Hemorheological risk factors of acute chest syndrome and painful vaso-occlusive crisis in children with sickle cell disease

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ABSTRACT

Background

Little is known about the effects of blood rheology on the occurrence of acute chest syndrome and painful vaso-occlusive crises in children with sickle cell anemia and hemoglobin SC disease.

Design and Methods

To address this issue, steady-state hemorheological profiles (blood viscosity, red blood cell deformability, aggregation properties) and hematologic parameters were assessed in 44 children with sickle cell anemia and 49 children with hemoglobin SC disease (8-16 years old) followed since birth. Clinical charts were retrospectively reviewed to determine prior acute chest syndrome or vaso-occlusive episodes, and rates of these complications were calculated.

Results

Multivariate analysis revealed that: 1) a higher steady-state blood viscosity was associated with a higher rate of vaso-occlusive crises in children with sickle cell anemia, but not in children with hemoglobin SC disease; 2) a higher steady-state red blood cell disaggregation threshold was associated with previous history of acute chest syndrome in children with hemoglobin SC disease and boys with sickle cell anemia.

Conclusions

Our results indicate for the first time that the red blood cell aggregation properties may play a role in the pathophysiology of acute chest syndrome in children with hemoglobin SC disease and boys with sickle cell anemia. In addition, whereas greater blood viscosity is associated with a higher rate of vaso-occlusive crises in children with sickle cell anemia, no association was found in children with hemoglobin SC disease, underscoring differences in the etiology of vaso-occlusive crises between sickle cell anemia and hemoglobin SC disease.

Key words: sickle cell anemia, hemoglobin SC disease, red blood aggregation, blood viscosity, red blood cell deformability.

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Introduction

Sickle cell disease (SCD) is a severe genetic disease characterized by frequent painful vaso-occlusive crises (VOC) and acute chest syndrome (ACS), requiring patient hospitalization.[1] ACS is a major cause of death in 25% of Jamaican patients with homozygous sickle cell anemia (SCA).[1] The phenotypic expression of SCA varies considerably with age and between patients,[2,3] and both intrinsic and extrinsic factors may be responsible for the different phenotypes.[4,5]

Numerous hemorheological alterations are found in SCA patients.[6,7] Abnormal hemorheology may severely impact blood flow[8] as increased blood viscosity can raise vascular resistance both at the systemic and pulmonary levels.[9] Blood viscosity is modulated by the hematocrit, which can be very low in SCD due to hemolysis, and by the rheological properties of red blood cells (RBC), which are markedly altered in SCD by the presence of sickle hemoglobin, HbS. In addition, although poorly studied in SCD,[7] RBC aggregation may modulate blood flow in smaller blood vessels because increased RBC aggregation causes an accumulation of RBC in the central zone of flow (axial migration), resulting in a cell-poor layer near the vessel wall, and leading to decreased hematocrit of marginal blood streams (i.e. plasma skimming).[8] In addition, large RBC aggregates increase the resistance to blood flow in smaller vessels if they are not disaggregated before entering into the capillary network.[10] Previous studies in adult SCA patients demonstrated that increased blood viscosity[11,12] and increased RBC deformability[13] are risk factors for VOC. Whether these hemorheological parameters are also involved in the pathophysiology of VOC in SCA children is unknown. Furthermore, no study has focused on the association between blood rheology and ACS in SCA children.

Interestingly, very few studies have been devoted to hemoglobin SC disease (SCC), which has long been considered to be a variant of SCA, sharing some of the clinical complications but with milder severity and lower frequency.[14] However, Lionnet *et al.*[15] recently reported a prevalence of 36% VOC and 20% ACS in a cohort of SCC patients requiring hospitalization. The authors concluded that SCC should be considered as a distinct disease entity.[15] Although blood rheology has been characterized in adult SCC,[7] no study has investigated the possible contribution of blood viscosity and RBC rheological properties to VOC and ACS in children with SCC.

The aim of this study was to identify factors associated with painful VOC and ACS in both SCA and SCC children to gain insight into the pathophysiology of these diseases. Therefore, we analyzed and compared the hematologic and hemorheological profiles of SCA and SCC children according to the occurrence and rate (episodes per patient-year) of these two major complications.

Design and Methods

Patients

The study took place between January 2010 and January 2011, and included 93 children with SCD (n=44 SCA, n=49 SCC) between the ages of 8 and 16 years. This patient group represents 80% of the SCD pediatric cohort in this age range, followed since birth by the Sickle Cell Center at the Academic Hospital of Pointe-à-Pitre (Guadeloupe, French West Indies). As hydroxyurea therapy may modulate the hematologic and blood rheology parameters, children followed at the Sickle Cell Center who were treated with hydroxyurea for more than two weeks were not included in the study. However, 2 SCA children who had started hydroxyurea treatment less than two weeks before the start of the study were included.

All children had been identified by neonatal screening, and diagnosis was made by isoelectrofocusing (Multiphor II™ System, GE Health Care, Bucks., UK), citrate agar electrophoresis, and cation-exchange high performance liquid chromatography (VARIANT™, Bio-Rad Laboratories, Hercules, CA, USA); diagnosis was confirmed by DNA studies.[16] Polymerase chain reaction (Gap-PCR) was used to detect 6 common α-thalassemia deletions, including $\alpha^{-3.7}$and $\alpha^{-4.2}$alleles and triplication defects of the α-globin genes.[17,18]

Steady-state was defined as no blood transfusions in the previous three months and absence of acute episodes (infection, VOC, ACS, stroke, priapism, acute splenic sequestration) at least one month before inclusion in the study. On the basis of a few studies, it seems that hemorheological changes occurring in SCD patients during painful VOC normalize 5-20 days after the end of the crisis.[19,20]

Hemorheological and hematologic parameters were measured in blood; blood samples were taken at steady-state. Charts were retrospectively reviewed by 3 physicians to identify all ACS and VOC episodes from birth to the time of blood sampling. The study was conducted in accordance with the guidelines set out by the Declaration of Helsinki and was approved by the Regional Ethics Committee (CPP Sud/Ouest Outre Mer III, Bordeaux, France, registration number: 2009-A00211-56). All children and their parents were informed about the purpose and procedures of the study, and gave their written consent.

Rates of painful vaso-occlusion crisis and acute chest syndrome

An acute event was considered a VOC if the painful episode lasted for more than 4 h, the patient felt that the pain was typical of that of vaso-occlusion, no other etiology of pain could be identified by the physicians, and the patient was admitted to the Accident and Emergency Pediatric Department to treat the pain with parenteral opioids (with or without non-steroidal anti-inflammatory drugs, NSAID). ACS, splenic or hepatic sequestration, or exacerbations of chronic painful conditions such as avascular necrosis or leg ulcer were not considered as painful VOC episodes.

ACS was defined as the appearance of a new infiltrate on chest X-ray, associated with one or more clinical symptoms such as chest pain, respiratory distress, fever and cough.[1,21]

The rates of ACS and VOC were calculated for each child by dividing the total number of ACS or painful VOC episodes by the number of patient-years.[12] Due to the limited number of patients with a VOC rate higher than 1 in the SCA (n=5 of 44) and SCC groups (n=3 of 49), the classification according to Platt *et al.*[12] could not be used. Therefore, each SCD group was divided into tertiles according to the painful VOC rate (i.e. episodes per patient-year). LSCA (0.04±0.05 episodes per patient-year) and LSCC (0±0 episodes per patient-year) corresponded to SCA and SCC patients with lower VOC rate, ISCA (0.23±0.10 episodes per patient-year) and ISCC (0.14±0.05 episodes per patient-year) corresponded to SCA and SCC patients with intermediate VOC rate, and HSCA (0.99±0.45 episodes per patient-year) and HSCC (0.65±0.48 episodes per patient-year) corresponded to the patients with higher VOC rate. For ACS, each SCD group was classified in those patients who had never experienced ACS (ACS rate=0; NACS-SCA or NACS-SCC) and those who had (rate>0; ACS-

SCA=0.24±0.16 episodes per patient-year or ACS-SCC=0.12±0.08 episodes per patient-year).

Although the constitution of SCA and SCC subgroups was made according to VOC or ACS rates, none of the subgroups can be considered to be clinically severe because mean VOC and ACS rates were not very high. Among the SCA children receiving hydroxyurea who were excluded from the analysis, several of them were treated for recurrent VOC and/or ACS and thus presented a more severe clinical course of the disease than children not treated with hydroxyurea included in the present study. Accordingly, our cohorts of children refer to mild to moderate forms of SCA and SCC.

Hematologic and hemorheological measurements

Venipuncture was performed between 8 a.m. and 10 a.m. and EDTA blood samples were immediately used for measurements of hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocytes (RET), red blood cell (RBC), platelets (PLT) and white blood cell (WBC) counts (Max M-Retic, Coulter, USA). Serum lactate dehydrogenase (LDH) concentration was determined by a standard colorimetric method.

All hemorheological measurements[22] were carried out within 4 h of the venipuncture to avoid rheological alterations[23] and after complete oxygenation of the blood.[22] The guidelines for international standardization in blood rheology techniques/measurements and interpretation were strictly adhered to.[22] Between 8 and 16 years old, hemorheological characteristics have been demonstrated to be quite stable in a healthy population.[24] Blood viscosity was determined at native Hct, at 25°C and at a shear rate of 90 s^{-1} using a cone/plate viscometer (Brookfield DVII+ with CPE40 spindle, Brookfield Engineering Labs., Natick, MA, USA). RBC deformability was determined at 37°C at two shear stresses (3 and 30 Pa) by laser diffraction analysis (ecktacytometry), using the Laser-assisted Optical Rotational Cell Analyzer (LORCA, RR Mechatronics, Hoorn, The Netherlands). RBC aggregation was determined at 37°C via syllectometry, (i.e. laser backscatter *vs.* time, using the LORCA) after adjustment of Hct to 40%, and was reported as the aggregation index (AI), which is calculated by the LORCA. The disaggregation threshold (γ), i.e. the minimal shear rate needed to prevent aggregation or to break down existing aggregates, was determined using a re-iteration procedure.[25]

Statistical analysis

Results are presented as means ± standard deviation (SD). ANOVA (and Tukey *post hoc* test) or unpaired Student's t-test and χ^2 test were used for continuous covariates and for categorical covariates, respectively, to compare hematologic and hemorheological parameters between the different groups.

To identify risk factors associated with painful VOC in SCA and SCC children, we used an ordinal multivariate logistical model as the variable, VOC, was defined by three ordered categories: low, intermediate, high. To identify factors associated with ACS in SCA and SCC children, we used a binary (i.e. presence or absence of ACS) multivariate logistical model. All variables at $P<0.2$ by univariate analysis were included as covariates in the multivariate regression models. $P<0.05$ was considered significant. Analyses were conducted using SPSS software (version 20, IBM SPSS Statistics, Chicago, IL, USA).

Table 1. Demographical, hematologic and hemorheological data of SCD children.

	SCA (n = 44)	SCC (n = 49)
Sex ratio (M/F)	23/21	28/21
alpha-thalassemia (%)	36.4	34.7
Age (years)	11.4±2.4	11.9±2.3
VOC rate	0.41±0.48	0.26±0.39
ACS rate	0.12±0.17	0.03±0.06***
HbF (%)	8.7±6.5	2.9±2.9***
WBC (10^9/L)	11.0±2.7	7.3±2.8***
RBC (10^{12}/L)	2.9±0.7	4.4±0.5***
PLT (10^9/L)	454±122	280±135***
Hb (g.dL-1)	7.9±1.3	11.2±1.0***
Hct (%)	23.1±4.2	31.7±2.8***
MCV (fl)	80.6±7.8	71.7±5.8***
MCH (pg)	27.7±2.0	25.3±2.4***
RET (10^9/dL)	295±115	129±45***
LDH (IU)	579±179	299±81***
Blood viscosity (mPa.s^{-1})	7.1±2.3	8.5±1.9**
RBC deformability at 3 Pa (a.u.)	0.16±0.06	0.17±0.03
RBC deformability at 30 Pa (a.u.)	0.38±0.11	0.45±0.05***
AI (%)	49.3±10.4	44.8±8.4*
γ (s^{-1})	270±93	285±118

*VOC: painful vaso-occlusive crisis; ACS: acute chest syndrome; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference between the two groups (*P<0.05, **P< 0.01, ***P<0.001).*

Results

Comparisons of hematologic and hemorheological profiles, and VOC and ACS rates between SCA and SCC children

Results are reported in Table 1. Gender distribution, prevalence of α-thalassemia and age were similar between the SCA and SCC groups. As expected, HbF levels, WBC counts, PLT counts, MCV, MCH, RET counts and LDH were significantly higher ($P<0.001$) in SCA than in SCC children while RBC counts, Hb and Hct levels were significantly lower ($P<0.001$) in SCA than in SCC children.

Blood viscosity and RBC deformability at 30 Pa were significantly lower in SCA than in SCC children ($P<0.001$) while there was no significant difference in RBC deformability at 3 Pa and γ between the two groups. SCA children had greater AI than SCC children ($P<0.05$).

Although the painful VOC rate was lower in SCC than in the SCA group, the difference did not reach statistical significance ($P<0.1$). SCA children showed greater ACS rates ($P<0.001$) than SCC children.

Association of the hematologic and hemorheological parameters with VOC and ACS rate in SCA children

Table 2 shows the results of the hematologic and hemorheological parameters in the SCA subgroups categorized according to the VOC rate. No difference was seen in the hematologic parameters between the three groups, except for the Hb levels that were lower in LSCA than in ISCA and HSCA children ($P<0.05$), and the Hct which was higher in the HSCA group ($P<0.05$) compared to the LSCA group, although no significant difference was seen between

the ISCA and LSCA groups (P<0.1). There was no difference in LDH and RBC counts between the three groups but ANOVA analysis showed P<0.20.

Blood viscosity and RBC deformability at 30 Pa were greater in HSCA and ISCA children than in LSCA children (P<0.05). In contrast, RBC deformability at 3 Pa was only increased in HSCA children (P<0.05). No difference between the three groups was observed in AI (P<0.20) and γ. In the SCA group, no association was found between the painful VOC rate and the ACS rate categories by the χ^2 test.

An ordinal multivariate logistical model was used that included blood viscosity, RBC deformability at 30 Pa, AI, LDH levels, and Hb levels as covariates. Hct and RBC counts were not included to avoid co-linearity effects with Hb levels. The overall model was statistically significant (χ^2 test=17.05; df=5; P<0.01). However, only blood viscosity was significantly associated with the VOC rate categories with an odds ratio of 1.57 (95% CI: 1.14-2.17; P<0.01).

As shown in Table 3, there was a significant difference in gender distribution between the two groups (P<0.05), with more boys in the ACS-SCA group. The MCV (P<0.01) and MCH values (P<0.05) were lower in the ACS-SCA group than in the NACS-SCA group. There was no difference in any of the other hematologic and hemorheology values between the two groups of SCA children.

While there was no difference in AI, α-thalassemia frequency and RBC counts between the two groups, unpaired Student's t-test showed P<0.2; these parameters were, therefore, included as covariates in a binary multivariate logistical model along with MCV, MCH and gender. The overall model was not statistically significant (χ^2= 11.60; df=6; P=0.07). Nevertheless, because P values for gender and MCV in the multivariate model were less than 0.1, we performed new univariate and multivariate analyses after grouping children by gender.

In SCA boys, there was no difference by univariate analysis between NACS-SCA and ACS-SCA children for any of the parameters except for γ for which a statistically significant difference was detected between the two groups (Table 4; P<0.01).

As PLT counts and RET counts reached P<0.2 by Student's t-test, they were included as covariates in the new binary multivariate logistical model along with γ. The overall model was statistically significant (χ^2=9.07; df=3; P<0.05) and γ was the only parameter significantly associated with the ACS rate categories in boys with an odds ratio of 1.02 (95% CI: 1.0-1.05; P<0.05).

For SCA girls, a comparison between NACS-SCA and ACS-SCA groups showed no difference in age, HbF levels, WBC, PLT and RET counts, Hb and Hct levels, LDH, RBC deformability at 3 and 30 Pa, AI and γ (Table 5). There was no statistical difference in blood viscosity and α-thalassemia frequency between the two groups but differences reached a P<0.20 by unpaired Student's t-test. MCV and MCH were lower in the ACS-SCA than in the NACS-SCA girls (P<0.05).

MCV, MCH, RBC counts, blood viscosity and α-thalassemia were used as covariates in a binary multivariate

Table 2. Comparison of hematologic and hemorheological parameters of SCA children classified according to painful VOC rates.

	LSCA (n = 15)	ISCA (n = 15)	HSCA (n = 14)
Sex ratio (M/F)	7/8	7/8	9/5
alpha-thalassemia (%)	33.3%	33.3%	42.9%
Age (years)	11.5±2.5	11.4±2.4	11.1±2.3
HbF (%)	6.0±2.8	10.7±7.6	8.1±5.9
WBC (10^9/L)	11.5±2.6	10.6±3.2	10.9±2.3
RBC (10^{12}/L)	2.7±0.6	2.9±0.7	3.2±0.6
PLT (10^9/L)	487±131	440±145	433±79
Hb (g.dL^{-1})	7.2±1.1	8.1±1.5*	8.4±1.1*
Hct (%)	21.0±3.5	23.5±4.7	25.1±3.4*
MCV (fl)	80.0±8.3	81.5±6.8	80.2±8.5
MCH (pg)	27.7±3.0	28.4±2.8	27.0±3.3
RET (10^9/dL)	314±127	261±114	310±104
LDH[§] (IU)	658±163	547±174	507±182
Blood viscosity (mPa.s-1)[‡‡]	6.0±1.8	8.0±2.2*	7.5±2.3*
RBC deformability at 3 Pa (a.u.)	0.14±0.04	0.17±0.08	0.19±0.05*
RBC deformability at 30 Pa (a.u.)	0.33±0.07	0.40±0.12*	0.42±0.10*
AI (%)[§]	47.0±8.6	47.7±10.5	53.6±11.3
γ (s^{-1})	265±92	261±120	284±60

*VOC: painful vaso-occlusive crisis; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference compared to LSCA children (*P<0.05, **P<0.01, ***P<0.001), [§]additional parameters included in the multivariate analysis (P<0.20), [‡‡]significant association after the multivariate analysis (P<0.01).*

Table 3. Comparison of hematologic and hemorheological parameters of SCA children classified according to the occurrence of ACS.

	NACS-SCA (n = 22)	ACS-SCA (n = 22)
Sex ratio (M/F)	8/14	15/7*
alpha-thalassemia (%)[§]	27.3	45.5
Age (years)	10.9±2.4	11.8±2.2
HbF (%)	9.6±7.2	6.9±3.9
WBC (10^9/L)	11.2±3.0	10.7±2.4
RBC (10^{12}/L)[§]	2.7±0.4	3.1±0.8
PLT (10^9/L)	473±140	436±100
Hb (g.dL^{-1})	7.8±1.2	8.0±1.4
Hct (%)	22.9±3.9	23.4±4.6
MCV (fl)	83.8±6.5	77.4±7.7**
MCH (pg)	28.8±2.5	26.6±3.1*
RET (10^9/dL)	287±135	302±95
LDH (IU)	609±193	548±163
Blood viscosity (mPa.s^{-1})	7.2±2.3	7.1±2.2
RBC deformability at 3 Pa (a.u.)	0.17±0.07	0.16±0.06
RBC deformability at 30 Pa (a.u.)	0.39±0.10	0.38±0.12
AI (%)[§]	48.9±8.7	49.7±12.0
γ (s^{-1})	266±110	274±74

*ACS: acute chest syndrome; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference between the two groups (*P<0.05, **P<0.01, ***P<0.001), [§]additional parameters included in the multivariate analysis (P<0.20)*

logistical model. The overall model was not statistically significant (χ^2=7.78; df=5; *P*=0.17) and neither did any of the covariates tested show significance.

Associations of hematologic and hemorheological parameters with VOC and ACS rate in SCC children

As shown in Table 6, there was no significant difference in the parameters tested between the three SCC groups. χ^2 test demonstrated lack of association between the VOC rate category and the ACS rate class in the SCC children.

Gender and AI (*P*<0.20 by ANOVA) were included in the ordinal multivariate logistical model with AI as covariate and gender as factor. The model was not statistically significant (χ^2=0.21; df = 2; *P*=0.90).

As shown in Table 7, PLT counts (*P*<0.05), blood viscosity (*P*<0.05) and γ values (*P*<0.05) were significantly higher in ACS-SCC children than NACS-SCC children. RBC deformability at 30 Pa was lower in the ACS-SCC group compared to the NACS-SCC group (*P*<0.05).

The binary multivariate logistical model, including PLT counts, blood viscosity, RBC deformability at 30 Pa and γ, was statistically significant (χ^2=12.02; df=4; *P*<0.05) and γ was independently associated with the ACS rate category with an odds ratio of 1.01 (95% CI: 1.0-1.02; *P*<0.05).

Discussion

The present study demonstrates: i) an association between the RBC disaggregation threshold and the rate of ACS occurrence in SCC children; ii) no association between any of the hemorheological factors and VOC rate in SCC children; iii) an association between increased RBC disaggregation threshold and ACS occurrence in SCA boys, but not in SCA girls; iv) an association between increased blood viscosity and higher painful VOC rate in children with SCA. Until now, no study had investigated the role of hemorheological factors (i.e. blood viscosity, RBC deformability and RBC aggregation properties) in the pathophysiology of ACS and VOC in the SCC population.

Hemoglobin SC disease has long been considered to be a mild form of SCA; however, a recent study demonstrated that it is in fact a distinct disease entity.[15] Lionnet *et al.* reported a greater prevalence of retinopathy and sensorineural otological disorders in SCC patients than in SCA patients.[15] The authors suggested that these findings could be attributed to the greater blood viscosity observed in SCC patients,[15] as observed in the present study. Lionnet *et al.*[15] also showed a high prevalence of VOC and ACS in their SCC population.

SCC children who had previously developed ACS had higher blood viscosity, lower RBC deformability and greater RBC disaggregation threshold than SCC children who had never experienced ACS. Each of these hemorheological parameters may affect the pulmonary vasculature. In animal models, increased blood viscosity and reduced RBC deformability have been shown to increase pulmonary vascular resistance,[9,26] and impaired RBC aggregation properties were demonstrated to impact microcirculation.[10] Multivariate analysis demonstrated that the RBC disaggregation threshold was the only parameter associated with the occurrence of ACS in SCC children. This is the first time that a component of RBC aggregation is identified as a risk factor for ACS in the

Table 4. Comparison of hematologic and hemorheological parameters of SCA boys classified according to the occurrence of ACS.

	NACS-SCA (n=8)	ACS-SCA (n=15)
alpha-thalassemia (%)	25.0	40.0
Age (years)	11.6±2.8	11.7±2.5
HbF (%)	9.5±8.9	5.8±3.2
WBC (10^9/L)	10.7±1.8	10.2±2.3
RBC (10^{12}/L)	2.8±0.4	3.1±0.9
PLT (10^9/L)§	492±136	415±111
Hb (g.dL^{-1})	7.3±1.2	7.5±1.6
Hct (%)	22.1±3.7	22.5±5.0
MCV (fl)	79.9±7.2	76.3±8.6
MCH (pg)	27.1±2.7	26.3±3.3
RET (10^9/dL)§	251±66	315±105
LDH (IU)	590±168	549±117
Blood viscosity (mPa.s^{-1})	7.5±2.4	6.4±2.3
RBC deformability at 3 Pa (a.u.)	0.15±0.06	0.15±0.06
RBC deformability at 30 Pa (a.u.)	0.39±0.06	0.37±0.12
AI (%)	45.8±9.7	47.8±12.1
γ (s^{-1})‡	200±54	288±66**

*ACS: acute chest syndrome; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference between the two groups (*P<0.05, **P<0.01, ***P<0.001), §additional parameters included in the multivariate analysis (P<0.20), ‡significant association after the multivariate analysis (P<0.05).*

Table 5. Comparison of hematologic and hemorheological parameters of SCA girls classified according to the occurrence of ACS.

	NACS-SCA (n = 14)	ACS-SCA (n=7)
alpha-thalassemia (%)§	28.6	57.1
Age (years)	10.5±2.2	12.0±1.6
HbF (%)	11.1±7.9	9.2±4.5
WBC (10^9/L)	11.0±3.4	10.8±1.9
RBC (10^{12}/L)§	2.6±0.5	3.1±0.7
PLT (10^9/L)	461±147	482±53
Hb (g.dL^{-1})	7.5±1.3	7.7±1.4
Hct (%)	22.7±4.2	23.4±3.9
MCV (fl)	85.2±5.6	78.3±5.6*
MCH (pg)	29.7±1.9	27.3±3.0*
RET (10^9/dL)	308±160	274±65
LDH (IU)	621±214	547±252
Blood viscosity (mPa.s^{-1})§	7.1±2.4	8.4±1.3
RBC deformability at 3 Pa (a.u.)	0.18±0.07	0.16±0.06
RBC deformability at 30 Pa (a.u.)	0.40±0.12	0.39±0.11
AI (%)	50.7±7.8	53.8±11.5
γ (s^{-1})	293±121	244±86

*ACS: acute chest syndrome; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference between the two groups (*P<0.05, **P<0.01, ***P<0.001), §additional parameters included in the multivariate analysis (P<0.20)*

SCC population. Elevated RBC disaggregation is thought to increase flow resistance at the entry of capillaries as RBC aggregates need to be completely dispersed before they can enter and negotiate small capillaries.[10] The reasons for which the increased threshold necessary to disaggregate RBC aggregates in a subset of SCC children are unknown, but this is probably not related to fibrinogen concentration, which is known to be an RBC pro-aggregating agent.[7] Further studies will be needed to understand why RBCs from SCC children with a previous history of ACS are stickier than RBCs from children who have never experienced ACS.

Surprisingly, we could not show that blood viscosity was associated with the VOC rate in SCC children. Statistical analyses revealed no significant difference in any of the hematologic or hemorheological parameters tested between LSCC, ISCC and HSCC children. In addition, although SCC children had far greater blood viscosity levels than SCA children, the VOC rate was lower in SCC than in SCA children. The determinants of clinical severity in SCC patients are still poorly understood,[1] but Mohan *et al.*[27] recently demonstrated that vascular function is better preserved in SCC patients than in SCA patients. As vascular resistance depends on hemorheological factors and vascular function, one may hypothesize that the preserved vascular function in SCC children can adjust better to the elevated blood viscosity than the vascular function in SCA children. This could account for the decrease in VOC occurrence in SCC children.

Our study also demonstrated that SCA children with a higher VOC rate have higher steady-state Hb levels, Hct, blood viscosity and RBC deformability values than children with lower VOC rates. These findings agree well with those described in SCA adults by several authors who observed that higher VOC frequency was associated with higher Hct,[12] blood viscosity[11] and RBC deformability.[13,28] Ballas *et al.*[13] proposed that the more sickle RBC are deformable, the greater their adherence to vascular endothelium will be, and the more they may cause VOC. This agrees with a previous study in which the authors demonstrated that irregularly shaped, deformable sickle RBCs were more adherent than rigid, irreversibly sickle RBCs.[29] Nevertheless, multivariate analysis showed that only blood viscosity remained associated with the VOC rate categories. The lack of association between RBC deformability and VOC categories may be due to the very wide inter-individual variability of this parameter, as previously shown by Ballas *et al.*[13] Taken altogether, our results indicate that blood viscosity may be a useful marker of increased risk of VOC in SCA children, as it is in SCA adults.[11]

Castro *et al.*[30] previously reported that reduced steady-state Hb and increased HbF levels decreased the ACS rate in SCA patients (adults and children together). In the present study, we did not find any significant difference in the Hb, Hct or HbF levels between ACS-SCA and NACS-SCA children. However, the univariate analysis indicated that there was a difference in gender distribution between the ACS-SCA group and the NACS-SCA group, prompting us to analyze the data from boys and girls separately. Whereas no hematologic or hemorheological parameters were associated with ACS in girls, multivariate analysis

Table 6. Comparison of hematologic and hemorheological parameters of SCC children classified according to painful VOC rates.

	LSCC (n=17)	ISCC (n=16)	HSCC (n=16)
Sex ratio (M/F)[§]	7/10	12/4	8/8
alpha-thalassemia (%)	44.4	40.0	18.8
Age (years)	12.4±2.0	12.1±2.4	11.3±2.3
HbF (%)	3.6±3.5	2.8±3.5	2.4±1.6
WBC (10^9/L)	7.5±3.1	7.0±2.5	7.3±2.9
RBC (10^{12}/L)	4.5±0.5	4.5±0.7	4.3±0.5
PLT (10^9/L)	262±113	293±153	288±146
Hb (g.dL^{-1})	11.1±1.2	11.3±1.0	11.2±0.8
Hct (%)	31.8±3.1	32.1±3.0	31.2±2.3
MCV (fl)	71.1±6.3	71.5±6.4	72.6±5.0
MCH (pg)	24.8±2.5	25.3±2.6	26.0±2.1
RET (10^9/dL)	121±44	127±43	139±48
LDH (IU)	277±90	322±90	301±57
Blood viscosity (mPa.s^{-1})	8.6±1.8	8.3±2.1	8.6±2.1
RBC deformability at 3 Pa (a.u.)	0.17±0.03	0.17±0.04	0.18±0.02
RBC deformability at 30 Pa (a.u.)	0.46±0.05	0.45±0.05	0.45±0.05
AI (%)[§]	46.7±10.6	41.1±5.4	46.9±5.9
γ (s^{-1})	305±153	245±72	301±103

*VOC: painful vaso-occlusive crisis; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference compared to LSCC children (*P<0.05, **P<0.01, ***P<0.001) [§]additional parameters included in the multivariate analysis (P<0.20).*

Table 7. Comparison of hematologic and hemorheological parameters of SCC children classified according to the occurrence of ACS.

	NACS-SCC (n = 38)	ACS-SCC (n = 11)
Sex ratio (M/F)	20/18	8/3
alpha-thalassemia (%)	36.8	27.3
Age (years)	11.9±2.3	12.1±2.2
HbF (%)	3.1±3.3	2.3±1.6
WBC (10^9/L)	7.1±3.0	7.9±2.1
RBC (10^{12}/L)	4.5±0.5	4.3±0.6
PLT (10^9/L)	256±121	360±152*
Hb (g.dL^{-1})	11.2±1.0	11.0±0.9
Hct (%)	31.9±2.8	31.1±2.8
MCV (fl)	71.3±5.6	73.1±6.7
MCH (pg)	25.2±2.3	25.9±2.6
RET (10^9/dL)	127±44	137±49
LDH (IU)	288±71	330±105
Blood viscosity (mPa.s^{-1})	8.2±1.9	9.7±1.7*
RBC deformability at 3 Pa (a.u.)	0.18±0.03	0.17±0.03
RBC deformability at 30 Pa (a.u.)	0.46±0.04	0.42±0.05*
AI (%)	44.4±7.9	47.6±9.0
γ (s^{-1})[‡]	266±83	350±188*

*ACS: acute chest syndrome; HbF: fetal hemoglobin; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; RET: reticulocyte count; LDH: lactate dehydrogenase; AI: red blood cell aggregation index; γ: red blood cell disaggregation threshold. Statistical difference between the two groups (*P<0.05, **P<0.01, ***P<0.001), [§]additional parameters included in the multivariate analysis (P<0.20), [‡]significant association after the multivariate analysis (P<0.05).*

showed that the RBC disaggregation threshold was significantly associated with ACS in boys, as it is in SCC boys and girls. This gender-related difference in the SCA population remains unexplained and further studies with a larger number of SCA children are needed to address this issue.

In conclusion, this is the first study devoted to the study of the relationship between the full hemorheological profile and clinical severity of SCD. The present data demonstrate an association between blood viscosity and VOC rate in SCA children, and suggest that the presence of RBC aggregates in the circulation could increase the risk of ACS occurrence in SCC children and SCA boys. Future longitudinal studies should be focused on the intra-patient evolution of hemorheological parameters (i.e. during steady-state, VOC or ACS, recovery periods) to better define the impact of blood rheology in the pathophysiology of these acute complications.

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