 HbSC/HbSβ⁺-thalassemia patients had PHT precluding between group statistical analysis (Table 1). No patients received blood transfusions in the weeks before study entry, use of hydroxyurea was not different between patients with or without PHT and no patients used anticoagulation, calcium antagonists, endothelin receptor blockers or sildenafil.

Table 1. Results echocardiography and coagulation studies

	HbSS/HbSβ⁰-thalassemia		*HbSC/HbSβ⁺-thalassemia*	
	Non-PHT (n=32)	*PHT (n=22)*	*Non-PHT (n=21)*	*PHT (n=3)*
Age (years)	33 (21-46)	30 (24-52)	29(23-39)	35 (26-55)
Male:female	6:26	6:16	12:9	1:2
Hemoglobin (mmol/L)	5.6 (4.9-6.0)	5.0 (4.3-5.9)	7.1 (6.7-7.8)	6.6 (6.3-9.6)
Fetal hemoglobin (%)	9.8 (5.6-17.6)	6.9 (2.2-13.4)	1.0 (1.0-1.6)	3.9 (1.0-4.0)
Lactate dehydrogenase (U/L)	389 (323-526)	543 (370-735)	233 (217-340)	257 (235-264)
Echocardiography TRV (m/s)	1.9 (1.3-2.3)*	2.6 (2.6-2.8)*	2.1 (1.3-2.2)	2.5 (2.5-2.5)
Coagulation activation				
Tat (µg/L)	4.7 (3.7-6.1)	3.9 (2.9-5.9)	3.6 (2.7-6.2)	3.7 (3.7-3.7)
F_{1+2} (pmol/L)	305 (245-368)	267 (173-401)	230 (205-311)	177 (162-192)
Fibrinolysis				
PAI-1 (IU/mL)	1.8 (0.9-2.2)	1.7 (0.9-2.7)	1.0 (0.8-1.2)	2.3 (0.9-3.6)
D-dimer (µg/L)	288 (248-373)	250 (162-475)	223 (127-331)	275 (117-433)
Tissue factor sTF (pg/mL)	177 (158-240)	221 (174-286)	168 (155-218)	194 (170-218)
Thrombin generation**: with 1 pmol TF and 4 µmol lipid				
ETP (nM-min)	1082 (920-1237)	1062 (935-1124)	1147 (1089-1393)	1315 (1052-1578)
with 1 pmol TF, µmol lipid and thrombomodulin				
ETP-TM (nM-min)	1054 (869-1173)	1012 (895-1086)	1076 (997-1318)	1267 (995-1538)
% change adding tm	3.1 (2.3-6.9)	4.6 (3.3-6.3)	6.7 (3.4-9.2)	4.0 (2.5-5.4)
with 1 pmol TF without 4 µmol lipid				
ETP-lipid (nM-min)	1030 (917-1151)	1017 (887-1072)	1132 (1049-1336)	1290.5 (989-1592)
% change without lip	3.7 (1.8-6.7)	5.6 (2.7-7.4)	2.9 (1.3-4.9)	2.6 (-0.9-6.0)

*All data are shown as median with corresponding interquartile range. *All significant ($p<0.05$) differences between PHT and Non-PHT groups are marked with *. PHT: pulmonary hypertension. **Normal values (means ± SD) in healthy controls: ETP 995 ±289 (nM-min) and decline ± 70% to 299±143 (nM-min) after adding thrombomodulin.[7]*

Table 2. Correlation studies between markers of hemolysis and the hypercoagulable state.

	All patients			HbSS/HbSβ⁰-thalassemia			HbSC/HbSβ⁺-thalassemia		
	Hb (mmol/L)	LDH (U/L)	Ret (%)	Hb (mmol/L)	LDH (U/L)	Ret (%)	Hb (mmol/L)	LDH (U/L)	Ret (%)
F_{1+2} (ρmol/L)	**-0.26 (0.02)**	**0.40 (<0.01)**	**0.38 (<0.01)**	-0.13 (0.35)	0.33 (0.02)	0.26 (0.08)	-0.02 (0.94)	0.18 (0.40)	**0.51 (0.01)**
TAT (μmol/L)	**-0.35 (<0.01)**	**0.44 (<0.01)**	**0.41 (<0.01)**	-0.26 (0.06)	0.35 (001)	**0.33 (0.02)**	-0.10 (0.66)	0.33 (0.13)	0.30 (0.17)
D-dimer (μg/l)	**-0.36 (<0.01)**	**0.41 (<0.01)**	**0.43 (<0.01)**	**-0.33 (0.02)**	**0.41 (<0.01)**	**0.45 (<0.01)**	-0.24 (0.27)	0.11 (0.62)	0.26 (0.23)
ETP (nM-min)	**0.28 (0.01)**	**-0.28 (0.01)**	-0.22 (0.06)	0.15 (0.28)	-0.17 (0.21)	-0.04 (0.76)	0.28 (0.19)	-0.13 (0.55)	-0.24 (0.27)
ETP-lipid (nM-min)	**0.34 (<0.01)**	**-0.33 (<0.01)**	**-0.30 (0.01)**	-0.2 (0.15)	-0.2 (0.15)	-0.10 (0.52)	0.44 (0.04)	-0.19 (0.38)	-0.35 (0.10)
ETP-TM (nM-min)	0.21 (0.07)	-0.20 (0.08)	-0.15 (0.21)	-0.11 (0.42)	-0.11 (0.42)	0.02 (0.87)	0.38 (0.08)	-0.11 (0.63)	-0.31 (0.15)

Data are shown as Spearman rank correlation coefficients with corresponding p values between brackets. Statistically significant correlations are depicted in bold. Hb: hemoglobin; LDH: lactate dehydrogenase, Ret: reticulocytes.

No differences could be detected between any of the measured parameters between patients with and without PHT (Table 1). Furthermore, there was no significant correlation of any parameter to the TRV (*data not shown*). These findings are in accordance with recent studies in SCD[8] and β-thalassemia[9] (also characterized by a high incidence of PHT). Ataga *et al.* failed to show a significant difference in markers of hemostasis in a comparable number of sickle cell patients, even though there was a trend to higher TAT, F1.2 and D-dimer levels in PHT patients.[8] However, they do not report on the severity of PHT in their patients, making comparison of data difficult. Nonetheless, based upon these data, a role of major importance of the hypercoagulable state in the early pathophysiology of SCD-related PHT seems unlikely.

In line with the recent report by Ataga *et al*.,[8] a modest but statistically significant correlation in especially HbSS/HbSβ⁰-thalassemia patients of the rate of hemolysis to haemostatic markers was detected (Table 2), identifying hemolysis as one of the potential driving forces of the hypercoagulable state in SCD, next to factors such as inflammation and endothelial tissue factor expression.[3] Conceding the fact that no race matched controls were included in this study, a marked *in vitro* resistance to thrombomodulin was detected (as compared to historical controls)[7] indicating functional protein C resistance, which is in accordance with reported reduced protein C and S levels in SCD.[10] In interpreting these data several factors need to be considered. As right heart catheterization remains the gold standard diagnostic test for PHT, we cannot exclude the possibility PHT may have been missed or over-diagnosed in some patients. However, given the strong correlation between pulmonary artery pressure and the TRV in SCD,[1] we do not feel that lack of right heart catheterization significantly influences our results.

We did not detect a significant difference in haemolysis between patients with and without PHT (even though LDH levels tended to be higher in PHT patients). In contrast to other studies,[1] we have not grouped the patients characterized by more profound haemolysis and a higher incidence of PHT (HbSS) together with patients with a lower hemolytic rate and a low PHT (HbSC) incidence for statistical analysis, which may in part explain this discrepancy. Also, the relatively small patient sample size may contribute, as well as the fact that our patients consisted mostly of mild PHT cases.

Taken together, even though hemolysis is one of the factors contributing to the hypercoagulable of SCD, and haemolysis is a risk factor for developing PHT, we could not demonstrate a direct link of the hypercoagulable state to mild SCD related PHT.

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