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Original Article

Immature reticulocyte fraction and erythrocyte indices in children with homozygous sickle cell disease during the interictal phase: a cross-sectional study in Lubumbashi.

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Abstract

Introduction:

Homozygous sickle cell disease (SS) is a hemoglobinopathy characterized by chronic hemolytic anemia and vaso -occlusive crises. In Lubumbashi (DRC), routine laboratory evaluation is often limited to a complete blood count, neglecting advanced reticulocyte parameters. This study aims to evaluate the profile of the immature reticulocyte fraction (IRF) and erythrocyte constants in children with SS sickle cell disease during the intercritical phase.

Methods:

A descriptive cross-sectional study was conducted on 49 children with SS sickle cell disease, aged 1 to 12 years, followed at the DeeServices Medical Laboratory. Hematological parameters, including hemoglobin, mean corpuscular volume (MCV), and IRF (sum of medium and high fluorescence fractions), were measured using the URIT 5160 analyzer. Statistical analysis was based on Spearman's rank correlation coefficient to assess the relationships between variables, in accordance with the principles of the Declaration of Helsinki.

Results:

The cohort presented with severe anemia, with a mean hemoglobin concentration of 7.33 g/ dL and impaired erythrocyte indices. The median IRF was 23.30%, reflecting significant reticulocyte stress and sustained compensatory erythropoietic activity. A positive and highly significant correlation (rho = 0.923, $p < 0.001$) was found between IRF and the absolute reticulocyte count.

Conclusion:

The intercritical phase in children with sickle cell disease (SS) does not correspond to a state of biological rest, but rather to active bone marrow regeneration. The IRF (erythropoietic response index) proves to be a relevant indicator for documenting the erythropoietic response in a cross-sectional setting.

Recommendation:

It is recommended that hospital laboratories in Haut-Katanga routinely integrate automated hematological analyzers capable of measuring IRF alongside absolute reticulocyte counts to accurately assess bone marrow regenerative activity during the interictal phase of pediatric sickle cell disease.

Keywords: *Sickle cell disease; Immature reticulocyte fraction (IRF); Compensatory erythropoiesis; Cross-sectional study; Interictal phase; Erythrocyte indices.*
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Introduction

Homozygous sickle cell disease (SS) is an autosomal recessive hemoglobinopathy, one of the most prevalent genetic diseases worldwide, with a particularly heavy epidemiological burden on the African continent (Diallo, 2008; OMS,2023). In the Democratic Republic of Congo, epidemiological studies estimate that 20 to 30% of the population carries the sickle cell trait AS, while 1.5 to 2% of live births correspond to the homozygous SS form (Mbenga et al., 2025). The disease progresses through two distinct clinical phases: the critical phase, marked by painful vaso - occlusive crises or infectious complications, and the intercritical phase, corresponding to a period of clinical stability associated with persistent chronic hemolytic anemia. (Thiam et al,2017) (Diqgne,2018).

In Lubumbashi, the capital of Haut-Katanga province, routine biological monitoring of sickle cell patients in the intercritical phase remains limited to the standard complete blood count (hemoglobin, MCV, MCH). These indices are static markers, unable to reflect the dynamics of bone marrow regeneration in real time. Relative reticulocyte counts themselves have limitations in interpretation, as they depend on the total mass of red blood cells destroyed by hemolysis (Adane et al., 2021).

Next-generation hematological analyzers such as the URIT 5160 allow for the measurement of the immature reticulocyte fraction (IRF), a parameter corresponding to young reticulocytes rich in ribonucleic acid, considered an early indicator of erythropoietic activity (URIT,2024). However, this biomarker remains underutilized in laboratories in Haut-Katanga, and no local reference data currently exist for the stable pediatric sickle cell population.

Study Objectives

- ✓ **Main objective**

To describe the IRF profile and erythrocyte indices in homozygous SS sickle cell children in the intercritical phase followed at the Medical Laboratory Deeservices (LMDS), and analyze the statistical associations between these hematological parameters.

- ✓ **Secondary objectives**

1. Describe the profiles of erythrocyte indices (MCV, MCHC, MCHC) and determine the central values of the IRF in the pediatric cohort studied;
2. Evaluate the statistical correlations between IRF, relative reticulocyte rate, and absolute reticulocyte count.

- ✓ **Main judgment criterion**

IRF value (%) measured on URIT 5160 analyzer at the time of the single blood sample taken from each patient

Materials and methods

Ethical considerations

This study was conducted in strict compliance with the fundamental ethical principles governing biomedical research involving human subjects, in accordance with the recommendations of the Declaration of Helsinki. All participants were minors between the ages of 1 and 12. A free and informed consent form was given to and explained orally in the local language to the legal guardian, who signed it before the sample was taken. No written consent from the children was required by the approved protocol. All patient data were anonymized using a unique numerical code; no personally identifiable information appeared in the study database. No additional samples were taken beyond the routine consultation; therefore, there was no additional risk to the participants.

Research framework

Our study is cross-sectional with an analytical focus. The DeeServices medical laboratory in Lubumbashi was selected as the experimental site for this study.

The choice of this location is based on several geographical and environmental factors. Lubumbashi, the second largest city in the Democratic Republic of Congo, is located at 12°19" South latitude and 27°28'51" East longitude, at an altitude of 1268m. Its climate is characterized by a six-month dry season followed by a six-month rainy season. The average annual temperature is 20°C.

By choosing the DeeServices medical laboratory in Lubumbashi, we therefore took into account both the geographical and environmental characteristics of the region. These factors contribute to the relevance and representativeness of the results obtained in the context of this scientific study.

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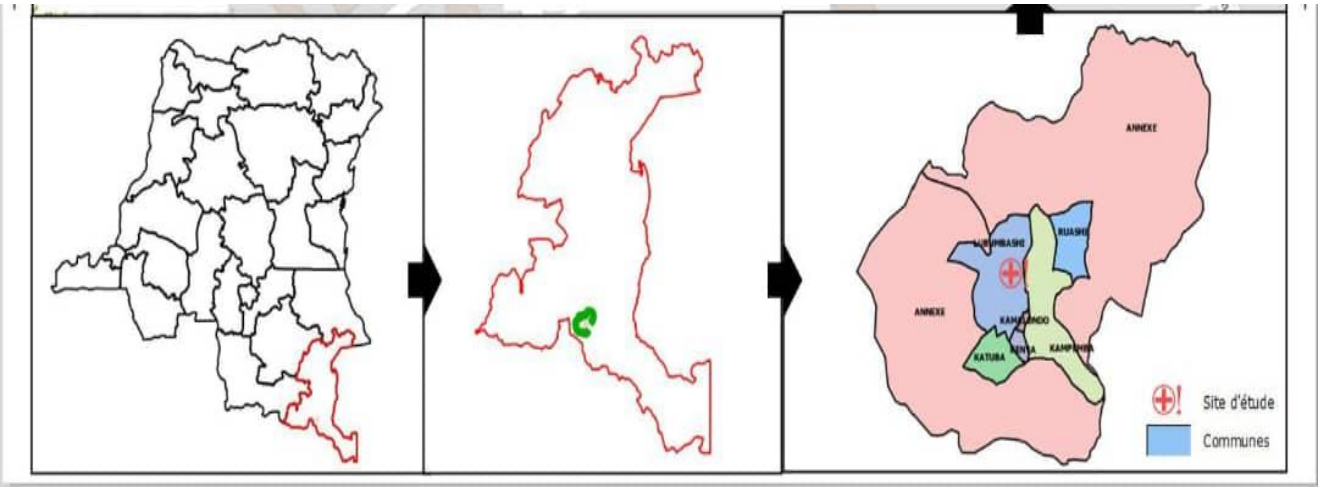

Figure 1: Geographic location map of the deeservices Medical Laboratory (LMDS), recruitment site for the pediatric cohort, central commune of Lubumbashi (Haut-Katanga, DRC). (DIVA-GIS / OpenStreetMap administrative boundaries, 2026)

Study topics

Sampling and sample size

Recruitment was based on non-probability convenience sampling, including all patients meeting all inclusion criteria and followed in the hematology and pediatrics departments of the LMDS during the recruitment window. The final sample size of 49 subjects corresponds to all eligible participants during the period; no prior statistical power calculations were performed, as this work constitutes an exploratory pilot study aimed at generating local reference values.

The recruitment across several communes of Lubumbashi does not allow for broad urban representativeness; convenience sampling induces limits of external validity; the results cannot be generalized to all children with sickle cell disease in the city outside the population followed at the LMDS.

Non-probability convenience sampling leads to a significant limitation in external validity: the generated IRF results and values cannot be generalized to all children with sickle cell disease in the city of Lubumbashi outside the population followed at the LMDS. Furthermore, the sample exhibits a marked sex imbalance (M/F sex ratio = 2.50), which may reduce the statistical power of inter-gender comparisons.

Inclusion and exclusion criteria

The inclusion criteria were:

1. Patient aged 1 to 12 years;
2. Confirmed diagnosis of homozygous SS sickle cell disease by hemoglobin electrophoresis;

intercritical phase according to the operational definition of the protocol;

4. Free and informed consent signed by the child's legal guardian.

Operational definition of the intercritical phase: Absence of painful vaso -occlusive crisis, acute chest syndrome, febrile infection or blood transfusion during the three months preceding the sampling; absence of any clinical signs of hemolytic decompensation on the day of the biological examination.

The exclusion criteria were:

1. Refusal of the legal guardian to participate in the study;
2. Patient in critical phase (painful crisis, acute infection, recent transfusion < 3 months);
3. Sickle cell genotype other than SS (SC, S-β-thalassemia);
4. Blood sample that is clotted, hemolyzed, or lipemic, incompatible with automated analysis

Informed consent from parents or legal guardians was obtained before patient inclusion. Patient selection was carried out without distinction of ethnic origin, tribe, or sex. Recruitment was based on non-probability convenience sampling, including all patients meeting all inclusion criteria between January and June 2026.

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Control of bias

To ensure the internal validity of the study, several measures were applied to minimize bias:

Selection bias: Participants were included without regard to race, tribal affiliation, or social class, and recruitment covered all communes of the city to ensure broad urban representation.

Classification bias: This bias was minimized by the use of standardized analysis protocols on the URIT 5160 analyzer, ensuring objective and reproducible classification of hematological and reticulocyte parameters.

Measurement bias: To limit this bias, the laboratory applied rigorous daily quality controls (QC), strict adherence to sampling conditions (EDTA tube, controlled temperature), and the use of a recognized analytical methodology (flow cytometry and impedance), thus minimizing human error during data counting and analysis. Some complementary biomarkers of hemolytic anemia (red blood cell distribution index [RDW], morphological examination of blood smears, and measurement of unconjugated and conjugated bilirubin) were not analyzed in this study. This limitation is due to a reduced protocol focused exclusively on evaluating the red blood cell distribution index (RBI) as the primary endpoint.

Data collection

Blood samples were obtained by strict venipuncture and collected in tubes containing the anticoagulant EDTA (Ethylenediaminetetraacetic acid). To prevent any morphological alteration or degradation of residual nucleic acids, the samples were stored at a controlled temperature between 2°C and 8°C. All analyses were performed within a strict timeframe not exceeding 24 hours post-collection, thus ensuring the stability of erythrocyte parameters and the reliability of the reticulocyte count. (Bossuyt & Boeynaems, 2001)

Parameters analyzed: methods, principles and interpretation

Parameters analyzed: The exploration focused on the complete blood count, with particular interest in erythrocyte constants (RBC count [RBC], Hemoglobin level [Hb], Mean Corpuscular Volume [MCV], Mean Corpuscular Hemoglobin Content [MCH], Mean Corpuscular Hemoglobin Concentration [MCHC]), as well as on the quantitative evaluation of reticulocytes and the fraction of immature reticulocytes (FRI / IRF).

Method and equipment: The analyses were performed using a *URIT 5160 multiparameter hematology analyzer.*

Principle and Procedure:

- **Analytical principle:** Cell counting and volume determination (MCV) are based on the principle of electrical impedance (the change in resistance as cells pass through a micro-orifice). Hemoglobin measurement uses spectrophotometry after lysis of red blood cells. Quantification of the immature reticulocyte fraction (IRF) uses flow cytometry combined with specific fluorochrome staining. The nucleic acids (RNA) of the reticulocytes are labeled with a fluorescent dye; the intensity of the emitted fluorescence allows the reticulocytes to be classified according to their degree of maturation (low, medium, and high fluorescence), with the IRF corresponding to the sum of the medium and high fluorescence fractions. (URIT,2024).
- **Procedure:** After adequate and gentle homogenization of the EDTA tubes on a roller shaker, the samples were automatically aspirated by the analyzer according to the laboratory's Standard Operating Procedures (SOPs).
- On this device, IRF is defined by the formula: IRF (%) = MFR (%) + HFR (%), where MFR = medium fluorescence reticulocytes, HFR = high fluorescence reticulocytes. This definition conforms to the URIT Medical technical manual. Electronic (2024).
- The absolute number of reticulocytes (/µL) is directly generated and exported by the analyzer, without secondary calculation from the relative rate and red blood cell count.

Interpretation of results

The values obtained were compared to international and local reference intervals, adjusted for age and sex. In the specific context of sickle cell disease in the intercritical phase, the combined analysis of erythrocyte constants and FRI made it possible to assess the level of compensatory bone marrow erythropoietic activity and to monitor the patients' chronic hemolytic status. (El Youssoufi,2016)

Internal quality control and analytical rigor

To ensure the accuracy, precision, and reproducibility of the study's analytical data, a strict Internal Quality Control (IQC) protocol was established.

- The calibration of the PLC was rigorously verified in accordance with the manufacturer's specifications.

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- Hematology Control Lot 240512 controls with three levels (low, normal, pathological) were analyzed daily before any series of patient samples included in the study. Levey-Jennings diagrams and Westgard rules were systematically completed; patient results were validated only if the controls were within ± 2 standard deviations. No additional analytical validation procedures were implemented specifically for this project. (BOUKLOUZE & CHERRAH, 2012)

statistical analysis

Data entry, coding and cleaning

Raw data from clinical data collection forms and laboratory results were initially entered and centralized using **Microsoft Excel 2016.** A rigorous coding phase for qualitative variables was performed to facilitate their electronic transfer. Before any analysis, data cleaning was carried out to identify and correct data entry errors, outliers, and missing data, thus ensuring the internal consistency of the database. (Kaur et al., 2018)

Descriptive statistical analysis and normality test

Statistical analysis was performed using **SPSS (Statistical Package for the Social Sciences), version 20.0** (IBM Corp., Armonk, NY, USA). Quantitative variables were expressed as means with standard deviations, while qualitative variables were presented as frequencies and percentages. The normality of the distribution of quantitative data (erythrocyte constants and FRI) was systematically assessed using the **Shapiro- Wilk test**. This test determined, for each variable, whether the distribution followed a normal distribution ($p > 0.05$) or a non-normal distribution ($p < 0.05$), thus guiding the choice of subsequent statistical tests.

Inferential and bivariate statistical analysis

Due to the non-normal distribution observed for most hematological parameters, the inferential analysis favored the use of non-parametric tests. The **Mann-Whitney U test** was used to compare the mean ranks between the different groups in order to identify statistically significant differences. The significance threshold was set at $p < 0.05$.

Graphical representations and correlations

Fraction of immature reticulocytes (FRI) correlation analyses were performed. The strength and direction of these associations were illustrated by scatter plots, supplemented with trend lines to visualize erythropoietic dynamics. (Bouyer, 2017)

Results

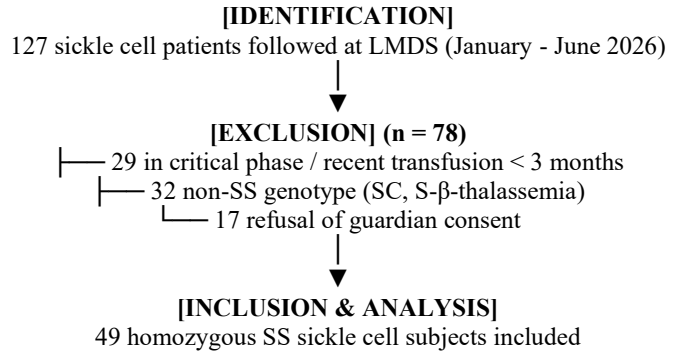

Figure 2: Standardized flow diagram (STROBE) describing the complete process of identification, inclusion, and exclusion of SS sickle cell patients for the cross-sectional study (January-June 2026)

The results of the descriptive and inferential analysis of the sample (N = 49 SS sickle cell patients in the intercritical phase) are detailed below.

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Socio-demographic characteristics of the study population

The demographic variables of interest (age and sex) were aggregated into a single table of basic characteristics (Table 1).

Table 1: Demographic profile of the study sample (N = 49)

Features	Indicators / Categories	Number (n)	Frequency (%) / [Min - Max]
Sex	Female	14	**28.6%**
	Male	35	**71.4%**
Age (Years)	Mean ± Standard deviation	**4.51 ± 2.82**	**95% CI: [3.69 - 5.33]**
	Values Extremes	—	**[1.00 – 12.00 years]**

The analysis highlights a clear male predominance within our study population with a sex ratio (M/F) of 2.50. The average age of the children is **4.51±2.82** years, reflecting a sample composed mainly of early and middle childhood, ranging from a minimum of 1.00 years (early childhood) to a maximum of 12.00 years.

Evaluation of the normality of hematological parameter distributions

In order to guide the choice between parametric and non-parametric tests, the normality hypothesis was tested by the Shapiro- Wilk algorithm.

Table 2: Statistical tests of normality of hematological variables (N = 49)

Clinical and Biological Variables	Shapiro-Wilk		
	Statistical	**Ddl**	**ρ-value**
Rate of Hemoglobin (Hb)	0.98	49	**0.44**
Reticulocytes (%)	0.98	49	**0.43**
Red Blood Cells (RBCs)	0.93	49	**0.01**
MCV (Global Volume) AVERAGE)	0.71	49	**0**
TCMH	0.85	49	**0**
CCMH	0.67	49	**0**
Reticulocytes Absolute (ABS)	0.94	49	**0.02**
Immature Reticulocyte Fraction)	**0.78**	**49**	**0.00**

The Shapiro- Wilk test shows that only the hemoglobin level ($p = 0.44$) and the percentage of reticulocytes ($p = 0.43$) follow a normal distribution. All other parameters (RBC, MCV, MCH, MCHC, absolute reticulocytes, IRF) show an asymmetric distribution ($p < 0.05$). Therefore, Hb and reticulocyte % are reported as mean ± standard deviation; all other variables as median [IQR].

Profile of erythrocyte constants and median IRF

The erythrocyte and reticulocyte regeneration profile was synthesized below by applying the estimators recommended by the assessment of normality.

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Table 3: Erythrocyte and reticulocyte characteristics of the population (N = 49)

Settings Hematological	Central Tendency and Dispersion Indicators	Confidence Interval [95% CI] or IQR Space	Values Extremes [Min - Max]
IRF (%)	**Median : 23.30%**	**IQR: [19.95% - 26.36%] %]**	**16.94% - 48.07 %**
Hemoglobin (g/ dL)	Average: 7.33 ± 0.83	95% CI: [7.09 - 7.57]	**5.60 - 8.80**
Global Volume Average (VGM, fL)	Median: 88.10	IQR: [86.15 - 89.95]	**69.70 - 125.30**
TCMH (pg)	Median: 25.40	IQR: [24.50 - 26.85]	**19.32 - 33.56**
CCMH (g/ dL)	Median: 33.30	IQR: [32.35 - 34.10]	**23.60 - 34.80**
Red Blood Cells (M/µL)	Median: 2.89	IQR: [2.47 - 3.30]	**2.25 - 3.60**
Reticulocytes (%)	Average: 4.39 ± 1.24	95% CI: [4.04 - 4.75]	**1.70 - 6.71**
Reticulocytes Absolute (/ µL)	**Median: 230,000**	**IQR: [195,000 - 250 000]**	**149,000 - 363,000**

The mean hemoglobin level of 7.33 g/ dL confirms stable severe anemia in all participants. The median RFR of 23.30% is significantly higher than normal values for healthy subjects (10 to 40% for the analyzer's reference range, with typical physiological values below 10% in children without chronic hemolytic anemia). The wide MCV range (69.70 to 125.30 fL) indicates significant heterogeneity in red blood cell volumes among the different patients in the sample.

Inter-sex comparison of hematological parameters (Mann-Whitney test)

The application of the Mann-Whitney U test assesses whether biological fluctuations are influenced by gender.

Table 4: Inductive statistics for inter-sex comparison (Mann-Whitney test)

Constants Analyzed	Ranks (MeanRank)	Means	U of	W of	Z-	ρ-value
	Female (n=14)	**Male (n=35)**	Mann-Whitney	Wilcoxon	Score	**(bilateral)**
IRF (%)	26.93	24.23	218.00	848.00	-0.598	**0.55**
Hemoglobin (Hb)	30.71	22.71	165.00	795.00	-1.77	**0.08**
Red Blood Cells (RBCs)	30.64	22.74	166.00	796.00	-1.75	**0.08**
VGM	23.39	25.64	222.50	327.50	-0.49	**0.62**
TCMH	21.11	26.56	190.50	295.50	-1.21	**0.23**
CCMH	30.36	22.86	170.00	800.00	-1.66	**0.09**
Reticulocytes (%)	21.64	26.34	198.00	303.00	-1.04	**0.29**
Reticulocytosis is Absolutes	**29.50**	**23.20**	**182.00**	**812.00**	**-1.39**	**0.16**

Inductive analysis shows that no statistically significant difference is detected between boys and girls for all the parameters studied ($p > 0.05$): IRF ($p = 0.55$), Hb ($p = 0.08$), GR ($p = 0.08$), MCV ($p = 0.62$), MCHC ($p = 0.23$), MCHC ($p = 0.09$), reticulocytes % ($p = 0.29$), absolute reticulocytes ($p = 0.16$).

Correlations between IRF, relative and absolute reticulocytes (Spearman)

In order to analyze the correlation between the immature reticulocyte fraction (IRF) and the overall reticulocyte

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contingent (expressed in % and in absolute value), the Spearman correlation matrix was calculated.

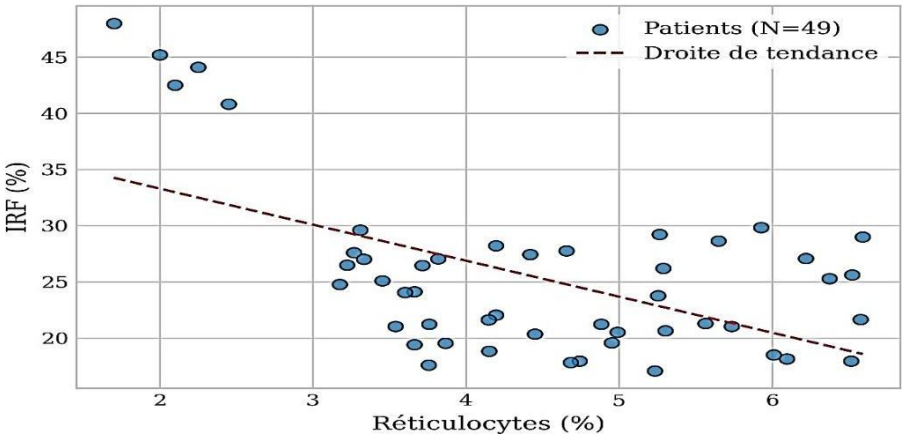
Table 5: Spearman non-parametric correlation matrix (N = 49)

Integrated Variables		Reticulocytes (%)	Reticulocytes Absolutes	IRF (%)
Reticulocytes (%)	Coefficient (rho)	1.00	**-0.581**	**-0.54**
Reticulocytes Absolutes	Coefficient (rho)	**-0.58**	1.0	**0.92**
IRF (%)	**Coefficient (rho)**	**-0.54**	**0.92**	**1.00**

The correlation is significant at the 0.01 level (two-sided).

1. IRF vs absolute reticulocytes: ρ = 0.923; 95% CI [0.861; 0.958]; p < 0.001 → very strong positive association;
2. IRF vs reticulocytes (%): ρ = -0.54; p < 0.001 → moderate negative association;
3. Reticulocytes (%) vs absolute reticulocytes: ρ = -0.58; p < 0.001 → moderate negative association.

Graph 1: Scatter plot illustrating the negative correlation between IRF (%) and relative reticulocyte count (%) in 49 children with sickle cell disease (SS) in the intercritical phase, Spearman analysis ρ = -0.54; p < 0.001. Graphic production software: SPSS 20.0



IRF/Reticulocyte Relationship (%): As illustrated in Figure 1, there is a surprisingly **moderate negative correlation** (rho = -0.545; ρ < 0.001). Statistically, this paradox is explained by the fact that the percentage is a relative fraction indexed to the total number of circulating erythrocytes, which is itself subject to the effects of hemolysis and transfusions. In conclusion, for the objective assessment of bone marrow regenerative capacity in homozygous SS sickle cell patients, the absolute value of reticulocytes combined with IRF constitutes a much more reliable and robust tool than the simple relative percentage. Clinically, this equation proves that interpretation based solely on the percentage, impacted by hemolysis, can mask the patient's true bone marrow response.

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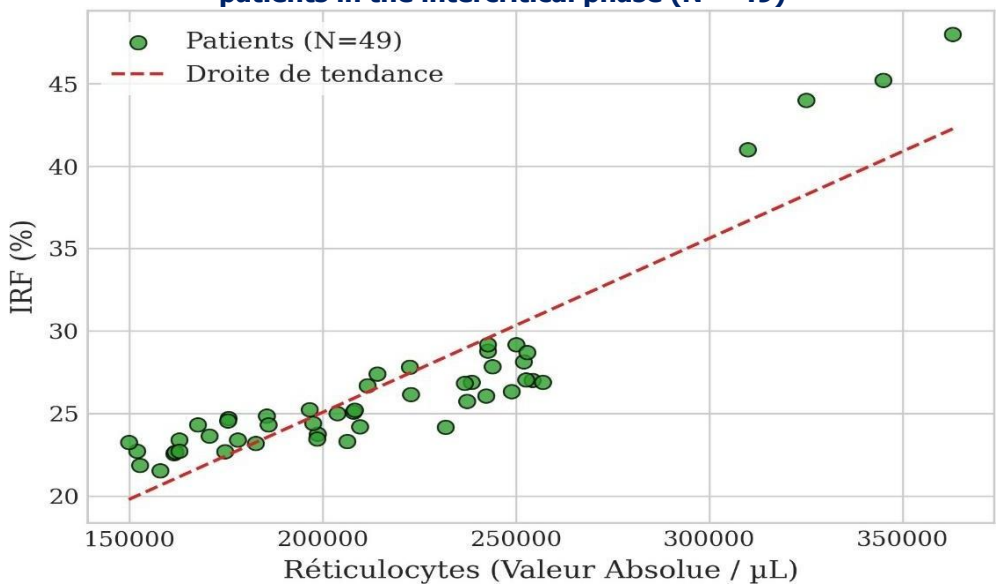
Figure 2 Scatter plot illustrating the positive correlation between the fraction of immature reticulocytes (IRF in %) and the absolute value of reticulocytes (per µl) in SS sickle cell patients in the intercritical phase (N = 49)



IRF/Absolute Reticulocyte Interaction: As illustrated in Figure 2, a nearly perfect and highly significant positive correlation is observed (rho = 0.923; $\rho < 0.001$). The profile demonstrates that the absolute quantitative increase in reticulocyte production is predominantly driven by the release of immature cells highly loaded with RNA (young fraction). This is a direct marker of the level of erythroid hyperactivity and regeneration in the intercritical phase. Clinically, the equation shows that the analyzer is reliable: the total increase in reticulocytes corresponds to the release of immature fractions.

Discussion

IRF profile and compensatory erythropoiesis

Our results show a consistently elevated IRF in children with sickle cell disease (SS) during the intercritical phase, with a median of 23.30%. This observation is consistent with persistent bone marrow reticulocyte stress linked to the accelerated destruction of sickle cells by chronic hemolysis (Kocko et al., 2016; Levy et al, 2001). Tissue hypoxia induced by anemia stimulates erythropoietin secretion, which prompts the bone marrow to massively produce immature reticulocytes that are prematurely released into the bloodstream before their intramedullary maturation is complete. These young, RNA-rich cells correspond precisely to the fraction measured by the IRF.

These data are consistent with work carried out in Niger (Mounkaila et al., 2015), which reported erythrocyte overproduction in SS patients in the stable phase; our study complements these results by characterizing the immature part of this bone marrow production using IRF.

In healthy children without chronic hemolytic anemia, the reference ranges for IRF measured on the URIT 5160 analyzer are below 10%. Our median of 23.30% significantly exceeds this physiological threshold, confirming the state of permanent spinal cord stimulation associated with sickle cell hemolysis. To date, no African study has provided specific pediatric IRF reference ranges for a stable sickle cell population; this work constitutes the first local comparative database for laboratories in Haut-Katanga equipped with the same instrument.

Associations between IRF and reticulocyte parameters

The strong positive association between IRF and the absolute number of reticulocytes observed in our sample suggests that any increase in total production of young red

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blood cells is primarily due to highly immature reticulocytes loaded with RNA. This correlation does not constitute proof of the analyzer's analytical reliability nor a demonstration of the clinical superiority of IRF; it merely reflects a monotonic association in our cohort.

The moderate negative correlation between the IRF and the relative reticulocyte count is explained by the relative nature of the percentage, calculated on the variable mass of circulating red blood cells. In sickle cell patients, cell lysis causes the denominator of the ratio to fluctuate, distorting the isolated interpretation of the reticulocyte count without an association with the absolute number. This result suggests that the combination of IRF and absolute reticulocyte count provides a more nuanced assessment of bone marrow activity than the simple relative count in the Congolese context.

This strong positive correlation between IRF and absolute reticulocytes confirms the analytical consistency of the measurements of the URIT 5160 analyzer on our cohort; however, this statistical association does not constitute a formal demonstration of diagnostic or predictive superiority of IRF in routine clinical practice.

Erythrocyte indices and inter-patient cellular heterogeneity

The mean hemoglobin level of 7.33 g/ dL corresponds to data reported in Morocco and Senegal for sickle cell disease (SS) patients in the intercritical phase (Dahmani et al., 2016; Doupa et al., 2017), confirming the characteristic of severe, stable anemia associated with this disease in sub-Saharan Africa. The median MCV of 88.10 fL places the majority of children within the normocytic range, but the very wide range of variation reflects significant interindividual heterogeneity in cell sizes. Without RDW measurement or morphological analysis of blood smears, we cannot attribute this dispersion to intraindividual anisocytosis related to macroreticulocytes and sickle cell debris. (Varet,2012)

Inter-sex comparison and statistical limitations

No significant hematological differences were observed between boys and girls in our sample. This finding should be qualified by the significant imbalance in the number of males and females, which reduces the statistical power of the Mann-Whitney test. Studies on sex-balanced samples will be necessary to confirm the absence of gender-based modulation of reticulocyte parameters in children with sickle cell disease.

Generalizability and External Validity

Because this study utilized a non-probability convenience sampling method restricted to patients monitored at the DeeServices Medical Laboratory in central Lubumbashi, the external validity of these findings is constrained. The resulting reference metrics and median IRF values cannot be broadly generalized to all pediatric sickle cell patients across the wider urban or rural settings of Haut-Katanga without broader multicenter validation. Furthermore, the observed male-to-female sex ratio imbalance (2.50) limits the statistical power required for definitive gender-stratified inferences.

Conclusion

This pilot cross-sectional study, conducted on 49 homozygous SS sickle cell children aged 1 to 12 years in the intercritical phase in Lubumbashi, describes the local profile of the immature reticulocyte fraction and erythrocyte indices measured using an automated URIT 5160 analyzer. In this Congolese pediatric cohort, the immature reticulocyte fraction (IRF) had a high median of 23.30%, and a very strong, statistically significant positive association was observed between the IRF and the absolute reticulocyte count. These biological observations are consistent with permanent compensatory erythropoiesis induced by the chronic hemolysis characteristic of stable sickle cell disease. This work provides local reference values that can be used by laboratories in Haut-Katanga equipped with the same hematological analyzer. However, this cross-sectional design does not allow us to demonstrate the diagnostic superiority, predictive value, or clinical benefit of IRF compared to conventional hematological parameters. Multicenter longitudinal studies including the monitoring of vaso -occlusive crises and the collection of therapeutic and infectious covariates are needed to further explore the clinical applications of IRF in the monitoring of Congolese sickle cell patients.

Limitations of the study

1. Cross-sectional design: A single sample per patient does not allow the temporal dynamics of IRF to be studied, nor can its predictive value for the occurrence of vaso -occlusive crises be established; longitudinal follow-ups are essential to explore this axis.

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2. Convenience sampling: The sample is not representative of all children with sickle cell disease in Lubumbashi, limiting the generalization of the reference values obtained.
3. Absence of confounding covariates: No data on hydroxyurea treatment, previous transfusions, parasitic infections, or nutritional deficiencies were collected during recruitment; we cannot adjust the correlations for these factors known to modify erythropoiesis.
4. Lack of complementary parameters: RDW, complementary electrophoresis, and seizure monitoring were not integrated into the protocol, restricting the morphological and clinical interpretation of erythrocyte indices.
5. Single analysis platform: IRF values are specific to the URIT 5160 analyzer; multicenter studies with different analyzers are needed to harmonize local reference thresholds.

Recommendations

1. To the health facilities of Haut-Katanga: Equip the province's hospital laboratories with automated hematological analyzers capable of measuring IRF, to have a complementary tool for assessing bone marrow activity in sickle cell patients during the intercritical phase. Integrate the collection of treatment data (hydroxyurea, transfusions) into the patients' biological monitoring records.
2. To local research teams: Implement longitudinal studies to analyze variations in IRF before the transition to the critical phase, and carry out probabilistic sampling balanced by age and sex to establish generalizable population references.
3. To pediatric clinicians: Integrate the IRF + absolute reticulocytes pair into the interpretation of the hematological assessment of children with sickle cell disease, in addition to the classic hemoglobin and MCV indices, to better appreciate the intensity of bone marrow regeneration in the stable phase.

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Conflict of interest

This work was carried out impartially and with complete independence of mind. No conflict of interest has been declared.

Data availability statement

Anonymized datasets and the dictionary of hematological variables can be provided to qualified researchers upon reasoned request addressed to the corresponding author, in accordance with Congolese medical confidentiality rules. No datasets are currently deposited in a public data repository.

List of abbreviations

- **CI:** Confidence Interval
- **Hb:** Hemoglobin
- **IRF:** Immature Reticulocyte Fraction
- **IQR:** Interquartile Range
- **LMDS:** DeeServices Medical Laboratory (*Laboratoire Médical DeeServices*)
- **MCH:** Mean Corpuscular Hemoglobin
- **MCHC:** Mean Corpuscular Hemoglobin Concentration
- **MCV:** Mean Corpuscular Volume
- **RBC:** Red Blood Cell
- **SCD:** Sickle Cell Disease
- **SS:** Homozygous Sickle Cell Genotype
- **URIT:** URIT Medical Electronic Co., Ltd.

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Author contribution

Systematic review of scientific literature, bibliographic review, correction of references.
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