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## ORIGINAL PAPER

# Association of haemolysis markers, blood viscosity and microcirculation function with organ damage in sickle cell disease in sub-Saharan Africa (the BIOCADRE study)

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## Summary

Sickle cell anaemia (SCA) is a monogenic disease with a highly variable clinical course. We aimed to investigate associations between microvascular function, haemolysis markers, blood viscosity and various types of SCA-related organ damage in a multicentric sub-Saharan African cohort of patients with SCA. In a cross-sectional study, we selected seven groups of adult patients with SS phenotype in Dakar and Bamako based on the following complications: leg ulcer, priapism, osteonecrosis, retinopathy, high tricuspid regurgitant jet velocity (TRV), macro-albuminuria or none. Clinical assessment, echocardiography, peripheral arterial tonometry, laboratory tests and blood viscosity measurement were performed. We explored statistical associations between the biological parameters and the six studied complications. Among 235 patients, 58 had high TRV, 46 osteonecrosis, 43 priapism, 33 leg ulcers, 31 retinopathy and 22 macroalbuminuria, whereas 36 had none of these complications. Multiple correspondence analysis revealed no cluster of complications. Lactate dehydrogenase levels were associated with high TRV, and blood viscosity was associated with retinopathy and the absence of macroalbuminuria. Despite extensive phenotyping of patients, no specific pattern of SCA-related complications was identified. New

Mor Diaw and Abdoul Karim Dembele contributed equally.

Clinical Trial registration: [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03114137) identifier NCT03114137.

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biomarkers are needed to predict SCA clinical expression to adapt patient management, especially in Africa, where healthcare resources are scarce.

#### KEY WORDS

blood viscosity, haemoglobinopathies, haemolysis, sickle cell anaemia, sub-Saharan Africa, vascular complications

## INTRODUCTION

Sickle cell disease (SCD) is the most common monogenic disease in the world due to a single mutation in the  $\beta$ -globin gene. Under deoxygenated conditions, the abnormal haemoglobin S (HbS) polymerizes and causes the red blood cells (RBCs) to distort into a crescent-like shape.<sup>1</sup> The loss of RBC deformability participates in vaso-occlusion events. Still, other key cellular actors, such as activated vascular endothelium, adherent reticulocytes, activated neutrophils, monocytes and platelets, and mastocytes, are involved in the pathophysiology of SCD.<sup>2,3</sup> In addition, chronic haemolytic anaemia leads to reduced oxygen-carrying capacity and tissue hypoxia. Through its effects on vascular function, inflammation and oxidative stress,<sup>4</sup> increased chronic haemolysis may play a role in progressive multi-organ damage, such as cerebral vasculopathy, pulmonary hypertension, kidney disease, leg ulcers, and priapism.<sup>5</sup> The high inter-individual variability of SCD clinical manifestations is notorious, even in patients of similar genotypes.

More than 230 000 children with SCD are born each year in sub-Saharan Africa, compared to 2600 in North America and 1300 in Europe.<sup>6</sup> In Africa and India, the numbers of newborns with sickle cell anaemia (SCA), the most frequent and severe phenotype due to the homozygous SS genotype, are projected to increase by 30% by 2050.<sup>6</sup> Until recently, it was estimated that 50%–90% of these children died before 5 years of age because of the lack of newborn screening programs and appropriate clinical care.<sup>7</sup> However, mortality is decreasing dramatically in Africa, at least in the urban population,<sup>8</sup> allowing patients to reach adulthood and develop long-term organ damage.

Although genetic, epigenetic, and environmental factors may be involved in SCD phenotype variability,<sup>9–13</sup> very few studies have been conducted in sub-Saharan Africa, where the socio-economic, environmental, and medical contexts are dramatically different from European countries and the United States, where most studies have been conducted. We built the collaborative CADRE (Coeur, Artères et DRépanocytose) study as a large cohort of patients with SCD in five countries of West and Central Africa (Cameroon, Gabon, Ivory Coast, Mali and Senegal) to gather reliable data on SCD-related organ damage. The main objectives of the CADRE cohort were (1) to estimate the incidence of micro- and macrovascular complications of SCD and (2) to identify predictive factors of these complications in these countries. Between 2011 and 2013, 3627 patients, 3 years of age and over, were included and investigated in the five centres.<sup>14,15</sup>

Our project combines an epidemiological approach to identify specific patterns of organ damage with experimental studies. For the 5-year follow-up of the CADRE patients, we designed a cross-sectional study, BIOCADRE, nested in cohort CADRE, based on severe phenotypes of SCA in two African countries, Mali and Senegal. We aimed to identify biological markers and functional tests associated with vascular disease, particularly those related to haemolysis. Here, we present the association of the main SCD-related organ damage with blood viscosity, classical plasma markers of haemolysis and peripheral arterial tonometry in the sub-Saharan context.

## PATIENTS AND METHODS

### Patient selection and biological analysis

BIOCADRE is a cross-sectional study nested in the CADRE cohort. Two centres participating in the CADRE cohort were selected for the BIOCADRE study: the “Centre National de Transfusion Sanguine” in Dakar, Senegal and the “Centre de Recherche et de Lutte contre la Drépanocytose” in Bamako, Mali. Dakar and Bamako were chosen among the five participating countries for practical reasons. First, BIOCADRE required local medical doctors or scientists ready to be trained in France for a few weeks on the special experimental techniques which had to be carried out in the field. Second, the laboratory where the experiments took place had to be close to the clinical setting so that the blood samples could be processed very quickly.

The CADRE inclusion database contained 699 patients from Bamako, including 377 with a SS phenotype and 719 patients from Dakar, including 662 with a SS phenotype. First, we identified subsets of adult patients with SS phenotype and specific organ complications: five groups of 40 patients (20 in each centre) with at least one of the following complications at inclusion: tricuspid regurgitant jet velocity (TRV)  $>2.5$  m/s (which may indicate pulmonary hypertension), albuminuria/creatininuria  $>300$  mg/g, leg ulcer, priapism and aseptic osteonecrosis. We selected the youngest patients with binary (i.e. present or absent) clinical complications or the patients with the most severe phenotype for quantitative traits (TRV and macroalbuminuria). We also selected the oldest 20 adult patients from each centre without any previous complications among those who had been extensively investigated. The total numbers of patients from Bamako and Dakar diagnosed with each of these complications at the inclusion in the

CADRE study are available in [Table S1](#). These 240 patients were recalled for a specific medical visit, that took place 5–7 years after their inclusion in the CADRE study.

Patients were investigated at steady-state, that is, 15 days or more after a vaso-occlusive crisis, 8 days or more after fever or infectious disease, and 3 months after the last blood transfusion. Laboratory tests included complete blood counts, reticulocyte counts, foetal haemoglobin (HbF) levels, serum bilirubin and lactate dehydrogenase (LDH) concentrations, and urine creatinine and albumin concentrations. The estimated glomerular filtration rate was calculated using the CKD epi formula without correction for the African origin.<sup>16</sup> Microalbuminuria and macroalbuminuria were defined by a urinary albumin/creatinine ratio >30 and >300 mg/g, respectively. Echocardiography was also performed using a predefined protocol. Retinopathy was evaluated based on dilated funduscopy in Bamako only, because of the lack of ophthalmologists available in Dakar to participate in the study. The leg ulcer and priapism diagnosis were based on clinical inquiry and examination. Osteonecrosis was suspected in the case of chronic articular pain and confirmed by radiological criteria (Arlet and Ficat classification). Patients were categorized in the “no complication” group only if they had no missing data regarding the five complications of selection.

Local investigators were trained by the French teams to measure blood viscosity and study microcirculation function. Blood viscosity was measured with fresh blood samples using a cone-plate viscometer (Pro DV-II+, with CPE40 spindle; Brookfield) at native haematocrit, 37°C and several shear rates (4.5, 11.5, 22.5, 45, 90 and 225 s<sup>-1</sup>). Microcirculation function was assessed by measuring changes in index finger pulse amplitude during reactive hyperaemia, using non-invasive peripheral arterial tonometry (EndoPAT2000®; Itamar Medical). Low scores of the Reactive Hyperaemia Index (RHI), an endothelial vasodilatation marker, reflected endothelial dysfunction. The reactive endothelial response was measured in a quiet room in low light, at 20–25°C.

Relevant ethics committees approved the BIOCADRE protocol: Mali (008/MSHP-CNESS/2014) and Senegal (35/MSAS/DPRS/CNERS).

## Statistical methods

The sample size was designed to demonstrate a difference of at least one standard deviation of the quantitative biological values between groups: at least 30 patients with each specific condition were required.

The investigated parameters were described in the studied population, country and complication group. We assessed potential clusters of clinical complications and biomarkers by multiple correspondence analyses. Then, different multivariate logistic regression analyses assessed the association between the presence of each vascular complication and the studied markers, with adjustment for age, sex and centre. Quantitative explanatory variables were standardized

(divided by their mean) to ensure comparability of the odds ratios.

Statistical significance was defined as  $p < 0.05$ . All analyses were two-sided and conducted using R version 3.5.2.

## RESULTS

We recalled 240 patients selected in the inclusion CADRE database: 17 were not reachable, and 21 were deceased. Among the 202 patients who could attend the BIOCADRE visit, 62 had developed at least one additional complication ([Table S1](#)). In particular, among the oldest 37 patients of the CADRE cohort with no complications who were investigated after the initial selection, 31 had developed one of the six studied complications between the inclusion and BIOCADRE visits. Therefore, we recalled 56 other adult patients without any complications mentioned in the CADRE inclusion database and 36 were eventually able to join the control group of patients. The oldest patient without any complication was 43 years old in Bamako and 53 years old in Dakar. Finally, 235 SCA adult patients were investigated, 107 from Bamako and 128 from Dakar.

The clinical and biological characteristics of the included patients are reported in [Table 1](#). Their average age was 28.8 years ( $\pm 11.0$ ). Among them, 58 had a high TRV ( $> 2.5$  m/s), 46 had aseptic osteonecrosis, 43 had priapism, 33 had leg ulcers, 87 had microalbuminuria, 22 had macroalbuminuria, and 31 had retinopathy, whereas 36 patients had none of these vascular complications.

Among 199 patients with at least one complication, 86 (43%) had more than the one they were selected for ([Table 2](#)).

Multiple correspondence analysis of the whole cohort did not reveal any cluster of complications ([Figure S1](#)). For example, 42 of 57 (74%) patients exhibiting a so-called “hyper-viscous” phenotype (retinopathy or osteonecrosis) also had at least one “hyper-haemolytic” complication (high TRV, albuminuria, leg ulcer, or priapism). Conversely, 42 of 120 (36%) patients with a “hyper-haemolytic” phenotype also had at least one “hyper-viscous” complication.

The association between the various SCD-related vascular complications and the investigated markers is shown in [Table 3](#) and graphically summarized in [Figure 1](#). Although higher levels of LDH were associated with elevated TRV (OR 2.07 [1.02;4.3],  $p = 0.025$ ), neither LDH nor bilirubin levels were associated with the other complications usually assigned to the “hyper-haemolytic” phenotype (albuminuria, priapism or leg ulcer), nor with osteonecrosis or retinopathy. Lower haemoglobinemia was associated with microalbuminuria (OR 0.7 [0.01;0.62],  $p = 0.011$ ). Osteonecrosis was associated with higher haemoglobinemia (OR 4.78 [1.70;27.7],  $p = 0.027$ ) and lower leucocyte counts (OR 0.04 [0.00;0.41],  $p = 0.007$ ).

High HbF percentage was only associated with the absence of leg ulcers (OR 0.14 [0.04;0.42],  $p = 0.002$ ).

Blood viscosity was positively associated with retinopathy (for example, at 11.5 s<sup>-1</sup>: adjusted OR 3.04 [1.10;11.54],

**TABLE 1** Clinical and biological characteristics of patients with SCA selected in Bamako, Mali and Dakar, Senegal.

	All N = 235	Bamako N = 107	Dakar N = 128	N
Age (mean, SD)	28.8 (11.0)	28.8 (10.6)	28.0 (11.3)	235
Female sex	63 (49%)	63 (58%)	53 (41%)	235
Osteonecrosis	46 (22%)	26 (25%)	20 (19%)	208
Retinopathy	31 (44%)	31 (44%)	NA	77
TRV > 2.5 m/s	58 (27%)	12 (14%)	46 (37%)	208
Priapism (males only)	43 (41%)	21 (50%)	22 (34%)	106
Leg ulcers	33 (16%)	22 (21%)	11 (10%)	211
Microalbuminuria	86 (49%)	70 (71%)	26 (33%)	177
Macroalbuminuria	22 (15%)	17 (18%)	6 (8%)	177
eGFR < 60 mL/min	7 (4%)	2 (2%)	5 (4%)	216
No vascular complication	36 (16%)	6 (6%)	30 (25%)	214
More than one complication	86 (38%)	60 (56%)	26 (21%)	235
Haemoglobin (g/dL)	8.25 [7.5;9.4]	8.55 [7.80;9.78]	8.09 [7.30;8.89]	219
Leukocytes (G/L)	11.2 [8.9;13.8]	10.7 [8.68;12.6]	12.3 [9.20;15.8]	219
Platelets (G/L)	470 [363;556]	494 [383;582]	453 [339;512]	216
Reticulocytes (G/L)	270 [200;358]	270 [200;358]	NA	108
Lactate dehydrogenase (iu/L)	897 [676;1023]	1097 [776;1438]	659 [504;861]	208
Foetal haemoglobin (%)	10.9 [7.20;15.3]	10.6 [7.40;15.7]	11.2 [6.80;14.0]	119
RHI	1.53 [1.30;1.88]	1.43 [1.29;1.76]	1.56 [1.36;2.02]	211
Log RHI	0.42 [0.26;0.63]	0.36 [0.25;0.57]	0.45 [0.31;0.70]	211
Viscosity (cP)				
4.5 s <sup>-1</sup>	11.8 [7.41;16.1]	11.0 [7.41;16.1]	12.5 [11.2;13.2]	213
11.5 s <sup>-1</sup>	8.60 [5.52;9.57]	7.10 [5.52;9.57]	9.9 [9.1;11.8]	216
22.5 s <sup>-1</sup>	7.02 [4.96;7.74]	5.82 [4.96;7.74]	8.54 [7.84;10.1]	216
45 s <sup>-1</sup>	6.14 [4.25;6.30]	5.07 [4.25;6.30]	7.24 [6.53;8.88]	216
90 s <sup>-1</sup>	5.70 [4.09;5.75]	4.70 [4.09;5.75]	6.66 [6.19;7.20]	216
225 s <sup>-1</sup>	4.81 [3.90;5.25]	4.41 [3.90;5.25]	5.21 [5.06;5.84]	216

Note: Quantitative variables are presented as median [interquartile range].

Abbreviations: eGFR, estimated glomerular filtration rate; SCA, sickle cell anaemia; TRV, tricuspid regurgitant jet velocity.

**TABLE 2** Matrix of micro- and macro-vascular complications in the BIOCADRE population.

	Age (years)	Osteonecrosis	Retinopathy <sup>a</sup>	TRV > 2.5 m/s	Priapism <sup>b</sup>	Leg ulcer	Microalbuminuria
	Median (Q1–Q3)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Osteonecrosis (n = 46)	32 (22–42)	46 (100%)	9 (47%)	12 (29%)	3 (20%)	7 (16%)	16 (49%)
Retinopathy (n = 31)	31 (25–40)	9 (29%)	31 (100%)	2 (6%)	4 (44%)	9 (30%)	17 (59%)
TRV > 2.5 m/s (n = 58)	28 (22–37)	12 (23%)	2 (29%)	58 (100%)	11 (34%)	8 (14%)	15 (37%)
Priapism (n = 43)	26 (21–31)	3 (7%)	4 (40%)	11 (28%)	43 (100%)	8 (19%)	21 (58%)
Leg ulcers (n = 33)	30 (23–38)	7 (22%)	9 (53%)	8 (25%)	8 (47%)	33 (100%)	15 (60%)
Microalbuminuria (n = 86)	27 (21–35)	16 (20%)	17 (39%)	15 (20%)	21 (58%)	15 (19%)	86 (100%)
Macroalbuminuria (n = 22)	26 (22–35)	2 (10%)	4 (57%)	2 (10%)	2 (29%)	4 (20%)	22 (100%)

Abbreviations: Q1, first quartile; Q3, third quartile; TRV, tricuspid regurgitant jet velocity.

<sup>a</sup>In Bamako only.

<sup>b</sup>In males only.

**TABLE 3** Associations between classical blood biomarkers, viscosity and clinical complications of sickle cell anaemia: odds ratios and their 95% confidence intervals after adjustment for age, sex and centre.

Complication	Haemoglobin (g/dL)	Bilirubin (µmol/L)	LDH (iu/L)	Leucocyte (G/L)	Foetal haemoglobin (%)	Blood viscosity (cP) <sup>a</sup>	RHI
Osteonecrosis	<b>4.78 [1.70;27.7]</b>	0.76 [0.37;1.34]	0.94 [0.45;1.82]	<b>0.04 [0.00;0.41]</b>	1.89 [0.84;4.33]	1.36 [0.50;3.75]	0.72 [0.16;3.07]
Retinopathy	1.14 [0.03;38.4]	0.86 [0.22;1.85]	1.37 [0.6;3.19]	1.04 [0.10;16.46]	1.41 [0.43;4.77]	<b>3.04 [1.1;11.54]</b>	4.6 [0.4;61.36]
TRV > 2.5 m/s	0.59 [0.08;4.17]	1.33 [0.86;2.10]	<b>2.07 [1.02;4.3]</b>	1.70 [0.67;5.34]	1.40 [0.57;3.42]	1.22 [0.34;4.30]	0.97 [0.28;3.32]
Priapism	0.83 [0.09;7.57]	0.76 [0.37;1.34]	1.26 [0.53;3.01]	0.53 [0.04;2.43]	0.51 [0.19;1.26]	2.35 [0.55;11.41]	1.12 [0.26;4.85]
Leg ulcers	0.26 [0.02;2.90]	1.13 [0.5;2.13]	1.15 [0.53;2.29]	0.92 [0.08;4.42]	<b>0.14 [0.04;0.42]</b>	0.90 [0.30;2.57]	1.37 [0.26;7.08]
Microalbuminuria	<b>0.07 [0.01;0.62]</b>	0.95 [0.5;1.73]	1.11 [0.5-2.31]	1.07 [0.18;3.95]	0.38 [0.13;1.05]	0.90 [0.34;2.43]	1.19 [0.26;5.62]
Macroalbuminuria	0.22 [0.01;4.09]	0.98 [0.34;2.19]	0.72 [0.42;1.77]	0.97 [NA;1.04]	0.35 [0.8;1.37]	<b>0.32 [0.04;0.92]</b>	17.29 [1.56;141.1]
No complication	0.21 [0.01;2.24]	0.61 [0.28;1.10]	0.60 [0.17-1.6]	0.34 [0.03;1.79]	1.96 [0.96;4.81]	0.53 [0.08;2.98]	0.71 [0.15;3.15]

Note: Odds ratios and 95% confidence intervals are issued from multivariate logistic regression analysis with adjustment for age, sex and centre. Statistically significant odds ratios ( $p < 0.05$ ) are in bold. All variables have been standardized (divided by their mean) to ensure comparability of the odds ratios.

Abbreviations: LDH, lactate dehydrogenase; RHI, Reactive Hyperaemia Index measured by peripheral arterial tonometry; TRV, tricuspid regurgitant jet velocity measured by echography.

<sup>a</sup>Blood viscosity measured at 11.5 s<sup>-1</sup>.

$p = 0.043$ ) and negatively associated with macro-albuminuria (OR 0.32 [0.04;0.92],  $p = 0.047$ ).

The microvascular dysfunction assessed by peripheral arterial tonometry was not associated with any of the selected complications.

## DISCUSSION

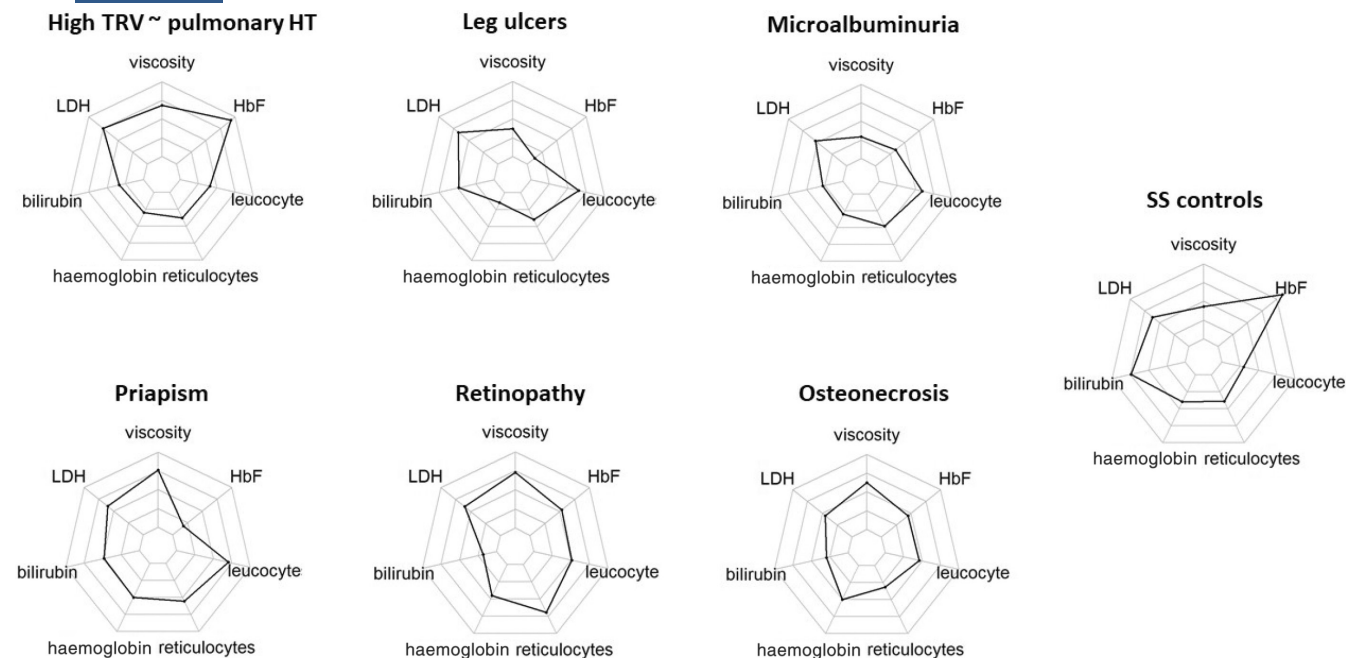
The extreme variability of the clinical expression of SCD in patients has been known for decades. Our study produced the first clinical report with a fine characterization of long-term organ damage in a large cohort of African patients with SCA. While selected on the presence of one specific type of organ damage, most patients had at least one other complication.

The BIOCADRE data confirmed previous observations made in the CADRE study, using different experimental processes and cross-sectional patient inclusion data,<sup>17</sup> that failed to detect any cluster of patients based on the different types of vascular complications.

The BIOCADRE study also failed to support the “hyper-haemolysis phenotype paradigm” previously proposed by Kato et al.<sup>5</sup> Indeed, we did find an association between plasma LDH levels and the occurrence of high TRV, a poorly specific surrogate marker of pulmonary hypertension,<sup>18</sup> as previously reported.<sup>19,20</sup> However, LDH levels were neither associated with other “hyper-haemolytic” complications (ulcers, priapism or nephropathy) nor inversely associated with “hyper-viscous” complications (retinopathy or osteonecrosis). Other indirect classical markers of haemolysis did not correlate with a history of vascular complications either. Another recent study performed on 166 patients with SCA from central Africa (Democratic Republic of Congo) could not find an association between indirect haemolysis markers in the blood (reticulocytes, LDH and bilirubin) and a global clinical severity score.<sup>21</sup>

One explanation may be that haemolytic markers like LDH are sensitive to infectious diseases (such as viral hepatitis or malaria) and may lose specificity in African populations where the infectious burden is high. BIOCADRE patients were included without overt clinical signs of infection, but the presence of subclinical infection (e.g. parasitaemia) was not verified because of the limited resources available. In future studies, it will be important to confront the studied parameters with signs of infectious diseases.

One hypothesis regarding the development of SCD vasculopathy driven by chronic haemolysis is related to the release of cell-free haemoglobin responsible for a decreased NO bioavailability, endothelial dysfunction and excessive vasoconstriction.<sup>22,23</sup> To investigate this hypothesis, we assessed microvascular reactivity using peripheral arterial tonometry. Using this method, a low RHI ( $\leq 1.67$  according to Itamar information) indicates endothelial dysfunction<sup>24</sup> and is a risk factor for vascular complications.<sup>25</sup> In our study, 61% of patients exhibited an RHI < 1.67. Our observation corroborates the only other publication reporting RHI data in patients with SCD, which found an RHI < 1.67 in two third of



**FIGURE 1** Radar plots of the main biological parameter distribution in patients with a vascular complication of sickle cell disease. For each group of patients with a specific clinical complication, median values of biological parameters were plotted on a scale between the first and third quartiles of the same parameters in the whole population, except for retinopathy for which only the patients from Bamako were considered. The number of patients in each group were: 36 with no complication, 33 with leg ulcers, 86 with microalbuminuria, 43 with priapism, 58 with high Tricuspid Regurgitation Velocity, 46 with osteonecrosis, 31 with retinopathy. Note that patients may have more than one clinical complication.

36 paediatric patients with SCA from the Western world.<sup>26</sup> Although previous work suggested impaired endothelium-dependent vasodilation in the finger of SCA patients,<sup>27</sup> the extent of vasomotor reactivity in fingertips was not associated with any studied vascular complications.

It may be noted that we also failed to identify a subgroup of hyper-haemolytic complications in the same population, using more elaborate markers of haemolysis, namely the measurement of circulating red blood cell-derived microparticles.<sup>28</sup>

On the other hand, blood viscosity was not inversely correlated to complications commonly associated with “hyper-haemolysis”, such as high TRV or leg ulcers, nor positively correlated to osteonecrosis, frequently considered a “hyper-viscous complication”. The lack of association between blood viscosity and osteonecrosis agrees with a previous study from our group in a French Caribbean cohort.<sup>29</sup> We found a positive association between blood viscosity and sickle cell retinopathy, in agreement with earlier reports in patients with SCA<sup>30,31</sup> or sickle cell-haemoglobin C disease.<sup>31</sup> In addition, our results confirm a previous study showing a negative association between blood viscosity and macro-albuminuria.<sup>32</sup>

Finally, we investigated the associations between organ damage and HbF levels. In our study, the mean HbF level was around 11% in Mali and Senegal, consistent with the wide predominance of the Senegal *HBB* haplotype in West African countries. Indeed, although HbF is increased in nearly all HbS homozygotes, each *HBB* haplotype is associated with a distinctive mean HbF level. In sub-Saharan Africa, adult patients with Senegal haplotype have an HbF

level of ~10%, whereas patients with Benin, Bantu, and Cameroon haplotypes have lower HbF levels.<sup>33</sup> The erythrocyte HbF concentration modifies the rate and extent of polymerization. It is noteworthy that blood HbF level or F-cell percentage are poor predictors of disease severity for an individual because neither accounts for the variable distribution of HbF levels among F-cells. The number of F-cells with polymer-inhibiting concentrations of HbF is likely to be a more important determinant of the features of SCA.<sup>33</sup>

Nevertheless, high HbF levels were strongly associated with fewer acute painful episodes, acute chest syndromes and lower mortality.<sup>34</sup> Therefore, higher HbF expression appears to have the most beneficial to acute clinical events that result directly from vaso-occlusions. However, HbF has also been associated with a reduced risk of leg ulcers,<sup>35</sup> which are classically part of the “hyper-haemolysis phenotype”,<sup>5</sup> as we observed in our study. Therefore, the protective role of higher HbF expression in SCA cannot be fully explained by the opposition between hyper-viscous and hyper-haemolysis sub-phenotypes.

We acknowledge some weaknesses in our study. First, because of the cross-sectional design, we could only assess patients who have survived the disease to date, leading to mortality bias. Although the mean age was similar in both centres, we observed differences in the prevalence of some clinical complications between the two centres, in accordance with our previous observations in the same countries.<sup>15,17</sup> We performed sensitivity analyses in each country and adjusted our tests to the centre to overcome this heterogeneity. We also deplore a high proportion of missing data in one of the centres concerning

three complications due to technical issues: retinopathy (100% missing data in Dakar), TRV (15% missing data in Bamako), and microalbuminuria (33% missing data in Dakar).

Our study encompassed many unique features and strengths. First, the large CADRE cohort allowed us to screen SCA patients with extreme phenotypes: we selected patients with the most severe quantitative phenotypes (i.e. albuminuria or elevated TRV), the youngest patients with a given qualitative phenotype (i.e. leg ulcers, retinopathy, osteonecrosis and priapism) and the oldest patients without any of those complications. As a consequence, the prevalence and characteristics of the complications studied in the BIOCADRE study were not representative of those of the whole CADRE cohort, but those patients represented the best candidates to manifest distinct biological patterns.

Furthermore, to our knowledge, the BIOCADRE study is the first to assess an extensive panel of biomarkers and functional tests in SCA patients who live in sub-Saharan Africa. Most of the previous literature focused on one type of SCD-related organ damage and/or looked for bivariate associations between specific clinical complications and a restricted number of biomarkers. These strategies are more likely to favour the publication of positive associations only. In contrast, BIOCADRE explored the relationship between multiple SCD-related vascular complications and various biological or functional tests, and we adjusted analyses for country and age since most complications become more prevalent with ageing.

## CONCLUSION

The BIOCADRE study provided the first comprehensive biological and functional characterization of a large selection of sub-Saharan African patients with specific SCD-related long-term organ damages.

It reveals that the measurement of routine haemolysis markers, endothelial-dependent vasomotor reactivity or blood viscosity does not facilitate segmenting patients with SCA into clinically relevant clusters in Sub-Saharan Africa. New predictive markers of long-term complications of SCD are needed to optimize the follow-up of these patients. To be clinically useful in low resources settings, biomarkers need to be easily and reproducibly measurable, even under non-optimal measuring conditions (high temperature and humidity, frequent electricity shortages, long time to ship the biological samples to the lab, presence of dust and insects, etc.). They also have to be cheap, which reduces considerably the number of good candidates. Moreover, given the absence of a clear pattern of complications combinations, it is unlikely that one marker will be able to predict the global severity of SCD. Therefore, several markers specific to one complication pathological process probably have to be combined to build a global prognostic score.

Since some determinants of SCA severity may be specific to the African context, we would also like to highlight the importance of acquiring datasets with comprehensive phenotypes of patients living in Africa in addition to the existent Caribbean, European and American cohorts.

## AUTHOR CONTRIBUTIONS

Brigitte Ranque, Marc Romana, Philippe Connes, Sylvain Le Jeune, Olivier Blanc-Brude, Xavier Jouven, Pierre-Louis Tharaux and Caroline Le Van Kim designed the research study. Brigitte Ranque, Marc Romana, Philippe Connes, Sylvain Le Jeune, Yves Colin-Aronovicz, Claudine Lapoumeroulie and Caroline Le Van Kim trained the African investigators. Dapa Diallo and Saliou Diop organized the recruitment of the patients, the clinical assessment and the research in Mali and Senegal respectively. The research was performed by Mor Diaw and Serigne Mourtalla Gueye (in Senegal), Abdoul Karim Dembele and Oumar Tessougue (in Mali), Claudine Lapoumeroulie, Marc Romana and Abdoul Karim Dembele (in France). Brigitte Ranque and Lucile Offredo did the statistical analyses. Brigitte Ranque wrote the first draft of the paper. Brigitte Ranque, Pierre-Louis Tharaux, Marc Romana, Philippe Connes, Sylvain Le Jeune, Olivier Blanc-Brude and Caroline Le Van Kim analysed the data and contributed to the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The datafile of the study is available upon request (to the corresponding author: [brigitte.ranque@aphp.fr](mailto:brigitte.ranque@aphp.fr)).

## ETHICS STATEMENT

Relevant national ethics committees approved the BIOCADRE protocol: Mali (008/MSHP-CNESS/2014) and Senegal (35/MSAS/DPRS/CNERS).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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