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# Hematological And Hemostasis Profile, Thrombotic Risk In Sickle Cell Patient In Cameroon : An Analytical Cross-Sectional Study

Romaric Tuono De Manfouo[1,2*], Josué Simo Louokdom[1,2], Rita Paule Tchouanmo Ngamgwe[1,2], Maryline Seuko Njopwouo[3], Pierre René Fotsing Kwetche[1,2,4]

[1]*Higher Institute of Health Sciences: Université des Montagnes, Bangangté, West-Cameroon*
[2]*Biomedical Sciences, Faculty of Health Sciences: Université des Montagnes, Bangangté, West-Cameroon*
[3]*District Hospital of Bangangté, West-Cameroon*
[4]*Laboratory of Cliniques Universitaires des Montagnes*

***Corresponding Author**: Romaric Tuono De Manfouo, Biomedical Sciences, Faculty of Health Sciences: Université des Montagnes, Bangangté, West-Cameroon.

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## Abstract

**Background :** Thrombosis is a disease characterized by the formation of blood clots inside the vessels. It is a situation commonly encountered during certain pathologies in general and sickle cell disease in particular during which it is associated with a very high risk of ischemic stroke. The purpose of this study is to determine the profile of blood count and thrombotic risk in sickle cell patients.

**Methodology :** We conducted an analytical cross-sectional study at the Bafoussam Regional Hospital over 03 months. The study population consisted of consecutively recruited homozygous sickle cell and sickle cell traits. Blood samples from the latter were collected and sent to the laboratory for the completion of the blood count and hemostasis profile. The results obtained were analyzed using statistical software R version 4.1.1. Univariate logistic regression analyses were used to identify factors associated with thrombotic risk in our study population.

**Results :** The size of our population was 148 patients including 80 sickle cell patients and 68 with sickle cell trait AS. The frequency of thrombotic risk in the population was 35.1% with 52,5% in SS and 14.7%% in AS ($p$=0.002). The mean fibrinogen in SS and AS was 5.32±0.50 g/L and 4.63±0.00 g/L respectively ($p$<0.001). The mean Quick Time was 11.8±1.94 s for SS and 12.2±2.72 s for AS ($p$=0.336). The mean cephalin time and activator were 23.3±5.90 s for SS and 24.3±7.89 s for AS with and ($p$=0.395), respectively. The mean D-dimer in SS was 1.88±2.09 mg/L and 1.12±2.40 mg/L in AS ($p$=0.045). The mean hemoglobin in SS was 7.13±1.41 g/dL and in AS was 14.4±2.57 g/dL ($p$<0.001). Thrombocytosis is positively associated with thrombotic risk (Or = 2.52, 95% CI = [1.13; 5.63] ; ($p$=0.022)). Moreover, 'AS' status is beneficial for the occurrence of thrombotic risk unlike SS (Or = 0.16, 95% CI = [0.07; 0.35]; ($p$=0.001). In addition, elevated fibrinogen is a risk factor for thrombotic risk (OR=2.50, 95% CI = [4.99; 1.25]; $p$=0.009).

**Conclusion :** Given the weight of sickle cell disease and its complex pathophysiology, knowledge, and understanding of thrombotic risk is imperative to guide therapeutic strategies in the management and clinical management of patients. These data highlight the need for regular and systematic hemostasis assessment in sickle cell patients to prevent possible risks of thrombosis.

***Keywords:*** *thrombotic risk, hematological profile, hemostasis profile, sickle cell patient.*

## Introduction

Thrombosis is a disease characterized by the formation of blood clots inside the vessels. These clots, also called thrombus, can form in veins or arteries [1]. This problem is of particular concern in several cardiovascular diseases such as diabetes, and even genetic diseases such as sickle cell anemia. Indeed, sickle cell anemia, an autosomal

recessive disease, results in a structural alteration of hemoglobin, causing chronic lysis of red blood cells and leading to various clinical consequences [2,3]; Among which stand out vaso-occlusive crises, priapism, anemia, aplastic seizures, and splenic sequestration, all elements that significantly alter the quality of life of patients [4–6]. The prevalence of sickle cell disease is marked by substantial figures: in the United States, between 70,000 and 100,000 individuals are affected by this pathology, mainly of black or African descent [6]. In Brazil, approximately 3,500 children are born each year with sickle cell disease, giving this genetic disease worldwide recognition as one of the most widespread and impactful on morbidity and mortality [7,8]. In addition, Cameroon annually records about 4,000 births of sickle cell patients, according to WHO data [9].

The complex pathophysiology of this genetic disease causes abnormal activation of the coagulation and fibrinolysis cascade, which contributes to the increased risk of thrombotic manifestations [10]. In particular, vaso-occlusive crises result from the obstruction of small blood vessels because of venous stasis by deformed red blood cells; this results in poor oxygenation of specific organs. In addition, venous stasis is also reported to promote hypercoagulability and the formation of thrombosis. Studies conducted by several authors have detailed the mechanisms underlying this hypercoagulability in the context of sickle cell disease [11,12]. However, despite the possible low mortality rate of thrombosis, it requires vigilant medical attention due to its potentially serious complications, including venous thromboembolism (VTE), pulmonary embolism (PE), and deep vein thrombosis (DVT). This concern is noted by the work of Noubiap et *al*. (2018), highlighting the increased risk of venous thromboembolism in sickle cell patients [1]. In addition, sickle cell disease also influences arterial circulation, which can lead to devastating consequences, including strokes and silent heart attacks [1].

Given the magnitude of sickle cell disease and its complex pathophysiology, a better understanding of thrombotic risk is of paramount importance to guide therapeutic strategies and clinical management of patients. However, despite this importance, data on thrombotic risk in sickle cell patients remains to be completed. Therefore, the present study aims to comprehensively characterize the profile of blood count and hemostasis in individuals with sickle cell disease, to accurately assess their level of thrombotic risk.

## Methodology

### *Study Design*

We conducted an analytical cross-sectional study over 03 months from August 1 to October 30, 2022, at the Bafoussam Regional Hospital. The recruitment of patients was carried out in the hematology and internal medicine departments of the hospital. The study population consisted of people with sickle cell disease and patients with sickle cell trait who were regularly followed in the hospital. Sampling was consecutive and participants whose consent was obtained were included in the study. Patients on anticoagulant therapy were not included. A questionnaire was developed to collect the socio-demographic and clinical-biological characteristics of participants.

### *Biology Casework Analysis*

A volume of 3-4 mL of blood was collected in tubes containing EDTA (Ethylene Diamine Tetra Acetic) and 3.2% citrate tubes in participants enrolled in the study. Each tube of blood sample collected was sent to the Hematology laboratory within one hour for analysis.

### *Exploration of hemostasis and thrombotic risk*

First of all, the determination of D-dimer was carried out according to the 'Finecare' method using the principle based on immunofluorescence. Subsequently, the determination of fibrinogen and Quick's Time were carried out by automatic method using the coagulmeter 'Sinothinker' according to the kit 'CYPRESS' and Biolabo respectively. The prothrombin level was derived from the quick time from the Thivolle calibration curve and then the patient's INR was inferred according to 'INR = (TQ patient (in s) / TQ control (in s)) $^{ISI}$. ISI = International Sensitivity Index. Finally, the determination of the Cephalin Time and Activator was carried out by automatic method according to the 'Biolabo' kit.

***NB:*** *The thrombotic risk was defined for D-dimer values greater than 0.5 mg/L.*

### *Hematological parameters*

Cellulose acetate band hemoglobin electrophoresis was performed to confirm the status of each participant. The study of hematological parameters was made on a hematology automaton 'Sinothinker' with nineteen (19) parameters. The blood count was systematically accompanied by blood smears to assess the quality of blood cells and the vital staining to classify cases of anemia and determine their origin. The slides colored with May Grunwald Giemsa and bright cresyl blue were read using the Olympus

binocular microscope. The results obtained were interpreted according to WHO's usual values.

### *Data processing and statistical analysis*

The data collected was saved in Microsoft Excel 2016 software. Statistical analysis of the data was performed using the statistical tool R version 4.1.1. The variables studied were sociodemographic and clinical characteristics: age, sex, clinical and medical history, thrombotic risk (defined for D-dimer values greater than 0.5 mg/L); biological variables: prothrombin level, Quick Time, Cephalin Time and Activator, D Dimers, fibrinogen, International Normalized Ratio, platelets, hemoglobin, red blood cells, white blood cells, average blood cell volume, lymphocytes, monocytes, granulocytes. Qualitative variables were presented as frequency while quantitative variables were presented as a mean ± standard deviation. The comparison of proportions was made with the chi$^2$ test when the expected numbers were greater than 5 and the Fisher test when not. Student's test was used to compare the mean between different groups in our study population. Univariate logistic regression analyses were used to identify factors associated with thrombotic risk in our study population. All these tests were done at a risk threshold α=5%.

### *Ethical considerations*

This study was approved by the Institutional Committee of Ethics and Research of the University of the Mountains (AUTHORIZATION Nº. 2022/142/UdM/PR/CIE). Authorization to collect data and analyze samples from eligible patients has been obtained from Bafoussam Regional Hospital (Nº. 870/L/MINSANTE/SG/DRSPO/HRB/D). Before starting the study, participants were given a newsletter about the objectives of the study, its benefits, and risks. For eligible participants, we obtained their consent by signature. The confidentiality of the research results was respected by the use of a unique code for each patient.

## Results

This study involved 148 patients of whom 68 (45.9%) were AS and 80 (54.1%) were SS. The average age of the total population was 22.6±17.8 of which 36.5±17.4 for AS and 11±5.4 for SS. Our study population was mainly from the Mifi-Ouest Cameroon department, i.e. 70 (87.5%) for SS and 62 (91.2%) for AS patients. All the sickle cell population received frequent blood transfusions. The sex ratio was 0.76 in favor of women, of which 0.62 were in favor of AS and 0.9 in favor of SS.

### Distribution of the study population by sociodemographic and clinicobiological characteristics.

### By socio-demographic characteristics

The following table describes the socio-demographic characteristics of the study population.

***Table 1.*** Sociodemographic characteristics of sickle cell patients who visited HRB during the study period.

| *Parameters* | *SS N= 80 n (%)* | *AS N= 68 n (%)* | *Total N= 148 n (%)* | *P-Value* |
|---|---|---|---|---|
| **Sex** | | | | 0,33 |
| **Women** | 42 (52.5) | 42 (61.8) | 84 (56.8) | |
| **Men** | 38 (47.5) | 26 (38.2) | 64 (43.2) | |
| **AGE, Mean±sd** | 11.1±5.48 | 36.7±17.4 | 22.9±17.8 | <0.001 |
| **Departments** | | | | 0.017 |
| **Mifi** | 70 (87.5) | 62 (91.2) | 132 (89.2) | |
| **Hauts plateaux** | 4 (5) | 2 (2.9) | 6 (4.1) | |
| **Koung-Khi** | 2 (2.5) | 0 (0.00) | 2 (1.30) | |
| **Menoua** | 2 (2.5) | 0 (0.00) | 2 (1.30) | |
| **Noun** | 2 (2.5) | 4 (5.9) | 6 (4.1) | |

***n= effective; %= frequency**; M: Mean, **Sd**: Standard deviation; Student test; patients from the following departments were not received during the study: Bamboutos, Haut Kam, Nde, * significant difference at p < 0.05.*

### According to clinicobiological characteristics

The table above describes the clinicobiological characteristics of the study population :

***Table 2 :*** Clinicobiological characteristics of the population

| *Parameters* | *SS N= 80 n (%)* | *AS N= 68 n (%)* | *Total N= 148 n (%)* | *P-Value* |
|---|---|---|---|---|
| **Medical history** | 4 (5.00) | 4 (5.88) | 8 (5.41) | 1.000 |
| **Family history** | | | | |
| **Diabetes** | 2 (2.50) | 0 (0.00) | 2 (1.35) | 0.525 |
| **Surgery** | 8 (10.0) | 14 (20.6) | 22 (14.9) | |
| **Oedema** | 14 (17.5) | 6 (8.82) | 20 (13.5) | |
| **Obesity** | 0 (0.00) | 2 (2.94) | 2 (1.35) | |
| **Lung diseases** | 10 (12.5) | 2 (2.94) | 12 (8.11) | |
| **Inflammatory diseases** | 2 (2.50) | 2 (2.94) | 4 (2.70) | |

***n= effective ; %= frequency ;***

It emerges from this table that 5.00 % of SS had a medical history compared to 5.88 % of AS.

### Profile and disorders of hemogram

The mean hemoglobin in SS patients was 7.28±1.58 g/dL and 14.8±4.00g/dL in AS patients ($p$<0.001). The mean platelet in SS patients was 818±334 G/L and in AS patients 380±153G/L ($p$<0.001). The following table presents the profile and disorders of hemogram in the population according to the red and platelet lineage on the one hand (Table III) and the white lineage on the other hand (Table IV):

***Table 3 :*** Profile and disorders of hemogram in the population by red lineage

| *Parameters* | | *SS N= 80 n (%)* | *AS N= 68 n (%)* | *Total N= 148 n (%)* | *P-Value* |
|---|---|---|---|---|---|
| **RBC, Moy±sd (T/L)** | | 2.45±0.52 | 7.28±14.7 | 4.70±10.3 | 0.009 [a] |
| **RBC** | **Low** | 76 (97.4) | 4 (5.88) | 80 (54.8) | <0.001 [b] |
| | **Normal** | 2 (2.56) | 56 (82.4) | 58 (39.7) | |
| | **High** | 0 (0.00) | 8 (11.8) | 8 (5.48) | |
| **HGB, Mean±sd (g/dl)** | | 7.13±1.41 | 14.4±2.57 | 10.5±4.14 | <0.001 [a] |
| **Hb** | **Low** | 80 (100) | 8 (11.8) | 88 (59.5) | <0.001 [b] |
| | **Normal** | 0 (0.00) | 60 (88.2) | 60 (40.5) | |
| **HTC, Mean ±sd (%)** | | 23.6±3.74 | 41.3±5.86 | 31.6±10.0 | <0.001 [a] |
| **HTC** | **Low** | 80 (100) | 8 (12.1) | 88 (60.3) | <0.001 [b] |
| | **Normal** | 0 (0.00) | 54 (81.8) | 54 (37.0) | |
| | **High** | 0 (0.00) | 4 (6.06) | 4 (2.74) | |
| **MCV, Mean±sd (fL)** | | 94.8±11.3 | 87.0±7.66 | 91.2±10.5 | <0.001 [a] |
| **MCV** | **Low** | 10 (12.5) | 4 (6.06) | 14 (9.59) | <0.001 [b] |
| | **Normal** | 36 (45.0) | 62 (93.9) | 98 (67.1) | |
| | **High** | 34 (42.5) | 0 (0.00) | 34 (23.3) | |
| **MCH, Mean±sd (g/dl)** | | 28.8±2.98 | 29.5±3.82 | 29.1±3.39 | 0.253 [a] |
| **MCH** | **Low** | 18 (22.5) | 8 (12.1) | 26 (17.8) | 0.197 [b] |
| | **Normal** | 62 (77.5) | 58 (87.89) | 120 (82.15) | |
| **MCHC, Mean±sd (pg/C)** | | 30.4±2.42 | 34.0±1.96 | 32.1±2.85 | <0.001 [a] |
| **MCHC** | **Low** | 64 (80.0) | 10 (15.2) | 74 (50.7) | <0.001 [b] |
| | **Normal** | 16 (20.0) | 56 (84.8) | 72 (49.3) | |

*RBC: red blood cells, HB: hemoglobin, MCH: Mean Corpuscular Hemoglobin Concentration, MCHC: Mean Corpuscular Hemoglobin Content, MCV: Mean Corpuscular Volume, HTC: hematocrit, WBC: white blood cells, LYM: lymphocytes, GRAN: granulocytes; [a]= student test; Fisher test; Sd: Standard deviation ; * significant difference at p < 0.05.*

***Table 4 :*** Profile and disorders of hemogram in the population by white and platelet lineage

| *Parameters* | | *SS N= 80 n (%)* | *AS N= 68 n (%)* | *Total N= 148 n (%)* | *P-Value* |
|---|---|---|---|---|---|
| **WBC, Mean ±sd (G/L)** | | 17.9±12.6 | 6.28±2.66 | 12.6±11.0 | <0.001 [a] |
| **WBC** | **Low** | 0 (0.00) | 4 (5.88) | 4 (2.70) | |
| | **Normal** | 14 (17.5) | 62 (91.2) | 76 (51.4) | <0.001 [b] |
| | **High** | 66 (82.5) | 2 (2.94) | 68 (45.9) | |
| **LYM, Mean ±sd (10*3/µL)** | | 12.7±13.4 | 2.91±1.51 | 8.23±11.0 | <0.001 [a] |
| **LYMPH** | **High** | 72 (92.3) | 6 (9.09) | 78 (54.2) | <0.001 [b] |
| | **Normal** | 6 (7.69) | 60 (90.9) | 66 (45.8) | |
| **Monocytes, Mean ±sd (10*3/µL)** | | 2.97±1.91 | 1.65±2.77 | 2.37±2.43 | 0.001 [a] |
| **Monocytes** | **High** | 54 (69.3) | 6 (9.09) | 60(41.7) | <0.001 [b] |
| | **Normal** | 24 (30.8%) | 60 (90.9%) | 84 (58.3%) | |
| **GRAN, Mean ±sd (10*3/µL)** | | 5.18±2.48 | 3.61±7.76 | 4.46±5.60 | 0.120 [a] |
| **GRAN** | **Low** | 0 (0.00) | 28 (42.4) | 28 (19.4) | |
| | **Normal** | 68 (87.2) | 36 (54.5) | 104 (72.2) | <0.001 [b] |
| | **High** | 10 (12.8) | 2 (3.03) | 12 (8.33) | |
| **Platelets, Mean ±sd (G/L)** | | 809±329 | 375±150 | 609±340 | <0.001 [a] |
| **PLT:** | **Normal** | 8 (10.0) | 38 (55.9) | 46 (31.1) | <0.001 [b] |
| | **Thrombocytosis** | 72 (90.0) | 30 (44.1) | 102 (68.9) | |

*WBC: white blood cells, LYM: lymphocytes, GRAN: granulocytes; [a]= student test; [b]=Fisher test; * significant difference at p < 0.05; PLT= platelets; Sd: Standard deviation ;*

It appears from the above table that 100% of SS patients and 11.8 % of AS patients had anemia ($p$<0.001) ; 90.0% of SS patients and 44.1% of AS patients experienced thrombocytosis ($p$<0.001).

**Distribution of our population according to thrombotic risk and profile of hemostasis disorders**

Thrombotic risk was assessed in patients. 52 (35.1%) of the population had a thrombotic risk, including 42 (52.5%) SS and 10 (14.7%) AS patients ($p$=0.002). The following table presents the profile and disorders of hemostasis parameters in the population :

***Table 5 :*** Haemostasis disorders in sickle cell patients

| *Parameters* | | *SS N= 80 n (%)* | *AS N= 68 n (%)* | *Total N= 148 n (%)* | *P-Value* |
|---|---|---|---|---|---|
| **QT (s) *M±Sd*** | | 11.8±1.94 | 12.2±2.72 | 12.0±2.33 | 0.336 [a] |
| **QT(s)** | **Low** | 26 (32.5) | 20 (29.4) | 46 (31.1) | |
| | **Normal** | 50 (62.5) | 44 (64.7) | 94 (63.5) | 0.924 [b] |
| | **High** | 4 (5.00) | 4 (5.88) | 8 (5.41) | |
| **Ratio TP (s) *M±Sd*** | | 1.16±0.19 | 1.19±0.27 | 1.17±0.23 | 0.336 [a] |
| **Ratio QT(s)** | **Normal** | 10 (12.5) | 10 (14.7) | 20 (13.5) | 0.881 [b] |
| | **High** | 70 (87.5) | 58 (85.3) | 128 (86.5) | |
| **TCA (s) *M±Sd*** | | 23.3±5.90 | 24.3±7.89 | 23.7±6.88 | 0.395 [a] |
| **TCA(s)** | **Low** | 50 (62.5) | 46 (67.6) | 96 (64.9) | |
| | **Normal** | 30 (37.5) | 18 (26.5) | 48 (32.4) | 0.042 [b] |
| | **High** | 0 (0.00) | 4 (5.88) | 4 (2.70) | |
| **Ratio TCA (s) *M±Sd*** | | 1.10±0.28 | 1.15±0.37 | 1.13±0.33 | 0.395 [a] |
| **Ratio TCA (s)** | **Low** | 4 (5.00) | 4 (5.88) | 8 (5.41) | |
| | **Normal** | 58 (72.5) | 44 (64.7) | 102 (68.9) | 0.575 [b] |
| | **High** | 18 (22.5) | 20 (29.4) | 38 (25.7) | |
| **Fibrinogen (g/l)** | | 5.32±0.50 | 4.63±0.00 | 5.00±0.50 | <0.001* [a] |
| **Fibrinogen (g/l)** | **Low** | 2 (2.50) | 0 (0.00) | 2 (1.35) | |
| | **Normal** | 6 (7.50) | 0 (0.00) | 6 (4.05) | 0.020 [b] |
| | **High** | 72 (90.0) | 68 (100) | 140 (94.6) | |
| **D-Dimer (mg/L) *M±Sd*** | | 1.88±2.09 | 1.12±2.40 | 1.53±2.27 | 0.045 [a] |
| **D-Dimer (mg/l)** | **Normal** | 38 (47.5) | 58 (85.3) | 96 (64.9) | <0.001 [b*] |
| | **High** | 42 (52.5) | 10 (14.7) | 52 (35.1) | |

*QT: Quick Time, TP: Prothrombin Level, TCA: Activated Cephalin Time; [b]=Fisher test; M: Mean, Sd: Standard deviation; [a]= student test ; * significant difference at p < 0.05.*

It emerges from this table that 32.5% of SS patients and 29.4% of AS presented a shortened Quick Time; 62.5% of SS and 67.6% of AS had a shortened ACT ($p$=0.042); 52.5% of SS and 14.7% of AS had high D-dimer levels ($p$<0.001). In addition, the mean fibrinogen (g/L) in SS patients was 5.32±0.50 g/L and 4.63±0.00 g/L in AS patients ($p$<0.001)

**Factors associated with thrombotic risk**

The frequency of thrombotic risk was 35.1%. The following table describes the factors associated with thrombotic risk in the population during the study:

***Table 6 :*** Factors associated with thrombotic risk

| | *Parameters* | *Yes N=52 n(%)* | *No-N=96 n(%)* | *Total N=148 n(%)* | *OR [IC 95%]* | *p-value_OR* | *p-value* |
|---|---|---|---|---|---|---|---|
| **Status:** | **SS** | 42 (80.8) | 38 (39.6) | 80 (54.1) | Ref. | Ref. | <0.001 |
| | **AS** | 10 (19.2) | 58 (60.4) | 68 (45.9) | 0.16 [0.07;0.35] | <0.001 | |
| **SEX:** | **Women** | 28 (53.8) | 56 (58.3) | 84 (56.8) | Ref. | Ref. | 0.725 |
| | **Men** | 24 (46.2) | 40 (41.7) | 64 (43.2) | 1.20 [0.61;2.37] | 0.603 | |
| **QT:** | **Low** | 18 (34.6) | 28 (29.2) | 46 (31.1) | Ref. | Ref. | |
| | **High** | 2 (3.85) | 6 (6.25) | 8 (5.41) | 0.52 [0.09;2.86] | 0.487 | 0.734 |
| | **Normal** | 32 (61.5) | 62 (64.6) | 94 (63.5) | 0.80 [0.39;1.67] | 0.560 | |
| **TCA** | **Low** | 38 (73.1) | 58 (60.4) | 96 (64.9) | Ref. | Ref. | |
| | **High** | 0 (0.00) | 4 (4.17) | 4 (2.70) | 0.00 [0.00;.] | 0.142 | 0.179 |
| | **Normal** | 14 (26.9) | 34 (35.4) | 48 (32.4) | 0.63 [0.30;1.32] | 0.226 | |
| **Fibrinogen, Mean±sd** | | 5.15±0.55 | 4.92±0.45 | 5.00±0.50 | 2.50 [4.99;1.25] | 0.009 | 0.013 |
| **Fibrinogen:** | **Low** | 46 (88.5) | 94 (97.9) | 140 (94.6) | Ref. | Ref. | |
| | **High** | 4 (7.69) | 2 (2.08) | 6 (4.05) | 4.09 [0.72;23.1] | 0.123 | 0.040 |
| | **Normal** | 2 (3.85) | 0 (0.00) | 2 (1.35) | . [.;.] | 0.113 | |
| **D-Dimer, Mean ±sd** | | 3.56±2.87 | 0.43±0.23 | 1.53±2.27 | . [.;0.00] | 0.983 | <0.001 |
| **D-Dimer:** | **High** | 52 (100) | 0 (0.00) | 52 (35.1) | Ref. | Ref. | <0.001 |
| | **Normal** | 0 (0.00) | 96 (100) | 96 (64.9) | 0.00 [0.00;.] | 0.000 | |
| **WBC, Mean ±sd** | | 18.1±15.4 | 9.55±5.92 | 12.6±11.0 | 1.14 [1.21;1.07] | <0.001 | <0.001 |

| | Parameters | Yes N=52 n(%) | No-N=96 n(%) | Total N=148 n(%) | OR [IC 95%] | p-value_OR | p-value |
|---|---|---|---|---|---|---|---|
| W | WBC: Low | 0 (0.00) | 4 (4.17) | 4 (2.70) | Ref. | Ref. | <0.001 |
| | WBC: High | 38 (73.1) | 30 (31.2) | 68 (45.9) | . [.;.] | 0.045 | |
| | WBC: Normal | 14 (26.9) | 62 (64.6) | 76 (51.4) | . [.;.] | 0.456 | |
| | RBC, Mean±sd | 2.70±0.86 | 5.80±12.7 | 4.70±10.3 | 0.39 [0.56;0.28] | <0.001 | 0.020 |
| | RBC: Low | 44 (84.6) | 36 (38.3) | 80 (54.8) | Ref. | Ref. | |
| | RBC: High | 0 (0.00) | 8 (8.51) | 8 (5.48) | 0.00 [0.00;.] | 0.003 | <0.001 |
| | RBC: Normal | 8 (15.4) | 50 (53.2) | 58 (39.7) | 0.13 [0.06;0.31] | <0.001 | |
| | HGB, Mean ±sd | 8.37±2.86 | 11.6±4.30 | 10.5±4.14 | 0.80 [0.89;0.73] | <0.001 | <0.001 |
| | HB: Low | 46 (88.5) | 42 (43.8) | 88 (59.5) | Ref. | Ref. | <0.001 |
| | HB: Normal | 6 (11.5) | 54 (56.2) | 60 (40.5) | 0.10 [0.04;0.26] | <0.001 | |
| | PLT, Mean ±sd | 746±373 | 535±298 | 609±340 | 1.00 [1.00;1.00] | 0.001 | 0.001 |
| | PLT: Normal | 10 (19.2) | 36 (37.5) | 46 (31.1) | Ref. | Ref. | 0.035 |
| | PLT: Thrombocytosis | 42 (80.8) | 60 (62.5) | 102 (68.9) | 2.52 [1.13;5.63] | 0.022 | |

*QT: Quick Time, TP: Prothrombin Level, TCA: Activated Cephalin Time; RBC: red blood cells, HB: hemoglobin, WBC: white blood cells, **LYM**: Lymphocytes, PLT: platelets; Sd: Standard deviation, univariate analysis, * significant difference at p < 0.05; Or=Odd ratio.*

From the table above, thrombocytosis is positively associated with thrombotic risk (Or=2.52, 95% CI=[1.13; 5.63] ; (*p*=0.022)). Moreover, AS status is beneficial for the occurrence of thrombotic risk unlike SS (Or = 0.16, 95% CI = [0.07; 0.35]; (*p*=0.001). In addition, elevated fibrinogen is a risk factor for thrombotic risk (Or=2.50, 95% CI = [4.99; 1.25]; *p*=0.009).

## Discussion

The purpose of the present study was to assess thrombotic risk in homozygous and heterozygous sickle cell patients and to determine the hematological profile in the latter. This shows a significant prevalence of thrombotic risk, including 52.5% in SS patients and 14.7% in SA patients (*p* = 0.002). This high frequency of thrombotic risk is explained by a frequency of painful episodes correlated with fibrinolytic activity, reflected by the high circulating level of D-dimers; This suggests that this parameter could help predict the occurrence of vaso-occlusive seizures [13]. Quari et *al*. (2015) reported a higher frequency (71%) of thrombotic risk in SS [14]. This difference could be explained by a higher frequency of patients in crisis during their study.

The size of our study population was 148 participants including 80 SS and 68 AS. The average age was 11±5.4 years in the SS and 36.5±17.4 years in the AS patients (Table I). This reflects a relatively young age of SS compared to AS. Indeed, the proportion of sickle cell patients decreases with age, This could be explained by the intrinsic factors linked to the disease that promote early mortality [15]. This result is close to that of Kengne et *al*. (2021) which also reports an average age of SS (9.5±7.1 years) lower than that of AS (25±16 years) [16]. Indeed, during a study by Houwing et *al*. (2019), more than half of sickle cell patients die before the age of 5 in sub-Saharan Africa [15]. These sightings were also reported by Dahamani et *al.* [17]. Patients had a history of infectious crisis, history of anemia, history of vasooclusive, and history of cardiovascular disease (Table I). These described signs and symptoms are characteristic and commonly encountered in sickle cell patients. These observations were also described in the review by Houwing et *al*. which provides a summary of the pathophysiology and management of sickle cell disease and encompasses the characteristics and complications of the disease; as well as that of Wonkam et *al*. (2016) [15,18]. The sex ratio was 0.76 in favor of women, of which 0.62 were in favor of AS and 0.9 in favor of SS. The sex ratio among SS men reflects an almost equal distribution of sickle cell disease between boys and girls. this has a genetic basis because the transmission of tare is independent of sex. These same observations were made by Dahmani et *al*. (2016) and Doupa et *al*. (2017) [17,19].

Complete blood counts were performed to determine the blood count profile in our study population; 97.4% erythrocytopenia was reported in SS patients and 5.88% in AS (*p*<0.001). In addition, 100% anemia was reported in SS patients for a mean hemoglobin of 7.13±1.41 g/dL; and next to it a mean hemoglobin of 14.4±2.57 g/dL in AS patients for 11.8% anemia (*p*<0.001). This difference between the 02 groups could be explained by the fact that SS patients even outside seizures are subjected to

hemolysis and decreased erythrocyte survival (12 and 14 days) [20]. These results are similar to those of Kengne et *al*. (2021) in a study of sickle cell patients who reported erythrocytopenia in SS for a mean red blood cell of 2.98±0.68 T/L and 4.77±0.84 T/L in AS patients ($p$<0.001) [16]. Fatima Dahmani et *al*. (2016) reported a hemoglobin level of 7.59±0.91dL in SS patients [17]. In addition, the mean white blood cell in SS was 17.9±12.6 G/L for 82.5% hyperleukocytosis and 6.28±2.66 in AS for 2.94% leukocytosis ($p$<0.001). Several studies also report hyperleukocytosis in sickle cell patients [21,22]. Sickle cell disease is indeed an inflammatory disease whose one of the markers is leukocytosis. The increase in the number and activation of leukocytes are important mediators of inflammation in sickle cell disease. Leukocytes can adhere to each other, sickle and non-sickle erythrocytes, platelets, and the vascular endothelium. In addition, in acute hemolysis, strong bone marrow regeneration is responsible for erythroblastosis causing false hyperleukocytosis; since erythroblasts because of their nucleus are counted as leukocytes by automata. Indeed, this uncorrected leukocytosis is more important in sickle cell patients [17,23]. The mean platelet count was 809±329 g/L in SS and 375±150 G/L in AS (p<0.001). The higher PLT count seen in patients with SCD could be attributed to possible splenic sequestration, reduction, or absence of spleen resulting from hyposplenism in SCD [24] or autosplenectomy [25], as well as the underlying chronic inflammation. Reports indicate that there is a correlation between PLT count and SCD, which corroborates the higher counts of PLTs among SCD patients in this study, although a correlation was not determined in this study. Findings from the current study on increased steady PLT count in SCD patients agree with the work of Freedman and Karpatkin who followed eight adult SCD patients over a [26] period of 6 months and found an elevation in steady-state PLT counts. Omoti, in his study, also recorded high PLT counts among SCD patients with vaso-occlusion as well as those in steady state [27].

Hemostasis tests were performed on our study population to assess thrombotic risk. 52.5% of SS had a high D-dimer level compared to 14.7% in AS ($p$=0.002). Indeed, high plasma levels of D-dimer indicate increased degradation of cross-linked fibrin plasmin and are therefore an indirect indication of increased thrombin activity and fibrin formation; this elevation of D-dimer can be explained by the fact that in sickle cell patients there is accelerated coagulation and hypofibrinolysis [12]. The cause of the increased coagulation activity includes several possibilities. Loss of phospholipid asymmetry in the membranes of sickle red blood cells [28] and in the membranes of the vesicles shed from the cells [29], with exposure of phosphatidylserine (PS) on the membrane surface, would contribute to hypercoagulability. Although the loss of asymmetry, which is associated with deoxygenation of sickle red cells and production of membrane-shed vesicles, would be less likely to occur in Hb AS red cells than in Hb SS or Hb SC red cells, loss of asymmetry can occur because deoxygenation of red cells is observed in individuals with Hb AS [30]. Another important clotting factor is a significant increase in platelet number and micro-aggregate [31]. Still, other authors justify hypercoagulability by the presence of a factor accelerating the conversion of fibrinogen into fibrin implying that increased concentrations of fibrinogen and factor VIII would help anticipate hypercoagulability in the plasma [26]. This author considers that this term could be used in the presence in plasma of increased activities or concentrations of various procoagulants or coagulation factors. In patients with this condition, thrombosis occurs with increased frequency [1,11,32]. It is, therefore, very likely that hypercoagulability as well as hypofibrinolysis found during crises in patients with SS disease favour the development of thrombosis provided that another factor will initiate the blood coagulation. In SS disease the painful crises are produced by vascular occlusion by sickled red cells [5]. The platelet-fibrin thrombi formed within the occluded vessels seem to be a secondary phenomenon due to the stasis and the presence of hypercoagulability and hypofibrinolysis. However, the thrombi formed are a factor probably affecting the duration and severity of the crises as these in the favorable environment of the circulating hypercoagulable blood may be extended to produce thrombosis of large vessels [1,33].

Qari et *al*. (2012) also report high levels of D-dimer in SS sickle cell patients although their proportion is higher than ours (71%) [14]. This difference would be explained by the large proportion of SS patients in crisis during this study, vaso-occlusive crises being an aggravating factor of thrombotic risk and therefore of the elevation of D dimers. Moreover, the level of fibrinogen has been evaluated; the mean fibrinogen in SS was 5.32±0.50 g/L and 4.63±0.00 g/L in AS ($p$<0.001). This is explained by the fact that during sickle cell disease, the painful crisis is associated with a significant increase in plasma fibrinogen and whole blood viscosity [34]. Fibrinogen is an acute phase

reactant, meaning that fibrinogen concentrations may rise sharply in any condition that causes inflammation or tissue damage. Elevated concentrations of fibrinogen are therefore not specific, however, several reports have linked the hypercoagulable state in SCD to increased levels of fibrinogen concentration [35]. The mechanism of the increase in this factor has been attributed to increased metabolism, primarily as a result of the disease, though, it does not necessarily exclude the fact that it might still be associated secondarily with cryptogenic infection [34]. It is therefore suggested that the raised fibrinogen level observed in the HbSS patients may be due to increased production, as a reactive process probably in response to the chronic hemolytic process. If fibrinogen increases in steady-state SS disease, then we would expect an even higher increase during SCD crisis, and fibrinogen level estimation could be used as a sensitive parameter to monitor the progression of sickle cell pain crises [36]. Several authors also report an elevation of fibrinogen in the SS [26,30,35].

In addition, we evaluated the Quick Time (QT) in our population; the mean QT was 11.8±1.94 s for SS and 12.2±2.72 s for AS ($p$=0.336). Moreover, 32.5% of SS patients and 29.4% of AS presented a shortened Quick Time. Indeed, during sickle cell disease, this may be a reflection of the reduced factor X levels observed in these patients and probably other coagulation factors not measured in this study, such as factors V and IX [10,35,37]. This reduction in Factor V concentration contributes to the shortening of Quick's Time in sickle cell disease [34]. These results are similar to those of Shet et *al*. (2020) in a study of sickle cell patients who reported an average quick time of 13.4±1.4s in SS and 13.1±1.1s in AS [38]. In addition, the determination of Cephalin Time and Activator allowed us to obtain low TCA in SS compared to AS patients ($p$=0.572). It has been previously reported that there is an elevation of Factor VIII in SCD in the absence of significant elevation of other coagulation factors, this isolated elevation of Factor VIII concentration may explain the significantly shorter APTT found in the HbSS patients we studied [24]. Several authors also report a low TCA in the SS compared to the SA [37,38].

After univariate analysis by logistic regression, 'AS' status is protective against thrombotic risk (Or = 0.16, 95% CI = [0.07;0.35]; ($p$=0.001). This would be explained by the fact that the low proportion of hemoglobin 'S' in the AS patient allows the hemoglobin of the latter to perform its functions fairly properly thus protecting him from the main clinical signs commonly encountered in the SS. Much more, unlike AS, as described by several authors several elements have been reported elements contributing to an increase in the thrombotic risk including the heme effects to be pathologically relevant [39,40]. In addition, fibrinogen elevation and thrombocytosis were associated with the development of thrombotic risk in SS in our study respectively (Or=2.50, 95% CI = [4.99;1.25]; $p$=0,009) and (Or=2.52, 95% CI = [1.13;5.63] ; ($p$=0.022)). Indeed, fibrinogen is 'the factor I' not activated during coagulation phenomena and its elevation is a sign of procoagulant hyperactivity in the body [11]. Much more as described above, platelets in SCD patients because of their activation are found to be more adhesive to the vascular endothelium than those found in normal people. This consequently could worsen the process of occlusion caused by these sickled RBCs [41].

## Conclusion

The purpose of this study was to determine the profile of blood count and hemostasis and to assess thrombotic risk in patients with homozygous sickle cell disease and sickle cell trait. There is a high frequency of thrombotic risk in the SS population compared to the AS population ($p$=0.002). Evaluation of hemostasis parameters showed an increase in fibrinogen and D dimer concentration in SS compared to AS patients; followed by a shortening of Quick's Time in the SS. These data highlight the need for regular and systematic hemostasis assessment in sickle cell patients to prevent possible risks of thrombosis.

## Declarations

### Data Availability

All data generated or analyzed during this article are included within the article. The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Authors' Contributions

Rita Paule Tchouanmo Ngamgwe carried out the data collection, and analysis of the biological samples and drafted the manuscript. Romaric De Manfouo Tuono conducted item retention, data interpretation, and statistical analysis, and major contribution to manuscript writing. Maryline Seuko Njopwouo contributed to data interpretation and manuscript writing. Pierre René Fotsing Kwetche and Josué Simo Louokdom contributed to the revision and supervised the work. All authors read and approved

the final manuscript.

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**Ethical statement**

Not applicable to this study.

**Orcid**

**Romaric De Manfouo Tuono: ID** https://orcid.org/0000-0002-2867-0538

**Josué SIMO LOUOKDOM: ID** https://orcid.org/0000-0002-0833-8167

## References

1. Noubiap, J. J., Temgoua, M. N., Tankeu, R., Tochie, J. N., Wonkam, A., & Bigna, J. J. (2018). Sickle cell disease, sickle trait and the risk for venous thromboembolism: a systematic review and meta-analysis. Thrombosis journal, 16, 1-8.
2. Es, K., & Eih, M. (2017). Article original Article original. *Med Trop*, 21:18–23.
3. Sundd P, Gladwin MT, Novelli EM. (2019). Pathophysiology of Sickle Cell Disease. Annu. Rev. Pathol. Mech. Dis. 14:263-292.
4. Ofori-Acquah, S. F. (2020). Sickle cell disease as a vascular disorder. Expert Review of Hematology, 13(6), 645-653.
5. Neonato, M. G., Guilloud-Bataille, M., Beauvais, P., Bégué, P., Belloy, M., Benkerrou, M., ... & French Study Group on Sickle Cell Disease. (2000). Acute clinical events in 299 homozygous sickle cell patients living in France. European journal of haematology, 65(3), 155-164.
6. Kato, G. J., Steinberg, M. H., & Gladwin, M. T. (2017). Intravascular hemolysis and the pathophysiology of sickle cell disease. The Journal of clinical investigation, 127(3), 750-760.
7. Santos, M. N. N., Bezerra, M. A. C., Domingues, B. L. T. B., Zaccariotto, T. R., Oliveira, D. M., Costa, F. F., ... & Sonati, M. D. F. (2011). Haptoglobin genotypes in sickle-cell disease. Genetic Testing and Molecular Biomarkers, 15(10), 709-713.
8. Barbosa, L. C. P., Miranda-Vilela, A. L., de Oliveira Hiragi, C., Ribeiro, I. F., Daldegan, M. B., Grisolia, C. K., & dos Santos-Neto, L. L. (2014). Haptoglobin and myeloperoxidase (− G463A) gene polymorphisms in Brazilian sickle cell patients with and without secondary iron overload. Blood Cells, Molecules, and Diseases, 52(2-3), 95-107.
9. Tchente, N. C., Brulet, C., Njiengwe, E., Nana, T. N., Tsingaing, J. K., & Chantraine, F. (2016). Diagnostic anténatal de la drépanocytose au Cameroun: L'expérience du staff de diagnostic anténatal de Douala. Journal de la SAGO, 17(2), 33.
10. Liesner, Mackie, Cookson, McDonald, Chitolie, Donohoe, ... & Machin. (1998). Prothrombotic changes in children with sickle cell disease: relationships to cerebrovascular disease and transfusion. British journal of haematology, 103(4), 1037-1044.
11. Rees, D. C., & Gibson, J. S. (2012). Biomarkers in sickle cell disease. British journal of haematology, 156(4), 433-445.
12. Westerman, M. P., Green, D., Gilman-Sachs, A., Beaman, K., Freels, S., Boggio, L., ... & Williamson, P. (2002). Coagulation changes in individuals with sickle cell trait. American journal of hematology, 69(2), 89-94.
13. Gruel Y. (2017). La drépanocytose : une pathologie à risque thrombotique élevé. Thromboses Et Hémopathies ;07:27–8.
14. Qari, M. H., Dier, U., & Mousa, S. A. (2012). Biomarkers of inflammation, growth factor, and coagulation activation in patients with sickle cell disease. Clinical and Applied Thrombosis/Hemostasis, 18(2), 195-200.
15. Houwing, M. E., De Pagter, P. J., Van Beers, E. J., Biemond, B. J., Rettenbacher, E., Rijneveld, A. W., ... & SCORE Consortium. (2019). Sickle cell disease: clinical presentation and management of a global health challenge. Blood reviews, 37, 100580.
16. Bernard Kengne Fotsing, C., Anatole Pieme, C., Cabral Biapa Nya, P., Paul Chedjou, J., Ashusong, S., Njindam, G., ... & Gatsing, D. (2021). Haptoglobin gene polymorphism among sickle cell patients in West Cameroon: hematological and clinical implications. Advances in Hematology, 2021.
17. Dahmani F, Benkirane S, Kouzih J, Woumki A, Mamad H, Masrar A. (2016). Profil épidémiologique des hémoglobinopathies: étude transversale descriptive autour du cas index. Panafrican-med-journal ;8688:1–5.
18. Wonkam, A., Ngo Bitoungui, V. J., & Ngogang, J. (2015). Perspectives in genetics and sickle cell disease prevention in Africa: beyond the

preliminary data from Cameroon. Public health genomics, 18(4), 237-241.

19. Doupa, D., Djite, M., Gueye, P. M., Seck, M., Faye, B. F., Seck, S. M., ... & Diop, S. (2017). Profil biochimique et hématologique des patients drépanocytaires homozygotes en phase stationnaire au centre National de Transfusion Sanguine de Dakar. International Journal of Biological and Chemical Sciences, 11(4), 1706-1715.
20. Sombodi U, Wembonyama SO, Luboya O. Profil hématologique et nutritionnel du drépanocytaire homozygote SS âgé de 6 à 59 mois à Lubumbashi, République Démocratique du Congo. 2015;8688:1–6.
21. Um, S. S. N., Seungue, J., Alima, A. Y., Mbono, R., Mbassi, H., Chelo, D., & Koki, P. O. (2019). A cross sectional study of growth of children with sickle cell disease, aged 2 to 5 years in Yaoundé, Cameroon. Pan African Medical Journal, 34(1).
22. Makulo, J. R., Itokua, K. E., Lepira, R. K., Bundutidi, G. M., Aloni, M. N., Ngiyulu, R. M., ... & Lepira, F. B. (2019). Magnitude of elevated iron stores and risk associated in steady state sickle cell anemia Congolese children: a cross sectional study. BMC hematology, 19, 1-6.
23. Makani, J., Ofori-Acquah, S. F., Nnodu, O., Wonkam, A., & Ohene-Frempong, K. (2013). Sickle cell disease: new opportunities and challenges in Africa. The scientific world journal, 2013.
24. Davila, J., Manwani, D., Vasovic, L., Avanzi, M., Uehlinger, J., Ireland, K., & Mitchell, W. B. (2015). A novel inflammatory role for platelets in sickle cell disease. Platelets, 26(8), 726-729.
25. Davila, J., Manwani, D., Vasovic, L., Avanzi, M., Uehlinger, J., Ireland, K., & Mitchell, W. B. (2015). A novel inflammatory role for platelets in sickle cell disease. Platelets, 26(8), 726-729.
26. Freedman, M. L., & Karpatkin, S. (1975). Elevated platelet count and megathrombocyte number in sickle cell anemia.
27. Omoti CE. Hematological Values in Sickle Cell Anaemia in Steady State and During Vaso-occlusive Crisis in Benin City, Nigeria HAEMATOLOGICAL VALUES IN SICKLE CELL ANAEMIA IN STEADY STATE AND DURING VASO-OCCLUSIVE CRISIS IN BENIN CITY, NIGERIA. 2022;
28. Westerman, M. P., Cole, E. R., & Wu, K. (1984). The effect of spicules obtained from sickle red cells on clotting activity. British journal of haematology, 56(4), 557-562.
29. Choe, H. R., Schlegel, R. A., Rubin, E., Williamson, P., & Westerman, M. P. (1986). Alteration of red cell membrane organization in sickle cell anaemia. British journal of haematology, 63(4), 761-773.
30. Stathakis, N. E., Papayannis, A. G., Papayotas, H., Scliros, P., & Gardikas, C. (1975). Hypercoagulability and hypofibrinolysis in sickle-cell disease. Blut, 31, 355-360.
31. Kenny, M. W., George, A. J., & Stuart, J. (1980). Platelet hyperactivity in sickle-cell disease: a consequence of hyposplenism. Journal of clinical pathology, 33(7), 622.
32. Ofori-Acquah, S. F. (2020). Sickle cell disease as a vascular disorder. Expert Review of Hematology, 13(6), 645-653.
33. Ataga, K. I., Brittain, J. E., Desai, P., May, R., Jones, S., Delaney, J., ... & Key, N. S. (2012). Association of coagulation activation with clinical complications in sickle cell disease. PloS one, 7(1), e29786.
34. Emokpae MA, Uadia PO, Gadzama AA. Correlation of oxidative stress and inflammatory markers with the severity of sickle cell nephropathy. Ann Afr Med. 2010;9:141–6.
35. Buseri, F. I., Jeremiah, Z. A., & Shokunbi, W. A. (2006). Plasma levels of some blood coagulation parameters in Nigerian homozygous sickle cell patients (HbSS) in steady state. Hematology, 11(5-6), 375-379.
36. Baralić, M., Robajac, D., Penezić, A., Miljuš, G., Šunderić, M., Gligorijević, N., & Nedić, O. (2020). Fibrinogen Modification and Fibrin Formation in Patients with an End-Stage Renal Disease Subjected to Peritoneal Dialysis. Biochemistry (Moscow), 85, 947-954.
37. Ajuwon, M. D., Olayemi, E., & Benneh, A. A. (2014). Plasma levels of some coagulation parameters in steady state HBSC disease patients. Pan African Medical Journal, 19(1).
38. Shet, A. S., Lizarralde-Iragorri, M. A., & Naik, R. P. (2020). The molecular basis for the prothrombotic state in sickle cell disease. Haematologica, 105(10), 2368.
39. Delesderrier, E., Cople-Rodrigues, C. S., Omena, J., Kneip Fleury, M., Barbosa Brito, F., Costa Bacelo, A., ... & Citelli, M. (2019). Selenium status and hemolysis in sickle cell disease patients. Nutrients, 11(9), 2211.

40. Setty, B. N. Y., Betal, S. G., Zhang, J., & Stuart, M. J. (2008). Heme induces endothelial tissue factor expression: potential role in hemostatic activation in patients with hemolytic anemia. Journal of Thrombosis and Haemostasis, 6(12), 2202-2209.
41. Alkindi S, Almufargi SS, Pathare A. Clinical and laboratory parameters, risk factors predisposing to the development of priapism in sickle cell patients. 2019;1–5.