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Evaluation of the diagnostic accuracy of a hemoglobin S and C screening test: Sickle Scan

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ABSTRACT

New tools for the rapid diagnosis of hemoglobinosis could encourage the extension of their screening in Africa. Our goal was to assess the analytical performances of a rapid hemoglobin S and C detection test, the Sickle Scan. This was a cross-sectional study carried out in March 2019 at the Yopougon Teaching Hospital. The subjects followed for hemoglobinosis as well as the subjects seeking out an electrophoresis of their hemoglobin were included. We carried out the hemogram, the electrophoresis of hemoglobin at alkaline pH (reference method) coupled with the metabisulfite sickling test (Emmel test) and the rapid detection test to be evaluated. This immunochromatographic test is capable of detecting hemoglobins A, S, and C, and to infer the hemoglobin phenotype from there. The study recruited 191 individuals. The test detected hemoglobins S and C with a sensitivity of 99.4% and 97.7% respectively; a specificity of 93.3% and 99.3%. The positive likelihood ratio for hemoglobins S and C was 15 and 144 respectively. The negative likelihood ratio was 0.01 for hemoglobin S, and 0.02 for hemoglobin C. The intrinsic characteristics obtained make this test an interesting screening tool for hemoglobinosis S and C.

Keywords: Diagnostic test, hemoglobinosis, Abidjan, sensitivity, specificity.

INTRODUCTION

Hemoglobinopathies are a public health issue in large parts of the world such as Sub-Saharan Africa. Indeed, more than half of the sickle cell patients worldwide live in that region (Piel et al., 2013). Sickle cell anemia is characterized by the presence of hemoglobin (Hb) S which results from a structural abnormality in the Hb. People with sickle cell syndrome suffer from chronic anemia with acute and chronic complications of the disease (Gueye et al., 2014; Diallo et al., 2018). Subjects with the heterozygous form are called sickle cell trait carriers. They have little to no clinical manifestations. The combination of a Hb S with another hemoglobinopathy can lead to major sickle cell syndromes as seen with the combination of Hb S and Hb C that promotes the dehydration of the red blood cells and the increase in the concentration of Hb S.

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Quantitative abnormalities stemming from Hb synthesis deficiencies have been initially described in populations of the Mediterranean Basin. However, β-thalassemia is also very widespread in West Africa (De Montalembert, 2008). In Côte d'Ivoire, the overall frequency of sickle cell disease is estimated at about 12.5% (Piel et al., 2013; N'draman-Donou et al., 2015) while the frequency of β-thalassemia varies from 2.3 to 11% depending on the region considered (Tolo-Diebkilé et al., 2012). In developed countries, the standard of care for diagnosis requires three distinct phenotypic tests including at least one electrophoretic technique (Mario et Sala, 2016). In our limited resources settings, the diagnosis uses only one technique. In addition, the diagnosis is not always financially and geographically accessible. In this context, the recent development of rapid detection tests (RDTs) that do not require some advanced equipment nor electricity, could extend the screening of hemoglobinopathy in Africa. The objective of the current study was to assess the analytical performance of the Sickle Scan Hb S and Hb C RDT.

MATERIALS AND METHODS

Study design

A cross-sectional study was carried out in March 2019 at the University Hospital of Yopougon, Côte d´Ivoire.

Population

All subjects carrying a hemoglobinopathy and followed-up at the University Hospital, were included. On top of them, subjects unaware of their hemoglobin status for whom an Hb electrophoresis analysis had been requested by their attending physician were also included. Oral consent from the patient or the accompanying parent (for children) was secured. All eligible subjects who had been transfused in the last three months prior to the start of the study were excluded.

Laboratory testing methods

Whole blood samples were collected by venipuncture into an ethylene diamine tetra-acetate (EDTA) anticoagulant tube. For each participant, a hemogram was performed using a multiple parameters automated hematology analyzer, the Abott's Cell-Dyn Rubby System. A Hb electrophoresis analysis at alkaline pH coupled with the metabisulfite sickling test (Emmel test) was also carried out, and served as the gold standard for comparison (Assoumanou et al., 2010). And the Sickle Scan RDT (BioMedomics Inc., Durham, NC, United States of America), was independently run following the manufacturer's instructions by two different operators.

The Sickle Scan test

The Sickle Scan RDT is a qualitative, cassette-based RDT using the principle of the non-competitive sandwich immunochromatography. It takes advantage of immobilized anti-Hb A, S, and C monoclonal antibodies to infer the Hb phenotype (AA2, AS, AC, SS, SC and CC) of each patient tested. Of note, one critical limit of the test is its inability to detect Hb F as highlighted in the Sickle Scan RDT insert sheet.

Statistical analysis

The analytical precision of the Sickle Scan RDT was assessed in comparison to the Gold Standard of Hb electrophoresis + Emmel test. The intrinsic features of the Sickle Scan RDT such as its sensitivity, its specificity, its positive likelihood ratio and its negative likelihood ratio were determined (Nendaz et Perrier, 2004; Delacour et al., 2009; Steichen et al., 2013; Djimadoum et al., 2018).

RESULTS

The study recruited 191 subjects with an average age of 17 years (1 year to 80 years). The sex ratio was 0.66. The study population was composed of 178 subjects (93.2%) that knew their Hb phenotype and 13 subjects (6.8%) that did not know it. The $SSFA_2$ phenotype was mainly found, followed by the SFA_2 phenotype (Table 1). The frequency of the various Hb fractions is shown in Table 2. With regard to the detection of the diverse Hb fractions, the intrinsic features of the Sickle Scan RDT are reported in Table 3. Table 4 summarized the cross-comparison of the Hb phenotypes detected by Hb electrophoresis

with those detected by the Sickle Scan RDT. As expected, Hb electrophoresis properly identified the phenotypes of two subjects as CAF and SCAF phenotype whereas the Sickle Scan RDT characterized them as respectively AC (or CA) and SC (or CS). All the sickle cell trait subjects (n = 4) were properly typed as such by the RDT. However, 87.5% (21/24) of the $SAFA_2$ subjects were wrongly identified as AS and 8.3% (2/24) as SS by the Sickle Scan RDT. The intrinsic features of the rapid test are summarized in Table 5.

Table 1: Hemoglobin phenotype identified using the reference standard method (Hb electrophoresis analysis at alkaline pH).

Phenotype	Number of sample (n)	Frequency (%)
$SSFA_2$	63	33.0
SFA_2	46	24.1
SC	39	20.4
$SAFA_2$	24	12.6
AA_2	9	4.7
AS	4	2.1
CC	4	2.1
CAF	1	0.5
SCF	1	0.5
Total	191	100.0

Table 2: Hemoglobin variant identified using the reference standard method.

Hemoglobin	Number of sample (N=191)	Frequency (%)
S	177	92.67
A	84	43.98
F	72	37.70
C	45	23.56

Tableau 3: Sickle Scan detection analysis by hemoglobin Variant.

	Hemoglobin		
	A	**S**	**C**
Sensibility	94.6% (CI 95%* : 87-100)	99.4% (CI 95%* :98-100)	97.7% (CI 95%* :93-100)
Specificity	99.3% (CI 95%* : 98-100)	93.3% (CI 95%* : 81-100)	99.3% (CI 95%* : 98-100)
Positive likelihood ratio	146	15	144
Negative likelihood ratio	0.05	0.01	0.02

* 95% Confidence Intervals

Table 4: Matrix between results achieved by the reference method and the Sickle Scan device, to determine the hemoglobin phenotype.

		ELECTROPHORESIS									
		AA	**AS**	**CAF**	**CC**	**SAFA2**	**SC**	**SCF**	**SFA2**	**SS**	**TOTAL**
SICKLE SCAN	**AA**	9	-	-	-	1	-	-	-	-	10
	AC	-	-	1	-	-	-	-	-	-	1
	AS	-	4	-	-	21	-	-	-	-	25
	CC	-	-	-	4	-	-	-	-	-	4
	SC	-	-	-	-	-	38	1	-	-	39
	SS	-	-	-	-	2	1	-	46	63	112
TOTAL		**9**	**4**	**1**	**4**	**24**	**39**	**1**	**46**	**63**	**191**

Table 5: Sickle Scan detection analysis by hemoglobin phenotype : $SSFA_2/SFA_2$, SC et CC.

Phenotype	Sensibility (CI : 95%*)	Specificity (CI : 95%*)
$SSFA_2/SFA_2$	100%	96.34 (CI 95% : 92-100)
SC	97.5 (CI 95% : 93-100)	100%
CC	100%	100%

* 95% Confidence Intervals

DISCUSSION

Overall, the evaluation of the analytical performance of the Sickle Scan RDT focused first on the ability of the test to detect the types of Hb present in the blood sample of people living in Côte d'Ivoire, Western Africa. We demonstrated that the test showed good sensitivity and specificity (> 93.0%) regardless of the type of Hb detected (A, S and C) (Table 3). In addition, the positive likelihood ratios of the RDT for Hb S and C were greater than 10 and the negative likelihood ratios were less than 0.02. The higher the positive likelihood ratio and the lower the negative likelihood ratio, the greater the diagnostic gain of the test (Delacour et al., 2013; Steichen et al., 2013).

Sickle Scan RDT performances have been studied both in developed countries and in resources-limited countries. In 2016, a study carried out in the United States of America on 139 samples noted a sensitivity and a specificity of respectively 99.5% and 92.5% for the detection of the Hb S (Mcgann et al., 2016). As for Hb C, the study found a sensitivity of 100% and a specificity of 100%. These values are similar to ours (Table 3). Based upon the types of Hb detected with the RDT, we inferred the phenotypes accordind to the manufacturer's guidelines. Thus, for the determination of the $SSFA_2$ / SFA_2, SC, CC phenotypes, the sensitivity and specificity of the RDT were good and above 96% (Table 5). Kanter et al. (2015) reported a sensitivity of 99% for the detection of the $SSFA_2$ and SFA_2 phenotypes, Mc Gann et al. (2016) and Nwegbu et al. (2017) reported 98.4% and

100% respectively, similar to our finding (Table 5). The specificity of the RDT on our hands for the detection of the $SSFA_2$ and SFA_2 phenotypes was lower than the ones reported by these same authors, 99.0%, 98.6% and 98.2% respectively. Likewise, the "Drepatest" study conducted in West Africa (Segbena et al., 2018) using the same RDT noted in Togo a sensitivity and specificity of 97.6% and 99.6% respectively. The sensitivity of the test was 97.5% (93-100) for the detection of the SC phenotype. Kanter et al. (2015) found 99%; Mc Gann et al. (2016) and Nwegbu et al. (2017) found a sensitivity of 100%; Segbena et al. (2018) obtained 97.6%. The specificity of the RDT for that phenotype was 100% in our study; similar to what Mc Gann et al. (2016) and Segbena et al. (2018) reported.

The 4 subjects carrying the sickle cell trait were correctly detected by the Sickle Scan RDT. However, it should be noted that in 21 other people previously known as $SAFA_2$, the test had displayed "AS" as a phenotype (Table 4). SFA_2 and $SAFA_2$ type thalasso-sickle-cell anemia are clinical forms of hemoglobinopathy in sub-Saharan African countries where sickle-cell anemia and β-thalassemia coexist. The Sickle Scan RDT is designed with a lower limit of detection of Hb A greater than 25% which is higher than that of Hb S and C (Kanter et al., 2015). As a result, $SAFA_2$ subjects with a Hb A fraction of less than 25% will be phenotyped as $SSFA_2$ or SFA_2. While subjects with sickle cell trait, with their Hb A fraction greater than 40% will be correctly classified as AS. Previous studies (Kanter et al., 2015; Mcgann et al., 2016; Nwegbu et al., 2017; Nguyen-Khoa et al., 2018; Segbena et al., 2018) had not assessed the ability of the Rapid Sickle Scan RDT in subjects with the $SAFA_2$ thalasso-sickle cell form. However, McGann et al. (2016) noted that the distinction between the sickle cell trait and the β + Thalassemia associated with sickle cell anemia ($SAFA_2$) can prove difficult or even impossible. However, Nguyen et al. (2018) found that Hb A and Hb S were detectable with the same RDT even when these Hbs were present at less than 1% and 2% respectively.

The population of our study was mainly in-patients with known hemoglobinopathy. In fact, 90% of the subjects recruited were carriers of Hb S (Table 1). The aim of this work was to study the intrinsic characteristics of the test, namely the sensitivity, the specificity and the likelihood ratios. The frequency of hemoglobinosis in the study population (Tables 1 and 2), was much higher than the national prevalence of 12.5% (Piel et al., 2013), but it does not constitute a bias. For the intrinsic characteristics of a screening test do not depend on the frequency of the event studied (Delacour et al., 2013; Steichen et al., 2013). On the other hand, the extrinsic characteristics of the RDT such as the predictive values (positive and negative) that are influenced by prevalence of the event studied could not be evaluated. There is a need of new studies to update the prevalence of hemoglobinopathies in the general population. Those studies will be useful to properly assess both the intrinsic characteristics (sensitivity, specificity, likelihood ratios), and the extrinsic characteristics (positive and negative predictive values) of candidate RDTs. It would also be necessary to assess the ease of use and social acceptability of theses RDTs for the integration of a diagnostic tool into a clinical process requires the evaluation of all those aspects.

Conclusion

The present study was carried out in Abidjan (Côte d'Ivoire). The results showed some good intrinsic characteristics of the Sickle Scan RDT in identifying Hbs S and C. However, the exact determination of the Hb phenotypes requires confirmation with the classical diagnostic tests such as Hb electrophoresis at alkaline pH.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

AL carried out the testing using the RDT. AL and MM performed the data mining. AL and YV wrote the manuscript. YA carried

out and interpreted the results of the Hb electrophoresis test, the Emmel test, and the hemogram. BS monitored the follow-up of the participants to the study. DS conceived, initiated and supervised the entire study.

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