Impaired blood rheology plays a role in the chronic disorders associated with sickle cell-hemoglobin C disease

Lionnet *et al.*[1] recently reported a high prevalence of retinopathy (RET) and otologic disorders (OTD) in patients with sickle cell-hemoglobin C disease (SC), while a significant number of patients had renal diseases, mainly glomerulopathy (GLO) and osteonecrosis (OST). The pathophysiological processes of these complications in SC are not well defined, although blood hyperviscosity has been suspected, but to the best of our knowledge never tested, as responsible for several chronic complications in SC disease.[1,2] The aim of this study was to analyze the associations between hematologic and hemorheological parameters and chronic complications in adult SC patients.

Ninety consecutive adults with SC were enrolled in the study: 40 males, 50 females; mean age 38±13 years. All patients were at steady state at study entry, i.e. no phlebotomy or blood transfusions in the previous three months, and absence of acute episodes (infection, vaso-occlusive crisis (VOC), acute chest syndrome (ACS), stroke, priapism) at least three months before enrollment. Pregnancy or breast feeding were also exclusion criteria. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Regional Ethics Committee (*registration n. 2010-A00244-35*). Written informed consent was obtained from all participants.

History, presence of chronic disorders, and occurrence of an acute event during the previous year of study were obtained from a retrospective chart review by 2 physicians. Patients under regular phlebotomy protocols, but without any phlebotomy in the three months preceding the study were identified. Phlebotomy is usually performed in symptomatic SC patients to avoid recurrent acute events[3] and has been prescribed by some when hemoglobin and/or hematocrit rise above 11 g/dL or 32%, respectively, to prevent complications with suspected blood hyperviscosity.[1,4] The optimal target hemoglobin level to reach under phlebotomy is unknown but our Sickle Cell Centre usually tries to reduce hemoglobin to 9.5-10.5 g/dL. In our study, SC patients with values greater than 11 g/dL and 32% but no clinical assessment of blood viscosity were categorized as having "theoretical hyperviscosity".

Measurements of hematologic and hemolytic parameters (bilirubin, lactate dehydrogenase, aspartate aminotransferase) were performed using standard methods.[5] Blood viscosity, red blood cell (RBC) deformability, aggregation and disaggregation threshold (i.e. RBC aggregate strength) were measured as described.[6,7]

Unpaired Student's t-test and χ^2 or Kappa coefficient test were used for continuous and categorical covariates, respectively. Association between several parameters was tested by Pearson correlation. The hemolytic component value was derived from hemolytic markers (bilirubin, lactate dehydrogenase, aspartate aminotransferase and reticulocytes) by principal component analysis.[8]

The most prevalent chronic complications in our SC cohort were RET (60%), GLO (micro/macro-albuminuria 40%), OST (31%), and OTD (20%). Leg ulcers, pulmonary hypertension and cerebral vasculopathy/stroke were extremely rare (2% each), as were ACS (2%) and VOC (5%) during the study period. Few males (8%) had a history of priapism.

Patients with OTD (OTD^+) had higher RBC count ($P<0.05$), and a tendency to higher hemoglobin level than patients without OTD (OTD^-; $P<0.1$) (Table 1). Blood viscosity was increased by 9.1% in OTD^+ compared to OTD^- patients ($P<0.05$). SC RET^+ patients had lower RBC deformability than RET^- ($P<0.05$) (Table 1). No association was observed between hematologic or hemorheological parameters and OST or GLO. Nevertheless, OST^+ and GLO^+ patients were older than OST^- and GLO^- individuals ($P<0.001$ and $P<0.01$, respectively). An association was found between RET and OST, with higher frequency of OST^+ in the RET^+ (38%) than in the RET^- (18%; $P<0.05$) groups. No association was found between the other complications.

In our cohort, 43% SC patients underwent phlebotomy, 74% of them on a regular basis (every 3 months). There was no difference in the frequency of RET^+ and RET^-

Table 1. Hematologic and hemorheological determinants of otologic disorders, retinopathy, osteonecrosis and glomerulopathy in SC patients.

	Otologic disorders (OTD)		Retinopathy (RET)		Osteonecrosis (OST)		Glomerulopathy (GLO)	
	OTD- (n = 64)	OTD+ (n = 16)	RET- (n = 33)	RET+ (n = 49)	OST- (n = 62)	OST+ (n = 28)	GLO- (n = 51)	GLO+ (n = 34)
Age (years)	40±13	37±14	39±14	39±13	35±12	45±13***	36±13	43±13**
White blood cells ($x10^9$L)	7.4±2.6	7.0±2.4	7.4±2.4	7.4±2.6	7.6±3.0	7.0±2.4	7.4±2.9	7.6±2.4
Platelet count ($x10^9$L)	306±154	292±151	298±166	302±145	308±156	278±139	301±152	308±152
Red blood cells ($x10^{12}$L)	4.3±0.6	4.7±0.7*	4.3±0.7	4.4±0.7	4.4±0.7	4.4±0.6	4.4±0.6	4.3±0.6
Hemoglobin (g/dL)	11.2±1.1	11.8±1.3$	11.3±1.2	11.4±1.2	11.4±1.3	11.3±1.1	11.5±1.1	11.2±1.2
Hematocrit (%)	31.0±2.7	32.1±3.8	31.0±2.9	31.3±3.1	31.1±3.2	31.4±2.9	31.4±2.8	30.8±3.1
Hemolytic index (relative unit)	0.01±0.98	-0.21±0.96	-0.12±1.07	0.02±0.93	0.02±0.99	-0.03±1.03	-0.06±0.94	0.08±1.08
Blood viscosity (cP)	7.38±1.17	8.10±0.85*	7.60±1.10	7.28±1.32	7.40±1.14	7.50±1.42	7.51±1.22	7.34±1.16
RBC deformability (a.u)	0.43±0.07	0.41±0.06	0.45±0.06	0.41±0.05*	0.43±0.07	0.42±0.07	0.42±0.07	0.43±0.07
RBC aggregation (%)	49±8	47±11	50±8	48±10	47±8	50±10	47±10	50±8
RBC disaggregation threshold (s^{-1})	320±126	312±132	317±101	319±140	306±115	330±141	312±144	314±91

*Means ± SD. - = absence of complication and + = presence of complication. Sensorineural OTD (requiring hospitalization or not) were recorded using previously defined criteria[1]. Criteria used to define VOC and ACS events (within the previous year of study) were similar to those used in previous studies performed in SCA adults[7]. Criteria used to define osteonecrosis and glomerulopathy were similar to those used in previous studies[5,15]. Difference between groups (*P<0.05; **P<0.01; ***P<0.001). $Statistical trend (P<0.1).*

patients treated by phlebotomy as 57.6% RET$^+$ patients had phlebotomy *versus* 42.4% RET$^-$ patients. Only a trend towards greater phlebotomy use was observed in OTD$^+$ patients (64%; P<0.1). Most patients (88.6%) with "theoretical hyperviscosity" (hemoglobin>11g/dL / hematocrit >32%) were phlebotomized (P<0.01). Comparing patients with or without theoretical hyperviscosity demonstrated no significant difference in blood viscosity (7.50±1.03 *vs.* 7.22±0.35 cP, respectively), while hemoglobin and hematocrit were higher in the theoretical hyperviscosity group (11.8±1.0 *vs.* 11.2±1.3 g/dL and 32.3±2.5 *vs.* 30.6±3.2%, respectively; P<0.01), as expected. The cohort was divided according to the median measured viscosity, and patients with blood viscosity greater than median value were considered as having "true hyperviscosity". No significant association was found between theoretical and true hyperviscosity (Kappa coefficient=0.09), or between blood viscosity and hemoglobin or hematocrit, (r=0.19 and r=0.18, respectively; P=0.16 in both cases).

Elevated blood viscosity was hypothesized to cause ischemia at the labyrinthine artery level, leading to cochlear damage.[10] This is in agreement with our cohort in which OTD$^+$ patients had higher blood viscosity than OTD$^-$. However, OTD$^+$ had only a trend towards higher hemoglobin and similar hematocrit as OTD$^-$. Blood viscosity is influenced by several factors, including hematocrit and hemoglobin, hence their clinical use to prescribe phlebotomy. However, this relationship was not significant in our cohort. Blood viscosity depends also on the rheological properties of RBCs, i.e. deformability and aggregation. For any given hemoglobin level, increased RBC deformability lowers blood viscosity while increased RBC aggregation causes a rise. The complex contribution of each hemorheological factor on blood viscosity suggests that blood viscosity may be elevated in some patients despite 'normal' hematocrit and hemoglobin levels.

More importantly, most patients with "theoretical hyperviscosity" did not have high blood viscosity, and only 44% of patients with measured "true hyperviscosity" were phlebotomized. Thus, as periodic phlebotomy could be useful to decrease blood viscosity in hyperviscous SC patients (as it is in patients with polycythemia vera[11] or cyanotic congenital heart disease[12]), our findings strongly suggest that blood viscosity measurements would allow better identification of SC patients at risk for OTD.

In contrast to OTD, RET was not associated with blood hyperviscosity. Instead, RBC deformability was decreased by 10% in RET$^+$ compared to RET$^-$ patients. RBC deformability is critical for optimal tissue perfusion and adequate blood flow in the micro-/macro-circulation,[13] and reduced RBC deformability is associated with diabetic retinopathy.[14,15] The effects of phlebotomy on RBC deformability in SC patients with RET have never been investigated, calling for further studies to address this question.

In conclusion, our study provides new data on the pathophysiology of several frequent chronic complications in SC disease. They clearly show that the clinical use of hemoglobin and hematocrit as surrogates for high blood viscosity in SC patients is not satisfactory for establishing treatment or determining risk for OTD. A prospective study to evaluate the relationships between blood rheology and the occurrence of acute complications is warranted.

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