RESEARCH ARTICLE

Validity of simple clinical and biological parameters as screening tool for sickle cell anemia for referral to tertiary center in highly resource constraints

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Background: In the Democratic Republic of Congo, the incidence of sickle cell anemia (SCA) is estimated around 40 000 neonates per year. However, it is notoriously difficult to perform conventional electrophoresis in all hospitals and laboratories, especially at peripheral levels and rural area. A panel of multiple clinical and laboratory features that would enhance sickle cell disease were assessed for the detection of the disease in highly resource-scarce settings.

Methods: A prospective study was conducted in Kinshasa. Venous blood samples were drawn from each study participant in order to determine the hematologic parameters, the peripheral smears, and the hemoglobin electrophoresis. We used Cohen's κ statistic to examine the agreement of each variable and diagnosis of sickle cell disease.

Results: A total of 807 patients were screened for sickle cell disease. Among these 807 children, 36 (4.5%) were homozygous for Hb S disease. The presence of at least 8% erythroblasts (PPV: 91%, NPV: 99%, sensitivity: 83.3%, specificity: 99.6%, κ value: .86) and sickle cells (PPV: 100%, NPV: 98%, sensitivity: 50%, specificity: 100%, κ value: .66) in the peripheral blood smear had an acceptable agreement for sickle cell disease.

Conclusion: These two biological markers may guide the clinician in the decision-making to initiate the management of the children as a sickle cell patient, pending confirmation of the disease by electrophoresis techniques.

KEYWORDS

Africa, children, diagnosis, highly resource constraints, Kinshasa, severe anemia, sickle cell disease, The Democratic Republic of Congo

1 | INTRODUCTION

Sickle cell disease (SCD) remains a genetic disease of global health concern with the heaviest burden of disease in resource-limited settings of sub-Saharan Africa.¹

Approximately, 305 800 neonates per year globally are born with HIV and 90% of these neonates are found in sub-Saharan Africa.¹

The Democratic Republic of Congo (DRC) has the third highest population of SCA patients in the world after Nigeria and India. The incidence of SCA is estimated around 40 000 neonates per year.¹ Without access to early diagnosis and adequate management including prevention of infection through the penicillin prophylaxis, pneumococcal vaccination, and health education, from 50% to 90% of these unknown SCD neonates die by the age of 5 years from several

complications.²⁻⁵ An increase in access to early diagnosis is critical for improving these children's survival and quality of life.⁶⁻¹⁰

In most resource-limited settings such as the DRC, SCD is conventionally diagnosed by hemoglobin electrophoresis. It is, however, notoriously difficult to perform conventional electrophoresis in all hospitals and laboratories, especially at peripheral levels and rural area; and some techniques are not recommended for infants <6 months of age.¹¹ In addition, the test is expensive and most of the families are poor and because a free healthcare facility is not available in the DRC, these techniques are not affordable to many of them.^{12,13}

We have been interested in the use of clinical and laboratory parameters as markers for sickle cell diagnosis and early management of patients, especially because of the general absence of neonatal screening for sickle cell disease in the health policy of the Democratic Republic of Congo.^{4,5,12} This feature is one of the innovative notions that we have proposed in our study. This information will give the whole view of patients and may serve to rule out general public health and in-hospital health policy in poor resources settings.

This article reports on a cross-sectional study that was conducted in children <3 years in Kinshasa, the large city of the DRC. The aim of this study was to carry out the evaluation of the value of the clinical and laboratory parameters easy to perform, which predict the diagnosis of sickle cell disease in absence of hemoglobin (Hb) electrophoresis in poor resources settings.

2 | METHODS

2.1 | Subjects and study design

A prospective study was conducted from May 2011 to August 2011 in four hospitals in Kinshasa, namely the General Hospital of Makala in the Funa district, the Kalembe Lembe Pediatric Hospital in Lukunga, Saint Joseph's Hospital in Mont Amba, and the Centre Hospitalier de Kingasani in Tshangu. Kinshasa is the largest city in the DRC with a prevalence rate of 1.4% of newborns with SCA, out of the estimated 3000 births/y.¹⁴

We consecutively recruited children with positive symptoms and signs of severe anemia aged between 3 months and 15 years requiring admission for blood transfusions, if their parents provided written informed consent for their child to participate. The exclusion criteria were as follows: a current crisis or history of sickle cell crisis, previous diagnosis of SCA, or blood transfusions in the 3 months before the study.

We carried out a complete clinical history and physical examination and recorded the patients' age, sex, medical history, and clinical feature. The clinical presentation report to what the physician had seen when examining the patient at our institution. We also recorded the outcome of laboratory investigations, including hemoglobin, blood count, mean corpuscular hemoglobin, mean corpuscular volume, electrophoresis of hemoglobin, and thick and thin blood film for malarial parasites.

2.2 | Data collection procedure and blood analysis

After obtaining informed consent from the parents, each child's caregiver was interviewed using a structured standard project

questionnaire to obtain a detailed history of the participants' disease. A complete physical examination was carried out on each child, which focused on detecting anemia, jaundice, hepatomegaly, and splenomegaly. Venous blood samples (5 mL EDTA) were drawn from each study participant in order to determine the hematologic parameters, the peripheral smears, and the hemoglobin electrophoresis. Blood to anticoagulant ratio should be in the right proportion. Samples were sent to the laboratory as soon as possible. Samples were analyzed within 2 hours of blood collection.

The thick and thin films were fixed with Giemsa for malarial parasite screening and identification.

Slide preparation was done by trained medical laboratory technologist and residents in hematopathology of the University Hospital of Kinshasa, who can ensure quality slides for microscopy. The slides were independently re-examined by three hematopathologists in the department to confirm the morphology of the blood cells. Where there was disparity in the interpretation, conference review was carried out until a consensus diagnosis was agreed upon.

Abnormal variations in cell size, shape, color, presence of intracellular inclusions, and pathologic arrangement of the cells were suggestive of abnormalities.

An amount of 100 μ L of blood was mixed with 100 μ L of the brilliant cresyl blue solution 1% in a tube. The mixture was placed in the incubator at 37°C for 15-20 minutes before taking a drop and made a thin smear on a slide. The blade was dried in the open air and the reading was made under the microscope objective 100 with the immersion oil. In all, 1000 red cells were counted to determine the percentage of reticulocytes by a training physician.

The sickle cell screening was performed using an isoelectric focusing technique with the Multiphor II apparatus (GE Healthcare, Little Chalfont, Buckinghamshire, England).

2.3 | Definition of concept

Anemia was defined as Hb <11.0 g/dL, as severe (Hb <7.0 g/dL), moderate (Hb between 7.0 and 10.0 g/dL), and mild (>10 g/dL Hb <11 g/dL).¹⁵ SCA (hemoglobin SS) was diagnosed based on the presence of mostly hemoglobin S (β_6 (A3) Glu→Val; HBB: c.20A>T) with no hemoglobin A ($\alpha_2\beta_2$).

Mean corpuscular volume was used to define microcytic anemia (<80 fL), normocytic anemia (80-100 fL), and macrocytic anemia (>100 fL); mean corpuscular hemoglobin was used to define hypochromic anemia (<27 pg) and normochromic anemia (27-35 pg); and the reticulocytosis count was used to define aregenerative anemia ($\leq 100\ 000/\text{mm}^3$) and regenerative anemia (>150 000/ mm^3). Elevated leukocytosis was defined as a leukocyte count more than $15 \times 10^9/\text{L}$ and thrombocytosis as a platelet count more than $500 \times 10^9/\text{L}$.

Anisocytosis was described as a wide variation in cell size. Abnormalities of cell size can be microcyte (smaller) or macrocyte (larger RBC). Target cells were described as red cells with central staining with precipitated hemoglobin. Sickle cells were described as spindle-shaped morphology of RBCs.

Reticulocytes were described as polychromatic cells on stained slides and erythroblasts as circulating nucleated red cells.

The interval scale categorizes the following parameters: (i) Age (≤ 6 months, 7-36 months, 37-60 months, > 60 months); (ii) presence of SCA in siblings (≤ 2 and > 2); (iii) history of transfusion in siblings (≤ 2 and > 2); (iv) history of deaths in siblings before the age of 5 years (≤ 2 and > 2); (v) number of blood transfusion (≤ 2 and > 2); and (vi) duration of disease (≤ 3 days and > 3 days).

2.4 | Data management

The data were analyzed using Epi info version 3.5.1 (CDC, Atlanta, GA, USA). After data cleaning, to control for quality and coherence, they were exported on SPSS 10.0 (SPSS for Windows, version 10.0; SPSS Inc, Chicago, IL, USA) for further analysis. Entered data were double checked for discrepancies. Data are represented as mean $\pm$ standard deviations (SD) when the distribution was normal and median with ranges when the distribution was not normal. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated for the sickle cell diagnosis. Finally, we used Cohen's κ statistic ranges from -1 to $+1$ and can be categorized as none agreement ($r = .01-.19$), minimal ($r = .20-.49$), weak ($r = .50-.59$), moderate ($r = .60-.79$), and very strong ($.80-.90$ or perfect agreement ($r > .90$) to examine the agreement of each index and diagnosis of sickle cell disease.¹⁶

3 | RESULTS

3.1 | Study population

Data on 807 patients, 402 males (53.2%) and 345 females (46.8%), were screened for sickle cell disease. Overall mean age at presentation was 42.7 months $\pm$ 29.7 months (range: 4-172 months);

3.2 | Clinical parameters

3.2.1 | Positive predictive values

Positive predictive values were from 6.5% to 60% for clinical variables (Table 1).

3.2.2 | Negative predictive values

Positive predictive values were from 95.9% to 99% (Table 1).

3.2.3 | Sensitivity

In this study, the observed sensitivity values were more than 90% for only the first blood transfusion < 36 months of age.

3.2.4 | Specificity

The observed specificity values were good ($> 90\%$) for medical history of more than two transfusions, antecedent of hand foot syndrome, absence of fever, and jaundice.

TABLE 1 Sociodemographic and epidemiological characteristics of study children

Parameter	Patients (n=807)
Age, mo	
Mean (SD)	42.7 (29.7)
Age distribution, mo	
< 6 mo, n (%)	31 (3.8%)
7-36 mo, n (%)	363 (45%)
37-60 mo, n (%)	252 (31.2%)
> 60 mo, n (%)	161 (20%)
Sex	
Female, n (%)	402 (57.2%)
Male, n (%)	345 (42.8%)
History of sickle cell disease in siblings, n (%)	20 (2.4%)
History of transfusion in siblings, n (%)	315 (39%)
History of death < 5 y in siblings, n (%)	201 (24.9%)
Personal history of hand-foot syndrome, n (%)	30 (4.1%)
Personal history of previous blood transfusion, n (%)	211 (26.1%)
Median number of transfusion (range)	0 (0-9)
Median age of first transfusion (range), mo	29 (4-159)

3.2.5 | Kappa values

In this report, no clinical parameters showed acceptable agreement with sickle cell disease. (Table 1).

3.3 | Biological parameters

3.3.1 | Sensitivity

In this study, the observed sensitivity values were more than 90% for hyperleukocytosis $\leq 15.10^3/\text{mm}^3$ and thrombocytosis (Table 2).

3.3.2 | Specificity

The observed specificity values were good ($> 90\%$) for regenerative anemia, target cells, sickle cell, and erythroblasts $\geq 8\%$ (Table 2).

3.3.3 | Kappa values

Kappa values varied from .05 to .86. The presence of sickle cells and the presence of at least 8% erythroblasts in the peripheral blood smear had a good and excellent agreement, respectively, with sickle cell disease (Table 2).

4 | DISCUSSION

The high prevalence of sickle cell disease in the DRC and the limitations of the currently available diagnostic and control methods

TABLE 2 Concordance values of clinical parameters and the diagnostic of sickle cell disease

Variables	PPV (%)	NPV (%)	Se (%)	Sp (%)	k	Agreement
Medical history of more than two transfusions	21.4	97.5	50	91.4	.25	Minimal
1st transfusion received before the age of 36 mo	6.5	99	91.7	38.1	.04	None
Antecedent of hand-foot syndrome	52.4	96.8	30.6	98.7	.37	Minimal
Absence of fever	50	95.9	8.3	99.6	.13	None
Current episode of illness ≤ 3 d	7.1	97.1	58.3	64.2	.05	None
Jaundice	60	96.6	25	99.2	.05	None

PPV, Positive predictive values; NPV, negative predictive values; Se, sensitivity; Sp, specificity.

require the development of complementary approaches to assist in the sustainable detection of the disease in children with severe anemia. Hemolytic anemia is a common clinical condition in patients with sickle cell disease and there are conflicting reports on clinical and laboratory indices useful in predicting those patients who are likely to sickle cell disease (Table 3).

In this cohort, no clinical parameters showed acceptable agreement with sickle cell disease.

In our midst, the peripheral blood smear examination provided the most consistent evidence with sickle cell disease in children suffering from severe anemia. According to the results, the presence of erythroblasts $\geq 8\%$ (PPV: 91%, NPV: 99%, sensitivity: 83.3%, specificity: 99.6%, κ value: .86) was found to be the most predictive factor for diagnosis of SCD in children with severe anemia in our midst. The presence of erythroblasts has been previously reported in the hematological profile of Congolese patients with homozygous sickle cell disease during periods of remission.¹⁷ Normally, red blood cell population on a peripheral blood smear is uniform in shape (biconcave disc) in size (7–8 μm) and coloring. In Hemolytic anemia, abnormalities of forms (poikilocytosis) and size (anisocytosis) are reported. It also contains circulating nucleated red cells (erythroblasts), the expression of an overactive bone marrow.¹⁸

Peripheral destruction of sickled erythrocytes is a cardinal feature of SCA, leading to the presence of high numbers of erythroblasts in

the peripheral blood. This presence is a reflection of the hypoxic stress and inadequate erythropoiesis described in the disease.¹⁹

In the standard blood smear, the sickle cells in the peripheral blood smear standard are often inconsistent. They are best observed when the red blood cells are deprived of oxygen (sickle cell test). These sickling tests do not differentiate homozygous SS subjects and the carrier of sickle cell trait. In our series, sickle cells (PPV: 100%, NPV: 98%, sensitivity: 50%, specificity: 100%, κ value: .66) were also found to be the most predictive factor for diagnosis of SCD. This finding reported in our cohort was similar to those reported by previous studies.^{20–22}

In the DRC, malaria is holoendemic and characterized by a high and stable transmission. *Plasmodium falciparum* is the most prevalent species and contributes around 97% of episodes of malaria.²³ Previous studies showed that the proportion of sickle cell anemia in the group with negative thick blood smear was significantly higher than that observed in anemic with positive thick smear.²⁴ These results were confirmed and extended by the findings of a recent study in our midst.⁵ Various hypotheses have been advanced to explain this phenomenon such as the invasion of red cells by *Plasmodium* causes sickling and the destruction of red blood cells by the spleen,²⁵ impaired parasite egress from dehydrated erythrocytes,²⁶ the difficulty for the *Plasmodium* to develop properly under reduced O_2 tension in a sickle red cell,^{26,27} and inhibition of parasite replication by human erythrocyte miRNAs.^{27,28}

TABLE 3 Concordance values of biological parameters and the diagnostic of sickle cell disease

Variables	PPV (%)	NPV (%)	Se (%)	Sp (%)	k	Agreement
Hemoglobin < 5 g/dL	6.9	98.4	83.3	47.1	.05	None
Normochromic anemia	18.6	98.2	66.7	86.4	.24	Minimal
Normocytic or macrocytic anemia	10	97.9	66.7	72	.1	None
Regenerative anemia	50	96.2	16.7	99.2	.23	Minimal
Leucocytosis $\leq 15 \times 10^9/\text{L}$	17.7	99.5	91.7	80.2	.24	Minimal
Thrombocytosis	17	99.5	91.7	79.1	.23	Minimal
Target cells	40	99.2	83.3	94.2	.5	Weak
Anisopoikilocytosis	27.8	99.1	83.3	89.9	.37	Minimal
Sickle cells	100	97.7	50	100	.66	Moderate
Erythroblasts $\geq 8\%$	90.6	99.2	83.3	99.6	.86	Strong
Negative thick films for plasmodies	12.7	98.1	66.7	78.6	.15	None

PPV, Positive predictive values; NPV, negative predictive values; Se, sensitivity; Sp, specificity.

Despite these hypothesis, our results show that negative thick films for plasmodium (PPV: 13%, NPV: 98%, sensitivity: 67%, specificity: 79%, κ value: .15) were worse predictor of sickle cell disease in children with severe anemia.

On the other hand, hemoglobin was a worse predictor of sickle cell disease and was similar to those described elsewhere.²²

5 | CONCLUSION

This worthwhile study will be important to identify early children suffering probably from SCA, especially for health professionals in a resource-limited setting. The presence of erythroblasts ($\geq 8\%$) and sickle cell on peripheral blood smear may be considered as indicators of the disease in children with severe anemia in our midst. These two biological markers may guide the clinician in the decision-making to initiate the management of the children as a sickle cell patient, pending confirmation of the disease by electrophoresis techniques.

5.1 | ETHICAL APPROVAL

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

ACKNOWLEDGMENTS

The authors thank all the children, guardians, and parents who participated in this study. We thank all our colleagues involved in the collection of samples, all the nurses and technicians of the four hospitals for the support given for the present study.

CONTRIBUTOR'S STATEMENT

BTK, JLG, and MNA conceived and designed the study protocol; BTK and JTB carried out the clinical assessment; BTK, JLG, MNA, and FIM analyzed and interpreted these data. BTK and MNA drafted the manuscript; MNA and JLG critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. BTK is guarantor of the paper.

Source of support: none.

Disclosure of funding received for this work: none.

Honorarium, grant, or other form of payment to produce the manuscript: none.

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How to cite this article: Kadima BT, Gini-Ehungu JL, Mbutiwi FIN, Bahati JT, and Aloni MN. Validity of simple clinical and biological parameters as screening tool for sickle cell anemia for referral to tertiary center in highly resource constraints. *J Clin Lab Anal*. 2017;31:e22145. <https://doi.org/10.1002/jcla.22145>