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Rheology of red blood cells in patients with HbC disease

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Abstract. Patients with hemoglobin C disease (CC) usually do not develop severe complications in comparison with individuals with sickle cell anemia (SS) or with sickle cell hemoglobin C disease (SC). The present study compared the hematological, biochemical, hemorheological and clinical characteristics of CC patients to those of SS, SC and healthy individuals (AA). Blood viscosity was measured at 225 s^{-1} with a cone plate viscometer. The hematocrit-to-blood viscosity ratio (HVR), i.e. an index of red blood cell (RBC) oxygen transport effectiveness, was calculated. RBC deformability was determined at 30 Pa by ektacytometry, and RBC aggregation properties by syllectometry. CC and SC had higher blood viscosity and lower HVR than AA. Nevertheless, HVR was higher in CC compared to SS and tended to be higher than in SC. The CC group exhibited very rigid hyperchromic RBC compared to the three other groups. RBC aggregation abnormalities were observed in CC: low RBC aggregation index and high RBC aggregates strength. Despite these hemorheological abnormalities, CC never had hospitalized painful vaso-occlusive crisis or acute chest syndrome. In contrast, all of them had splenomegaly. Of note, 2 out of 7 CC developed retinopathy or otologic disorders. Whether the blood hyperviscosity and decreased RBC deformability are responsible for these complications is unknown. The higher oxygen transport effectiveness (i.e., HVR) of CC compared to SS is probably at the origin of the very low risk of medical complication in this population.

Keywords: HbC disease, blood viscosity, red blood cell deformability, sickle cell disease

1. Introduction

While hemoglobin S (HbS) polymerizes under deoxygenation, hemoglobin C (HbC) crystallizes in its oxy configuration. Homozygous patients for HbS (i.e., sickle cell anemia or SS) are prone to frequent severe vaso-occlusive painful crises and may experience other acute or chronic complications such as acute chest syndromes, osteonecrosis, leg ulcers, pulmonary hypertension, priapism and glomerulopathy. Patients with both HbS and HbC (i.e., sickle cell SC disease; SC) are less prone to acute vaso-occlusive episodes but may develop retinopathy and otologic disorders [19]. We and others have shown that blood rheological abnormalities participate to the occurrence of these complications [2–4, 7–9, 11, 14–18, 24].

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Very few studies focused on HbC disease (homozygous patients for HbC; CC) because, from a clinical point of view, patients do not develop severe complications [23]. Individuals with HbC disease have hematological (moderate anemia, hyperchromic microcytosis) and clinical evidence of their hemoglobinopathy but appear to have a normal lifespan and few signs or symptoms besides splenomegaly that are attributable to their disease [23]. They do not develop vaso-occlusive events typical of SS patients, and to a lesser extent SC patients, because of the absence of HbS into the red blood cells (RBC). Nevertheless, this benign condition could seem paradoxical since RBC from CC patients are very dehydrated, dense and rigid [6].

The present study compared the hematological, biochemical, hemorheological and clinical characteristics of CC patients to those of SS and SC patients and healthy individuals (AA) [9, 16]. The aim of the present study was to describe all the hemorheological characteristics of CC patients and to better understand how, despite the biological abnormalities, this population is not frequently exposed to adverse events.

2. Materials and methods

2.1. Patients

Although HbC disease is not frequently diagnosed in the Caribbean, 7 CC (56 ± 11 yr) adult patients were recruited. Their biological and clinical data were compared to 82 SC (37 ± 13 yr) and 97 SS patients (35 ± 13 yr). A group of 150 healthy individuals of the same ethnic origin than the other groups (52 ± 18 yr) was also composed for the hemorheological parameters. All patients were at steady state at the time of the study, 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.

Charts were retrospectively reviewed by two physicians to record history of acute complications or presence of chronic disorders, as previously done [9, 16]. The study was conducted in accordance with the guidelines set by the Declaration of Helsinki, and was approved by the Regional Ethics Committee (CPP Sud/Ouest Outre-Mer III, Bordeaux, France, registration number: 2010-A00244-35). All patients were informed about the purpose and procedures of the study, and gave their consent.

2.2. Biological and biochemical parameters

Venipuncture was performed between 7 : 00 and 9 : 00 a.m., and blood samples were used immediately for analyses. Hematocrit (Hct), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and reticulocytes count (RET) were determined using hematology analyzer (Max M-Retic, Coulter, USA). Measurements of the level of total bilirubin (BIL), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were performed using standard biochemistry in CC, SS and SC groups. Analyzed together, RET, AST, BIL and LDH are relevant markers of hemolysis [9, 22].

Measurements of blood viscosity was determined at native hematocrit and a shear rate of 225 s^{-1} at 25°C, using a cone/plate viscometer (Brookfield DVII+with CPE40 spindle, Brookfield Engineering Labs, Natick, MA). The hematocrit-to-viscosity ratio (HVR) was calculated as follows: Hct/blood viscosity at 225 s-1 [25]. HVR reflects a balance between blood oxygen carrying capacity (*i.e.,* hematocrit) and blood flow resistance (*i.e.,* blood viscosity): the highest the HVR, the greatest the RBC oxygen transport

effectiveness [1, 10, 25]. RBC deformability (Elongation Index) was determined at 37°C at 30 Pa by laser diffraction analysis (ektacytometry), 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 versus time, using the LORCA) after adjustment of the hematocrit to 40%. RBC disaggregation threshold, i.e., RBC aggregates strength or the minimal shear rate needed to breakdown existing RBC aggregates, was determined using a re-iteration procedure [12]. The guidelines for international standardization in blood rheology techniques/measurements were strictly followed [5].

2.3. Statistical analysis

Results are presented as means ± SD. A one way analysis of variance (ANOVA) with *post-hoc* test (Newman-Keuls) was used to compare the different groups. Significance level was defined as $p < 0.05$. Analyses were conducted using SPSS (v. 20, IBM SPSS Statistics, Chicago, IL).

3. Results

3.1. Biological parameters

Blood viscosity was higher in SC than in AA and SS groups ($p < 0.001$) and CC exhibited greater blood viscosity than AA (Fig. 1A; $p < 0.05$). Blood viscosity was not different between CC and SC or SS. Hct was reduced in SS compared to the three other groups ($p < 0.001$), and in CC and SC compared to AA (Fig. 1B; $p < 0.001$). HVR was lower in SS compared to the three other groups (CC: $p < 0.05$; SC and AA: $p < 0.001$), and in CC and SC compared to AA (Fig. 1C; $p < 0.001$). CC tended to have higher HVR than SC ($p < 0.1$). RBC deformability was severely decreased in CC compared to the three other groups (SS: $p < 0.01$; SC an AA: $p < 0.001$). Both SC and SS had reduced RBC deformability compared to AA ($p < 0.001$) but the reduction was more important in SS than in SC ($p < 0.001$; Fig. 1D). RBC aggregation was lower in CC than in SS ($p < 0.01$) and AA ($p < 0.001$), and lower in both SC and SS in comparison with AA ($p < 0.001$). In addition, RBC aggregation was decreased in SC compared to SS ($p < 0.001$; Fig. 1E). RBC aggregates strength was greater in CC than in the three other groups ($p < 0.001$). SC and SS had higher RBC aggregates strength than AA ($p < 0.001$; Fig. 1F).

Table 1 shows the results on MCHC, MCV and biochemical parameters. MCV of the SC and CC patients was not different and both were decreased compared to AA and SS ($p < 0.001$). MCHC was higher in CC and SC than in the two other groups ($p < 0.001$). RET levels detected in SC and CC were not different, and both groups exhibited lower RET levels to those of SS patients ($p < 0.001$) but higher compared to AA individuals ($p < 0.001$). SS exhibited greater RET than AA ($p < 0.001$). BIL, LDH and ASAT were not different between SC and CC; both groups had lower values than SS ($p < 0.001$).

3.2. Clinical parameters

None of the CC patients previously developed hospitalized painful vaso-occlusive crisis (VOC) or acute chest syndrome (ACS) since birth. In contrast, 14.4% SS were hospitalized for VOC in the previous year of the present study ($p < 0.05$ vs. CC). Few SC patients had VOC within the same period (4%; $p > 0.05$ vs. CC). We noted that 2 SC and 9 SS patients experienced ACS within the same period too (3% and 9% vs. CC, respectively, $p > 0.05$). Two CC (29%) developed retinopathy vs. 55% SC ($p < 0.001$) and 34% SS

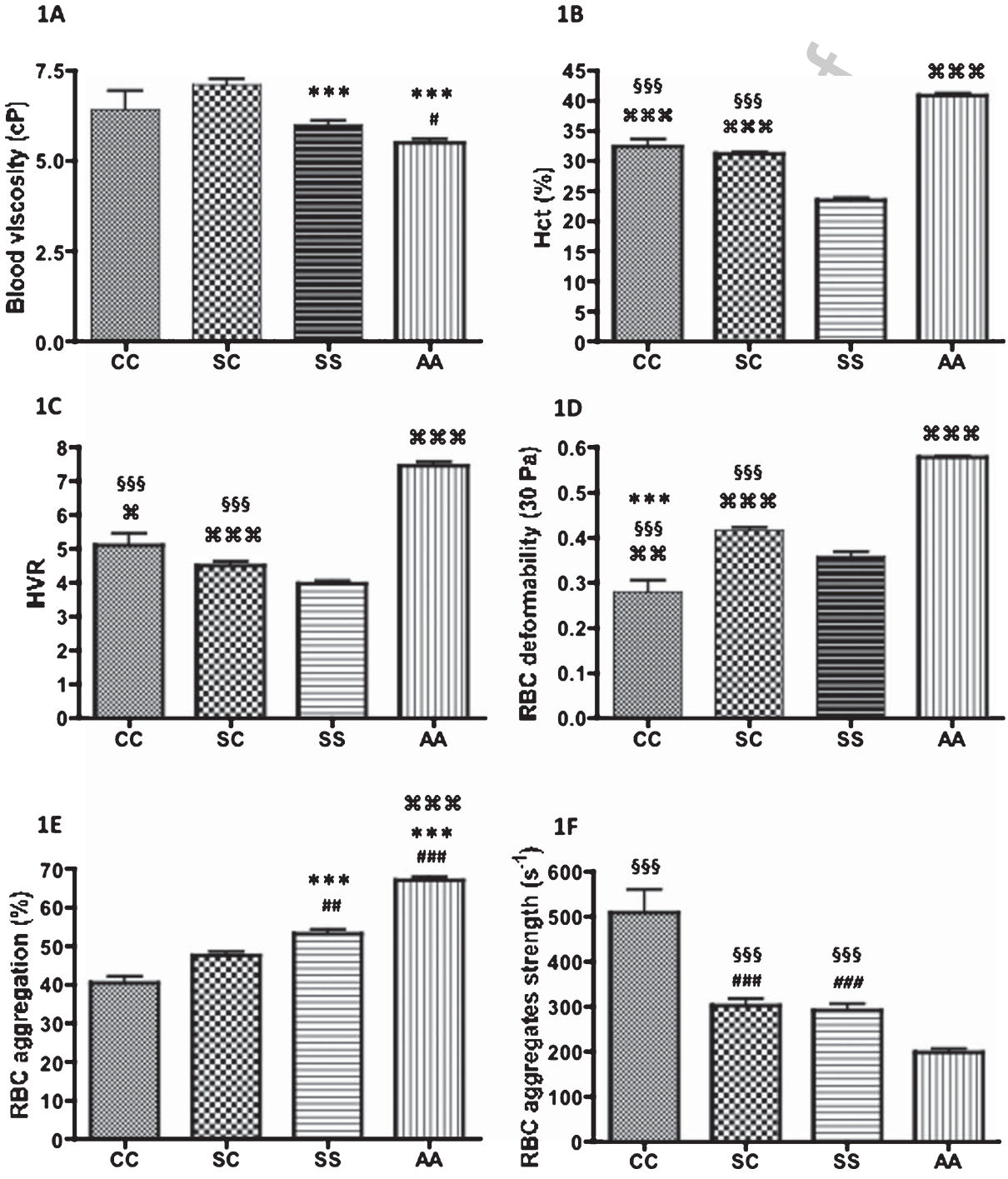

Fig. 1. Hemorheological parameters in patients with HbCC disease (CC), sickle cell hemoglobin SC disease (SC) and sickle cella anémia (SS), and in healthy individuals (AA). Hct, hematocrit; HVR, hematocrit-to-blood viscosity ratio; RBC, red blood cell. Different from SC ($^{***}p < 0.001$); different from CC ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$); different from AA ($^{\S\S\S}p < 0.001$); different from SS ($^{\text{⌘}}p < 0.05$; $^{\text{⌘⌘}}p < 0.01$; $^{\text{⌘⌘⌘}}p < 0.001$).

Table 1
Hematological and biochemical parameters in patients with HbCC disease (CC), sickle cell hemoglobin SC disease (SC) and sickle cella anémia (SS), and in healthy individuals (AA)

	CC $n=7$	SC $n=82$	SS $n=97$	AA $n=150$
MCV (fl)	68.4 ± 3.6	72.0 ± 6.5	$82.9 \pm 8.2^{***\#\#\#\S\S}$	$87.0 \pm 4.7^{***\#\#\#}$
MCHC (g/dL)	35.9 ± 1.9	36.4 ± 2.6	$30.3 \pm 3.8^{***\#\#\#}$	$31.2 \pm 2.3^{***\#\#\#}$
RET (10^9/L)	149 ± 38	109 ± 45	$213 \pm 77^{***\#\S\S\S}$	$49 \pm 19^{***\#\#\#}$
BIL (μmol/L)	28.7 ± 16.8	24.8 ± 16.1	$59.0 \pm 42.1^{***\#\#}$	-
LDH (U/L)	217 ± 32	286 ± 79	$494 \pm 153^{***\#\#\#}$	-
ASAT (U/L)	22.0 ± 7.6	25.0 ± 10.1	$38.7 \pm 13.5^{***\#\#\#}$	-

MCV, mean cell volume; MCHC, mean corpuscular hemoglobin concentration; RET, reticulocytes count; BIL, total bilirubin; LDH, lactate dehydrogenase; AST, aspartate aminotransferase (AST). Different from SC ($^{***}p<0.001$); different from CC ($^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$); different from AA ($^{\S\S}p<0.01$; $^{\S\S\S}p<0.001$).

($p>0.05$). Two CC (29%) had otologic disorders vs. 14 SC (17%; $p>0.05$). Otologic disorders were not accurately quantified in this study for SS patients. Only 1 CC patient had osteonecrosis (14%) while this complication was present in 32% SC and 31% SS ($p<0.001$). Percentage of proteinuria was not different between SC and CC (14% vs. 14%, respectively) while 33% of SS had this complication ($p<0.001$). Leg ulcer was present in 2 SC and in none of the CC individuals but in 22% SS patients ($p<0.05$). Finally, 100% of CC individuals exhibited splenomegaly.

4. Discussion

Very few studies focused on the hemorheological characteristics of patients with HbC disease [6, 20]. The present work describes the full hemorheological profile of this population. We confirmed the presence of very dense and rigid hyperchromic RBCs. In addition, we reported for the first time the presence of RBC aggregation abnormalities and blood hyper-viscosity in HbC disease. Nevertheless, despite these abnormalities, the oxygen transport effectiveness (i.e., HVR) was better than the one of SS patients.

Although patients homozygous for HbC have hematological evidence of their disease (moderate anemia, low hemolytic rate, hyperchromic microcytosis), their clinical status is usually benign and the main complication described so far is splenomegaly. Most of their hemorheological characteristics are similar to SC patients but microcytosis probably compensates for the very low RBC deformability (lower than SS individuals) and allows RBC to flow easily in the microcirculation. For instance, HbC disease cells caused only a 10–20% increase in peripheral resistance when studied in a rat mesoappendix model [23]. Indeed, patients with HbC disease are not prone to acute vaso-occlusive events. Moreover, abnormal adhesion of RBC to the vascular wall [13] is required to trigger VOC in SS. The ability of HbC RBC to adhere to the vascular wall of venules is unknown but one may speculate that it should be very low, in comparison with RBC containing HbS.

In SS, increased blood viscosity is a risk factor for vaso-occlusive events [15, 21] while in SC, increased blood viscosity is associated with systemic arterial hypertension and otologic disorders [16, 18]. Here, we observed elevated blood viscosity in CC (similar to the one of SC) compared to AA. Since vaso-occlusive events are not a characteristic of HbC disease, one may suggest that the vascular function would be well preserved in this disease in comparison with SS, and to a lesser extent, SC. The clinical significance

of the increased blood viscosity in CC is unknown and further studies on larger cohorts are needed to define whether increased blood viscosity in HbC disease could participate to the occurrence of retinopathy and/or otologic disorders. Nevertheless, it was suggested that the increased blood viscosity and decreased RBC deformability in CC patients could increase the risks for spleen sequestration and the occurrence of splenomegaly [20].

The sample size of the CC group is limited in comparison with the other groups and larger cohorts should be studied in multicentric protocols. Nevertheless, we observed two CC patients with a clinical picture slightly different from the other CC: one had retinopathy, splenomegaly and proteinuria (age: 67 yrs), and the other one had retinopathy, splenomegaly and osteonecrosis (age: 60 yrs). The reasons of this slightly greater severity are unknown and the deterioration of the vascular function occurring with age probably played a role. Nevertheless, one may note that these two patients also had the lowest RBC deformability of the CC group. Whether the reduced RBC deformability is responsible for the occurrence of retinopathy in CC patients requires further studies but could be agreement with the recent study by Lemonne et al. [16] in SC patients.

In conclusion this works reported all the hemorheological abnormalities of individuals with HbC disease for the first time. Despite these severe abnormalities, this population do not develop severe complications, as it can be the case in the other hemoglobinopathies. However, few studies focused on large cohorts of patients with HbC disease and multicentric works are needed to address this question.

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