Elevated hypercoagulability markers in hemoglobin SC disease

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ABSTRACT

Hemoglobin SC disease is a very prevalent hemoglobinopathy; however, very little is known about this condition specifically. There appears to be an increased risk of thromboembolic events in hemoglobin SC disease, but studies evaluating the hemostatic alterations are lacking. We describe the findings of a cross-sectional observational study evaluating coagulation activation markers in adult patients with hemoglobin SC, comparing them with those in sickle cell anemia patients and healthy controls. A total of 56 hemoglobin SC and 39 sickle cell anemia patients were included in the study, all in steady state, and 27 healthy controls. None of the patients was taking hydroxyurea. Hemoglobin SC patients had a significantly up-regulated relative expression of *tissue factor*, as well as elevations in thrombin-antithrombin complex and D-dimer, in comparison to controls (*P*<0.01). Hemoglobin SC patients had lower *tissue factor* expression, and thrombin-antithrombin complex and D-dimer levels when compared to sickle cell anemia patients (*P*<0.05). Markers of endothelial activation (soluble thrombomodulin and soluble vascular cell adhesion molecule-1) and inflammation (tumor necrosis factor-alpha) were both significantly elevated in hemoglobin SC patients when compared to controls, being as high as the levels seen in patients with sickle cell anemia. Overall, in hemoglobin SC patients, higher hemolytic activity and inflammation were associated with a more intense activation of coagulation, and hemostatic activation was associated with two very prevalent chronic complications seen in hemoglobin SC disease: retinopathy and osteonecrosis. In summary, our results demonstrate that hemoglobin SC patients have a hypercoagulable state, although this manifestation was not as intense as that seen in sickle cell anemia.

Introduction

Hemoglobin SC (HbSC) disease is a heterozygous condition in which similar concentrations of hemoglobin S (HbS) and hemoglobin C (HbC) coexist. The unique pathogenic characteristic of HbSConline disease is that HbC has the ability to induce erythrocyte dehydration and intracellular crystal formation.[1,2] Erythrocytes containing HbC have high K-Cl cotransport activity with high K$^+$ and water efflux, leading to cellular dehydration; this results in erythrocytes that are denser than normal red blood cells, microcytic, hyperchromic and have a markedly increased mean corpuscular hemoglobin concentration.[1-4] While HbC in homozygosity produces only a mild hemolytic anemia, the coexistence of HbC and HbS results in a significantly more serious disorder, as the dehydration induced by HbC enhances the pathogenic properties of HbS.[1,2] Hemoglobin SC disease is considered to be a milder hemoglobinopathy than sickle cell anemia (SCA), the homozygous state of HbS (HbSS). All of the clinical complications seen in SCA are also present in HbSC disease, but in the latter they generally occur less frequently and are less severe.[1,5]

There appears to be an increased risk of thromboembolic events in HbSC disease. Studies of sickle cell disease, including both HbSS and HbSC patients in their study cohorts, have shown elevated rates of venous thromboembolism, especially pulmonary embolism.[6,7] An autopsy study showed that pulmonary emboli/thrombi are the second most important cause of mortality in HbSC patients, responsible for 13.6% of deaths, and are a more frequent cause of death in HbSC disease than in SCA.[8] Arterial thromboembolic events are also prevalent, with a higher risk of ischemic stroke in patients than in the general population.[9] There is clear evidence of a hypercoagulability state in SCA, with nearly every element of hemostasis altered in the pro-coagulant direction.[10-17] While coagulation abnormalities have been extensively studied in SCA, very little is known about the hemostatic state specifically in HbSC disease. The aim of the present study was to evaluate coagulation activation markers in HbSC disease and compare them with those in HbSS patients and normal controls. We also evaluated associations of hypercoagulability markers with clinical complications and with markers of endothelial activation, hemolysis and inflammation.

Methods

Patients

This was a cross-sectional observational study that included adult HbSC and HbSS patients (aged >18 years) who consecutively attended the Hematology and Hemotherapy Center of the University of Campinas (UNICAMP, Brazil) during the period from September 2011 to January 2013. Only patients in steady state who had not had any type of painful crisis, hospitalization or blood transfusions in the preceding 3 months were included. None of the HbSC and HbSS patients

included in the study was being treated with hydroxyurea. Patients who were on anticoagulation were excluded. A total of 56 HbSC patients were compared to a population of 39 HbSS patients, of whom 15 were described in a previous study.[17] We also selected 27 healthy age-matched HbAA controls. The University's ethics committee approved the study and all of the patients included signed a written informed consent form.

Venous blood samples for all of the study analyses, including peripheral blood counts and hemolysis markers, were obtained simultaneously.

Tissue factor *mRNA expression in leukocytes*

Tissue factor (TF) expression was analyzed by real time quantitative polymerase chain reaction (qPCR) assays performed on the ABI 7500 Sequence Detection System (Applied Biosystems). cDNA samples were reverse transcribed from total leukocyte RNA, isolated using Trizol Life Reagents (Invitrogen). The reverse transcription reaction was performed using a RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas). Maxima Syber green qPCR master mix (MBI Fermentas) was used for real-time detection of amplification. The *TF* gene was analyzed in parallel with the β-*Actin* gene, used as an endogenous control, and all samples were run in triplicate. A sample from one of the controls was used as the calibrator. Relative quantification of *TF* mRNA was normalized by the cycle threshold (Ct) of β-*Actin* (ΔCT), calculated using the equation, $2^{-\Delta\Delta CT}$.[18] The dissociation protocol was performed at the end of each run to check for non-specific amplification. Primers for *TF* and β-*Actin* were both designed using Primer Express.

Markers of thrombin generation, endothelial activation and inflammation

Thrombin-antithrombin complex (TAT) and D-dimer are known thrombin generation markers and were measured as final markers of coagulation activation in plasma. Endothelial activation was evaluated through quantification of the levels of soluble thrombomodulin and soluble vascular cell adhesion molecule-1 (sVCAM-1). Tumor necrosis factor-alpha (TNF-α) and interleukin 8 (IL-8) were assessed as pro-inflammatory markers. All of these markers were measured in duplicate, using commercially available enzyme-linked immunosorbent assay kits: TAT (Enzygnost®, Siemens, Marburg, Germany), D-dimer (Sekisui Imuclone® D-dimer, Stamford, CA, USA), soluble thrombomodulin (IMU-BIND®, American Diagnostica Inc., Stamford, CT, USA), sVCAM-1 (Quantikine®, R&D Systems, Minneapolis, MN, USA) and ultrasensitive TNF-α and IL-8 (TNF-α US and IL-8 US, Invitrogen, Camarillo, CA, USA).

Statistical analyses

The expression of *TF* mRNA, TAT, D-dimer, soluble thrombomodulin, sVCAM-1, IL-8 and TNF-α were compared among the two groups of patients and the control group using Mann-Whitney U tests. Categorical variables were compared by the Fisher exact test. Spearman rank correlation coefficient was used to analyse bivariate associations between *TF* expression, TAT, D-dimer, soluble trombomodulin, sVCAM-1, IL-8, TNF-α, white blood cell counts, hemolysis markers, and age.

Statistical analyses were performed using Prism 6 (GraphPad Software). P values ≤ 0.05 were considered statistically significant.

Results

Patients' characteristics

A total of 56 steady state HbSC and 39 HbSS patients

were included in the study. Clinical and laboratory data were collected from all patients (Table 1). None of the patients in either of the groups was being treated with hydroxyurea. Patients in the HbSC group were older than the HbSS patients. Histories of retinopathy and osteonecrosis were much more frequent in the HbSC group. Pulmonary hypertension and autosplenectomy (absence of a spleen visible by ultrasound) were more prevalent in the HbSS patients. The prevalence of other clinical sickling complications was similar in the two groups of patients, probably due to the fact that only HbSS patients without an indication for hydroxyurea (who represent a subset of HbSS patients with a milder phenotype) were included in the study. As expected, when compared to HbSS patients, HbSC patients had significantly higher hemoglobin and hematocrit levels, lower levels of hemolysis markers (lactate dehydrogenase, indirect bilirubin, reticulocyte counts) and lower leukocyte, monocyte and platelet counts.

Tissue factor *expression is elevated in HbSC disease*

Quantitative PCR analysis showed an up-regulation of *TF* mRNA relative expression in the leukocytes of HbSC patients, in comparison to that in the leukocytes of healthy controls (1.7 in HbSC patients *versus* 0.9 in controls, $P=0.005$). When compared to leukocytes from HbSS patients, those from HbSC patients had lower *TF* relative

Table 1. Clinical and laboratory characteristics of the hemoglobin SC and sickle cell anemia patients.

	SC n=56	SS n=39	P[1]
Clinical characteristics:			
Age (years)[2]	40 (33, 49)	36 (26, 43)	0.004
Gender: male/female[3]	25(45) / 31(55)	10(26) / 29(74)	0.08
α-thalassemia trait[3,4]	13 (31)	8 (28)	0.8
Clinical sickling complications[3]			
Acute chest syndrome	7 (13)	3 (8)	0.7
Stroke	2 (4)	3 (8)	0.4
Leg ulcers	2 (4)	5 (13)	0.1
Retinopathy[5]	32 (65)	7 (24)	0.0009
Osteonecrosis	22 (39)	4 (10)	0.004
Pulmonary hypertension[6]	4 (9)	8 (27)	0.05
Autosplenectomy[7]	21 (45)	21 (84)	0.002
Laboratory characteristics[2]:			
Lactate dehydrogenase (U/L)	453 (403, 571)	1092 (850, 1365)	<0.0001
Indirect bilirubin (mg/dL)	1.0 (0.8, 1.3)	3.1 (1.7, 5.1)	<0.0001
Hemoglobin(g/dL)	12 (10.5, 12.9)	7.4 (6.8, 8.5)	<0.0001
Hematocrit (%)	33.9 (30.4, 37.3)	21.5 (19.5, 24.3)	<0.0001
Mean corpuscular volume (fL)	78 (74, 83)	90 (86, 96)	<0.0001
Absolute reticulocyte count (x10⁹/L)	177.5 (138, 251)	306 (249, 429)	<0.0001
Absolute leukocyte count (x10⁹/L)	8.1 (6.6, 9.8)	10.9 (9, 12.7)	<0.0001
Absolute neutrophil count (x10⁹/L)	4.7 (3.4, 5.9)	5.2 (4.1, 6.6)	0.07
Absolute monocyte count (x10⁹/L)	0.5 (0.3, 0.7)	1.0 (0.6, 1.3)	<0.0001
Platelet count (x10⁹/L)	343 (185, 439)	420 (337, 530)	0.003

SC: hemoglobin SC patients; SS: hemoglobin SS patients. [1]All P values are two-tailed. The patients' ages and laboratory results were compared using the Mann-Whitney U test. The categorical variables were compared using the Fisher exact test. [2]Data presented as: median (25th, 75th percentiles). [3]Data presented as: number (%). [4]α-thalassemia trait: -3.7kb deletion (-α 3.7). Results were available for 42 patients in the SC group and 29 patients in the SS group. [5]Data regarding retinopathy were available for 49 patients in the SC group and 29 patients in the SS group. [6]Pulmonary hypertension was screened by echocardiogram and defined by a tricuspid regurgitation velocity greater than 2.5 m/s. Data regarding pulmonary hypertension were available for 45 patients in the SC group and 30 patients in the SS group. [7]Autosplenectomy was defined as absence of a spleen visible by ultrasound. Data regarding autosplenectomy were available for 47 patients in the SC group and 25 patients in the SS group.
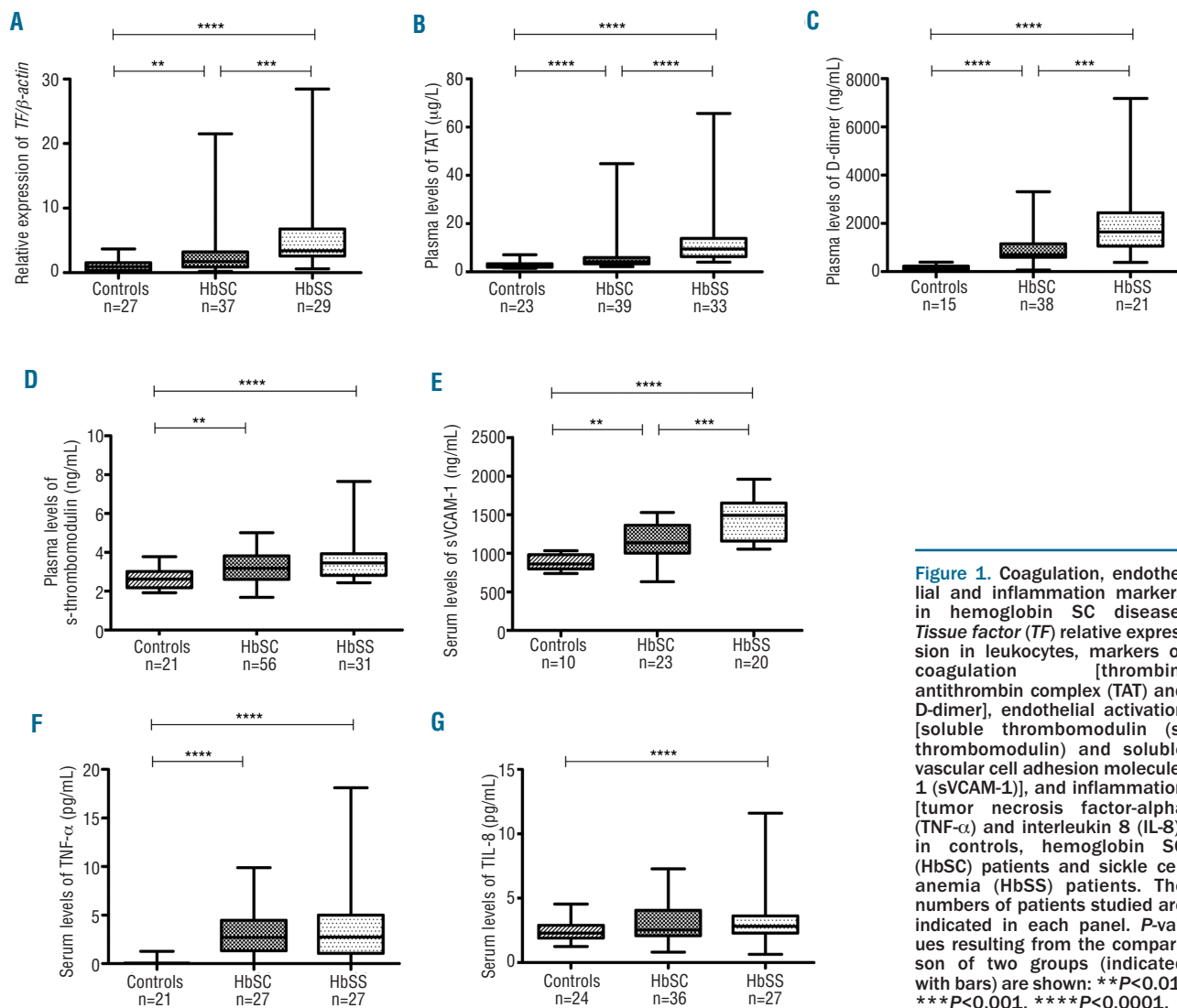
Figure 1. Coagulation, endothelial and inflammation markers in hemoglobin SC disease. *Tissue factor (TF)* relative expression in leukocytes, markers of coagulation [thrombin-antithrombin complex (TAT) and D-dimer], endothelial activation [soluble thrombomodulin (s-thrombomodulin) and soluble vascular cell adhesion molecule-1 (sVCAM-1)], and inflammation [tumor necrosis factor-alpha (TNF-α) and interleukin 8 (IL-8)] in controls, hemoglobin SC (HbSC) patients and sickle cell anemia (HbSS) patients. The numbers of patients studied are indicated in each panel. *P*-values resulting from the comparison of two groups (indicated with bars) are shown: **$P<0.01$, ***$P<0.001$, ****$P<0.0001$.

expression (1.7 in HbSC patients *versus* 3.4 in HbSS patients, $P=0.0001$) (Figure 1A, Table 2).

Elevation of coagulation activation markers in HbSC disease

The thrombin generation markers TAT and D-dimer were both significantly elevated in HbSC patients, in comparison to those in controls (TAT: 4.2 μg/mL in HbSC patients *versus* 2.4 μg/mL in controls, $P<0.0001$; D-dimer: 710 ng/mL in HbSC patients *versus* 150 ng/mL in controls, $P<0.0001$). As expected, TAT and D-dimer levels were lower in HbSC than in HbSS (TAT: 4.2 μg/mL in HbSC patients *versus* 9.5 μg/mL in HbSS patients, $P<0.0001$; D-dimer: 710 ng/mL in HbSC patients *versus* 1643 ng/mL in HbSS patients, $P=0.0004$) (Figures 1B,C; Table 2).

Raised levels of markers of inflammation and endothelial activation markers in HbSC disease

Plasma levels of soluble thrombomodulin, a marker of endothelial activation, were significantly elevated in HbSC patients, compared to those in healthy controls (3.2 ng/mL *versus* 2.6 ng/mL, respectively, $P=0.004$) and similar to the levels observed in HbSS patients (3.2 ng/mL in

HbSC patients *versus* 3.5 ng/mL in HbSS patients, $P=0.1$) (Figure 1D). Similarly, sVCAM-1 serum levels were higher in HbSC patients than in controls (1137 ng/mL *versus* 862 ng/mL, respectively, $P=0.002$), but lower than in HbSS patients (1137 ng/mL in HbSC patients *versus* 1492 ng/mL in HbSS patients, $P=0.0007$) (Figure 1E; Table 2).

The inflammatory markers evaluated were TNF-α and IL-8. Serum TNF-α levels were significantly higher in HbSC patients than in controls (2.7 pg/mL *versus* 0 pg/mL, respectively, $P<0.0001$), being as high as the levels seen in HbSS (2.7 pg/mL in HbSC patients *versus* 2.7 pg/mL in HbSS patients, $P=1.0$) (Figure 1F). On the other hand, serum IL-8 levels were similar in HbSC patients and controls (2.5 pg/mL *versus* 2.3 pg/mL, respectively, $P=0.2$), and also similar to the levels in HbSS (2.5 pg/mL in HbSC patients *versus* 2.8 pg/mL in HbSS patients, $P=0.5$) (Figure 1G; Table 2).

Correlations of coagulation markers with hemolysis, inflammation and endothelial activation in HbSC patients

We evaluated the associations between the hypercoagulability markers (*TF* mRNA expression, TAT, D-dimer) and hemolysis (hemoglobin, hematocrit, lactate dehydro-

Table 2. *Tissue factor* expression, markers of coagulation, endothelial and inflammation activation in controls, hemoglobin SC and sickle cell anemia patients.

	n	Controls Median (2th, 75th percentiles)	n	SC Median (25th, 75th percentiles)	n	SS Median (25th, 75th percentiles)	P[1] SC vs. Controls	P[1] SC vs. SS
Tissue factor[2]	27	0.9 (0.4, 1.5)	37	1.7 (0.9, 3.2)	29	3.4 (2.6, 6.8)	0.005	0.0001
TAT (µg/mL)	23	2.4 (1.9, 3.3)	39	4.2 (3.3, 5.9)	33	9.5 (6.4, 13.9)	<0.0001	<0.0001
D-dimer (ng/mL)	15	150 (104, 225)	38	710 (596, 1155)	21	1643 (1058, 2440)	<0.0001	0.0004
sVCAM-1 (ng/mL)	10	862 (798, 982)	23	1137 (1002, 1364)	20	1492 (1158, 1651)	0.002	0.0007
sThrombomodulin (ng/mL)	21	2.6 (2.2, 3)	56	3.2 (2.6, 3.8)	31	3.5 (2.8, 3.9)	0.004	0.1
TNF-α (pg/mL)	21	0 (0, 0.06)	27	2.7 (1.3, 4.5)	27	2.7 (1.1, 5.0)	<0.0001	1.0
Interleukin-8 (pg/mL)	24	2.3 (1.9, 2.9)	36	2.5 (2.1, 4.1)	27	2.8 (2.3, 3.6)	0.2	0.5

SC: patients with hemoglobin SC disease; SS: patients with hemoglobin SS (sickle cell anemia); TAT: thrombin-antithrombin complex; sVCAM-1: soluble vascular cell adhesion molecule-1; sThrombomodulin: soluble thrombomodulin; TNF-α: tumor necrosis factor-alpha. All P values are two-tailed and calculated using the Mann-Whitney U test. [2]Tissue factor relative expression evaluated by quantitative PCR.

genase, indirect bilirubin, reticulocyte counts), endothelial activation (soluble thrombomodulin, sVCAM-1), inflammation markers (TNF-α, IL-8, leukocyte, neutrophil and monocyte counts), and age in the HbSC cohort (Table 3). Significant positive correlations were identified between: *TF* mRNA levels and sVCAM-1 and monocyte counts; TAT and lactate dehydrogenase, reticulocyte, leukocyte, monocyte and platelet counts; D-dimer and lactate dehydrogenase, reticulocyte and monocyte counts. Overall, higher hemolytic activity and inflammation were associated with more intense activation of coagulation in HbSC patients.

Associations of coagulation markers and clinical complications in HbSC patients

In the HbSC cohort, we analyzed the associations between coagulation markers (*TF* mRNA expression, TAT, D-dimer) and history of clinical complications. We compared the levels of each marker in subjects with or without the following clinical complications: stroke, acute chest syndrome, retinopathy, osteonecrosis, pulmonary hypertension, leg ulcers and autosplenectomy (Table 4). HbSC patients with retinopathy had higher levels of TAT and D-dimer than those in patients without this complication (TAT: 4.7 µg/mL *versus* 3.9 µg/mL, *P*=0.04; D-dimer: 852 ng/mL *versus* 600 ng/mL; *P*=0.01). TAT levels were also higher in patients with a history of osteonecrosis than in patients without this manifestation (4.6 µg/mL *versus* 3.9 µg/mL, *P*=0.04). Patients with autosplenectomy had higher levels of TAT and D-dimer (TAT: 4.8 µg/mL *versus* 3.8 µg/mL, *P*=0.005; D-dimer: 870 ng/mL *versus* 616 ng/mL; *P*=0.006). The prevalence of some of the clinical complications, such as stroke, acute chest syndrome, pulmonary hypertension and leg ulcers, was very low in this cohort, which might have affected this analysis.

Discussion

This study demonstrates that patients with HbSC disease have a hypercoagulable state. When compared to normal controls, HbSC patients showed up-regulation of leukocyte *TF* expression, the main physiological initiator of coagulation *in vivo*. Confirming the biological relevance of *TF* up-regulation, levels of thrombin generation markers TAT and D-dimer were also significantly higher in the circulation of HbSC patients than in normal controls.

Table 3. Correlations of *TF* expression and coagulation markers with hemolysis, endothelial activation and inflammation markers in hemoglobin SC patients.

	TF[1] r (*P*)[2]	TAT (µg/mL) r (*P*)[2]	D-dimer (ng/mL) r (*P*)[2]
sVCAM-1 (ng/mL)	*0.64 (0.02)*	-0.02 (0.96)	-0.37 (0.17)
sThrombomodulin (ng/mL)	-0.31 (0.87)	-0.21 (0.20)	-0.21 (0.19)
Interleukin-8 (pg/mL)	0.24 (0.26)	0.36 (0.06)	0.07 (0.76)
TNF-α (pg/mL)	-0.04 (0.88)	0.03 (0.88)	-0.07 (0.76)
Lactate dehydrogenase (U/L)	-0.21 (0.44)	*0.63 (<0.0001)*	*0.50 (0.001)*
Indirect bilirubin (mg/dL)	-0.30 (0.67)	0.20 (0.28)	0.01 (0.99)
Hemoglobin (g/dL)	0.08 (0.65)	-0.26 (0.11)	0.06 (0.72)
Hematocrit (%)	-0.18 (0.33)	-0.30 (0.06)	-0.02 (0.89)
Absolute reticulocyte count (x10⁹/L)	-0.11 (0.51)	*0.40 (0.01)*	*0.45 (0.004)*
Absolute leukocyte count (x10⁹/L)	0.04 (0.83)	*0.50 (0.001)*	0.20 (0.21)
Absolute monocyte count (x10⁹/L)	*0.40 (0.01)*	*0.39 (0.01)*	*0.32 (0.04)*
Absolute neutrophil count (x10⁹/L)	-0.08 (0.65)	0.20 (0.22)	0.06 (0.73)
Platelet count (x10⁹/L)	0.15 (0.37)	*0.49 (0.001)*	0.19 (0.24)
Age	0.18 (0.28)	0.03 (0.88)	-0.02 (0.89)

TF: tissue factor; TAT: thrombin-antithrombin complex; sVCAM-1: soluble vascular cell adhesion molecule-1; sThrombomodulin: soluble thrombomodulin; TNF-α: tumour necrosis factor-alpha. [1]TF relative expression evaluated by quantitative PCR. [2]Spearman rank correlation coefficient (r) was used to analyze bivariate associations and all P values are two-tailed. Statistically significant results are shown in italics.

Although levels of *TF* expression, TAT and D-dimer were significantly elevated, demonstrating that there is activation of coagulation in HbSC disase, these parameters were not as elevated as those seen in HbSS patients; when compared to HbSS patients, HbSC patients had lower *TF* expression, and lower TAT and D-dimer levels. It is important to emphasize that the HbSS patients included in this study were not taking hydroxyurea, so there was no interference from this treatment with coagulation activation parameters. We have previously shown that hydroxyurea treatment is associated with reductions in hypercoagulability markers in SCA patients[17] and, thus, the inclusion of patients on hydroxyurea therapy would have affected the comparison of results.

Previous studies have evaluated hypercoagulability markers in sickle cell disease, including both patients with SCA and those with HbSC disease.[10,11,13-16,19-22] In some of these studies, the authors described differences between

Table 4. Associations between coagulation markers and clinical complications in hemoglobin SC disease.

Variable	Clinical complication	Yes		No		P^1
		N.	Median	N.	Median	
Tissue factor[2]	Stroke	0	NA	37	1.7	NA
	Acute chest syndrome	4	1.3	33	1.7	0.8
	Pulmonary hypertension[3]	2	1.0	28	1.8	NA
	Leg ulcers	2	3.5	35	1.7	NA
	Osteonecrosis	16	1.9	19	1.7	0.2
	Retinopathy	22	1.6	12	2.1	0.3
	Autosplenectomy[4]	12	2.2	20	1.4	0.2
TAT (μg/mL)	Stroke	2	3.7	37	4.2	0.5
	Acute chest syndrome	2	3.5	37	4.2	0.4
	Pulmonary hypertension[3]	2	5.2	31	4.2	0.8
	Leg ulcers	2	5.6	37	4.2	0.3
	Osteonecrosis	17	4.6	20	3.9	*0.04*
	Retinopathy	26	4.7	13	3.9	*0.04*
	Autosplenectomy[4]	18	4.8	19	3.8	*0.005*
D-dimer (ng/mL)	Stroke	2	519	36	778	0.1
	Acute chest syndrome	5	1135	33	699	0.1
	Pulmonary hypertension[3]	3	666	30	707	0.8
	Leg ulcers	1	2220	37	709	NA
	Osteonecrosis	16	777	19	684	0.4
	Retinopathy	22	852	11	600	*0.01*
	Autosplenectomy[4]	17	870	13	616	*0.006*

TAT: thrombin-antithrombin complex; NA: not applicable. [1]All P values are two-tailed and calculated using the Mann-Whitney's U test. [2]Tissue factor *relative expression evaluated by quantitative PCR.* [3]*Pulmonary hypertension was screened by echocardiogram and defined by a tricuspid regurgitation velocity > 2.5m/s.* [4]*Autosplenectomy was defined as absence of a visible spleen by ultrasound. Statistically significant results are shown in italics.*

HbSC and SCA patients, but the numbers of HbSC patients included were always very low (n<18) and the results were very variable.[10,11,19-22] Since the focus of these studies was not HbSC disease, only a few compared the HbSC patients with normal controls.[11,19,21,22] When this comparison was made, the results were variable and no conclusions could be drawn. It is important to emphasize that, since our study was focused specifically on HbSC disease, we included a large cohort of these patients (n=56), whereas only very small numbers were included in the previous studies (<18 cases).[10,11,19,21,22] Since we included a larger number of patients, we were able to evaluate associations of hyperoagulability markers with clinical complications in HbSC patients.

Our results suggest that hemostatic activation may be involved in two very prevalent chronic complications seen in HbSC disease; retinopathy and osteonecrosis. Patients with these manifestations had higher levels of thrombin generation markers. This might reflect a causal relationship between hypercoagulability and the development of these complications or, alternatively, simply reflect more severe disease in these patients. Another finding in our HbSC cohort was that patients with autosplenectomy had raised TAT and D-dimer levels, probably due to the faster rate of intravascular hemolysis and elevated leukocyte and platelet counts.

In our cohort of HbSC patients, endothelial activation, evaluated by the levels of soluble thrombomodulin and sVCAM-1, was very intense, almost comparable to that found in SCA patients. In previous studies, similar sVCAM-1 levels were found in these two groups of patients, with lower soluble P-selectin and E-selectin in HbSC disease.[19,23] Despite the findings of a significant degree of endothelial activation in HbSC patients, our correlation analyses showed a positive correlation of sVCAM-1 and *TF* expression, but did not reveal any association between soluble thrombomodulin levels and coagulation activation markers. This finding differs from that in SCA, in which *TF* expression was significantly, positively correlated with soluble thrombomodulin level.[17]

We assessed the inflammatory activity in HbSC patients by measuring serum TNF-α and IL-8 levels. With regards to IL-8, no difference was found between levels in the two groups of patients or controls. On the other hand, TNF-α levels were significantly higher in HbSC patients, reaching the same levels seen in SCA patients, and both groups of patients had higher levels than those in controls. More importantly, our correlation analyses demonstrated that inflammation, indicated by leukocyte and monocyte counts, is positively associated with coagulation activation markers and possibly contributes to the development of the hypercoagulability state in patients with HbSC disease. Although HbSC patients were significantly older than HbSS patients, coagulation activation markers, including *TF* expression, TAT and D-dimer levels, were not significantly correlated with age.

A faster rate of hemolysis, demonstrated by higher reticulocyte counts and lactate dehydrogenase levels, also correlated positively with higher levels of coagulation activation markers in HbSC patients. The pathophysiological link between hemolysis and hemostatic activation is probably heme, a product of intravascular hemolysis, which is capable of inducing TF expression by endothelial cells and inducing neutrophil extracellular trap formation in sickle cell disease.[24,25] Similarly to observations in SCA, the hypercoagulability state encountered in HbSC disease seems to be associated with inflammation and hemolysis.[17,19,26] Thus, our results demonstrate similarities between the pathophysiological mechanisms involved in the activation of coagulation in HbSC disease and SCA. However,

we believe that there are probably additional mechanisms that are specific to HbSC disease and require further investigation, such as hyperviscosity and other aspects unique to HbSC disease.

In order to obtain an accurate evaluation of the association between *TF* expression with functional coagulation activation markers, as well as between additional coagulation, inflammation and hemolysis markers, we only included data obtained from samples collected at the same timepoint for each individual. While this characteristic of our study design allowed us to obtain a more precise picture of the relationship between hypercoagulability, inflammation and hemolysis, it also precluded us from performing all assays in all patients, because of limited availability of mRNA, plasma and serum. Since the exclusion of samples from some of the assays was completely at random, we do not expect that this introduced any bias in our analysis.

In summary, our results demonstrate the presence of a hypercoagulable state in HbSC patients, although this hypercoagulability is less intense than that seen in SCA.

As in SCA, inflammation and hemolysis seem to have causative roles in the hemostatic activation in HbSC disease, but additional unique factors may also be involved. Given the high prevalence and morbidity of thrombotic complications in this population, we believe that future studies should focus on a better understanding of the pathophysiology of hypercoagulability in HbSC disease.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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