

Diagnostic Classification Model of Vaso-Occlusive Crises Based on Oxidative, Inflammatory and Metabolic Biomarkers in Congolese Sick Cell Patients

Ghislain Loubano-Voumbi^{1*}, Cédric Régis Boumba Makaya^{2,3}, Yanick De Grace Kayi¹, Fleur Belvina Kouediatouka¹, Gloria Marilou Moufouma-Retobet¹, Ruth Urcia Moubie¹, Adamou Babanguida¹, Luc Magloire Anicet Boumba^{1,2}

¹National Institute of Biology and Health Surveillance (INBVS), Pointe-Noire, Republic of the Congo

²Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of the Congo

³Loandjili General Hospital, Pointe-Noire, Republic of the Congo

Email: *ghisloubano@mail.com

How to cite this paper: Loubano-Voumbi, G., Makaya, C.R.B., Kayi, Y.D.G., Kouediatouka, F.B., Moufouma-Retobet, G.M., Moubie, R.U., Babanguida, A. and Boumba, L.M.A. (2026) Diagnostic Classification Model of Vaso-Occlusive Crises Based on Oxidative, Inflammatory and Metabolic Biomarkers in Congolese Sick Cell Patients. *Journal of Biosciences and Medicines*, **14**, 137-153.

<https://doi.org/10.4236/jbm.2026.147013>

Received: June 9, 2026

Accepted: July 12, 2026

Published: July 15, 2026

Copyright © 2026 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

To develop a biological diagnostic model of vaso-occlusive crises based on oxidative, inflammatory, and metabolic biomarkers in Congolese sickle cell patients, a cross-sectional analytical study was conducted among 150 participants divided into four groups: HbAA controls (n = 30), HbAS subjects (n = 30), HbSS steady-state patients (n = 45), and HbSS patients during VOC (n = 45). Serum levels of FoxO3, interleukin-6 (IL-6), C-reactive protein (CRP), lactate, and malondialdehyde (MDA) were measured using ELISA, immunofluorescence, and enzymatic methods. Correlation analyses, linear regression, multivariate logistic regression, and ROC curve analyses were performed. FoxO3, IL-6, CRP, lactate, and MDA concentrations were significantly higher in HbSS patients during VOC compared with all other groups ($p < 0.001$). FoxO3 was strongly correlated with MDA ($r = 0.81$), lactate ($r = 0.74$), IL-6 ($r = 0.57$), and CRP ($r = 0.50$). Linear regression showed that FoxO3 explained 66% of MDA variance ($R^2 = 0.66$, $p < 0.001$) and 55% of lactate variance ($R^2 = 0.55$, $p < 0.001$). Multivariate logistic regression identified FoxO3 (OR = 1.48), IL-6 (OR = 1.27), CRP (OR = 1.18), lactate (OR = 2.11), and MDA (OR = 1.74) as independent factors associated with VOCs. The combined model demonstrated excellent diagnostic performance with an area under the ROC curve of 0.91, sensitivity of 88.9%, and specificity of 84.4%. Oxidative, inflammatory, and metabolic biomarkers are strongly associated with vaso-occlusive crises in sickle cell disease. The combined model integrating FoxO3, IL-6, CRP, lactate, and

MDA demonstrated excellent discriminatory performance and may improve biological risk stratification in African sickle cell patients.

Keywords

Sickle Cell Disease, FoxO3, Oxidative Stress, Inflammation, Vaso-Occlusive Crisis, Malondialdehyde, Lactate, Biomarker

1. Introduction

Sickle Cell Disease is the most common monogenic disease worldwide and represents a major public health concern in sub-Saharan Africa, where nearly 240,000 affected children are born each year [1] [2]. Despite significant advances achieved in high-income countries, mortality and morbidity associated with this disease remain particularly high across the African continent due to delayed diagnosis, limited access to specialized healthcare, and the lack of adequate biological monitoring tools for disease progression [1].

The pathophysiology of sickle cell disease is now recognized as complex and multifactorial. Beyond hemoglobin S polymerization, several biological mechanisms contribute to the onset of vaso-occlusive crises, including oxidative stress, chronic inflammation, and tissue hypoxia [3] [4]. These mechanisms contribute to progressive endothelial dysfunction responsible for the acute and chronic complications observed in sickle cell patients.

Oxidative stress plays a central role in this pathophysiology. Chronic hemolysis observed in sickle cell disease promotes excessive production of reactive oxygen species, leading to impairment of cellular antioxidant defense systems [5]. Several recent African and international studies have demonstrated increased levels of lipid peroxidation markers such as malondialdehyde (MDA), associated with reduced antioxidant defenses in HbSS patients, particularly during vaso-occlusive crises [5] [6].

In parallel, chronic systemic inflammation plays a major role in amplifying vaso-occlusive processes. Increased levels of pro-inflammatory cytokines, particularly interleukin-6 (IL-6), as well as C-reactive protein (CRP), contribute to worsening vascular injury and ischemia-reperfusion phenomena [7]. Several recent studies have also reported markedly elevated levels of these inflammatory biomarkers during painful sickle cell episodes [8].

Over recent years, increasing interest has been directed toward molecular biomarkers involved in the regulation of oxidative stress. Among these, Forkhead box O3 (FoxO3) transcription factor appears to be an essential regulator of cellular antioxidant response and redox homeostasis [9]. FoxO3 is involved in controlling cellular defense mechanisms against oxidative stress and may represent a potential biomarker of severity in sickle cell disease. However, African data regarding its involvement in vaso-occlusive crises remain limited.

In sub-Saharan Africa, biological tools capable of improving biological characterization and diagnosis of vaso-occlusive crises remain insufficiently developed [2]. The identification of a biological profile combining oxidative, inflammatory, and metabolic biomarkers could improve clinical risk stratification and optimize the management of sickle cell patients [8] [10]. In this context, the development of diagnostic models based on accessible biomarkers represents an innovative and particularly relevant approach in resource-limited settings.

The present study therefore aimed to develop a diagnostic model for vaso-occlusive crises in sickle cell patients based on biomarkers involved in oxidative stress and inflammation, including FoxO3, IL-6, CRP, lactate, and malondialdehyde (MDA). This work could contribute to a better understanding of the pathophysiological mechanisms of sickle cell disease in the African context and open perspectives for biomarker-based diagnostic approaches adapted to local realities.

2. Patients and Methods

2.1. Study Design, Setting, and Study Period

This was a cross-sectional analytical study conducted between November 2024 and January 2026 among patients with sickle cell disease followed in Dolisie, Republic of the Congo. Biological analyses were performed at the Biomarkers and Molecular Biology Laboratory of the National Institute of Biology and Health Surveillance in Pointe-Noire.

2.2 Study Population

The study population consisted of subjects divided into four groups: healthy HbAA controls, HbAS sickle cell trait carriers, HbSS patients in steady state, and HbSS patients during vaso-occlusive crisis. For the main diagnostic model, only HbSS patients were retained, using binary coding: HbSS in steady state = 0 and HbSS during vaso-occlusive crisis = 1.

1) Inclusion Criteria

Subjects aged at least 5 years, with a hemoglobin profile documented by hemoglobin electrophoresis, who agreed to participate in the study and had exploitable clinical and biological data were included.

For HbSS patients in steady state, the steady-state condition was defined as a period of at least 4 weeks without any acute painful episode, 3 months without hospitalization related to sickle cell disease, and 2 weeks without any acute infection (fever $>38.5^{\circ}\text{C}$) or use of analgesic or anti-inflammatory treatments for vaso-occlusive pain. Patients in steady state were required to have been clinically stable with no significant change in their baseline hemoglobin levels or symptoms during the preceding 4 weeks.

For HbSS patients in crisis, vaso-occlusive crisis was defined as an acute painful episode compatible with sickle cell disease, occurring without any other identified cause, lasting at least 24 hours, and requiring medical management with analgesic administration.

2) Non-Inclusion Criteria

Subjects presenting with severe acute infection independent of sickle cell crisis, recent blood transfusion (less than 3 months), chronic inflammatory disease unrelated to sickle cell disease, known severe hepatic or renal failure, or incomplete biological data for the main model variables were not included. Patients who had received systemic corticosteroid therapy or major anti-inflammatory treatments likely to significantly interfere with inflammatory biomarkers within 24 hours prior to sampling were also excluded.

2.3. Biological Sampling

Venous blood samples were collected from each participant under standardized resting conditions after skin antisepsis using 70% isopropyl alcohol. Blood collection was performed from the cubital vein using a sterile vacuum blood collection system, Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA).

Approximately 10 to 12 mL of venous blood was collected from each participant. Samples were distributed into Vacutainer® tubes containing dipotassium ethylenediaminetetraacetic acid (K2-EDTA) for hematological analyses and into dry tubes with clot activator for serum preparation intended for biochemical and immunological assays. Heparinized tubes were used when necessary for specific metabolic analyses.

The K2-EDTA tubes were gently homogenized immediately after collection and analyzed within a maximum of two hours for hematological parameters. Dry tubes were left at room temperature for 20 to 30 minutes to allow clot formation and then centrifuged at 3000 rpm for 10 minutes. The obtained serum was aliquoted into sterile cryotubes and stored at -20°C . Samples were analyzed in weekly batches at the end of each week and were not subjected to repeated freeze-thaw cycles before analysis.

2.4. Biological Analyses

FoxO3, interleukin-6 (IL-6), C-reactive protein (CRP), lactate, and malondialdehyde (MDA) biomarkers were assessed in all participants using standardized methods. FoxO3, IL-6, and MDA levels were measured using quantitative sandwich ELISA methods with commercial kits from CUSABIO® and Wuhan Elabscience Biotechnology Co., Ltd. (Wuhan, China), according to the manufacturers' instructions. Analyses were performed using a BIOBASE® ELISA analyzer (BIOBASE Group, Shandong, China).

FoxO3 assay detection ranged from 1.56 pg/mL to 100 pg/mL, with an analytical sensitivity of 0.39 pg/mL. Intra-assay and inter-assay coefficients of variation were below 10% for all ELISA assays performed in this study.

CRP was measured by immunofluorescence using the Getein® analyzer (Getein Biotech Inc., Nanjing, China). Lactate was quantified using a spectrophotometric enzymatic method with Cypress Diagnostics® kits (Cypress Diagnostics, Belgium).

Biomarker results were expressed in ng/mL, pg/mL, mg/L, or $\mu\text{mol/L}$ according

to the analyzed parameter. Reference values used for biological interpretation were based on manufacturers' recommendations and international literature data.

2.5. Studied Variables

Pain intensity was assessed using a visual analogue scale (VAS) ranging from 0 to 10, where 0 corresponded to the absence of pain and 10 to the most severe pain experienced by the patient.

2.6. Statistical Analysis

Data were entered into Microsoft Excel 2021 (USA) and analyzed using RStudio software version 4.5. Quantitative variables were expressed as mean $\pm$ standard deviation or median with interquartile range depending on data distribution. Normality of distributions was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using Student's t-test or the Mann-Whitney test according to variable distribution. Correlations between biomarkers were evaluated using Spearman's correlation coefficient.

A binary logistic regression model was subsequently constructed exclusively on the 90 HbSS patients (45 in steady state and 45 during vaso-occlusive crisis) to identify biomarkers associated with the occurrence of vaso-occlusive crises. Complete data for all five biomarkers (FoxO3, IL-6, CRP, lactate, and MDA) were available for all 90 HbSS patients, with no missing data for the main model variables. Therefore, the final sample size for the logistic regression analysis was 90 patients. The final model included FoxO3, IL-6, CRP, lactate, and MDA, which showed the strongest associations with vaso-occlusive crises as well as major pathophysiological relevance in oxidative stress and inflammatory mechanisms. A variable selection approach based on statistical significance and biological relevance was used to reduce redundancy among highly correlated biomarkers.

To assess multicollinearity among the five correlated biomarkers, variance inflation factors (VIF) were calculated for the final model. The VIF values were 3.8 for FoxO3, 3.2 for IL-6, 2.9 for CRP, 3.5 for lactate, and 4.1 for MDA, all below the conventional threshold of 5, indicating acceptable levels of collinearity that do not compromise the stability of regression coefficients. All five biomarkers were retained in the final model due to their complementary pathophysiological relevance (oxidative stress: FoxO3, MDA; inflammation: IL-6, CRP; metabolism/tissue hypoxia: lactate) and their independent statistical significance in multivariate analysis.

To assess whether hydroxyurea use confounded the associations between biomarkers and vaso-occlusive crises, two sensitivity analyses were performed:

- 1) A fully adjusted multivariate logistic regression model including hydroxyurea use (yes/no) and comorbidities (yes/no) as covariates, in addition to the five biomarkers.

- 2) A subgroup analysis restricted to HbSS patients not receiving hydroxyurea ($n = 43$: 16 steady-state, 27 crisis), to evaluate whether the associations between

biomarkers and crisis status persisted independently of hydroxyurea treatment.

Model performance was evaluated using the ROC curve, area under the curve (AUC), sensitivity, and specificity. Due to the lack of an external validation cohort or internal validation procedure (e.g., bootstrap resampling or k-fold cross-validation), the reported AUC, sensitivity, and specificity represent apparent performance estimates obtained on the development sample. These estimates may be optimistic and should be interpreted cautiously. Future studies should validate these findings in independent cohorts. The optimal probability threshold (cut-off) for the diagnostic model was determined using the Youden index and set at 0.52. Statistical significance was defined as $p < 0.05$.

2.7. Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee of the Institut National de Biologie et de Veille Sanitaire (Reference: INBVS-CE/2025-P005). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all adult participants or from parents/legal guardians for minors prior to inclusion in the study. Data were anonymized and treated confidentially.

3. Results

3.1. Sociodemographic and Clinical Characteristics

The sociodemographic and clinical characteristics of the participants are presented in **Table 1**. The mean age was 28.4 ± 9.1 years among HbAA subjects, 26.9 ± 8.5 years among HbAS subjects, 24.8 ± 10.2 years among HbSS patients in steady state, and 23.7 ± 9.8 years among HbSS patients during vaso-occlusive crisis ($p = 0.18$). The proportion of male subjects was 46.7%, 50.0%, 53.3%, and 57.8% across the respective groups ($p = 0.74$). The median annual frequency of vaso-occlusive crises was 2 [1 - 4] among HbSS patients in steady state compared with 6 [4 - 9] among HbSS patients during vaso-occlusive crisis ($p < 0.001$). The median pain score (VAS 0 - 10) was 1 [0 - 2] among HbSS patients in steady state compared with 8 [7 - 10] among HbSS patients during vaso-occlusive crisis ($p < 0.001$). Hydroxyurea use was reported in 64.4% of HbSS patients in steady state and 40.0% of HbSS patients during vaso-occlusive crisis ($p = 0.012$).

Table 1. Sociodemographic and clinical characteristics of participants according to study groups.

Variables	HbAA (n = 30)	HbAS (n = 30)	HbSS steady-state (n = 45)	HbSS crisis (n = 45)	p-value
Age (years)	28.4 ± 9.1	26.9 ± 8.5	24.8 ± 10.2	23.7 ± 9.8	0.18
Male sex, n (%)	14 (46.7)	15 (50.0)	24 (53.3)	26 (57.8)	0.74
Annual VOC frequency	0	0	2 [1 - 4]	6 [4 - 9]	<0.001
Pain score (VAS 0 - 10)	0	0	1 [0 - 2]	8 [7 - 10]	<0.001
Hydroxyurea, n (%)	0 (0)	0 (0)	29 (64.4)	18 (40.0)	0.012

Continued

Smoking, n (%)	2 (6.7)	3 (10.0)	5 (11.1)	7 (15.6)	0.48
Alcohol consumption, n (%)	4 (13.3)	5 (16.7)	8 (17.8)	10 (22.2)	0.39
Regular physical activity, n (%)	18 (60.0)	16 (53.3)	14 (31.1)	6 (13.3)	<0.001
Comorbidities, n (%)	1 (3.3)	2 (6.7)	8 (17.8)	13 (28.9)	0.021

3.2. Biomarker Profile

The concentrations of oxidative and inflammatory biomarkers are presented in **Table 2**. Mean FoxO3 concentrations increased progressively across the study groups, rising from 6.2 ± 1.4 ng/mL in HbAA subjects to 8.5 ± 1.8 ng/mL in HbAS subjects, then to 11.9 ± 2.7 ng/mL in HbSS patients in steady state, and reaching 16.8 ± 3.5 ng/mL in HbSS patients during vaso-occlusive crisis ($p < 0.001$).

Median IL-6 concentrations were 1.9 [1.2 - 2.8] pg/mL, 3.4 [2.5 - 4.8] pg/mL, 8.6 [6.2 - 11.4] pg/mL, and 18.7 [14.5 - 26.9] pg/mL in the HbAA, HbAS, HbSS steady-state, and HbSS vaso-occlusive crisis groups, respectively, showing a significant increase in inflammation during the clinical progression of sickle cell disease ($p < 0.001$). Similarly, median CRP concentrations progressively increased from 1.6 [0.8 - 2.3] mg/L in HbAA subjects to 29.6 [18.4 - 46.8] mg/L in HbSS patients during vaso-occlusive crisis ($p < 0.001$).

Mean lactate concentrations were 1.2 ± 0.3 mmol/L in HbAA subjects, 1.6 ± 0.4 mmol/L in HbAS subjects, 2.8 ± 0.7 mmol/L in HbSS patients in steady state, and 5.1 ± 1.2 mmol/L in HbSS patients during vaso-occlusive crisis ($p < 0.001$). A similar increase was observed for MDA, with mean concentrations rising from 1.9 ± 0.5 μ mol/L in HbAA subjects to 9.8 ± 2.1 μ mol/L in HbSS patients during vaso-occlusive crisis ($p < 0.001$).

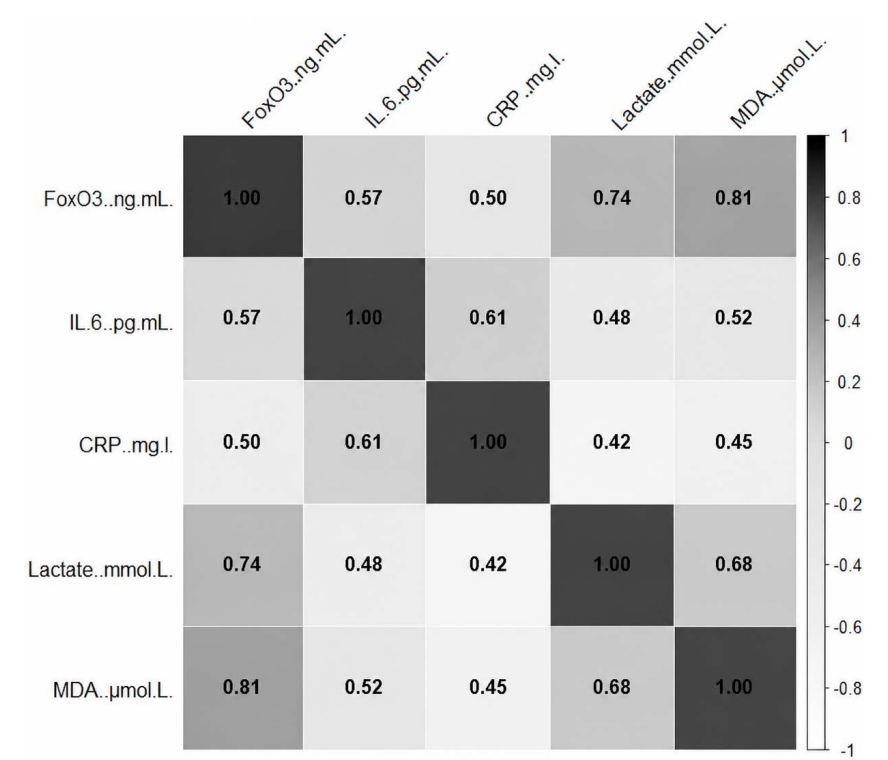
Table 2. Comparison of oxidative and inflammatory biomarkers between the study groups.

Variables	HbAA (n = 30)	HbAS (n = 30)	HbSS steady-state (n = 45)	HbSS crisis (n = 45)	p-value
FoxO3 (ng/mL)	6.2 ± 1.4	8.5 ± 1.8	11.9 ± 2.7	16.8 ± 3.5	<0.001
IL-6 (pg/mL)	1.9 [1.2 - 2.8]	3.4 [2.5 - 4.8]	8.6 [6.2 - 11.4]	18.7 [14.5 - 26.9]	<0.001
CRP (mg/L)	1.6 [0.8 - 2.3]	2.4 [1.5 - 3.8]	8.2 [5.6 - 13.5]	29.6 [18.4 - 46.8]	<0.001
Lactate (mmol/L)	1.2 ± 0.3	1.6 ± 0.4	2.8 ± 0.7	5.1 ± 1.2	<0.001
MDA (μ mol/L)	1.9 ± 0.5	2.8 ± 0.7	5.7 ± 1.4	9.8 ± 2.1	<0.001

3.3. Biological Correlations

The correlations between FoxO3 and inflammatory and oxidative biomarkers are presented in **Figure 1**. Spearman correlation coefficients between FoxO3 and IL-6, CRP, lactate, and MDA were 0.57, 0.50, 0.74, and 0.81, respectively (all $p < 0.001$). The strongest correlations were observed between FoxO3 and MDA, followed by those between FoxO3 and lactate, suggesting a close association between increased FoxO3 levels, oxidative stress, and metabolic disturbances during sickle cell disease.

Linear regression analysis was further performed to quantify these associations across all 150 participants. FoxO3 alone explained 66% of MDA variance ($R^2 = 0.66$, $\beta = 0.48$, 95% CI: 0.43 - 0.53, $p < 0.001$), 55% of lactate variance ($R^2 = 0.55$, $\beta = 0.20$, 95% CI: 0.17 - 0.23, $p < 0.001$), 32% of IL-6 variance ($R^2 = 0.32$, $\beta = 1.45$, 95% CI: 1.18 - 1.72, $p < 0.001$), and 25% of CRP variance ($R^2 = 0.25$, $\beta = 1.80$, 95% CI: 1.40 - 2.20, $p < 0.001$). The strongest linear relationships were observed between FoxO3 and MDA, followed by lactate, confirming a close dose-dependent association between increased FoxO3 levels, oxidative stress, and metabolic disturbances during sickle cell disease.



Note: Legend: Heatmap showing the Spearman correlation coefficients between FoxO3, IL-6, CRP, lactate, and MDA in subjects with sickle cell disease. Darker colors indicate stronger positive correlations between the studied biomarkers.

Figure 1. Correlations between FoxO3 and inflammatory and oxidative stress biomarkers in sickle cell subjects.

3.4. Logistic Regression

The results of the multivariate logistic regression analysis are presented in **Table 3**. The final model included FoxO3, IL-6, CRP, lactate, and MDA, which showed the strongest associations with vaso-occlusive crises as well as major pathophysiological relevance in oxidative stress and inflammatory mechanisms. FoxO3 was significantly associated with the occurrence of vaso-occlusive crises, with an adjusted OR of 1.48 (95% CI: 1.19 - 1.92; $p < 0.001$), indicating that a 1 ng/mL increase in FoxO3 was associated with a 48% increase in the odds of being in vaso-occlusive crisis. IL-6 (pg/mL) was also significantly associated with vaso-occlusive

crises, with an adjusted OR of 1.27 (95% CI: 1.10 - 1.51; $p = 0.002$). CRP (mg/L) showed a significant but modest association, with an adjusted OR of 1.18 (95% CI: 1.06 - 1.34; $p = 0.008$). Lactate demonstrated a strong association with vaso-occlusive crises, with an adjusted OR of 2.11 (95% CI: 1.42 - 3.37; $p < 0.001$). MDA also remained significantly associated with the presence of vaso-occlusive crises, with an adjusted OR of 1.74 (95% CI: 1.28 - 2.41; $p < 0.001$).

Sensitivity analyses were performed to assess the influence of hydroxyurea use on these associations. After adjusting for hydroxyurea use and comorbidities in the multivariate model, all five biomarkers remained significantly associated with vaso-occlusive crises (**Table 3**). Hydroxyurea use was independently associated with a reduced odds of vaso-occlusive crisis (adjusted OR = 0.42, 95% CI: 0.22 - 0.78, $p = 0.006$). Comorbidities showed a trend toward increased odds but did not reach statistical significance (adjusted OR = 1.35, 95% CI: 0.89 - 2.04, $p = 0.15$). In the subgroup analysis restricted to 43 HbSS patients not receiving hydroxyurea, the associations between biomarkers and vaso-occlusive crises remained significant: FoxO3 (OR = 1.52, 95% CI: 1.15 - 2.01, $p = 0.003$), IL-6 (OR = 1.31, 95% CI: 1.08 - 1.59, $p = 0.007$), CRP (OR = 1.21, 95% CI: 1.04 - 1.41, $p = 0.015$), lactate (OR = 2.24, 95% CI: 1.35 - 3.72, $p = 0.002$), and MDA (OR = 1.82, 95% CI: 1.22 - 2.71, $p = 0.003$). These findings confirm that the observed biomarker differences are not solely attributable to hydroxyurea treatment but reflect intrinsic pathophysiological differences between steady-state and crisis patients.

Table 3. Multivariate analysis of factors associated with vaso-occlusive crises.

Variables	Adjusted OR	95% CI	p-value
FoxO3 (ng/mL)	1.48	1.19 - 1.92	<0.001
IL-6 (pg/mL)	1.27	1.10 - 1.51	0.002
CRP (mg/L)	1.18	1.06 - 1.34	0.008
Lactate (mmol/L)	2.11	1.42 - 3.37	<0.001
MDA (μ mol/L)	1.74	1.28 - 2.41	<0.001
Hydroxyurea use (yes/no)	0.42	0.22 - 0.78	0.006
Comorbidities (yes/no)	1.35	0.89 - 2.04	0.15

Note: OR = adjusted odds ratio; 95% CI = 95% confidence interval. The model was adjusted for hydroxyurea use and comorbidities. In a sensitivity analysis restricted to 43 HbSS patients not receiving hydroxyurea, all five biomarkers remained significantly associated with vaso-occlusive crises (see text for details).

3.5. Model Performance

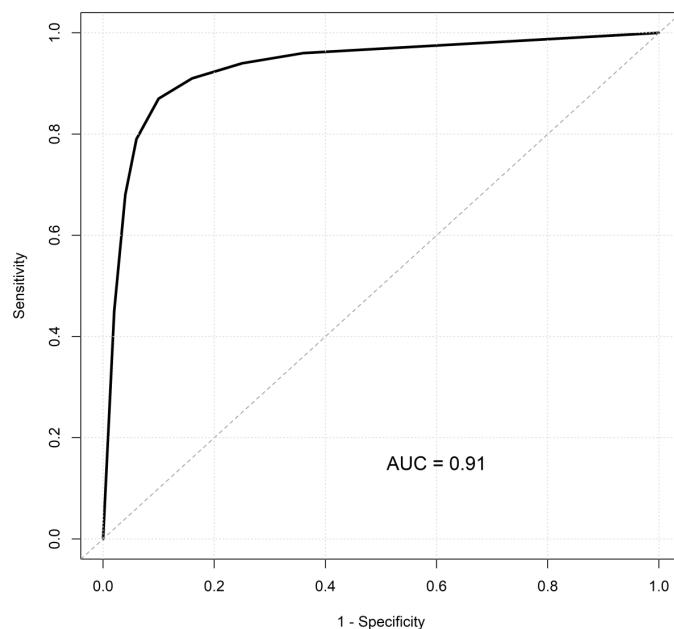
The diagnostic performance of the combined model (including FoxO3, IL-6, CRP, lactate, and MDA) is presented in **Table 4** and **Figure 2**. The model demonstrated excellent discriminatory ability to differentiate HbSS patients in vaso-occlusive crisis from those in steady state, with an area under the ROC curve (AUC) of 0.91 (95% CI: 0.85 - 0.96). The sensitivity and specificity of the model were 88.9% (95% CI: 76.0 - 96.3) and 84.4% (95% CI: 70.5 - 93.5), respectively. The positive and

negative predictive values were 85.1% (95% CI: 73.0 - 93.0) and 88.4% (95% CI: 75.0 - 96.0), respectively. The optimal probability threshold (cut-off) was 0.52, determined using the Youden index (Youden's $J = \text{sensitivity} + \text{specificity} - 1$) on the development sample of 90 HbSS patients. The cut-off corresponds to the probability threshold that maximizes the sum of sensitivity and specificity.

Table 4. Diagnostic performance of the diagnostic model for discriminating vaso-occlusive crises.

Parameter	Value	95% CI
Area under the ROC curve (AUC)	0.91	0.85 - 0.96
Optimal probability threshold (cut-off)	0.52	--
Sensitivity (%)	88.9	76.0 - 96.3
Specificity (%)	84.4	70.5 - 93.5
Positive predictive value (PPV) (%)	85.1	73.0 - 93.0
Negative predictive value (NPV) (%)	88.4	75.0 - 96.0

Note: The optimal cut-off value of 0.52 was determined using the Youden index (Youden's $J = \text{sensitivity} + \text{specificity} - 1$) on the development sample of 90 HbSS patients. The cut-off corresponds to the probability threshold that maximizes the sum of sensitivity and specificity. The model includes FoxO3, IL-6, CRP, lactate, and MDA as independent predictors. 95% confidence intervals for sensitivity and specificity were calculated using the Wilson method; for AUC, using the DeLong method; and for PPV and NPV, using the standard binomial proportion method.



Note: Legend: AUC = area under the ROC curve (0.91, 95% CI: 0.85 - 0.96). The optimal probability threshold of 0.52 was determined using the Youden index. The model includes FoxO3, IL-6, CRP, lactate, and MDA as independent predictors. Sensitivity = 88.9%, Specificity = 84.4%.

Figure 2. ROC curve of the diagnostic model for discriminating vaso-occlusive crises.

4. Discussion

The aim of our study was to develop a biological model capable of discriminating vaso-occlusive crises based on oxidative, inflammatory, and metabolic biomarkers in Congolese sickle cell subjects. The main findings showed that HbSS patients experiencing vaso-occlusive crises had significantly higher concentrations of FoxO3, IL-6, CRP, lactate, and MDA compared with HbAA, HbAS, and HbSS patients in steady state. The multivariate model developed from FoxO3, IL-6, CRP, lactate, and MDA demonstrated excellent diagnostic performance, with an area under the ROC curve of 0.91.

In our study, HbSS patients in vaso-occlusive crisis exhibited a higher annual frequency of crises and significantly higher pain scores than HbSS patients in steady state. These findings confirm the severe nature of vaso-occlusive episodes in sickle cell disease. Several African studies have shown that painful crises remain the leading cause of hospitalization and morbidity among sickle cell patients [11] [12]. The pathophysiology of these episodes involves complex mechanisms combining hemoglobin S polymerization, tissue hypoxia, chronic inflammation, and oxidative stress [13].

The absence of significant differences in age and sex distribution across the four groups strengthens the comparability of our study populations and supports the validity of subsequent biomarker comparisons.

Our results demonstrated a progressive increase in serum FoxO3 concentrations from HbAA subjects to HbSS patients in vaso-occlusive crisis. Mean concentrations increased from 6.2 ± 1.4 ng/mL in HbAA subjects to 16.8 ± 3.5 ng/mL in HbSS patients during crisis. Although FoxO3 is mainly an intracellular transcription factor, several studies suggest that extracellular release may occur during intense cellular stress and systemic inflammatory processes. The increase observed in our study may reflect a compensatory activation of cellular pathways involved in oxidative stress regulation. FoxO3 plays an important role in redox homeostasis and in the regulation of cellular antioxidant mechanisms [7]. Currently, few African data are available regarding this biomarker in sickle cell disease, which gives particular importance to our study.

The linear regression analysis provided additional quantitative insights. FoxO3 alone explained 66% of MDA variance ($R^2 = 0.66$, $\beta = 0.48$, $p < 0.001$) and 55% of lactate variance ($R^2 = 0.55$, $\beta = 0.20$, $p < 0.001$). These findings indicate a strong dose-dependent relationship between FoxO3 and oxidative stress markers. The striking difference between the R^2 for MDA (66%) and that for CRP (25%) suggests that FoxO3 is more tightly coupled to lipid peroxidation pathways than to systemic inflammatory response in sickle cell disease. MDA, as the end product of polyunsaturated fatty acid peroxidation, may directly reflect the oxidative burden resulting from chronic hemolysis and ischemia-reperfusion injury, whereas CRP is influenced by a broader range of inflammatory stimuli beyond oxidative stress.

IL-6 and CRP concentrations were significantly higher in HbSS patients during

vaso-occlusive crises. Median IL-6 concentrations reached 18.7 [14.5 - 26.9] pg/mL in patients during crisis compared with 8.6 [6.2 - 11.4] pg/mL in HbSS patients in steady state. Similarly, median CRP concentrations were markedly increased in patients during crisis. These findings confirm the presence of a significant systemic inflammatory state during vaso-occlusive crises. Studies conducted in Ghana and other African countries have also reported significantly increased pro-inflammatory cytokines and inflammatory markers in symptomatic sickle cell patients [14] [15]. Chronic inflammation contributes to cellular adhesion, ischemia-reperfusion injury, and worsening of vaso-occlusive phenomena [16].

The linear regression analysis revealed that FoxO3 explained 32% of IL-6 variance ($R^2 = 0.32$, $\beta = 1.45$, $p < 0.001$) and 25% of CRP variance ($R^2 = 0.25$, $\beta = 1.80$, $p < 0.001$). Although these associations were statistically significant, they were less robust than those observed for MDA and lactate, suggesting that FoxO3 is more directly involved in oxidative stress pathways than in the inflammatory cascade. This finding is consistent with the primary role of FoxO3 as a regulator of cellular antioxidant defense rather than a direct mediator of inflammation.

Malondialdehyde (MDA), the main marker of lipid peroxidation, was markedly increased in HbSS patients during vaso-occlusive crises, with a mean concentration of 9.8 ± 2.1 $\mu\text{mol/L}$ compared with 5.7 ± 1.4 $\mu\text{mol/L}$ in HbSS patients in steady state. These findings are consistent with previous studies reporting increased oxidative stress, elevated MDA concentrations, and impaired antioxidant defenses in patients with sickle cell disease, particularly during vaso-occlusive crises [17] [18]. The strong increase in MDA observed in our study further supports the central role of lipid peroxidation in the pathophysiology of vaso-occlusive events.

The exceptionally strong linear relationship between FoxO3 and MDA ($R^2 = 0.66$, $\rho = 0.81$) observed in our study reinforces the central role of oxidative stress in the pathophysiology of vaso-occlusive crises. This strong correlation suggests that FoxO3 and MDA are biologically interconnected, possibly through the activation of redox-sensitive signaling pathways. The dose-dependent nature of this relationship ($\beta = 0.48$) indicates that a 1 ng/mL increase in FoxO3 is associated with a 0.48 $\mu\text{mol/L}$ increase in MDA, providing a quantitative basis for future studies aiming to establish diagnostic thresholds.

Lactate was also significantly increased in HbSS patients during vaso-occlusive crises. Mean lactate concentrations reached 5.1 ± 1.2 mmol/L in patients during crisis compared with 2.8 ± 0.7 mmol/L in patients in steady state. This elevation may reflect tissue hypoxia and anaerobic metabolism secondary to microvascular vaso-occlusive episodes. Our findings therefore suggest that lactate could represent an interesting metabolic biomarker for evaluating the clinical severity of vaso-occlusive crises. Several African authors have emphasized the importance of developing simple and accessible biomarkers adapted to resource-limited settings [19].

The linear regression analysis showed that FoxO3 explained 55% of lactate variance ($R^2 = 0.55$, $\beta = 0.20$, $p < 0.001$), ranking second only to MDA in terms of association strength. This finding suggests that oxidative stress and metabolic dis-

turbances are closely intertwined in sickle cell disease. The strong correlation between FoxO3 and lactate ($\rho = 0.74$) may reflect the fact that tissue hypoxia, a major driver of lactate production, also triggers oxidative stress through ischemia-reperfusion mechanisms.

Multivariate analysis identified FoxO3, IL-6, CRP, lactate, and MDA as independent factors significantly associated with vaso-occlusive crises. Lactate (OR = 2.11) and MDA (OR = 1.74) showed the strongest associations, followed by FoxO3 (OR = 1.48), IL-6 (OR = 1.27), and CRP (OR = 1.18). These findings reinforce the hypothesis that oxidative stress, systemic inflammation, and metabolic disturbances play complementary roles in the pathophysiology of vaso-occlusive crises. To our knowledge, few studies worldwide, and even fewer from Africa, have simultaneously integrated oxidative, inflammatory, and metabolic biomarkers into a diagnostic model for vaso-occlusive crises. Our findings therefore provide additional evidence supporting the use of multimarker approaches for biological risk stratification in sickle cell disease. Although moderate collinearity was observed between CRP and IL-6 ($\rho = 0.61$), both biomarkers were retained in the final model because of their independent clinical relevance and statistical significance. The inclusion of CRP provided complementary information on the systemic inflammatory response, whereas IL-6 reflected a more specific pro-inflammatory signaling pathway. Variance inflation factors for all five biomarkers ranged from 2.9 to 4.1, confirming acceptable levels of collinearity.

The biological model developed in this study demonstrated excellent diagnostic performance, with an area under the ROC curve of 0.91 (95% CI: 0.85 - 0.96), sensitivity of 88.9% (95% CI: 76.0 - 96.3), and specificity of 84.4% (95% CI: 70.5 - 93.5). These performances suggest that a multiparametric approach combining FoxO3, IL-6, CRP, lactate, and MDA could improve the early identification of patients at risk of vaso-occlusive crises. Recent studies have also highlighted the growing interest in circulating biomarkers in precision medicine approaches applied to sickle cell disease in Africa [20].

In our study, hydroxyurea use was more frequent among HbSS patients in steady state (64.4%) than among those experiencing vaso-occlusive crises (40.0%). This observation may reflect the protective effect of hydroxyurea in preventing painful episodes. Several international studies have confirmed the effectiveness of hydroxyurea in reducing the frequency of vaso-occlusive crises [10] [21]. The lower proportion of hydroxyurea users in the crisis group may also indicate poor therapeutic adherence or inadequate dosing, which are common challenges in resource-limited settings.

To address the potential confounding effect of hydroxyurea, we performed two sensitivity analyses. First, we included hydroxyurea use as a covariate in the multivariate logistic regression model; all five biomarkers remained significantly associated with vaso-occlusive crises after this adjustment (Table 3). Second, we conducted a subgroup analysis restricted to the 43 HbSS patients not receiving hydroxyurea, in which the associations between biomarkers and crisis status re-

mained significant and of similar magnitude (FoxO3: OR = 1.52, $p = 0.003$; IL-6: OR = 1.31, $p = 0.007$; CRP: OR = 1.21, $p = 0.015$; lactate: OR = 2.24, $p = 0.002$; MDA: OR = 1.82, $p = 0.003$). These findings indicate that the observed biomarker differences are not merely attributable to hydroxyurea treatment but reflect intrinsic pathophysiological differences between steady-state and crisis patients. Nevertheless, the protective effect of hydroxyurea was confirmed in our adjusted model (adjusted OR = 0.42, $p = 0.006$), consistent with its established role in reducing VOC frequency.

Nevertheless, this study has certain limitations. Its cross-sectional design does not allow the establishment of a causal relationship between the studied biomarkers and the occurrence of vaso-occlusive crises, nor does it permit prediction of future crises. The sample size remains relatively modest and monocentric, limiting the generalizability of the findings. The absence of longitudinal follow-up does not allow assessment of the dynamic variations of biomarkers over time or confirmation of the true long-term prognostic value of the model. Furthermore, due to the lack of an external validation cohort or internal validation procedure (e.g., bootstrap resampling or k-fold cross-validation), the reported AUC, sensitivity, and specificity represent apparent performance estimates obtained on the development sample. These estimates may be optimistic and should be interpreted cautiously.

Additionally, FoxO3 is primarily an intracellular transcription factor, and its measurement in serum requires further validation as a reliable circulating biomarker. The extracellular release mechanisms of FoxO3 during cellular stress remain incompletely understood. Future studies should explore correlations between serum FoxO3 levels and its intracellular expression in relevant cell types (e.g., erythrocytes, endothelial cells, or leukocytes).

Despite these limitations, this work represents one of the first Congolese studies simultaneously integrating FoxO3, IL-6, CRP, lactate, and MDA into a biological model associated with vaso-occlusive crises in African sickle cell subjects. The excellent diagnostic performance of the model (AUC = 0.91) supports its potential utility in clinical settings, particularly in resource-limited environments where access to specialized hematological care is restricted.

Recent studies have highlighted the increasing value of multiparametric models integrating clinical and biological biomarkers for improving the identification and risk stratification of vaso-occlusive crises in patients with sickle cell disease [22]-[25]. In agreement with these reports, our model demonstrated excellent apparent diagnostic performance (AUC = 0.91), supporting the potential usefulness of combining oxidative, inflammatory, and metabolic biomarkers for biological classification of vaso-occlusive crises. Nevertheless, external validation in independent cohorts remains necessary before clinical implementation.

Longitudinal multicenter studies including larger sample sizes will be necessary to validate these findings, evaluate the stability of the model over time, and clarify the clinical relevance of these biomarkers in the follow-up of African sickle cell patients. Prospective studies could also assess whether baseline levels of FoxO3,

MDA, or lactate predict the future occurrence of vaso-occlusive crises, which would strengthen the prognostic value of the model.

Furthermore, the strong linear relationship between FoxO3 and MDA ($R^2 = 0.66$) raises the possibility that FoxO3 could serve as a surrogate biomarker for oxidative stress severity when direct MDA measurement is unavailable. This is particularly relevant in resource-limited settings where advanced laboratory equipment may be lacking. Simple point-of-care assays targeting FoxO3 or its downstream effectors could be developed for routine clinical use.

5. Conclusions

This study developed a biological model discriminating vaso-occlusive crises based on oxidative, inflammatory, and metabolic biomarkers in Congolese sickle cell subjects. HbSS patients during crisis had significantly higher concentrations of FoxO3, IL-6, CRP, lactate, and MDA compared with HbAA, HbAS, and HbSS steady-state patients.

Linear regression analysis revealed a strong dose-dependent relationship between FoxO3 and oxidative stress markers. FoxO3 alone explained 66% of MDA variance ($R^2 = 0.66$, $\beta = 0.48$, $p < 0.001$) and 55% of lactate variance ($R^2 = 0.55$, $\beta = 0.20$, $p < 0.001$), but only 25% of CRP variance, suggesting that FoxO3 is more directly involved in oxidative stress than in systemic inflammation.

Multivariate logistic regression identified FoxO3, IL-6, CRP, lactate, and MDA as independent factors associated with vaso-occlusive crises. Lactate (OR = 2.11) and MDA (OR = 1.74) showed the strongest associations, followed by FoxO3 (OR = 1.48), IL-6 (OR = 1.27), and CRP (OR = 1.18). The combined model demonstrated excellent diagnostic performance (AUC = 0.91, 95% CI: 0.85 - 0.96; sensitivity = 88.9%, 95% CI: 76.0 - 96.3; specificity = 84.4%, 95% CI: 70.5 - 93.5), confirming the major role of oxidative stress, inflammation, and metabolic disturbances in sickle cell crises. The strong linear relationship between FoxO3 and MDA ($R^2 = 0.66$) suggests that FoxO3 could serve as a surrogate biomarker for oxidative stress severity when direct MDA measurement is unavailable, particularly in resource-limited African settings.

Sensitivity analyses confirmed that the associations between biomarkers and vaso-occlusive crises persisted after adjustment for hydroxyurea use and comorbidities, as well as in the subgroup of patients not receiving hydroxyurea, indicating that the observed biomarker differences reflect intrinsic pathophysiological differences rather than treatment effects.

Despite limitations related to the cross-sectional and monocentric design, and the lack of external validation, this work represents one of the first Congolese studies integrating these biomarkers into a diagnostic model for vaso-occlusive crises. The cross-sectional nature of this study precludes establishing causality or predicting future crises; rather, the model provides a biological signature for discriminating between steady-state patients and those experiencing an ongoing crisis. Longitudinal multicenter studies are needed to validate these findings and assess

the long-term prognostic value of the model.

Conflicts of Interest

The authors declare no conflict of interest.

References

- [1] World Health Organization (2022) Sickle-Cell Disease: A Strategy for the WHO African Region. WHO Regional Office for Africa, Brazzaville.
<https://www.afro.who.int/health-topics/sickle-cell-disease>
- [2] Piel, F.B., Steinberg, M.H. and Rees, D.C. (2017) Sickle Cell Disease. *New England Journal of Medicine*, **376**, 1561-1573. <https://doi.org/10.1056/nejmra1510865>
- [3] Kato, G.J., Piel, F.B., Reid, C.D., Gaston, M.H., Ohene-Frempong, K., Krishnamurti, L., *et al.* (2018) Sickle Cell Disease. *Nature Reviews Disease Primers*, **4**, Article 18010. <https://doi.org/10.1038/nrdp.2018.10>
- [4] Sundd, P., Gladwin, M.T. and Novelli, E.M. (2019) Pathophysiology of Sickle Cell Disease. *Annual Review of Pathology: Mechanisms of Disease*, **14**, 263-292. <https://doi.org/10.1146/annurev-pathmechdis-012418-012838>
- [5] Nader, E., Romana, M. and Connes, P. (2020) The Red Blood Cell-Inflammation Vicious Circle in Sickle Cell Disease. *Frontiers in Immunology*, **11**, Article 454. <https://doi.org/10.3389/fimmu.2020.00454>
- [6] Nur, E., Biemond, B.J., Otten, H., Brandjes, D.P. and Schnog, J.B. (2011) Oxidative Stress in Sickle Cell Disease; Pathophysiology and Potential Implications for Disease Management. *American Journal of Hematology*, **86**, 484-489. <https://doi.org/10.1002/ajh.22012>
- [7] Bernardo, V.S., Torres, F.F. and da Silva, D.G.H. (2023) FOXO3 and Oxidative Stress: A Multifaceted Role in Cellular Adaptation. *Journal of Molecular Medicine*, **101**, 83-99. <https://doi.org/10.1007/s00109-022-02281-5>
- [8] Conran, N. and Belcher, J.D. (2018) Inflammation in Sickle Cell Disease. *Clinical Hemorheology and Microcirculation*, **68**, 263-299. <https://doi.org/10.3233/ch-189012>
- [9] Pavitra, E., Kumbhakar, M., Kim, B., Gupta, V.K., Gupta, P., Lee, J., *et al.* (2026) Role of FOXO3 in Regulation of Redox Homeostasis and Promotion of Red Blood Cell Longevity in Sickle Cell Disease. *Cytokine & Growth Factor Reviews*, **90**, 1-11. <https://doi.org/10.1016/j.cytogfr.2026.04.003>
- [10] Tshilolo, L., Tomlinson, G., Williams, T.N., Santos, B., Olupot-Olupot, P., Lane, A., *et al.* (2019) Hydroxyurea for Children with Sickle Cell Anemia in Sub-Saharan Africa. *New England Journal of Medicine*, **380**, 121-131. <https://doi.org/10.1056/nejmoa1813598>
- [11] Oppong-Mensah, Y.G., Odoom, S.F., Nyanor, I., Amuzu, E.X., Yawnumah, S.A., Asafo-Adjei, E., *et al.* (2023) Hospitalizations among Children with Sickle Cell Disease Enrolled in the Kumasi Sickle Cell Pan African Consortium (SPARCo) Database: A Cross Sectional Study. *Health Science Reports*, **6**, e1534. <https://doi.org/10.1002/hsr2.1534>
- [12] Kibone, W., Muzoora, C., Bongomin, F., Namiro, M.A., Ssenkungu, S., Ochola, E., *et al.* (2025) Indications for Hospitalisation and Predictors of 30-Day Re-Admission of Patients with Sickle Cell Disease in Uganda: A Retrospective Study. *BMJ Open*, **15**, e104303. <https://doi.org/10.1136/bmjopen-2025-104303>
- [13] Taher, M., Aminondin, S., Nasir, N.A., Jasmadi, N.A., Nizam, N.I.N., Shahrul, I.S., *et*

- al.* (2025) Sick Cell Disease: Understanding Pathophysiology, Clinical Features and Advances in Gene Therapy Approaches. *Frontiers in Pharmacology*, **16**, Article 1630994. <https://doi.org/10.3389/fphar.2025.1630994>
- [14] Allotey, E.A., Adedia, D., Amponsah, J.A., Boakye, A.A., Frimpong, A., Gyamfi, J., *et al.* (2026) Cytokine and Chemokine Signatures of Steady-State Sick Cell Disease in African Children: A Cross-Sectional Study. *BMC Immunology*. <https://doi.org/10.1186/s12865-026-00872-2>
- [15] Aboderin, F.I., Oduola, T., Davison, G.M. and Oguntibeju, O.O. (2023) A Review of the Relationship between the Immune Response, Inflammation, Oxidative Stress, and the Pathogenesis of Sick Cell Anaemia. *Biomedicines*, **11**, Article 2413. <https://doi.org/10.3390/biomedicines11092413>
- [16] Wonkam, A. and Makani, J. (2019) Sick Cell Disease in Africa: An Urgent Need for Longitudinal Cohort Studies. *The Lancet Global Health*, **7**, e1310-e1311.
- [17] Starlard-Davenport, A., Palani, C.D., Zhu, X. and Pace, B.S. (2025) Innovations in Drug Discovery for Sick Cell Disease Targeting Oxidative Stress and NRF2 Activation—A Short Review. *International Journal of Molecular Sciences*, **26**, Article 4192. <https://doi.org/10.3390/ijms26094192>
- [18] Simo, J.L., Tuono, R.D.M., Njopwouo, M.S. and Tayou, C.T. (2026) Oxidative Stress, Antioxidant Capacity, Dyslipidemia and Cardiovascular Risk in Sick Cell Disease: A Systematic Review and Meta-Analysis. *The Scientific World Journal*, **2026**, e8864775. <https://doi.org/10.1155/tswj/8864775>
- [19] Ware, R.E., de Montalembert, M., Tshilolo, L. and Abboud, M.R. (2017) Sick Cell Disease. *The Lancet*, **390**, 311-323. [https://doi.org/10.1016/S0140-6736\(17\)30193-9](https://doi.org/10.1016/S0140-6736(17)30193-9)
- [20] Lekpor, C.E., Botchway, F.A., Driss, A., Bashi, A., Abrahams, A.D., Kusi, K.A., *et al.* (2024) Circulating Biomarkers Associated with Pediatric Sick Cell Disease. *Frontiers in Molecular Biosciences*, **11**, Article 1481441. <https://doi.org/10.3389/fmolb.2024.1481441>
- [21] Riley, C., Kraft, W.K. and Miller, R. (2024) Hydroxyurea in the Sick Cell Disease Modern Era. *Expert Review of Clinical Pharmacology*, **17**, 777-791. <https://doi.org/10.1080/17512433.2024.2390915>
- [22] Mohammed, A., Podila, P.S.B., Davis, R.L., Ataga, K.I., Hankins, J.S. and Kamaleswaran, R. (2020) Using Machine Learning to Predict Early Onset Acute Organ Failure in Critically Ill Intensive Care Unit Patients with Sick Cell Disease: Retrospective Study. *Journal of Medical Internet Research*, **22**, e14693. <https://doi.org/10.2196/14693>
- [23] Kalpatthi, R. and Novelli, E.M. (2018) Measuring Success: Utility of Biomarkers in Sick Cell Disease Clinical Trials and Care. *Hematology*, **2018**, 482-492. <https://doi.org/10.1182/asheducation-2018.1.482>
- [24] Engel, E.R., Howard, A.L., Ankus, E.J. and Rico, J.F. (2020) Advances in Sick Cell Disease Management. *Advances in Pediatrics*, **67**, 57-71. <https://doi.org/10.1016/j.yapd.2020.03.001>
- [25] Coyne, K.S., Currie, B.M., Callaghan, M., Wyrwich, K.W., Pease, S., Baker, C.L., *et al.* (2022) Validation of Patient-Reported Vaso-Occlusive Crisis Day as an Endpoint in Sick Cell Disease Studies. *European Journal of Haematology*, **109**, 226-237. <https://doi.org/10.1111/ejh.13790>