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► To cite this version:

Clément Lemaire, Yann Lamarre, Nathalie Lemonne, Xavier Waltz, Sadri Chahed, et al.. Severe proliferative retinopathy is associated with blood hyperviscosity in sickle cell hemoglobin-C disease but not in sickle cell anemia.: Sickle cell disease and retinopathy. *Clinical Hemorheology and Microcirculation*, 2013, 55 (2), pp.205-12. 10.3233/CH-2012-1622 . inserm-00849032

HAL Id: inserm-00849032

<https://inserm.hal.science/inserm-00849032>

Submitted on 29 Jul 2013

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Severe proliferative retinopathy is associated with blood hyperviscosity in sickle cell hemoglobin-C disease but not in sickle cell anemia

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Running title: Sickle cell disease and retinopathy

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Abstract

Little is known about the impact of blood rheology on the occurrence of retinopathy in sickle cell disease (SCD). Fifty-nine adult SCD patients in steady-state condition participated to the study: 32 with homozygous SCD (sickle cell anemia; SCA) and 27 with sickle cell hemoglobin-C disease (SCC). The patients underwent retinal examination and were categorized according to the classification of Goldberg: 1) no retinopathy (group 1), 2) non-proliferative or proliferative stage I-II retinopathy (group 2) and 3) proliferative stage III-IV-V retinopathy (group 3). Hematological and hemorheological (whole blood viscosity, RBC deformability and aggregation properties) measurements were performed for each patient. In the whole SCD group (SCA + SCC patients) and in SCC patients, the group 3 had higher platelets count than group 2 but the difference between group 3 and group 1 did not reach statistical significance. No difference was observed for the other parameters between the three groups. SCC patients from the group 3 exhibited higher whole blood viscosity than SCC patients from the group 1. No significant difference was observed between the three groups in SCA patients. This study revealed that severe sickle proliferative retinopathy is associated with blood hyperviscosity in SCC patients but not in SCA patients.

Key words: sickle cell disease, blood rheology, retinopathy.

Introduction

Retinal disease is the most common ocular complication in sickle cell disease (SCD). A decrease of microcirculatory flow at the retinal level may cause ischemia resulting in non-proliferative or proliferative retinopathy [17]. The National Institute of Health (NIH) recommends that all patients with SCD should have regular dilated retinal examinations by an ophthalmologist with expertise in retinal diseases [22]. Ocular fundus may reveal microcirculatory changes at the retinal level which are characteristics of retinopathy. Retinopathy is most frequent in patients with sickle cell-hemoglobin C disease (SCC) than in patients with sickle cell anemia (SCA) [9]. The greater **whole** blood viscosity usually reported in SCC patients in comparison with SCA patients has been hypothesized to increase the risk of SCC patients to develop retinopathy [12]. Nevertheless, this association has been poorly studied [21]. It seems that high hemoglobin concentration and low fetal hemoglobin level could increase the risks of SCA males and females, respectively, to develop sickle retinopathy [10, 13]. In SCC females, high mean cell volume and low fetal haemoglobin have been reported to be associated with retinopathy [10, 14].

Chronic hemorheological alterations have been reported in SCD [1, 3-4, 6-8, 18, 23, 25, 29] with the level of alterations being very different between SCA and SCC patients [29] and highly variable from one patient to another for a given β -genotype [23, 29-30]. Some of these hemorheological disorders are involved in the pathophysiology of SCD and the occurrence of acute vaso-occlusive accidents [4, 20, 23-24] but few data regarding the involvement of blood rheology in the development of sickle retinopathy is available in the literature. Serjeant et al. reported a greater **whole** blood viscosity (determined at high shear rate) in SCA males with sickle retinopathy than in SCA males without retinopathy [26]. The other hemorheological parameters investigated were not different between the two populations [26]. In SCC, no

association between blood rheology and sickle retinopathy has been reported [27]. However, the techniques available in the eighties to assess blood rheological characteristics were not as reproducible and accurate than the ones currently available [5]. Recent progresses in blood rheological techniques make the assessment of red blood cell (RBC) rheological properties easier that justify to re-analyze the association of SCD retinopathy with hemorheology.

We studied the blood rheological profile of 59 patients with SCD and compared each parameter between patients with either no retinopathy (group 1), non-proliferative or proliferative stage I-II retinopathy (group 2) or proliferative stage III-IV-V retinopathy (group 3).

Materials and Methods

Patients and protocol

Fifty-nine adult patients with SCD (32 SCA and 27 SCC) and followed annually by the Sickle Cell Unit of the Academic Hospital of Pointe à Pitre (Guadeloupe) were included between October 2010 and May 2011. All patients were in clinical steady state at the time of the study (i.e. without vaso-occlusive crisis, acute medical complication or blood transfusion/phlebotomies within the last 3 months). Any treatment potentially modifying the hemorheological characteristics of blood and any condition causing a peripheral proliferative retinopathy were exclusion criteria. Two independent ophthalmologists performed indirect ophthalmoscopy with a non-contact slip lamp lens (SuperField, Volk Optical, Mentor, OH, USA) and patients were classified into three groups as proposed by Goldberg et al. [11]: no retinopathy (group 1), non proliferative or proliferative stage I-II retinopathy (group 2) and proliferative stage III-IV-V retinopathy (group 3). This classification is more accurate than in previous studies which

investigated associations between blood rheology and sickle retinopathy [10, 26-27]. These previous works compared patients with no retinopathy to patients with retinopathy without taking into account the increasing severity of retinal ischemic and proliferative lesions. Blood was sampled in EDTA tubes for hematological and hemorheological measurements. This study received the ethic approval by the Regional Ethics Committee (CPP Sud-Ouest Outre-Mer III, Bordeaux, France). The experiments were performed in accordance with the guidelines set by the Declaration of Helsinki.

Hematological and hemorheological measurements

Hemorheological measurements were performed within 4 hours after blood sampling and were conducted in accordance with the recent international guidelines for hemorheological laboratory techniques after full re-oxygenation of blood for 10-15 min [5].

Whole blood viscosity (η_b) was measured using a cone-plate viscometer (Brookfield DVII+ with CPE40 spindle) at 25°C and at 90 s⁻¹. RBC deformability was determined at 7 increasing shear stresses (0.95, 1.69, 3.00, 5.33, 9.49, 16.87 and 30.00 Pa) by laser diffraction analysis (Ektacytometry, LORCA, RR Mechatronics, Hoorn, The Netherlands) and at 37°C. The system calculates an average RBC elongation index (EI). The higher this index, the more deformable the RBCs. RBC aggregation was determined at 37°C via syllectometry (i.e, laser backscatter versus time), using the LORCA (RR Mechtronics, Hoom, The Netherlands), after adjustment of hematocrit to 40%. The system calculates an RBC aggregation index (AI). The RBC disaggregation threshold (γ_{thr}) – i.e. the minimal shear rate needed to prevent RBC aggregation or to break down existing RBC aggregates – was determined using a re-iteration procedure.

Hematocrit (Hct) was measured after blood microcentrifugation (JOUAN-HEMA-C, Saint Herblain, France). Total counts of leukocytes, polynuclear neutrophils, monocytes, lymphocytes, eosinophils, platelets and RBCs, percentage of reticulocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and hemoglobin concentration (Hb) were determined using a hematology analyzer (Max M-Retic, Coulter, USA).

Statistics

Hematological and hemorheological parameters were studied in the whole SCD group (SCA + SCC patients) and separately in SCA and SCC patients. The hematological and hemorheological data were compared between the three groups (group 1 = no retinopathy, group 2 = patients with proliferative or non proliferative stages I, II retinopathy and group 3 = patients with proliferative stages III-IV-V retinopathy) using a one-way analysis of variance (ANOVA, with Tukey test for post-hoc comparisons). Statistical significance was set at $p < 0.05$. The results are presented as mean \pm standard error (SD). Analyses were conducted using Statistica (v. 5.5, Statsoft, Tulsa, OK, USA).

Results

Of the 59 SCD patients included, 17 (28.8 %) showed no retinopathy (8 SCC and 9 SCA). The ophthalmic examination of the fundus was normal for 29.6% and 28.1% of the SCC and SCA patients, respectively. Twenty-four (40.7 %) SCD patients had a non-proliferative or proliferative stages I or II retinopathy: 8 SCC (29.6%) and 16 SCA (50%). Proliferative stages III, IV or V retinopathy was found in 18 SCD patients (32.7%) with 11 SCC (40.7%) and 7 SCA (21.9%) subjects.

In the whole SCD group (i.e. SCA + SCC patients, table 1) and SCC patients alone (table 2), platelet count was significantly higher ($p < 0.05$) in the group 3 than in the group 2. The difference between group 1 and group 3 did not reach statistical significance. The other hematological, as well as hemorheological, parameters were not different between the three groups in **the whole SCD group** (table 1). **SCC patients from the group 3 had higher whole blood viscosity than SCC patients from the group 1, and group 2 had values comprised between those of the groups 1 and 3. No hematological and hemorheological difference were found between the three groups in SCA patients.**

Discussion

Few previous studies investigated the associations between sickle retinopathy and blood rheology [26-27]. However, the hemorheological techniques used at that time were much less sensitive than the current ones [5]. In addition, no distinction between the different stages of retinopathy was made in these studies [26-27]. The present study took into account some of these limitations to re-analyze the association between sickle retinopathy and blood rheology.

Our results clearly indicate that sickle retinopathy, and particularly the most severe form (proliferative stages III-IV-V), is more frequent in SCC than in SCA patients, which confirm previous study [10]. Regular dilated retinal examinations are clearly needed in SCD patients with a special attention devoted to SCC patients.

Since SCC patients have usually higher **whole** blood viscosity than SCA patients [19, 29] - that is also the case in the present study (tables 2 and 3) - it has been hypothesized that SCC patients could be prone to a greater risk for retinopathy than SCA patients [12]. SCC patients are clearly

at a greater risk than SCA patients to develop the most severe forms of retinopathy. However, previous results from Serjeant et al. showed no association between blood rheology and the development of proliferative retinopathy in SCC or in SCA patients [26-27]. In contrast, we clearly demonstrated that the group 3 of SCC patients had a 22% greater whole blood viscosity than group 1. The reasons of these divergences between our results and those from Serjeant et al. [26-27] remain unknown but one of the major differences is that the severity of the retinal ischemic and proliferative lesions was not taken into account in the studies from Serjeant et al. [26-27]. The higher whole blood viscosity observed in the group 3 may partly impair retinal perfusion and oxygenation, thus participating to the development of proliferative retinopathy in SCC patients.

Recently, Tripette et al. reported that SCC and SCA patients are characterized by RBC aggregation abnormalities [29]. Whereas RBC aggregation is lower than normal values in SCD patients, the strength needed to disperse RBC aggregates (i.e. RBC disaggregation threshold) is greater than normal values [29-30]. The pathophysiological role of the increased RBC disaggregation threshold in SCD has not been frequently assessed but one may suggest that it could impair blood flow in microcirculation where RBC aggregates need to be fully dispersed before entering into the capillaries [16, 28]. Recently, Lamarre et al. [19] also reported that SCD children at risk for acute chest syndrome (ACS) have higher RBC disaggregation threshold than SCD children who never developed ACS since birth. No study has tested the association between RBC aggregation and sickle retinopathy. The RBC disaggregation values found in the present study are in agreement with those previously published [19, 29-30]. However, we found no

association with sickle retinopathy suggesting that RBC aggregation properties do not play a role in this specific SCD complication.

Fox et al. previously shown that high hemoglobin concentration and low fetal hemoglobin level could increase the risks of SCA males and females, respectively, to develop sickle retinopathy [10]. In SCC females, high mean cell volume and low fetal hemoglobin have been reported to be associated with retinopathy [10]. We did not test these associations in function of gender because of our limited sample size for each subgroup and more patients are needed to address this issue. However, when both males and females were analyzed together, we found no difference for hemoglobin concentration, fetal hemoglobin (data not shown) or mean cell volume between the three groups categorized according to the retinopathy severity.

Platelets count was higher in the most severe SCD and SCC groups **in comparison with** the intermediate retinopathy group. One could suggest that the higher platelet count in the most severe patients could participate to the alterations of the retinal microcirculatory blood flow since they are able to adhere to the endothelium [15]. However, platelets count was not different between SCD or SCC patients with no retinopathy (groups 1) compared to patients with proliferative stages III-IV-V retinopathy (groups 3). That suggests that platelet count is probably not involved in the development or worsening of retinopathy in SCD.

One of the major limitations of the present study is the **lack of** fluorescein angiography **measurement which** is much more sensitive than indirect ophthalmoscope to detect and quantify retinopathy. However, routine exams are more often **performed** with indirect ophthalmoscope in SCD population. On the whole, our study suggests that blood rheology or hematology alone is

not a risk factor for the development of retinopathy in SCA patients. **In contrast, the most severe form of sickle retinopathy in SCC patients is associated with blood hyperviscosity.** The retinal occlusive complications in SCD are probably related to multi-cellular and molecular factors that require further studies. Longitudinal studies are needed to follow the evolution of blood rheology and the development or not of sickle retinopathy to **conclude** definitively **on the role, or not,** of blood rheology in this complication.

This study has been done in respect of the principles of ethical authorship for publication in *Clinical Hemorheology and Microcirculation* [2].

Table 1: Hematological and hemorheological parameters in SCD patients (SCA + SCC) categorized according to retinopathy severity

	Group 1 (n=17)	Group 2 (n=24)	Group 3 (n=18)
Leukocytes ($10^9/l$)	7.55 ± 1.90	9.42 ± 3.10	8.73 ± 2.61
RBCs ($10^{12}/l$)	3.58 ± 0.88	3.36 ± 1.05	3.56 ± 0.81
Hb (g/100 ml)	9.74 ± 1.80	9.40 ± 2.08	10.04 ± 1.28
MCV (fl)	77.95 ± 10.85	79.42 ± 9.07	79.83 ± 10.52
MCH (pg)	27.92 ± 4.27	28.80 ± 3.44	28.96 ± 3.88
Platelets count ($10^9/l$)	351.88 ± 124.99	299.88 ± 135.26	416.72 ± 149.62 [†]
Polynuclear Neutrophils ($10^9/l$)	3.67 ± 1.15	5.04 ± 1.90	4.80 ± 2.21
Eosinophils ($10^9/l$)	0.31 ± 0.26	0.28 ± 0.21	0.26 ± 0.15
Lymphocytes ($10^9/l$)	2.67 ± 0.66	2.93 ± 1.21	2.77 ± 1.00
Monocytes ($10^9/l$)	0.85 ± 0.37	1.11 ± 0.63	0.83 ± 0.37
Reticulocytes (%)	5.02 ± 2.80	5.80 ± 3.37	5.10 ± 3.57
Lactate dehydrogenase (UI/l)	359.94 ± 120.47	447.60 ± 147.44	348.47 ± 115.49
Hct (%)	29.54 ± 4.89	28.53 ± 6.24	28.94 ± 4.52
η_b at $90s^{-1}$ (mPa/s)	8.31 ± 2.28	7.86 ± 2.01	8.98 ± 2.21
EI at 0.95 Pa	0.06 ± 0.03	0.06 ± 0.04	0.07 ± 0.03
EI at 1.69 Pa	0.12 ± 0.03	0.10 ± 0.04	0.12 ± 0.03
EI at 3 Pa	0.19 ± 0.04	0.16 ± 0.05	0.17 ± 0.04
EI at 5.33 Pa	0.26 ± 0.05	0.22 ± 0.07	0.23 ± 0.06
EI at 9.49 Pa	0.33 ± 0.06	0.29 ± 0.08	0.30 ± 0.07
EI at 16.87 Pa	0.39 ± 0.07	0.34 ± 0.09	0.36 ± 0.08
EI at 30 Pa	0.43 ± 0.08	0.38 ± 0.10	0.40 ± 0.08
AI (%)	52.78 ± 10.05	50.88 ± 8.41	49.58 ± 9.24
γ_{thr} (s^{-1})	242.94 ± 74.69	277.41 ± 117.25	309.62 ± 173.74

Group 1: without sickle cell retinopathy, group 2: non-proliferative and proliferative stage I and II retinopathy, group 3: proliferative retinopathy of stage III, IV and V. Mean ± SD. RBCs = red blood cells, Hb = hemoglobin concentration, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, Hct = hematocrit, η_b = whole blood viscosity, EI = Elongation Index (i.e. RBC deformability), AI = RBC aggregation index, γ_{thr} = RBC disaggregation threshold. [†] Different from group 2 ($p < 0.05$).

Table 2: Hematological and hemorheological parameters in SCC patients categorized according to retinopathy severity

	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=11)
Leukocytes (10 ⁹ /l)	6.58 ± 1.54	8.49 ± 2.44	7.96 ± 2.26
RBCs (10 ¹² /l)	4.20 ± 0.48	4.44 ± 0.81	4.08 ± 0.54
Hb (g/100 ml)	11.18 ± 1.07	11.38 ± 1.40	10.74 ± 0.91
MCV (fl)	73.78 ± 4.78	71.00 ± 8.36	72.88 ± 4.81
MCH (pg)	26.72 ± 1.98	26.08 ± 3.50	26.55 ± 2.12
Platelets count (10 ⁹ /l)	330.63 ± 141.93	210.50 ± 101.26	424.00 ± 159.95 [†]
Polynuclear Neutrophils (10 ⁹ /l)	3.11 ± 0.66	5.17 ± 2.00	4.33 ± 1.71
Eosinophils (10 ⁹ /l)	0.35 ± 0.35	0.17 ± 0.09	0.24 ± 0.13
Lymphocytes (10 ⁹ /l)	2.39 ± 0.61	2.25 ± 0.74	2.66 ± 0.86
Monocytes (10 ⁹ /l)	0.69 ± 0.37	0.86 ± 0.67	0.66 ± 0.28
Reticulocytes (%)	2.88 ± 1.03	2.18 ± 0.86	3.14 ± 1.57
Lactate dehydrogenase (UI/l)	269.38 ± 53.56	371.67 ± 155.22	300.00 ± 68.50
Hct (%)	33.05 ± 3.25	33.25 ± 4.50	30.65 ± 3.71
η _b at 90s ⁻¹ (mPa/s)	7.39 ± 1.50	9.08 ± 1.45	9.47 ± 1.80 [*]
EI at 0.95 Pa	0.06 ± 0.01	0.07 ± 0.03	0.08 ± 0.02
EI at 1.69 Pa	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.02
EI at 3 Pa	0.19 ± 0.02	0.17 ± 0.02	0.17 ± 0.03
EI at 5.33 Pa	0.26 ± 0.03	0.24 ± 0.03	0.23 ± 0.05
EI at 9.49 Pa	0.34 ± 0.05	0.31 ± 0.04	0.30 ± 0.06
EI at 16.87 Pa	0.40 ± 0.06	0.37 ± 0.05	0.36 ± 0.07
EI at 30 Pa	0.45 ± 0.07	0.43 ± 0.05	0.40 ± 0.07
AI (%)	46.96 ± 9.17	47.38 ± 10.10	46.66 ± 6.50
γ _{thr} (s ⁻¹)	225.00 ± 66.82	297.14 ± 116.47	283.85 ± 98.86

Group 1: without sickle cell retinopathy, group 2: non-proliferative and proliferative stage I and II retinopathy, group 3: proliferative retinopathy of stage III, IV and V. Mean ± SD. RBCs = red blood cells, Hb = hemoglobin concentration, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, Hct = hematocrit, η_b = **whole** blood viscosity, EI = Elongation Index (i.e. RBC deformability), AI = RBC aggregation index, γ_{thr} = RBC disaggregation threshold. ^{*} **Different from group 1 (p < 0.05)**, [†] different from group 2 (p < 0.05).

Table 3: Hematological and hemorheological parameters in SCA patients categorized according to retinopathy severity

	Group 1 (n=9)	Group 2 (n=16)	Group 3 (n=7)
Leukocytes ($10^9/l$)	8.41 ± 1.83	9.89 ± 3.36	9.94 ± 2.83
RBCs ($10^{12}/l$)	3.02 ± 0.78	2.83 ± 0.67	2.75 ± 0.35
Hb (g/100 ml)	8.46 ± 1.26	8.42 ± 1.61	8.96 ± 0.99
MCV (fl)	81.66 ± 13.51	83.63 ± 6.08	90.74 ± 6.92
MCH (pg)	28.99 ± 5.51	30.16 ± 2.55	32.74 ± 2.78
Platelets count ($10^9/l$)	370.78 ± 112.98	344.56 ± 129.92	405.29 ± 143.30
Polynuclear Neutrophils ($10^9/l$)	4.18 ± 1.29	4.97 ± 1.91	5.53 ± 2.82
Eosinophils ($10^9/l$)	0.27 ± 0.15	0.33 ± 0.23	0.30 ± 0.18
Lymphocytes ($10^9/l$)	2.91 ± 0.63	3.27 ± 1.27	2.93 ± 1.24
Monocytes ($10^9/l$)	0.99 ± 0.33	1.23 ± 0.59	1.11 ± 0.35
Reticulocytes (%)	6.90 ± 2.47	7.61 ± 2.47	8.70 ± 3.44
Lactate dehydrogenase (UI/l)	440.44 ± 104.98	480.14 ± 136.76	437.33 ± 136.59
Hct (%)	26.41 ± 3.92	26.01 ± 5.61	26.50 ± 4.70
η_b at $90s^{-1}$ (mPa/s)	9.03 ± 2.59	7.21 ± 2.00	8.29 ± 2.69
EI at 0.95 Pa	0.06 ± 0.03	0.05 ± 0.04	0.05 ± 0.04
EI at 1.69 Pa	0.12 ± 0.04	0.10 ± 0.05	0.11 ± 0.05
EI at 3 Pa	0.19 ± 0.05	0.15 ± 0.06	0.17 ± 0.06
EI at 5.33 Pa	0.26 ± 0.06	0.22 ± 0.08	0.23 ± 0.07
EI at 9.49 Pa	0.32 ± 0.07	0.28 ± 0.10	0.30 ± 0.09
EI at 16.87 Pa	0.38 ± 0.08	0.32 ± 0.11	0.36 ± 0.10
EI at 30 Pa	0.42 ± 0.09	0.36 ± 0.11	0.40 ± 0.11
AI (%)	57.96 ± 8.03	52.51 ± 7.31	53.75 ± 11.39
γ_{thr} (s^{-1})	258.89 ± 81.50	268.20 ± 120.51	346.43 ± 251.29

Group 1: without sickle cell retinopathy, group 2: non-proliferative and proliferative stage I and II retinopathy, group 3: proliferative retinopathy of stage III, IV and V. Mean ± SD. RBCs = red blood cells, Hb = hemoglobin concentration, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, Hct = hematocrit, η_b = whole blood viscosity, EI = Elongation Index (i.e. RBC deformability), AI = RBC aggregation index, γ_{thr} = RBC disaggregation threshold.

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