ORIGINAL ARTICLE | SICKLE CELL DISEASE

Diagnosis of Sickle Cell Disease in Gabon Using Sickle SCAN®: A Point-of-Care Blood Test

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ABSTRACT

Background: Sickle cell disease (SCD) is the world’s most common genetic blood disorder. Most infant deaths from SCD occur in sub-Saharan Africa, and the mortality rate is exceptionally high in Gabon in Central Africa. In Gabon, there are limited resources for early and accurate detection of sickle cell or trait conditions. Most affected individuals reside in low-resource areas where access to diagnostic testing is often very limited. The most commonly used diagnostic methods require trained staff, adequate infrastructure, electric power, and enough time to perform the analysis. These requirements do not currently allow mass screening to be performed in remote areas of Gabon. The purpose of this pilot study is to develop a standardized screening procedure for SCD in order to determine its prevalence in the southeast area of Gabon by using a rapid and reliable test that does not require significant resources, such as the Sickle SCAN® device.

Methods: The accuracy of the Sickle SCAN® device was assessed based on testing 272 blood samples. The exact hemoglobin was identified in 100% of the samples. The test comprises three proprietary indicators that detect the presence of hemoglobin A, S, and C, thus allowing the user to rapidly distinguish between normal (HbAA, N=142), carrier (HbAS, N=41), and sickle cell disease (HbSS, N=88; HbSC, N=1) samples. Hemoglobin variants (S and C) were confirmed by capillary electrophoresis (MinicapR; Sebia). The sensitivity and specificity were calculated for each phenotype.

Results: The Sickle SCAN® test analysis revealed the following normal and abnormal phenotypes, including sickle cell trait (HbAS=14.71%) and SCD [HbSS (32.35%) and HbSC (0.74)]. There are no false positives in Sickle SCAN® result for the presence of hemoglobin variant, compared to gold standard approaches to capillary electrophoresis. The estimated sensitivity of the Sickle Scan® test was 92.2%, 100%, and 100% for HbSS, HbAS, and HbSC, respectively. The specificity exceeded 88.23% for all phenotypes (HbSS, HbAS, and HbSC).

Conclusion and Implications for Translation: The Sickle Scan® device was found to be reliable with a sensitivity of 92.2%, 100%, and 100% for HbSS, HbAS, and HbSC, respectively. The specificity exceeded 88% for all phenotypes.

Keywords: • Sickle Cell Disease • Phenotypic Diagnosis • Sickle SCAN® • Capillary Electrophoresis • Gabon
1. Introduction

1.1 Background of the Study

Sickle hemoglobin (HbS)\(^1\) is the most common pathological hemoglobin variant in humans in the world.\(^2\)\(^3\) It is estimated that more than 300,000 children are born each year with a severe inherited hemoglobin disorder and that approximately 80% of these births occur in low- or middle-income countries.\(^4\) The homozygous HbSS state is associated with clinical illness with many complications. The World Health Organization (WHO) has declared this disease a public health priority.\(^5\) Anemia, susceptibility to infections, and vaso-occlusive crises at multiple sites, which are often painful and responsible for associated complications,\(^6\) are ubiquitous occurrences in sickle cell disease clinics.\(^6\) The use of penicillin and neonatal screening has reduced mortality among sickle cell patients in developed nations.\(^7\) However, the prevalence of sickle cell disease (SCD) remains high in Africa, where it is a leading cause of death in children under 5 years of age in the absence of adequate medical care.\(^8\)

In Gabon, Central Africa, a number of studies carried out in urban areas has highlighted the serious health problem posed by sickle cell disease. In 1998,
the mortality rate of children due to sickle cell disease in a pediatric ward in Libreville, the capital of Gabon, was 7.2%.9 Recent studies carried out in urban areas by Vierin Nzame et al. in neonates revealed a 1.8% incidence of the homozygous state (SS).10,11 A study by Délicat-Loembet et al. in rural areas (in individuals aged at least 15 years) found that 21% of the population were carriers of the S-allele in the heterozygous state, with an absence of homozygous individuals over 15 years of age.12 Without an early diagnosis, most children born with SCD in settings with limited resources die within the first five years of life, usually before a diagnosis is even made, presumably due to severe anemia or infection.13 However, there are no studies that can be used to assess the prevalence of sickle cell trait or sickle cell disease among young children in urban and semi-urban area of south-east Gabon. Currently, two or three gold standard approaches have been validated for first-level neonatal screening and routine diagnosis of SCD: (1) high-performance liquid chromatography (HPLC); (2) capillary electrophoresis (CE), and (3) isoelectric focusing (IEF).

In the context of low-income countries, diagnosing sickle cell disease requires point-of-care (POC) testing.14 Indeed, the techniques used to date are neither sufficiently rapid nor easy to implement in resource-limited settings as they require a substantial financial investment in order to ensure regular restocking of key reagents, the availability of electricity, involvement of dedicated and trained technicians, transport and storage of biological samples, and adherence to strict laboratory standards. They also suffer from a high risk of loss to follow-up due to the long lag time between sample collection and the delivery of results, which often takes 4 to 6 weeks or more.15

The burden of SCD is becoming increasingly recognized on a global scale by large organizations with an identified urgent need for low-cost and accurate POC diagnostic devices for SCD in high-prevalence, low-resource areas. There are several types of POC for diagnosing sickle cell disease based on various analytical principles such as the Sickle SCAN® device from BioMedomics. This device, which is based on a sandwich-format chromatographic immunoassay developed for qualitative measurement of HbA, HbS, and HbC in whole blood samples, has been shown to have an excellent intrinsic performance to detect common variants of hemoglobin.16

1.2 Objectives of the Study
The objectives of this study were to (1) identify the methods of analysis suitable in the context of the Gabonese environment and the various SCD genotypes, and (2) evaluate the effectiveness of the Sickle SCAN® rapid diagnostic test.

2. Methods

2.1 Study Design
The study was conducted on 299 subjects in Haut-Ogooué in Gabon, a country in Central Africa. Gabon has a surface area of 267,667 km² (nearly 80% of which is covered by rainforest) Figure 1A.

2.2 Study Population and Sample Size
A multidisciplinary (pediatricians, nurses, epidemiologists, laboratory technicians, and health researchers) team conducted the survey for three days during a free medical screening drive organized by the Non-Governmental Organization Sickle Cell Disease Organization of Gabon in June 2018. In the beginning, 299 voluntary participants who had not received a recent blood transfusion were recorded and at the end, we selected participants whose age was not over 19 years old for further analyses.

2.3 Procedure
Two biological examinations were performed, Sickle SCAN® device and capillary zone electrophoresis (CEZ). The testing used venous blood samples in EDTA tubes. The collected blood samples were stored between 2 °C and 8 °C for a maximum of seven days. All of the experiments were performed in an anonymized manner using a unique identification number for each blood sample. The Sickle SCAN® test was performed according to the manufacturer’s protocol.17 A qualified laboratory staff member obtained all of the results which were then validated by the physician.
To confirm positive Sickle SCAN® results, we used CEZ (MinicapR; Sebia) to determine the types and percentages of normal and abnormal hemoglobin in each sample using a high-throughput analysis to detect different hemoglobin variants.\(^{18}\)

CEZ is a quantitative test that is a hybrid technique combining two principles to separate hemoglobin variants.\(^ {19}\) The relative amounts of hemoglobin in the fractions can be quantified and represented graphically as a pherogram. The results obtained using the Sickle SCAN® device (HbS and HbC) were compared with those obtained by capillary zone electrophoresis at Franceville Interdisciplinary Medical Research Center (CIRMF) medical analysis laboratory. The sensitivity and specificity to detect the presence of HbA, HbS, and HbC were calculated.

### 2.4 Statistical Analysis

Several explanatory variables were considered (sex, age, names, and surnames of the parents as well as their employment and their place(s) of origin) and the results of the diagnosis of each subject were reported on a survey sheet. The statistical analyses were performed using Epi Info™ software version 7.2 (public domain software) and StatView® software (version 5.0) (by SAS Institute Inc). The quantitative data were presented as minimum, maximum, and average values, and the standard deviation, while the qualitative data are presented as percentages. The Chi-square test was used to compare the categorical variables; Fisher’s exact test was used when the expected value in any cell was less than five and the Student test was used for quantitative variables. The p-value used for comparison was 0.05. The distribution of patients according to several variables was analyzed in terms of their age range, sex, the result of the Sickle SCAN®, and those of CEZ.

### 2.5 Ethical Approval

Informed consent was obtained, which for minors entailed the parents signing the informed consent to participate. Subjects whose parents refused to provide informed consent or who had a blood transfusion less than three months prior to the day...
of the analysis were excluded. The pharmacy and medicine executive body of Gabon approved the procedure of this medical screening initiative.

3. Results

3.1 Socio-demographic Characteristics of the Study Population

The database comprised 299 patients, aged 6 months to 33 years, with a median age of 7 years, the 10-year mode, the median was 7 years and the average was 7.748 years ± 5.643. All of the samples were venous blood samples. The study population came from various localities: 97.99% (293/299) were from the Haut-Ogooué province, 2% (6/299) were from the province of Ogooué-Lolo, 98.98% of all of them were Gabonese. In all, 272 individuals were selected for data analysis. Ultimately, they were 124 males (45.23%) and 148 females (54.61%), thus amounting to a sex ratio of 1.2.

The tests were carried out on subjects who were over 6 months of age. The distribution of the study population by age and sex showed that most of the children were in the 5-9 years age group (31.25%) followed by children in 2-4 years (27.94%) and followed by the group of 10-14 children aged (16.17%). Children aged from 15 to 19 years constituted the smallest age group in our study. The results of the analysis of this Sickle SCAN® point-of-care test are presented in Figure 1B. The Sickle SCAN® test analysis revealed the following normal and abnormal phenotypes including sickle cell trait (HbAS) and SCD (HbSS and HbSC). The homozygous HbAA phenotype was most prevalent (52.21%), followed by the homozygous S-allele (32.35%) and heterozygous AS (14.71%). There was also a small proportion of composite heterozygous SC (0.74%) as shown in Figure 1C.

3.2 Phenotype Identified by Sex and Age Range

In order to determine the sex and age range of children most affected by this disability, we represented the data taking into account age, sex, and the results obtained with Sickle SCAN®. The homozygous and heterozygous HbS (HbSS and HbAS) cases were more often female (17.27% for HbSS and 8.08% for HbAS) than male (15.07% for HbSS and 6.61% for HbAS), but it was not significant (p=0.67), while the number of composite heterozygous HbSC cases was the same for both sexes, at 0.36% each. The HbAS phenotype was more common in children aged 0-1 and 5-9 years (p=0.01), while the HbSC phenotype was found only in the 2-4 and the 10-14 years of age groups. Concerning the HbSS phenotype, Figure 1D shows that it was more prevalent in children between 5 and 9 and between 10 and 14 years of age.

3.3 Does Sex Affect the Proportion of SS in the Different Age Groups?

We examined whether the distribution of homozygous hemoglobin S (HbSS) in the various age groups varied according to sex. Figure 1D shows that females were more prevalent in the 5-9 years of age group and that males were more prevalent in the 10-14 years of age group but it was not significant (p=0.35). The least prevalent age group was the 15-19 years of the age group for males and the 0-1 years of the age group for females.

3.4 Phenotypes Identified Using Capillary Electrophoresis

Participants with a positive Sickle SCAN® result, with a hemoglobin variant (HbAS, HbSS, or HbSC), were asked to undergo a second test to confirm the result by CEZ. Out of all, 50 participants were eligible for the second test, but ultimately only 17 participants took part in this second round of screening. The remaining 33 had undergone a transfusion within the past three months and were excluded. Table 1 shows the phenotypes identified using the CEZ test. The homozygous HbSS phenotype was predominant at 82.35%, while the homozygous HbDD phenotype, the AS, and SC composite heterozygous phenotypes each represented 5.88%. Sickle SCAN® rapid test

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAS</td>
<td>1</td>
<td>5.88%</td>
</tr>
<tr>
<td>HbDD</td>
<td>1</td>
<td>5.88%</td>
</tr>
<tr>
<td>HbSC</td>
<td>1</td>
<td>5.88%</td>
</tr>
<tr>
<td>HbSS</td>
<td>14</td>
<td>82.35%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>17</td>
<td>100%</td>
</tr>
</tbody>
</table>
results were compared with those obtained with capillary electrophoresis for the 17 subjects, as shown in Table 2.

The presence of a new phenotype (HbDD) and a heterozygous HbAS (detected by Sickle SCAN®) became homozygous HbSS (detected with CEZ). There are no false positives in Sickle SCAN® result for the presence of the hemoglobin variant.

3.5 Sensitivity and Specificity of HbA, HbS, and HbC Detection by Sickle SCAN® Compared to Gold Standard Approaches (CEZ):

The sensitivity (the ability of the test to correctly identify patients with a disease) and the specificity (the ability of the test to correctly identify people without the disease) of Sickle SCAN® were calculated for each phenotype. The estimated sensitivities were 92.2%, 100%, and 100% for HbSS, HbAS, and HbSC, respectively. The specificity exceeded 88.23% for all of the phenotypes.

4. Discussion

The study found that the prevalence of sickle cell trait was 14.71%. In addition to being a Central African country, Gabon is also an endemic country for Plasmodium falciparum malaria, which is thought to be an agent responsible for the appearance of the S-allele. Our findings are consistent with what has been observed in the malaria transmission zone in Central Africa, where the prevalence of sickle cell trait ranges from 5% to 40% according to Diallo and Tchernia20 and WHO in 2006.

A high proportion of the S-allele in the homozygous state (32.35%) was observed. This is contrary to the results of Vierin Nzame et al.,11 who showed in a study on neonatal screening of sickle cell anemia in Gabon, carried out using isoelectric focusing and high-performance cation-exchange liquid chromatography, that 1.80% of newborns were homozygous SS. Another study of neonatal screening using the isoelectric focus carried out by Nga Motaze in 2013 in Cameroon found that the incidence of homozygous SS was 0.6%. Similarly, Akuete et al. carried out a study on 296 subjects who were at least 6 months old. They identified 45 heterozygous HbAS subjects and 41 homozygous HbSS subjects among the 209 cases that were tested. The type of analytical technique used could explain the difference between these results and ours. The recruitment of their subjects was done in medical centers while ours depended on the willingness and the availability of the subjects during the screening drive.

The low proportion of composite heterozygous SC is thought to be because it is a much more common phenotype in West Africa, its presence within the populations of Central Africa is thought to be due to the migration and mixing of the populations.

Children aged 5-9 and 10-14 years were found to be more concerned by sickle cell disease (homozygous HbSS phenotype) in the Haut-Ogooué province. This indicates that the distribution of phenotypes is not random in different age groups. Indeed, the disease exhibits a degree of clinical diversity between the ages of 5 and 9 years, with the appearance of new complications such as neurological impairments that continue until adolescence. Children aged 5 to 9 years are at greater risk of severe medical consequences. The infant mortality rate is also higher in this age group, as neurological manifestations are associated with infectious, hematological, and cardio-pulmonary manifestations. Stroke, for example, occurs before

Table 2: Comparison of the phenotypes identified by the Sickle SCAN® rapid diagnostic test and Capillary Electrophoresis

<table>
<thead>
<tr>
<th>Result CEZ=HbAS</th>
<th>Result CEZ=HbSS</th>
<th>HbSS and HbSC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result RDT=HbAS</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Result RDT=HbSS</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HbSC and HbSS</td>
<td>0</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>15</td>
<td>17</td>
</tr>
</tbody>
</table>

Legend: Rapid Diagnostic Test (RDT), Capillary Electrophoresis (CEZ)
the age of 18, generally around 14 years of age. This explains the small proportion of children over the age of 15 compared to the high proportion of children between the ages of five and 15 in this study.

Moreover, Renoux et al. showed that children aged 5 to 9 and 10 to 14 years have less deformable red blood cells and a high degree of erythrocyte aggregate formation, indicating their enhanced susceptibility to vaso-occlusive crises that cause vascular and tissue damage and that result in the observed complications. This could suggest that parents would become more aware of medical screening drives as a result of these issues.

In addition, children between 2 and 4 years of age are more affected than those 0–1 years of age. Indeed, sickle cell clinical signs most often only start at 3 months of age, which is when hemoglobin S begins to replace fetal hemoglobin. The severity is much greater in children 2 to 5 years of age, for whom infection is generally the cause of morbidity and mortality. The low incidence of children between 0 and 2 years of age was also observed by Pongombo Shongo et al. in a Democratic Republic of Congo (DRC) study of sickle cell disease in infants aged 6–59 months, in stationary-phase in Lubumbashi children. Generally, early sickle cell clinical signs would lead to the early discovery of sickle cell disease in the absence of routine neonatal screening. This would leave some parents with no clinical signs and the belief that their children are in good health.

Table 2 shows two specific cases involving subjects with phenotypes different from those detected with the rapid diagnostic test. The first case involved a homozygous HbSS phenotype (Sickle SCAN®) that became an HbDD phenotype (detected by CEZ). This result is explained by the fact that some hemoglobins migrate to the same position. Hemoglobin D and G migrate to the same level as hemoglobin S in gel electrophoresis. This confirms that the rapid diagnostic test only detects hemoglobin A, S, and C, irrespective of comigrating hemoglobin variants.

In the initial objective, given that the S-allele is most prevalent in Gabon, rapid diagnostic Sickle SCAN® tests were commissioned with the aim of detecting hemoglobin A, S, and C. Given that hemoglobin D and hemoglobin S migrate to the same position, the Sickle SCAN® test does not distinguish between hemoglobin D and hemoglobin S, and it is, therefore, important to perform a second-line test to confirm the results obtained with the Sickle SCAN® test.

For the second case, the HbAS phenotype (detected using Sickle SCAN®) turned out to be a homozygous SS (based on CEZ). In this case, the subject underwent a blood transfusion less than three months prior to the day of the screening. It is in fact recommended for any study of hemoglobin and for its interpretation that any blood transfusion is performed at least three months beforehand. By providing normal hemoglobin, a blood transfusion decreases the amount of hemoglobin S. The level of normal hemoglobin decreases and becomes essentially zero after three months, leaving only hemoglobin S, thus explaining the homozygous S-allele identified by capillary electrophoresis.

Results show that the Sickle SCAN® test can be used as a first-line test for mass screening, while its sensitivity and specificity need to be confirmed by a gold standard test. Determination and improvement of the life expectancy of sickle cell patients require the use of diagnostic methods that are suitable for use in low-resource countries. Studies in Bamako, Mali, and Togo, of a population over six months of age, have shown that the detection of different hemoglobin variants with the Sickle SCAN® test has 100% specificity and sensitivity.

4.1 Strengths and Limitations of the Study

However, there are limitations to this method regarding the results, including the fact that it is a qualitative test, which could result in a test result being interpreted as sickle cell trait (HbAS) in individuals with HbS/β+ thalassemia. Samples containing HbS and HbA at levels as low as 5–10% will result in a positive band for HbS and HbA and may hence be interpreted as sickle cell trait, whereas samples with HbA < 5–10% may only result in a single positive band for HbS and may, therefore, be falsely interpreted as SCD. The variable intensity of the test bands is another limitation that can lead to potential misinterpretation of the results. Nevertheless, the sensitivity and specificity of the test are close to perfection for the diagnosis of different phenotypes.
5. Conclusion and Implications for Translation

This study evaluated the Sickle SCAN® test with 272 blood samples and compared the results to those obtained by capillary electrophoresis. As a point-of-care test, the Sickle Scan® device was found to be reliable, with a sensitivity of 92.2%, 100%, and 100% for HbSS, HbAS, and HbSC, respectively. The specificity exceeded 88% for all phenotypes.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare that they have no competing interests. Financial Disclosure: Authors Lucrèce M. DELICAT LOEMBET, A.N.D. MABIALA, and Ulrich BISVIGOU do not hold any patents or have not received reimbursements, fees, funding, or salary from any organizations that in any way gain or lose financially from the publication of this manuscript now or in the future. These authors do not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, now or in the future. All Authors reported no financial disclosures.

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Key Messages

► Approximately 80% of sickle cell disease births occur in low- or middle-income of sub-Saharan African countries.
► Without an early diagnosis, most children born with SCD in settings with limited resources die within the first several years of life.
► In the context of low-income countries, diagnosing sickle cell disease requires point-of-care (POC) testing and Sickle cell can be considered a good choice for front-line diagnosis.

References


