Correlation between the Lactate Dehydrogenase Levels with Laboratory Variables in the Clinical Severity of Sickle Cell Anemia in Congolese Patients

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Abstract

Background
Sickle cell anemia is an inflammatory disease and is characterized by chronic hemolysis. We sought to evaluate the association of lactate dehydrogenase levels with specific clinical phenotypes and laboratory variables in patients with sickle cell anemia.

Methods
The present cross-sectional study was conducted in Sickle Cell Centre of Yolo in Kinshasa, the Democratic Republic of Congo. Two hundred and eleven patients with Sickle Cell Anemia in steady state were recruited. Seventy-four participants with normal Hb (Hb-AA) were selected as a control group.

Results
The average rates of hemoglobin, hematocrit, and red blood cells tended to be significantly lower in subjects with Hb-SS (p<0.001). The average rates of white blood cells, platelets, reticulocytes and serum LDH were significantly higher in subjects with Hb-SS (p<0.001). The average rates of Hb, HbF, hematocrit and red blood cells of Hb-SS patients with asymptomatic clinical phenotype were significantly higher than those of the two other phenotypes. However, the average rates of white blood cells, platelets, reticulocytes, and LDH of Hb-SS patients with symptomatic phenotype were significantly lower than those of the other two phenotypes.
patients with the severe clinical phenotype are higher than those of two other clinical phenotypes. Significant correlations were observed between Hb and white blood cell in severe clinical phenotype ($r_3 = -0.37^*$) between Hb and red blood cells in the three phenotypes ($r_1 = 0.69^* \ r_2 = 0.69, \ r_3 = 0.83^*$), and finally between Hb and reticulocytes in the asymptomatic clinical phenotype and severe clinical phenotype ($r_1 = -0.50^* \ r_3 = 0.45^*$). A significant increase in LDH was observed in patients with leg ulcer, cholelithiasis and aseptic necrosis of the femoral head.

**Conclusion**
The increase in serum LDH is accompanied by changes in hematological parameters. In our midst, serum LDH may be considered as an indicator of the severity of the disease.

**Introduction**

Sickle cell anemia (SCA) is one of the commonest genetic diseases worldwide [1]. SCA is an inflammatory disease and is characterized by chronic hemolysis [2–5].

The prevalence of sickle cell anemia is high in the Congolese population. The HBB’S allele frequency in neonates varies in the country, from 0.96% to 1.4% [6, 7]. However, there is no policy for early detection of the disease in the health care system and the diagnosis is delay in the majority of the patients [8]. It is well known that the early detection of SCA would provide the opportunity to implement adequate management to reduce the incidence of the acute crisis, transfusion rate and organ damage [9, 10].

An elevated serum of lactate dehydrogenase (LDH) was observed in the sickle cell patient population in steady state [11, 12]. The elevation of LDH was associated with hemolysis, pain crisis, pulmonary hypertension, leg ulcer, kidney damage and endothelial activation with elevated soluble vascular adhesion molecules [11–15]. However, SCA is clinically characterized clinically by a phenotypic polymorphism due to haplotype [16], genetics factors, fetal hemoglobin level [16, 17] and environmental factors [18]. All these factors may influence the severity of the disease and thus the level of LDH as shown in previous studies [13, 14, 19]. The identification of level of LDH may be considered as a marker of hemolysis and might be an important tool for the early detection of the severity of the disease in SCA individuals [13].

In the Democratic Republic of Congo (DRC), the Bantu haplotype is predominant and the Congolese SCA patients displayed low levels of fetal hemoglobin (HbF) and F-cells that contribute to the severity of SCA [20]. In addition, socio-economic conditions and the absence of a health care policy for the SCA patients exposed to severe forms [21, 22].

Despite this high prevalence and the risk severity of the disease, information about LDH in population suffering from SCA in DRC are unknown. The objective of this study was to investigate and determine the risk factors associated with clinical phenotypes among SCA individuals in steady state living in Kinshasa, the DRC.

**Materials and Methods**

**Ethical considerations**

All major participants provided written consent for study participation. Since some participants were minors, they provided oral assent and their legal guardians provided written
consent for study participation. This consent procedure and the study were reviewed and approved by the National Ethical Committee of the Public Health School of the University of Kinshasa, Kinshasa, and the DRC (ESP/CE/027B/2011), in compliance with the principles of the Helsinki Declaration II. The aim and the procedures of the study were explained to the participants. The participants were informed that they could withdraw anytime without further obligation. None of the authors collected samples. Samples were collected and sent to the authors by Research unit of Sickle cell Centre of Yolo. Anonymity of the participants was guaranteed and no personal details were recorded.

Study design and population

The present cross-sectional study was conducted in Sickle cell Centre of Yolo. These hospitals provide most of the non-private paediatrics beds in the DRC for sickle cell patients.

Study participants. The samples were collected from patients in steady state regularly followed up at the outpatient clinic of Sickle cell center of Yolo. All patients were free of pain for at one month and had not been hospitalized or transfused for at least 100 days before the study [23]. The starting number was randomly chosen from the first three in the section roll call. Every third patient was taken until the assigned number was reached.

We excluded subject with (i) initiated antibiotics treatment prior to seeking medical care; (ii) previous blood transfusion in the 3 months prior to the study (iii) under hydroxyurea (iv) under chronic transfusion program.

Data collection procedure and blood analysis

Five ml of venous blood sample was drawn from each study participant into an EDTA tube, used to determine hematologic parameters. Hematologic parameters were performed using an automate Sysmex XS—1000 i (Lincolnshire, USA).

Five ml of venous blood sample was drawn from each study participant into an EDTA tube, used to determine haemoglobin electrophoresis. Sickle cell screening was performed using semi-automated electrophoresis technique with the Hydrasis II apparatus (SEBIA, France). The electrophoresis technique separates hemoglobin in acid and alkaline agarose gel. SCA was diagnosed in presence of production of Hb S with no Hb-A. The concentrations were measured by an integrated densitometer.

For LDH assay, the samples were collected in dry tubes. The serum LDH assay was performed with a spectrophotometer at 340 μm with Thermo GENESYS 10S Bio apparatus (USA). The kit was provided by Cypress diagnostics (Landrop-Belgium). The reference values at 30°C were 160–320 U/L.

All analysis was performed at Institut National de Recherche Biomédicale (INRB) at Kinshasa, the DRC.

Case definitions

We conceive a clinical phenotype score built up by recording the individual scores related to the most relevant medical history parameters. The following definitions were applied: asymptomatic clinical phenotype (ACP) (score ≤ 5), moderate clinical phenotype (MCP) (score between 6 and 15), and severe clinical phenotype (SCP) (score ≥ 16) (Table 1).

Two hundred and eleven patients were suffering from SCA in steady state were recruited. All patients were homozygous for the β-globin S gene mutation (SS disease). Seventy-four patients with normal Hb (Hb-AA) were selected as a control group.
Results were manually entered into a microcomputer and analyzed using the Excel Version 2002 (CDC) and they were exported on SPSS 17.0 for further analysis. Data are represented as means ± SD when the distribution was normal and median with range when the distribution was not normal. The analysis of Student’s t-test was used for comparisons of means. ANOVA test were used to compare differences among categorical variables. Associations between variables and LDH was evaluated using chi-square and fisher exact test (for the cell with expected frequency less than 5 in two by two table more than 20%). Statistical significance level was set at p = 0.05.

### Results

#### Age

The mean age of the patients with SCA was 21.2 (SD = 10.7) years while that of the control group was 29.75 (SD = 15.4) years (Table 2). In the SCA group, the mean age of the patients with ACP was 25.9 (SD = 10.0) years while that of the MCP subgroup was 20.5 (SD = 11.2) years and 18.9 (SD = 9.14) for patient with SCP (Table 3).

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**Table 1. Clinical criteria and severity score based on phenotype.**

| Clinical criteria                           | Variables | Score (points) |
|--------------------------------------------|-----------|----------------|
| Days of hospitalization/year               | ≤ 1       | 0              |
|                                            | 2–7       | 2              |
|                                            | ≥ 8       | 5              |
| Severe vasoocclusive crisis/year           | 0         | 0              |
|                                            | 1–2       | 2              |
|                                            | ≥ 3       | 5              |
| Blood transfusion/year                     | 0         | 0              |
|                                            | 1–2       | 2              |
|                                            | ≥ 3       | 5              |
| Hip disease                                | Absent    | 0              |
|                                            | Present   | 5              |
| Leg ulcer                                  | Absent    | 0              |
|                                            | Present   | 5              |
| Hepatobiliary complications                | Absent    | 0              |
|                                            | Present   | 5              |
|                                            | Cholecystectomy | 2      |
| Neurologic events                          | Absent    | 0              |
|                                            | Present   | 5              |
| Renal disorders                            | Absent    | 0              |
|                                            | Present   | 5              |
| BMI                                        | 19–27     | 0              |
|                                            | < 19      | 2              |
| Total                                      | ≤ 5: ACP* (1) |              |
|                                            | 6–15: MCP** (2) |          |
|                                            | ≥ 16: SCP*** (3) |         |

*ACP: Asymptomatic clinical phenotype; MCP: Moderate clinical phenotype; SCP: Severe clinical phenotype; BMI = Body Mass Index.

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The mean rate of HbF in SCA group was 6.35%.

Gender

The sex-ratio male to female in the case group and control group was respectively 96/115 and 20/54 (Table 2). In the SCA group, the sex-ratio to female was respectively 11/32, 54/60 and 31/22 for patients with ACP, MCP and SCP (Table 3).

Relationship between haematological variables in both groups

The average rates of Hb, hematocrit, and red blood cells were significantly lower in subjects with Hb-SS than in Hb-AA subjects (Table 2). The average rates of white blood cells, platelets,

| Variables                  | Group 1 (Hb-SS) | Group 2 (Hb-AA) | p*  |
|----------------------------|-----------------|-----------------|-----|
| Age (years)                | 21.2±10.7       | 29.8±15.4       | 0.001|
| Hemoglobin (g/dL)          | 7.7±1.7         | 12.5±1.8        | 0.001|
| Hematocrit (%)             | 23.5±5.0        | 39.3±4.8        | 0.001|
| RBCs(x 10^6/μL)            | 3.0±0.8         | 4.9±0.6         | 0.001|
| WBCs (x 10^3/μL)           | 12.6±6.2        | 5.2±1.2         | 0.001|
| Reticulocytes (%)          | 15.5±10.5       | 0.8±2.3         | 0.001|
| Platelets (x 10^3/μL)      | 295.7±175.3     | 207.2±65.7      | 0.001|
| MCV (fl)                   | 80±11           | 81±7            | 0.6  |
| MCHC (g/dL)                | 33±2            | 32±1            | 0.001|
| LDH (U/L)                  | 827±296         | 283±92          | 0.001|

*Student test;
RBCs: Red Blood Cells; WBCs: White Blood Cells; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; LDH: Lactodehydrogenase

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| Variables                  | ACP n = 43      | MCP n = 115     | SCP n = 53     | p* (anova) |
|----------------------------|-----------------|-----------------|----------------|------------|
| Age (years)                | 25.9±10.0       | 20.5±11.2       | 18.9±9.1       | 0.003      |
| Hemoglobin (g/dl)          | 9.1±1.7         | 7.5±1.3         | 6.8±1.6        | 0.001      |
| Hb F (%)                   | 15.7±7.4        | 4.6±2.9         | 0.0            | 0.001      |
| Hematocrit                 | 28±5            | 23±4            | 21±5           | 0.001      |
| RBCs(x 10^6/μL)            | 3.7±0.9         | 3.0±0.7         | 2.6±0.7        | 0.001      |
| WBCs(x 10^3/μL)            | 8.9±4.1         | 13.0±6.3        | 15.0±6.0       | 0.001      |
| Reticulocytes (%)          | 11.3±9.1        | 16.2±10.3       | 17.6±11.3      | 0.009      |
| Platelets (x 10^3/μL)      | 249.1±104.4     | 288.4±157.4     | 349.1±236.9    | 0.02       |
| MCV (fl)                   | 79±12           | 79±10           | 84±12          | 0.01       |
| MCHC (g/dL)                | 33±1            | 33±2            | 33±2           | 0.96       |
| LDH (U/L)                  | 705±299         | 840±294         | 897±274        | 0.01       |

*Anova test;
ACP: Asymptomatic clinic phenotype; MCP: Moderate clinic phenotype; SCP: Severe clinic phenotype; HbF: Fetal hemoglobin; RBCs: Red Blood Cells; WBCs: White Blood Cells; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; LDH: Lactodehydrogenase

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reticulocytes and serum LDH were significantly higher in subjects with Hb-SS than in Hb-AA subjects (Table 2).

Relations between haematological variables in clinical phenotypes groups

The average rates of Hb, HbF, hematocrit and red blood cells of sickle cell patients with ACP clinical phenotype were significantly higher than those of the two other phenotypes MCP and SCP (Table 3). However, the average rates of white blood cells, platelets, reticulocytes, and LDH sickle cell patients with the SCP clinical phenotype SCP are higher than those of two other clinical phenotypes (Table 3).

Table 4 shows that significant correlations were observed between LDH and Hb in the ACP phenotype (r1 = -0.33 *) between the LDH and white blood cells in the ACP and MCP phenotypes (r1 = 0.51 **, * r2 = 0.30), between LDH and reticulocytes in the ACP phenotype (r1 = 0.37 *).

In relation with the Hb (Table 5), significant correlations were observed between Hb and white blood c in phenotype SCP (r3 = -0.37 *) between Hb and red blood cells in the three phenotypes (r1 = 0.69 *, r2 = 0.69, r3 = 0.83 *), and finally between Hb and reticulocytes in the ACP and SCP phenotypes (r1 = -0.50 *, r3 = 0.45 *).

Relationship between LDH and sickle cell complications

A significant increase in LDH was observed in patients with leg ulcer, cholelithiasis and aseptic necrosis of the femoral head in the SCP phenotype (Table 6).

Table 4. Correlation coefficients between LDH and haematological variables according clinical phenotype in the sickle cell study population.

| Variables             | ACP (r1) | MCP (r2) | SCP (r3) |
|-----------------------|----------|----------|----------|
| Hemoglobin (g/dl)     | -0.33*   | -0.11    | -0.09    |
| WBCs (x 10^3/μL)     | 0.51**   | 0.30*    | 0.25     |
| Platelets (x 10^3/μL)| -0.24    | 0.18     | -0.05    |
| Reticulocytes (%)     | 0.37*    | 0.06     | 0.003    |

**: significant correlation at the 0.01 level;
*: significant correlation at the 0.05 level;
WBCs: White blood cells; LDH: Lactodehydrogenase

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Table 5. Correlation coefficients between LDH and haematological variables according clinical phenotype in the sickle cell study population.

| Variables   | Correlation coefficient of Hb |
|-------------|------------------------------|
|             | ACP (r1) | MCP (r2) | SCP (r3) |
| WBCs (x 10^3/μL) | -0.19     | -0.15     | -0.37*    |
| RBCs (x 10^6/μL) | 0.69*     | 0.69*     | 0.83*     |
| Reticulocytes (%) | -0.50*    | -0.08     | 0.45*     |

*: Correlation significant at the 0.01 level;
WBCs: White blood cells; RBCs: Red blood cells

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Our study showed that the values of Hb, Ht and red blood cells of sickle cell patients are lower than those of non-SCA subjects. The low rate of Hb in sickle cell patients is due to chronic hemolysis [4, 11, 15].

However, between sickle cell phenotypes we observed a difference in these values. In addition, our study showed a significant correlation between Hb and the number of red blood cells in the three phenotypes. The high rate of Hb and red blood cells in patients with ACP phenotype can be due to the presence of fetal hemoglobin [24, 25]. HbF is a genetic factor that modulates the sickle cell phenotype. The presence of fetal hemoglobin, has the effect of reducing the concentration of HbS in the erythrocytes and in the sickling process [26–28]. This dual action of HbF has the advantage of increasing the residual rate of Hb and to increase the survival time of red blood cells [26, 27,29, 30].

The mean of HbF in our study population was 6.35%. This rate is similar to that reported in previous African studies [20, 24, 25, 31–33]. However, the rate of HbF in our cohort was slightly low in comparison with arabo-indian haplotypes [34, 35].

In this cohort, the number of white blood cells of sickle cell patients was higher compared to non-SCD subjects. The factor responsible for leukocytosis in SCD steady state is not known. The analysis of the three phenotypes showed that there was a disparity of the white blood cells values number. The values were significantly higher in the ACP clinical phenotype. It has been shown that the leukocyte is an important factor that leads to the hyperviscosity and the phenomenon of endothelial adhesion [36–38]. The leukocytosis is associated with increased morbidity in sickle cell disease and can explain the difference in clinical expression of the disease between the three phenotypes as reported in previous studies [39].

Sickle cell patients have a higher reticulocytosis compared to non-SCA subjects. The hyper reticulocytosis is the result of the chronic peripheral hemolysis [40, 41].

However, between the different clinical phenotypes, our study has shown that patients with the ACP phenotype had lower reticulocytes values than the other two clinical phenotypes. This low rate of reticulocytes could be probably due to the presence of HbF leading to reduce hemolysis and to increase the survival time of erythrocytes F cells. From a pathological view, reticulocytes are associated with a risk of vaso-occlusive accidents because of the risk of adhesion to vascular endothelium [42]. In our study, hyper reticulocytosis was associated with higher morbidity in patients with SCP clinical phenotype. Our results confirm and extend previous observations [11, 43].

Our study confirmed that the SCA had a higher platelet count than non-SCA subjects. The platelet activation is an important factor in the pathogenesis of vaso-occlusive crises [44, 45]. During their activation, platelets secrete thrombospondin involved in vaso-occlusion [46].

Table 6. Relationship between clinical phenotypes, LDH and sickle cell complications.

| Variables                | ACP n = 43 | MCP n = 115 | SCP n = 53 |
|--------------------------|------------|-------------|------------|
| LDH (U/l)                | 705±299    | 840±294     | 897±274    |
| VOC/year                 | < 1        | 1–3         | ≥ 4        |
| Transfusion/year         | < 1        | 1–2         | ≥ 3        |
| Cholelithiasis (%)       | 2          | 9.6         | 21.6       |
| Leg ulcers (%)           | 1.6        | 10.1        | 23.5       |
| Femoral head necrosis    | 3.6        | 15.9        | 36.0       |

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study showed that between the different clinical phenotypes, the SCP clinical phenotype had high morbidity in relation with platelets activation.

Our study confirmed that SCA patients in steady state had a higher level of serum LDH than non-SCA subjects. This elevation of serum LDH is due to oxidative stress associated with chronic hemolysis [13].

Between the different clinical phenotypes, our study has shown that patients with the ACP phenotype had lower LDH values than the other two clinical phenotypes. The low level of LDH in patients with ACP phenotype may be due to a reduction of the oxidative stress associated with hemolysis [11, 47]. Our results showed that the decrease in hemolysis of patients with the ACP clinical phenotype is characterized biologically by an increase of Hb, a high number of red blood cells and a lower reticulocytosis. In addition, modulation of fetal hemoglobin in clinical severity was also demonstrated. In contrast, elevated serum LDH is associated with the SCP clinical phenotype as shown in Table 3, with the decrease of Hb rate, red blood cells and with an elevation of white blood cells, reticulocytes and platelets count. These hematological variables are associated with a high risk for increased mortality [11, 13].

In contrast, we observed a significant correlation between LDH and Hb in the ACP phenotype ($r_1 = -0.33$) between LDH and white blood cells ($r_1 = 0.51$) and between LDH and reticulocytes ($r_1 = 0.37$). Several studies have shown that elevated levels of serum LDH was associated with hyperhemolysis, leg ulcers and acute chest syndrome [11, 13, 15].

Conclusion

The first study conducted in Central Africa carried out on the Bantu population showed that the severity of the disease can vary within a haplotype from one individual to another depending on modulating factors. Our study confirmed that SCA patients in steady state have a high rate of Serum LDH. In addition, there is a correlation between the level of LDH and the severity of the disease. In SCA patients, serum LDH may be considered as an indicator of the severity of the disease in our midst.

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Author Contributions

Conceived and designed the experiments: TMM PLT GML JJMT GM JMMM. Performed the experiments: TMM GML JJMT GM JMMM. Analyzed the data: TMM MNA PZA GM VR JMMM. Contributed reagents/materials/analysis tools: TMM JJMT GML JMMM VR GM. Wrote the paper: TMM GM VR PLT JMMM MNA.

References

1. Rees DC, Williams TN, Gladwin MT (2010) Sickle cell disease. Lancet 376: 2018–2031. doi: 10.1016/S0140-6736(10)61029-X PMID: 21131035
2. Bower SA, Reid HL, Greenidge A, Landis C, Reid M (2013) Blood Viscosity and the Expression of Inflammatory and Adhesion Markers in Homozygous Sickle Cell Disease Subjects with Chronic Leg Ulcers. Plos One 8: e68929. doi: 10.1371/journal.pone.0068929 PMID: 23922670
3. Hatzipantelis ES, Pana ZD, Gombakis N, Taparkou A, Tzimouli V, Kleta D, et al. (2013) Endothelial activation and inflammation biomarkers in children and adolescents with sickle cell disease. Int J Hematol 98:158–63. doi: 10.1007/s12185-013-1392-y PMID: 23807289
4. Conning V, Lamarré Y, Waltz X, Ballas SK, Lemonne N, Etienne-Julian M, et al. (2014) Haemolysis and abnormal haemorheology in sickle cell anaemia. Br J Haematol 164:279–39. doi:10.1111/bjh.12786

5. Saraf SL, Zhang X, Kania T, Lash JP, Molokie RE, Oza B, et al. (2014) Haemoglobinuria is associated with chronic kidney disease and its progression in patients with sickle cell anaemia. Br J Haematol 163:71–9. doi:10.1111/bjh.13690 PMID: 24329963

6. Agasa B, Bosungka K, Opara A, Tshilumba K, Dupont E, Vertongen F, et al. (2010) Prevalence of sickle cell disease in a northeastern region of the Democratic Republic of Congo: what impact on transfusion policy? Transfus Med 20:62–65. doi:10.1111/j.1365-3148.2009.00943.x PMID: 19712051

7. Tshilolo L, Aissi LM, Lukusa D, Kinsiaime C, Wembonyama S, Gulbis B, et al. (2009) Neonatal screening for sickle cell anaemia in the Democratic Republic of the Congo: experience from a pioneer project on 31 204 newborns. J Clin Pathol 62:35–38. doi:10.1136/jcp.2008.058958 PMID: 19103857

8. Aloni MN, Nkée L (2014) Challenge of managing sickle cell disease in a pediatric population living in kinshasa, democratic republic of congo: a sickle cell center experience. Hemoglobin 38:196–200. doi: 10.3109/03630269.2014.896810 PMID: 24669956

9. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN (2013) Global burden of sickle cell anaemia in children under five, 2010–2050: modelling based on demographics, excess mortality, and interventions. PLoS Med 10:e1001484. doi:10.1371/journal.pmed.1001484 PMID: 23874164

10. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN (2011) Sickle cell disease in Africa: a neglected cause of early childhood mortality. Am J Prev Med 41:S398–405. doi:10.1016/j.amepre.2011.09.013 PMID: 22099364

11. Taylor JG 6th, Nolan VG, Mendelsohn L, Kato GJ, Gladwin MT, Steinberg MH (2008) Chronic hyper-hemolysis in sickle cell anemia: association of vascular complications and mortality with less frequent vasoocclusive pain. PLoS One 3:e2095. doi: 10.1371/journal.pone.0002095 PMID: 18461136

12. Darbarin SD, Onyekweret O, Nouraie M, Minniti CP, Luchtman-Jones L, Rana S, et al. (2012) Markers of severe vaso-occlusive painful episode frequency in children and adolescents with sickle cell anemia. J Pediatr 160:286–290. doi:10.1016/j.jpeds.2011.07.018 PMID: 21890147

13. Kato GJ, McGonan V, Machado RF, Little JA, Taylor J, Morris CR, et al. (2006) Lactate dehydrogenase in Jamaica are important predictors of leg ulceration in sickle cell anaemia. Br J Haematol 133:570–578. doi:10.1111/j.1365-2457.2006.06074.x PMID: 16681647

14. Bonds DR (2005) Three decades of innovation in the management of sickle cell disease: the road to understanding the sickle cell disease clinical phenotype. Blood Rev 19:99–110. PMID:15603913

15. Bhatnagar P, Purvis S, Barron-Casella E, DeBaun MR, Casella JF, Arking DE, et al. (2011) Genome-wide association study identifies genetic variants influencing F-cell levels in sickle-cell patients. J Hum Genet 56:316–320. doi:10.15252/jhg.2011.09.013 PMID: 22099364

16. Smith WR, Coyne P, Smith VS, Mercier B (2003) Temperature changes, temperature extremes, and their relationship to emergency department visits and hospitalizations for sickle cell crisis. Pain Manag Nurs 4:106–11. PMID: 14566708

17. Van der Veer J, Vorstman J, Dijkhuizen MA, van der Meer R, van Engelen J, van der Grond J, et al. (2014) Vascular Reactivity of Sickle Cells and Hemoglobin S-Carbonyl Contents in In Vivo Situations. PLoS One 9:10.3109/03630263.2014.2295847 PMID: 22958547

18. Aloni MN, Nkée L (2014) Challenge of managing sickle cell disease in a pediatric population living in kinshasa, democratic republic of congo: a sickle cell center experience. Hemoglobin 38:196–200. doi: 10.3109/03630269.2014.896810 PMID: 24669956

19. Taylor JG 6th, Nolan VG, Mendelsohn L, Kato GJ, Gladwin MT, Steinberg MH (2008) Chronic hyper-hemolysis in sickle cell anemia: association of vascular complications and mortality with less frequent vasoocclusive pain. PLoS One 3:e2095. doi: 10.1371/journal.pone.0002095 PMID: 18461136

20. Darbarin SD, Onyekweret O, Nouraie M, Minniti CP, Luchtman-Jones L, Rana S, et al. (2012) Markers of severe vaso-occlusive painful episode frequency in children and adolescents with sickle cell anemia. J Pediatr 160:286–290. doi:10.1016/j.jpeds.2011.07.018 PMID: 21890147

21. Kato GJ, McGonan V, Machado RF, Little JA, Taylor J, Morris CR, et al. (2006) Lactate dehydrogenase in Jamaica are important predictors of leg ulceration in sickle cell anaemia. Br J Haematol 133:570–578. doi:10.1111/j.1365-2457.2006.06074.x PMID: 16681647

22. Bonds DR (2005) Three decades of innovation in the management of sickle cell disease: the road to understanding the sickle cell disease clinical phenotype. Blood Rev 19:99–110. PMID:15603913

23. Bhatnagar P, Purvis S, Barron-Casella E, DeBaun MR, Casella JF, Arking DE, et al. (2011) Genome-wide association study identifies genetic variants influencing F-cell levels in sickle-cell patients. J Hum Genet 56:316–320. doi:10.15252/jhg.2011.09.013 PMID: 22099364

24. Smith WR, Coyne P, Smith VS, Mercier B (2003) Temperature changes, temperature extremes, and their relationship to emergency department visits and hospitalizations for sickle cell crisis. Pain Manag Nurs 4:106–11. PMID: 14566708

25. Cumming V, King L, Fraser R, Serjeant G, Reid M (2008) Venous incompetence, poverty and lactate dehydrogenase in Jamaica are important predictors of leg ulceration in sickle cell anaemia. Br J Haematol 142:119–125. doi:10.1111/j.1365-2414.2008.07115.x PMID: 18477043

26. Tshilolo L, Summa V, Gregorj C, Kinsiaime C, Bazebos J, Avvisati G, et al. (2012) Foetal haemoglobin, erythrocytes containing foetal haemoglobin, and hematological features in congolese patients with sickle cell anaemia. Anemia 2012:105349. doi:10.1155/2012/105349 PMID: 22830000

27. De Montalembert M, Tshilolo L (2007) Is therapeutic progress in the management of sickle cell disease applicable in sub-Saharan Africa?. Med Trop (Mars) 67:612–6. PMID: 18300525

28. Wembonyama S, Mpaka S, Tshilolo L (2007) Medicine and health in the Democratic Republic of Congo: from Independence to the Third Republic. Med Trop (Mars). 67:447–57. PMID: 18225727

29. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN (2011) Sickle cell disease in Africa: a neglected cause of early childhood mortality. Am J Prev Med 41:S398–405. doi:10.1016/j.amepre.2011.09.013 PMID: 22099364

30. Aloni MN, Nkée L (2014) Challenge of managing sickle cell disease in a pediatric population living in kinshasa, democratic republic of congo: a sickle cell center experience. Hemoglobin 38:196–200. doi: 10.3109/03630269.2014.896810 PMID: 24669956
25. Omori CE (2005) The value of foetal haemoglobin level in the management of Nigerian sickle cell anemia patients. Niger Postgrad Med J. 12:149–54. PMID: 16160713

26. Akinsehaye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastiani P, et al. (2011) Fetal hemoglobin in sickle cell anemia. Blood 118:19–27. doi: 10.1182/blood-2011-03-325258 PMID: 21490337

27. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, editors. Disorders of Hemoglobin: Genetics, Pathophysiology, Clinical Management ( 2nd ed). Cambridge, United Kingdom: Cambridge University Press; 2009.

28. Rodgers GP, Steinberg MH (2001) Pharmacologic treatment of sickle cell disease and thalassemia: the augmentation of fetal hemoglobin. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, editors. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management ( 1st ed). Cambridge, United Kingdom: Cambridge University Press; p. 1028–1051. PMID: 12779271

29. Powars D, Weiss JN, Chan LS, Schroeder WA (1984) Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? Blood. 63:921–6. PMID: 6200161

30. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al (1994) Mortality in sickle cell disease Life expectancy and risk factors for early death. N Engl J Med. 330:1639–44. PMID: 7993409

31. Mouélé R, Galactéros F, Feingold J (1999) Haemoglobin F (HbF) levels in sickle-cell anaemia patients homozygous for the Bantu haplotype. Eur J Haematol. 63:136–7. PMID: 10480294

32. Enosolease ME, Ejele OA, Awodu OA (2005) The influence of foetal haemoglobin on the frequency of vaso-occlusive crisis in sickle cell anaemia patients. Niger Postgrad Med J. 12:102–5. PMID: 15997258

33. Olaniyi JA, Arinola OG, Odetunde AB (2010) Foetal Haemoglobin (HbF) status in adult sickle cell anemia patients in Ibadan, Nigeria. Ann Ib Postgrad Med. 8:30–3. PMID: 20562172

34. Rahimi Z, Karimi M, Haghsheenass M, Merat A (2003) Beta-globin gene cluster haplotypes in sickle cell disease patients from southwest Iran. Am J Hematol. 74:156–60. PMID: 14587041

35. Mukerjee MB, Surve RR, Gangakhedkar RR, Ghosh K, Colah RB, Mohanty D (2004) Beta-globin gene cluster haplotypes linked to the betaS gene in western India. Hemoglobin. 28:157–61. PMID: 15182059

36. Sakamato TM, Lanaro C, Ozelo MC, Garrido VT, Olalla-Saad ST, Conran N, et al. (2013) Increased adhesive and inflammatory properties in blood outgrowth endothelial cells from sickle cell anemia. Microvasc Res 90: 173–9. doi: 10.1016/j.mvr.2013.10.002 PMID: 24144783

37. Canalli AA, Franco-Penteado CF, Saad ST, Conran N, Costa FF (2008) Increased adhesive properties of neutrophils in sickle cell disease may be reversed by pharmacological nitric oxide donation. Haematologica 93:605–609. doi: 10.3324/haematol.12119 PMID: 18326523

38. Kasschau MR, Barabino GA, Bridges KR, Golan DE (1996) Adhesion of sickle neutrophils and erythrocytes to fibronectin. Blood 87:771–780. PMID: 855502

39. Sebastiani P, Nolan VG, Baldwin CT, Abad-Grau MM, Wang L, Adewoye AH, et al. (2007) A network model to predict the risk of death in sickle cell disease. Blood 110:2727–35. PMID: 17600133

40. Dhaliwal G, Cornell PA, Tierney LM Jr (2004) Hemolytic anemia. Am Fam Physician 69:2599–606. PMID: 15202694

41. Maier-Redelsperger M, Flahault A, Neonato MG, Girot R, Labie D (2004) Automated analysis of mature red blood cells and reticulocytes in SS and SC disease. Blood Cells Mol Dis. 33:15–24. PMID: 15233005

42. Sawadogo D, Tolo-Dilkébié A, Sangaré M, Aguéhoundé N, Kassi H, Latte T. (2004) Influence of the genotype of HbaS and HbaA1c for the vaso-occlusive crisis in sickle cell anaemia patients. Niger Postgrad Med J. 12:102

43. Novelli EM, Kato GJ, Ragni MV, Zhang Y, Hildesheim ME, Nouraie M, et al. (2012) Plasma thrombomodulin-1 is increased during acute sickle cell vaso-occlusive events and associated with acute chest syndrome, hydroxyurea therapy, and lower hemolytic rates. Am J Hematol 87:326–30. doi: 10.1002/ajh.22274 PMID: 22318901

44. Van der Land V, Peters M, Biemond BJ, Heijboer H, Hartveld CL, Fijnvandraat K. et al. (2013) Markers of endothelial dysfunction differ between subphenotypes in children with sickle cell disease. Thromb Res 132:712–7. doi: 10.1016/j.thromres.2013.10.006 PMID: 24182550