Haemolysis and abnormal haemorheology in sickle cell anaemia

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Summary

Although pulmonary hypertension, leg ulcers, priapism, stroke and glomerulopathy in sickle cell anaemia (SCA) result from the adverse effects of chronic haemolysis on vascular function (haemolytic phenotype), osteonecrosis, acute chest syndrome and painful vaso-occlusive crises are caused by abnormal vascular cell adhesion and increased blood viscosity (viscosity-vaso-occlusion phenotype). However, this model with two sub-phenotypes does not take into account the haemorheological dimension. We tested the relationships between the biological parameters reflecting the haemolytic rate (haemolytic component) and red blood cell (RBC) rheological characteristics in 97 adults with SCA. No significant difference in the proportion of patients with low or high haemolytic component in the low and high blood viscosity groups was observed. The RBC elongation index (i.e. deformability) was negatively correlated with the haemolytic component. The RBC aggregates strength (i.e. RBC aggregates robustness) was negatively correlated with RBC elongation index. Sickle RBCs with high density had lower elongation index and higher aggregates strength. In conclusion, (i) the ‘haemolytic’ phenotype is characterized by decreased RBC deformability and increased RBC aggregates strength and (ii) the viscosity-vaso-occlusion phenotype is characterized by increased RBC deformability but not always by increased blood viscosity. α-thalassaemia modulates the haemorheological properties but other factors seem to be involved.

Keywords: haemorheology, sickle cell disease, haemolysis.

The last four decades of basic, clinical and translational research in sickle cell anaemia (SCA) have witnessed the development of a new pathophysiological scheme of the disease. Several groups have proposed two main clinical/biological phenotypes, which could explain most of the acute and chronic complications of SCA (Kato et al., 2007; Gladwin & Vichinsky, 2008): (i) the haemolytic-endothelial dysfunction phenotype and (ii) the viscosity-vaso-occlusion phenotype.

The haemolytic-endothelial dysfunction phenotype has been defined as associated with a high rate of haemolysis, which would mainly impact on the vascular physiology both at the pre-capillary arteriolar level, as well as in large arteries (Kato et al., 2007; Connes et al., 2013a). The high amount of haemoglobin and arginase released into the plasma would react with nitric oxide (NO) and L-arginine, respectively, leading to the reduction of NO bioavailability. The drop in plasma NO content could cause endothelial dysfunction,
over-expression of vascular adhesion molecules and vasomotor tone impairment (Kato et al., 2005; Lin et al., 2005). In support of these mechanisms, plasma from patients with SCA contains cell-free oxyhaemoglobin, which stoichiometrically consumes micromolar quantities of NO and abrogates forearm blood flow response to NO donor infusion (Reiter et al., 2002). These biological alterations could be involved in the development of pulmonary hypertension (Gladwin et al., 2004), glomerulopathy (Nebor et al., 2010), leg ulcers (Bowers et al., 2013), priapism (Kato et al., 2007) and cerebral vasculopathy (Bernaudin et al., 2008; DeBaun et al., 2012; Connes et al., 2013a).

In the viscosity-vaso-occlusion phenotype, the haemolytic rate is less severe and these SCA patients have milder anaemia than in the first phenotype described. Patients with this phenotype would be prone to vaso-occlusive complications, such as painful vaso-occlusive crisis (VOC), acute chest syndrome (ACS) and osteonecrosis (Kato et al., 2007). The large Cooperative Study of Sickle Cell Disease (CSSCD) in the United States demonstrated that high haematocrit (Hct) increased the risks for VOC (Platt et al., 1991). Given that blood viscosity is Hct-dependent, it is generally assumed that high blood viscosity is mainly responsible for the occurrence of VOC and other VOC-like complications. However, in practice, blood viscosity is rarely measured in SCA and the contribution of abnormal haemorheology in this disease is ignored.

It is noteworthy that substantial criticisms have been addressed regarding the two clinical sub-phenotypes model and the involvement of haemolysis, and the subsequent decrease of NO bioavailability, in the development of SCA endothelial dysfunction (Bunn et al., 2010; Hebbel, 2011). Moreover, the two clinical/biological sub-phenotypes model developed in recent decades does not take into account the consequences of altered haemorheology in the development of several SCA complications (Kato et al., 2007; Gladwin & Vichinsky, 2008).

We recently investigated the relationships between haemorheological abnormalities and several acute and chronic complications of SCA (Nebor et al., 2011; Lamarre et al., 2012, 2013, In press-a; Connes et al., 2013b; Lemonne et al., 2013). Although increased Hct and red blood cell (RBC) deformability were found to be associated with two complications from the viscosity-vaso-occlusion phenotype: VOC (Lamarre et al., 2012) and osteonecrosis (Lemonne et al., 2013), we also reported that increased haemolytic rate/decreased Hct, reduced RBC deformability and abnormal RBC aggregation properties were associated with two complications associated with the haemolytic-endothelial dysfunction phenotype: leg ulcers (Connes et al., 2013b) and glomerulopathy (Lamarre et al., In press-a). Indeed, we suspect that the haemorheological profile and haemolytic profile of the patients could be inter-related to each other, thus contributing to the development of specific complications. We therefore investigated the relationships between haemorheological, haematological and haemolytic parameters in adults with SCA. Moreover, we also conducted rheological studies on RBC subpopulations in some patients with SCA to elucidate the associations between RBC deformability, RBC fragility and RBC aggregation.

Methods

Patients

This study used the biological data from the ‘Sickle cell haemorheology study’ (Lemonne et al., 2013; Lamarre et al., In press-a). Ninety-seven adults with SCA regularly followed by the Sickle Cell Centre at the Academic Hospital of Pointe-à-Pitre (Guadeloupe, French West Indies) were included in this study. All patients were in steady-state condition at the time of the study; no blood transfusions in the previous 3 months and absence of acute episodes (infection, VOC, ACS, stroke, priapism) for at least 2 months before inclusion into the study. Charts were retrospectively reviewed by two physicians to record all ACS and VOC episodes, as previously reported (Lamarre et al., 2012, 2013), within the previous year of blood sampling. The presence of proteinuria and osteonecrosis, as previously defined (Lemonne et al., 2013; Lamarre et al., In press-a), was also recorded for each patient. Finally, positive history of recurrent leg ulcers was also noted for each patient (Connes et al., 2013b). All patients were informed about the purpose and procedures of the study, and gave their written consent. 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).

Genetic parameters

Diagnosis of SCA was made by isoelectrofocusing (Multiphor II™ System, GE Healthcare, Buckinghamshire, UK), citrate agar electrophoresis, and cation-exchange high performance liquid chromatography (VARIANT™, Bio-Rad Laboratories, Hercules, CA, USA), and was confirmed by DNA studies (Tarer et al., 2006). Polymerase Chain Reaction (Gap-PCR) was used to detect six common α-thalassaemia deletions (Tan et al., 2001; Wang et al., 2009).

RBCs subpopulation

Plasma was removed and stored at 4°C after centrifugation of SCA blood collected in EDTA tubes, and the resulting RBC pellet was layered onto a discontinuous gradient of Percoll (GE Healthcare) with densities of 1.076 and 1.106. The RBC fraction between densities 1.076 and 1.106 (i.e. light fraction) corresponded to reticulocytes (20–30%) and discocytes (70–80%) while the RBC fraction at the bottom of the tube (density > 1.106; heavy fraction) was composed of intermediary dense cells and irreversibly sickled cells (Durpes 2014 John Wiley & Sons Ltd, British Journal of Haematology
et al, 2010). Each RBC fraction was washed three times in phosphate-buffered saline + 10 mmol/l glucose (pH 7.4 at 37°C, 300 mos mol/kg H2O) and resuspended in autologous plasma at adjusted Hct (40%) for haemorheological analysis.

Haematological and biochemical parameters

Blood samples were drawn after a 12-h overnight fast, between 8:00 a.m. and 10:00 a.m., and were immediately used for analyses.

Haematological parameters were determined using a haematology analyser (Max M-Retic, Coulter, Miami, FL, USA) and haemoglobin concentration (Hb) and reticulocytes were used for the purpose of the study. Measurements of the level of haemolytic markers (total bilirubin, lactate dehydrogenase [LDH], aspartate aminotransferase [AST]) were performed using standard biochemistry. A principal component analysis was used to derive a haemolytic component from the four haemolytic markers measured (i.e. total bilirubin, LDH, AST and reticulocytes expressed in percentage). This standard statistical data reduction approach uses conventional clinical measurements to explain the maximum-shared variance among these indirect measures of haemolysis (Nouraei et al, 2013). The haemolytic component has recently been demonstrated to reflect the rate of intravascular haemolysis assessed by measurements of the cell-free plasma haemoglobin (Nouraei et al, 2013).

Haemorheological parameters

Blood viscosity was measured after complete oxygenation of the blood, at native Hct, at 25°C and at a shear rate of 225/s using a cone/plate viscometer (Brookfield DVII+ with CPE40 spindle, Brookfield Engineering Labs, Natick, MA, USA) (Baskurt et al, 2009).

Red blood cell deformability was determined at 37°C at 30 Pascals (Pa) by laser diffraction analysis (ectacytometry), using the Laser-assisted Optical Rotational Cell Analyser (LORCA, RR Mechatronics, Hoorn, the Netherlands). The system has been described elsewhere in detail (Baskurt et al, 2009). Briefly, 25 ml of prepared blood suspension was mixed with 5 ml polyvinylpyrrolidone (PVP; viscosity = 30 cP) and sheared into a Couette system made of glass. The diffraction pattern was analysed by the computer and an elongation index was calculated. An increased elongation index indicates greater RBC deformability (Baskurt et al, 2009).

Red blood cell aggregation properties were determined at 37°C by laser backscatter method, using the LORCA, after adjustment of the Hct to 40% with autologous plasma (Hardeman et al, 2001; Baskurt et al, 2009). Blood was inserted into the Couette system of the LORCA and RBC aggregates strength was determined using a re-iteration procedure (Hardeman et al, 2001): seven separate pre-defined shear rates between 7.5/s and 800/s were applied on the RBC suspension, with or without alternating disaggregation shear rate, to locate the minimal shear rate needed to prevent RBC aggregation.

After RBC fractionation, each of the two RBCs subpopulations was also submitted to a stability test with RBCs being exposed to a constant shear stress (70 Pa) for 30 min: the RBC elongation index was measured every 2 min and the percentage of decrease of RBC elongation index between the beginning and the end of the stability test protocol was calculated, indicating the extent of RBC fragmentation (Baskurt et al, 2009). The guidelines for international standardization in blood rheology techniques/measurements were strictly followed (Baskurt et al, 2009).

Statistical analysis

Principal component analysis was used to derive the haemolytic component. Pearson correlation was used to test the relationship between the different biological parameters. For each biological parameter, the values were separated into two sub-sections: values < median (LOW) and values > median (HIGH): the chi-square test was used for categorical covariates to test the relationships between the LOW and HIGH categories of the different biological parameters. Unpaired student t test or Mann-Whitney test was used to compare the haemorheological parameters of the light and heavy RBC fractions. For both sub-groups with and without α-thalassaemia, Student t test was used to compare the biological parameters between patients with one of the complication studied and those without. Significance level was defined as \( P < 0.05 \). Analyses were conducted using SPSS (v. 20, IBM SPSS Statistics, Chicago, IL, USA).

Results

Relationships between haemolysis, haemoglobin and blood viscosity

As expected, there was a positive and significant relationship between Hb level and blood viscosity (\( r = 0.46; P < 0.001; \) Fig 1A). Significant relationships were also demonstrated between haemolytic component and Hb level (\( r = -0.47; P < 0.001; \) Fig 1B) or blood viscosity (\( r = -0.26; P < 0.05; \) Fig 1C). Nevertheless, no significant difference was found in the proportion of patients with LOW (< median) or HIGH (> median) haemolytic component in the LOW and HIGH blood viscosity groups (\( \chi^2 = 1.25; P = 0.26; \) 18.3% of patients had low haemolytic component but low blood viscosity, 31.7% had low haemolytic component and high blood viscosity, 24.4% had high haemolytic component and low blood viscosity and 25.6% had high haemolytic component but high blood viscosity. As previously described, α-thalassaemia was present in 41.3% of the present SCA cohort (Lemonne et al, 2013). While the frequency of α-thalassaemia was not different between the LOW and HIGH blood
Blood viscosity (cP) vs. Hb (g/l) for different groups of patients:

(A) Blood viscosity vs. Hb for SC and non-SC patients (r = 0.46; P < 0.001)

(B) Blood viscosity vs. Hb for SC patients with or without θ-thalassaemia (r = -0.47; P < 0.001)

(C) Blood viscosity vs. Hb for SC patients with or without θ-thalassaemia (r = -0.26; P < 0.05)

Fig 1. Relationships between (A) blood viscosity and haemoglobin (Hb) level, (B) Hb level and haemolytic component and (C) blood viscosity and haemolytic component.

Haemolysis and RBC elongation index (RBC deformability)

There was a significant and negative relationship between haemolytic component and RBC elongation index (r = -0.60; P < 0.001; Fig 2A). The frequency of θ-thalassaemia was higher in the HIGH RBC elongation index group (27.2% of the cohort) than in the LOW one (15.2% of the cohort; χ² = 3.86; P < 0.05). A previous comparison performed between patients with and without θ-thalassaemia (Lamarre et al., In press-a) showed higher RBC elongation in those with θ-thalassaemia (0.38 ± 0.08 vs. 0.34 ± 0.10; P < 0.05). The study of the haemorheological properties of the RBC fractions (n = 8 SCA patients) demonstrated that the light RBC fraction was more deformable than the heavy fraction (P < 0.001; Fig 2B). The same experiment was performed in five patients with Hb SC disease (SC) and in these cases, the light RBC fraction had greater elongation index than the heavy fraction (results not shown). The stability test was used to assess RBC fragmentation in the RBC fractions of 6/8 SCA patients (not determined in two SCA patients) and the five SC patients (Fig 2C,D). The raw decrease of the RBC elongation index over time was similar for both RBC fractions (Fig 2C). Nevertheless, when expressed as a percentage decrease from baseline, the heavy RBC fraction (i.e. with the lowest elongation index) exhibited a greater percentage of decrease of the RBC elongation index under shear stress exposure in comparison to the light RBC fraction (P < 0.05).

Relationship between RBC elongation index and RBC aggregates strength

A significant negative correlation between RBC elongation index and RBC aggregates strength was shown (r = -0.47; P < 0.001; Fig 3A). The previous comparison between patients with and without θ-thalassaemia (Lamarre et al., In press-a) showed lower RBC aggregates strength in those with θ-thalassaemia (264 ± 122 vs. 315 ± 147/s; P < 0.05). RBC aggregation experiments done on 5 SCA patients demonstrated a greater RBC aggregates strength in the heavy fraction than in the light fraction (P < 0.01; Fig 3B).

Relationships between biological parameters and clinical complications in patients with or without θ-thalassaemia

The whole population of SCA patients was divided into two subgroups according to θ-thalassaemia status. In each subgroup, we compared SCA patients with one of the studied complications to those without. The results are summarized in Table I. For both the θ-thalassaemic and non θ-thalassaemic patients, we found (i) a trend for SCA patients with a positive history of VOC to have higher RBC elongation index than SCA patients without (P < 0.1); (ii) higher Hb level, lower haemolytic component and higher RBC elongation index in patients with osteonecrosis compared to those without (P < 0.05 or P < 0.01; see Table I); (iii) lower RBC elongation index in patients with proteinuria compared to those without (P < 0.05). In addition, non θ-thalassaemic SCA patients with proteinuria had lower Hb and blood viscosity values, as well as increased RBC aggregates strength, than patients without proteinuria (P < 0.5). Although patients with θ-thalassaemia and recurrent leg ulcers had higher
haemolytic component, lower Hb level and decreased RBC elongation index \( (P < 0.05) \) than those without recurrent leg ulcers, no statistical difference was found between non \( \alpha \)-thalassaemic patients with recurrent leg ulcers and those without. The relationships between blood rheology and ACS were not investigated in this study because of the limited number of patients with recent positive history of ACS in each sub-group.

**Discussion**

The present study demonstrated that (i) patients with a high haemolytic component have decreased RBC elongation index (i.e. decreased deformability); (ii) both RBCs with the lowest elongation index (dense RBCs; i.e. irreversibly sickled cells) and RBCs with higher deformability (reticulocytes and reversibly sickled RBCs) are susceptible to fragmentation when exposed to high shear stress; (iii) RBCs with a low elongation index are prone to form resistant RBC aggregates; (iv) although blood viscosity, Hb level and haemolytic component may have high blood viscosity and patients with low haemolytic component may have low blood viscosity. Furthermore, our data suggests that haemorheological parameters could be modulated by unknown factors other than \( \alpha \)-thalassaemia, depending on the clinical complication considered.

Previous studies showed that patients with a high haemolytic rate could be prone to develop specific chronic complications, such as leg ulcers, priapism, cerebral vasculopathy, pulmonary hypertension and glomerulopathy (Gladwin *et al*., 2004; Kato *et al*., 2007; Bernaudin *et al*., 2008; Connes *et al*., 2013a,b; Lamarre *et al*., In press-a). The main mechanism proposed to date is that the release of a large amount of Hb and RBC arginase into the plasma of the patients would result in a significant decrease of NO bioavailability, causing impaired vasodilation and endothelial dysfunction (Kato *et al*., 2007). However, the present study is the first one to demonstrate that patients with high haemolytic rate (i.e. with high haemolytic component) also have decreased RBC deformability and increased RBC aggregates strength. The experiments on sickle RBCs of different densities showed...
that dense sickle RBCs have decreased elongation index and increased RBC aggregates strength. In addition, our results demonstrated that both dense and less dense sickle RBCs are susceptible to fragmentation under high shear stress exposure. These data suggest that increased haemolysis and decreased RBC deformability are related. These abnormalities may combine to develop the above complications by several processes: (i) decreased RBC deformability may cause mechanical obstruction at the entry of capillaries and decrease tissue perfusion. Baskurt et al (2004) previously demonstrated that a reduction of only 15% of RBC deformability causes a rise of vascular resistance of more than 75%; (ii) decreased RBC deformability has been demonstrated to cause increased mechanical stress applied on endothelial cells, promoting dysfunction and over-expression of vascular cell adhesion molecules, even in the absence of ischaemia and oxidative stress due to haemolysis (Mannino et al, 2012); (iii) increased RBC aggregates strength may increase vascular resistance, particularly at the level of microcirculation where RBC aggregates need to be dispersed to facilitate blood flow (Tripetto et al, 2009). Our data are in accordance with those recently reported by Bartolucci et al (2012) who demonstrated that the percentage of dense dehydrated RBCs was greater in patients with leg ulcers, priapism or renal dysfunction compared to those without. The authors reported significant positive relationships between the percentage of

Fig 3. (A) Relationship between RBC elongation index and RBC aggregates strength. (B) Comparison of RBC aggregates strength between high (heavy) and light density sickle RBCs.

Table 1. Relationships between biological parameters and clinical complications in patients with or without α-thalassemia.

| α-thalassemia sub-group | VOC + | PROT− | ULG− | OLG− | ULC+ | PROT− | ULG− | VOC+ | PROT− | OLC− | ULC− | VOC+ | PROT− | ULG− | VOC− | PROT− | OLC− | ULC− | VOC+ | PROT− | OLC− | ULC− | VOC+ | PROT− | OLC− | ULC− |
|------------------------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Blood viscosity (cP)   | 6.08  | 5.57  | 5.97  | 6.01  | 6.04 | 6.17  | 5.87  | 6.05  | 6.09  | 6.04 | 6.13  | 5.77  | 5.94  | 6.10  | 5.84  | 6.09  | 6.13  | 5.77  | 5.94  | 6.10  | 5.84  | 6.09  | 6.13  | 5.77  | 5.94  | 6.10  | 5.84  |
| RBC count (10^6/l)     | 4.32  | 4.79  | 4.32  | 4.32  | 4.32 | 4.32  | 4.32  | 4.32  | 4.32  | 4.32 | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  | 4.32  |
| Hemoglobin (g/l)       | 87    | 82    | 87    | 83    | 87   | 85    | 83    | 87    | 83    | 87   | 85    | 87    | 83    | 87    | 83    | 87    | 83    | 87    | 85    | 87    | 83    | 87    | 83    | 87    | 83    | 87    |
| Oxyhemoglobin (%)      | 66    | 63    | 66    | 63    | 66   | 65    | 63    | 66    | 63    | 65   | 66    | 63    | 66    | 63    | 66    | 63    | 66    | 63    | 65    | 66    | 63    | 66    | 63    | 66    | 63    | 66    |
| Elongation index (%)   | 80    | 77    | 80    | 77    | 80   | 77    | 80    | 77    | 80    | 77   | 80    | 77    | 80    | 77    | 80    | 77    | 80    | 77    | 80    | 77    | 80    | 77    | 80    | 77    | 80    | 77    |

Results are expressed as means (standard deviation). VOC, vaso-occlusive crisis; OSL, osteoartrosis; ULG, recurrent leg ulcers; PROT, proteinuria. The signs ‘<’ and ‘>’ define the absence or presence, respectively, of the complication. No information regarding recurrent leg ulcers was available for seven patients. For proteinuria, patients with micro-albuminuria were not included in the comparisons (see Lamarre et al (In press-a) for the definition used). Significant difference between patients with and without complications: *P < 0.05; **P < 0.01. Indicates a trend (P < 0.1).
dense RBCs and different haemolytic markers (LDH and bilirubin). The protective role of α-thalassaemia on several haemolytic complications, such as glomerulopathy (Nebor et al., 2010; Lamarrre et al., In press-a) or cerebral vasculopathy (Ballas, 2001; Bernaudin et al., 2008) may be explained, in part, by the positive effect of α-thalassaemia on RBC deformability and haemolysis. Nevertheless, our present study demonstrates that reduced RBC deformability may also be present in patients with α-thalassaemia and glomerulopathy. This finding indicates that factors other than α-thalassaemia can modulate the deformability of RBCs in SCA and play a role in the development of chronic complications. In addition, while we found higher haemolytic component, lower Hb level and reduced RBC deformability in non-α-thalassaemic patients with recurrent leg ulcers compared to those without this complication, we found no statistical difference between patients with recurrent leg ulcers and those without in the non-α-thalassaemic sub-group. The pathophysiology of leg ulcers is complex and others factors, such as venous incompetence (Clare et al., 2002) and inflammation (Bowers et al., 2013), may be involved in this complication. The reasons why sickle RBCs with decreased deformability have also increased aggregates strength are unknown but it was previously reported that, in healthy subjects, exposition of RBCs to oxidative stress lead to a drop in RBC deformability and a rise of RBC aggregates strength (Baskurt et al., 1998). The greater reduction of RBC deformability in patients without α-thalassaemia lead patients with glomerulopathy to also exhibit higher RBC aggregates strength than patients without proteinuria. Oxidative stress is usually enhanced in patients with SCA (Chirico & Pialoux, 2012) but further studies are required to test the impact of oxidative stress on sickle RBC rheology.

Patients with SCA and high blood viscosity are thought to be prone to vaso-occlusive-like complications such as VOC, ACS or osteonecrosis (Kato et al., 2007). Both Nebor et al. (2011) and Lamarrre et al. (2012) reported that both SCA adults and children exhibiting increased blood viscosity were at risk for painful VOC. Because vascular function is impaired in SCA, the vascular system may not cope well with an increase in blood viscosity (Lamarrre et al., In press-b), hence impairing blood flow (de Montalembert et al., 2007; Lamarrre et al., In press-b). In contrast, despite high Hct and Hb levels in SCA patients with osteonecrosis, blood viscosity was not different from that in patients without this complication (Lemonne et al., 2013). This lack of significant difference in blood viscosity between the two groups was due to compensation for the higher Hb level by the greater RBC deformability (due to the greater proportion of patients with α-thalassaemia) in the group with osteonecrosis (Lemonne et al., 2013). Although our present results confirm that blood viscosity is partly modulated by the Hb level ($R^2 = 0.21$) and the haemolytic rate ($R^2 = 0.07$), the associations were very weak (i.e. $R^2 < 0.5$). In addition, the stratification of our group of SCA patients into subgroups according to the median values showed that there is no difference in the proportion of patients with high blood viscosity (>median blood viscosity) between the subgroup with low haemolytic component (<median haemolytic component) and the subgroup with high haemolysis (>median haemolytic component). The decreased or increased RBC deformability in SCA patients with high or low haemolytic component, respectively, may partly offset the effects of decreased or enhanced Hb level, respectively, on blood viscosity. Indeed, complications of SCA considered to belong to the viscosity-vaso-occlusive phenotype (Kato et al., 2007) are not always characterized by increased blood viscosity, as previously indicated for osteonecrosis.

We have previously shown that increased RBC deformability could play a more significant role on vaso-occlusive like complications, such as VOC or osteonecrosis, than blood viscosity. Both Lamarrre et al. (2012) and Lemonne et al. (2013) found that increased RBC deformability was a significant and independent risk factor for VOC and osteonecrosis, respectively. While α-thalassaemia is clearly involved in the modulation of RBC deformability and in the development of these complications (Embury et al., 1982; Ballas et al., 1988; Lemonne et al., 2013), our results also suggest that increased RBC deformability could be a characteristic of non-α-thalassaemic patients at risk for VOC and osteonecrosis. The factors involved in the modulation of RBC deformability in non-α-thalassaemic patients remain to be determined. The implication of RBC deformability in VOC and osteonecrosis is poorly understood but Mohandas and Evans (1987) described that irregular deformable sickle discs had greater adhesion potential to endothelial cells than irreversibly rigid sickled RBCs (9% vs. 58% of adhering RBCs). Recently, numerical stimulations performed by Lei and Karniadakis (2013) on pre-existing data (Kaul et al., 1994) suggested that that young deformable sickle RBCs (mainly composed of reticuloocytes and discocytes) could be involved in the initiation phase of vaso-occlusion by interacting firmly with the vascular endothelium. However, it was also noted that as long as adherent RBCs remains deformable, they can be detached from the endothelial surface by the fluid forces in the microcirculation and the consequences of cell adhesion to vaso-occlusion could be minimal (Mohandas & Evans, 1987). If, however, the deformable adherent cells become deoxygenated before they are detached, they would lose their ability to deform and such cells could not be stripped away from the endothelial surfaces by fluid forces in the microcirculation. These adherent and undeformable cells indeed could have the potential to cause vaso-occlusion (Mohandas & Evans, 1987). Further studies are clearly needed to test the effects of RBC deformability on RBC vascular adhesion under flow.

In conclusion, our study brings new data that contribute to a better understanding of the SCA pathophysiological model developed in the past few years (Kato et al., 2007). We demonstrated that (i) the haemolytic biological phenotype is characterized by decreased RBC deformability and increased
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