Systematic Screening of Neonatal Sickle Cell Disease with HemoTypeSC™ Kit-Test: Case Study and Literature Review

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Abstract

HemoTypeSC™ test is a new cheap, faster, and appropriate screening method for neonatal diagnosis of sickle cell disease. The literature reports a few cases of its applicability. This study extends the cases study and reviews the available literature. The sample consisted of 99 subjects, including 87 newborns (36 girls and 51 boys; 1.9 - 4.9 kg BW) sampled among 566 babies bone at six hospitals in Kisangani city (Democratic Republic of Congo) during March-April 2019; height infant-adolescents (<18 years); and four adults. Duplicate blood samples of 75 newborns, spotted on filter paper, were transferred to Liège in Belgium for LC-MS test confirmation. Of 99 subjects, 74.74% tested HbAA, 24.26% HbAS and 1% HbSS. The prevalence of HbAS compared to the HbAA phenotype was 15/60 (20%) by HemoTypeSC™ and 14/61 (18.7%) by LC-MS. The concordance between the two methods was 98.3% or a discordance of 1.7%. The findings support the validity of the HemoTypeSC™ test as a sensitive, specific point of care test, cheap and reliable for poor African populations.

Keywords

Sickle Cell Disease, Hemoglobin Phenotype, HemoTypeSC™, Neonates

1. Introduction

Sickle cell disease (SCD) is an autosomal recessive inherited hemoglobinopathy due to the substitution of glutamic acid by valine at the sixth amino acid in the β-globin chain [1]-[6]. The resulting abnormal hemoglobin (HbS) is poorly soluble and rapidly polymerizes when deoxygenated [2]. SCD is probably the first genetic disease in the world [3] [7] and one of the most common high-prevalence hemoglobinopathies in sub-Saharan Africa [5] [8].

Although data on child mortality are not widely available today, several authors report that 50% to 90% of sickle cell children die before the age of 5, undiagnosed [5] [9] [10]. There is a consensus that early diagnosis of SCD in neonates is necessary for initial management [4] [5] [8] [11]. In many resource-rich areas, universal neonatal screening programs associated with prophylactic interventions have significantly reduced SCD morbidity and mortality in the first 20 years of patients' life [4] [5] [12] [13] [14]. However, in sub-Saharan Africa and central India, where more than 90% of sickle-cell births occur, neonatal screening programs are not systematically applied, if at all, mainly because of the cost and logistical burden of laboratory diagnostic tests [11] [13].

There are many rapid tests also called Point of care (POC) rapid tests on the market with bedside results (SickleScan, HemoTypeSC™, and Sickledex) [9] [13] [15] and others are quite robust but do not give immediate results (capillary electrophoresis, high-performance liquid chromatography (HPLC), mass spectrometry (MS) and PCR polymerase chain reaction) [9] [15]. There are intermediate tests such as the Lamp Human Hemoglobin S & C mutation Kit (LaCAR), which have demonstrated their effectiveness in the diagnosis of hemoglobinopathies. However, these tests are not as fast as the first ones [6]. Rapid tests exploit either solubility properties of HbS and the cell density, or immunological approaches [15] [16]. All POC rapid tests have the advantage of saving time in terms of results and facilitating application in rural areas without electricity or qualified personnel [9] [11]. Several studies report that robust tests are available in high-income countries where they are considered worldwide as a "gold standard" for the diagnosis of sickle cell disease [18]. Their sensitivities and specificities have been studied and evaluated at 100% [18]. Nevertheless, all these studies recognize some of the weaknesses of these robust technologies for implementing them in Africa [6] [11] [12] [13] [17] [18].

HemoTypeSC™ is a competitive side-flow immunoassay that uses monoclonal antibodies to detect hemoglobin A, S, and C in a 1.5 µL whole blood sample [11] [17]. HemoTypeSC™ provides point-of-care determination of hemoglobin phenotypes HbAA (normal), HbSS and HbSC (sickle cell disease), HbCC (hemoglobin C disease), and HbAS and HbAC (carrier or trait). It does not detect other hemoglobin variants (such as Hb D or E), these variants will give the same result as Hb A. Its diagnostic accuracy oscillates around 99 or even 100% when performed on a sample containing more than 80% of HbF [11] [17]. In January 2018, HemoTypeSC™ was utilized at Holy Family Hospital in Techiman, Ghana,

to screen over 400 newborns, infants, children, and adults for hemoglobins A, S, and C. All 14 carriers of hemoglobin C were successfully identified. The multicentric study by Steele *et al.* [11] and that of Charles Quinn [17] indicate that only the HemoTypeSC™ remains the rapid test mentioned for neonatal screening [11].

While all these considerations point to the use of simple, rapid, inexpensive, and easy-to-use diagnostic tools [13], the clinical evaluation of these tests remains necessary in many African countries. In the Democratic Republic of Congo (DRC), neonatal screening for sickle cell disease is not a common practice due to a lack of adequate infrastructure [19]. Only a few centers in Kinshasa or Lubumbashi practice it [4] [15] [20]. In Kisangani, updated data on neonatal screening for sickle cell disease are not available due to a lack of appropriate diagnostic tools [13] [15]. The available SickleSCAN assay (Biomedomics, Inc., Research Triangle Park, North Carolina) is better for screening beyond the age of 6 months and not before this period because of a high concentration of fetal hemoglobin (HbF) in the newborn's blood that would interfere with the outcome of this test [13] [21].

To implement a systematic neonatal diagnosis, we designed this preliminary study to explore the possibility of using HemoTypeSC™ as a systematic neonatal screening method in the Kisangani environment.

2. Methods

2.1. Study Design and Sample

To test the applicability of the test HemoTypeSC™ for the detection of SCD in the Kisangani case, we gathered 99 subjects, including 87 newborns, height infant-adolescents, and four adults apparently without a history of HbSS. The newborns were a random sample from 566 babies born in March-April 2019 in 6 maternity wards of general referral hospitals (GRH) and private clinics (CS) located in different heath zones: Makiso-Kisangani (GRH, CS IMANI, CS Clinic Stanley), Tshopo (GRH), and Kabondo (GRH, CS Clinic Saint Camille). We considered 15% HbAS prevalence and 95% precision.

$$N = \frac{1.96^2 * 0.15 * 0.85}{0.05^2} \quad (1)$$

Adjustment for 566 births in two weeks gave around 100 subjects.

Neonates transfused during the study period, and those whose mothers did not consent to participate did not enter the study. We collected blood samples in duplicate, and one part tested immediately on the point of care with HemoTypeSC™ immunoassay (Hemotype, AZUSA, CA, USA). The other samples part from neonates, adsorbed on filter paper (Whatman 903), were taken to the laboratory of the Centre Hospitalier Universitaire (CHU) of the University of Liege in Belgium to validate the result by Liquid chromatography-Mass spectroscopy (LC-MS) method.

2.2. Ethical Consideration

The ethics committee of the University of Kisangani approved the study, Ref. UNIKIS/CER/005/2018. Parents of the children included in the study gave informed consent.

2.3. Procedures for HemoTypeSC™

Following the manufacturer's instructions (Figure 1), a sample of 1.5 µL of blood was collected by a puncture in the heel from the neonates or in the finger from adults using a thin needle (lancet) regularly used locally for the blood sample.

1) Using Dropper Pipette, add six drops of water to Test Vial. Place Test Vial in a compatible rack. 2) Open Vial of Blood Sampling Devices, remove one Blood Sampling Device and reclose Vial. Obtain blood sample, a small drop is sufficient (1 to 2 microliters). Touch the white pad of the Blood Sampling Device to the blood sample, until the white pad absorbs the blood droplet. Ensure that the entire white pad has turned red. 3) Insert Blood Sampling Device into Test Vial water and swirl to mix (Check visually to ensure that water has become pink or light-red in color). 4) Open Vial of Test Strips, remove one Test Strip and reclose Vial. Insert HemoTypeSC™ Test Strip into Test Vial with arrows pointing down. 5) Wait 10 minutes. 6) Take HemoTypeSC™ Test Strip out of the Test Vial and read results. Compare Test Strip to Results Chart on the reverse side of this document for reference.

2.4. Procedures for LC-MS Method

In Belgium, they used the TQ5500 triple quadrupole mass spectrometer source (Sciex) system (AB Sciex, Njeuwerkerkaan den Ijssel, Netherlands) for LC-MS analysis following the manufacturer's technical specifications as described elsewhere [22]. Briefly, the blood samples are desorbed from the disc by gentle rotation with 200 µL deionized water for one hour in 90 microplates and then 100 µL of each plate transferred to fresh microplates. Following a 10 minutes' denaturation step with ACN 17 µL) and 1% aqueous formic acid (17 µL), proteins are

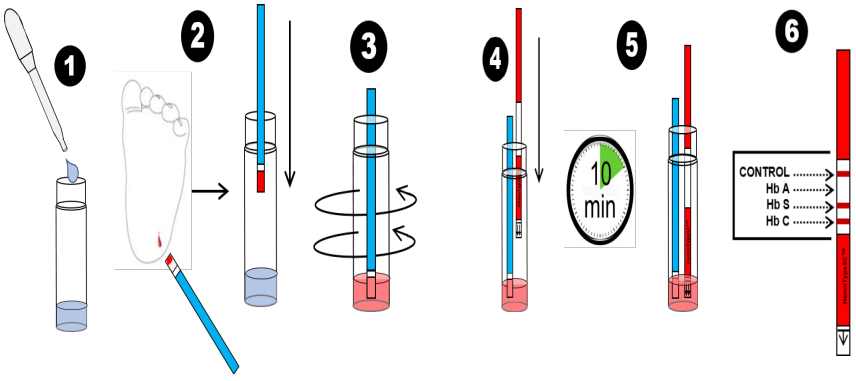

Figure 1. Procedure of the HemoTypeSC™ (From Manufacturer: Product # HT111, USA).

treated overnight with 10 µL of a TCPK-treated trypsin solution. After centrifugation and incubation at 37˚C, 20 µL of the digested solution is diluted with 180 µl of ACN/deionized water (1:1) with formic acid 0.1% and centrifuged again. This working solution is ready for injection into ACN: H_2O (50:50) with 0.1% formic acid mobile phase in Waters Acquity-UPLC system and directly introduced into the source without prior chromatographic separation.

The MS analysis operated in the multiple reaction monitoring (MRM) mode, with total acquisition time 60 seconds. The first tryptic peptide of the β globin chain (T1β) was analyzed to identify HbS and HbC variants while the twelfth tryptic peptide of the δ-globin chain (T12g) was selected to check the efficiency of digestion and to calculate HbA/HbF ratio. For each peptide, the system acquires four transitions and calculates cumulated MRM ratios variant/HbA, which allows Phenotype classification.

2.5. Statistical Analysis

We compared results from HemoTypeSC™ and LC-MS methods to measure the concordance between the two, using a paired t-test with 0.05 precision. The demographic characteristics concerning gender, time of birth, and weight of neonates were determined to anticipate the potential influence on the prevalence of HbAS and the discordance between the two methods with ANOVA.

3. Results

3.1. Phenotyping with HemoTypeSC™

Table 1 shows that out of 99 cases examined, 74 (74.75%) were HbAA, 24 (24.24%) HbAS and 1 (1.01%) HbSS. All cases combined, males represented 52.53% and females 47.47%. The unique case of HbSS was a male adolescent.

3.2. Phenotyping of 75 Neonates with HemoTypeSC™ and LC-MS

Table 2 shows that of 75 cases compared, only 1 case was discordant. The specificity of HemoTypeSC™ is roughly about 96.3% - 98.3%, and the accuracy 100%, with an expected error of 1.7%, compared to LC-MS. We anticipated factors that

Table 1. Phenotyping of the global sample with HemoTypeSC™.

Age	Gender	HbAA		HbAS		HbSS		Total	
		N	%	N	%	N	%	N	%
Neonate	Female	27	27.27	9	9.10	0	0.00	36	36.27
	Male	40	40.40	11	11.11	0	0.00	51	51.52
Infant	Female	5	5.05	2	2.02	0	0.00	7	7.16
	Male	0	0.00	0	0.00	1	1.01	1	1.01
Adult	Female	2	2.02	2	2.02	0	0.00	4	4.04
	Male	0	0.00	0	0.00	0	0.00	0	0.00
Total		74	74.75	24	24.24	1	1.01	99	100

Table 2. Concordance between HemoTypeSC™ (HTSC) and LC-MS (LCMS) results.

			HemoTypeSC™		LC-MS		Discordance
Covariates	Categories	Total	HbAA	HbAS	HbAA	HbAS	HTSC-LCMS
		N	N (%)	N (%)	N (%)	N (%)	
	All categories	75	60 (80.0)	15 (20.0)	61 (81.3)	14 (18.7)	1/75
Gender	Female	30	24 (80.0)	6 (20.0)	24 (80.0)	6 (20.0)	
	Male	45	36 (80.0)	9 (20.0)	37 (81.3)	8 (17.8)	1/45
Delivery	Term	55	42 (76.4)	13 (23.6)	43 (78.2)	12 (21.8)	1/55
	Pre Term	17	15 (88.2)	2 (11.8)	15 (88.2)	2 (11.8)	
	Post Term	3	3 (100)	0 (0)	3 (100)	0 (0)	
Weight	1.9 - 3 kg	27	24 (41.8)	13 (48.2)	25 (45.6)	12 (44.4)	1/27
	3.1 - 3.9 kg	39	27 (94.9)	2 (5.1)	27 (94.9)	2 (5.1	
	4 - 4.9 kg	9	9 (100)	0 (0)	9 (100)	0 (0)	

could predict the positivity of HbAS, such as sex, the timing of delivery, and newborn weight. It is evident that those factors do not predict the status but can perhaps affect the specificity of the test. The discordant subject was a male, born at term, weighing less than 3 kg. No statistical differences were found ($p > 0.05$).

4. Discussion

According to the literature point of view, the value of neonatal screening is the early detection of newborns with HbSS to receive penicillin prophylaxis between 2 - 3 months, preferably before the onset of SCD symptoms [8] [16] [23] [24] and immunization with the vaccine against encapsulated germs and mostly the pneumococcal vaccine [10] [23] [24] [25].

Satellite studies conducted in the DRC between 2007 and 2018 [4] [25] [26] found 18.7% - 20% of SCD in neonates, close to our results in Kisangani by HemoTypeSC™. Batina [25] found 23.3% in Kisangani in 2007 with another method; Shongo [4] found 15.6% in Lubumbashi, and Tshilolo [27] 16.9% in 2008. The prevalence of HbSS in this study was 1% for the small sample examined. One percent for a population of 100,000 people will be at least 1000 children suffering SCD. The best-recommended strategy to curve the tendencies of SCD in a population is counseling HbAS carriers not getting married between them. Thus, testing should also concern all adolescents before marriage. There is, therefore, a need for availing simple, specific, and sensitive testing methods.

The specificity of a clinical test refers to the ability of the test to exclude the event from unaffected subjects [24]. The multicenter study conducted by Cindy Steele *et al.* [11] showed the sensitivity and specificity of HemoTypeSC™ greater than 99%, which led the authors to qualify this test for neonatal screening of SCD. In Uganda, Nankanja *et al.* [28] emphasized that the value of sensitivity, specificity, positive, and negative value of HemoTypeSC™ is around 100%. The concordance in 98.3% of cases or 1.7% of discordance between HemoTypeSC™

and LC-MS method in our study supports the literature reports even though accuracy, positive and negative values of HemoTypeSC™ could not be provided here since the objective was not to validate a method (we did not use control samples). We added specificity and sensitivity in comparison with LC-MS result. This test falsely diagnosed a patient as sick when he was not, but a double check by HemoTypeSC™ would have been necessary to re-check the accuracy.

We anticipated factors that could predict the positivity of HbAS, such as sex, the timing of delivery, and newborn weight. It is evident that those factors do not predict the status but can perhaps affect the specificity of the test.

Other rapid tests exist, like Sickle SCAN and Sickledex, but they are not effective in neonates [17] [21]. The study by Luke *et al.* [19] reports that SickleSCAN™ was able to simultaneously detect SCD and anemia in children less than 21 years of age, while it was less valid in the newborns. The simplicity of HemoTypeSC™ lies in the fact that the blood sample is taken from the soles of the feet of newborns. Venous blood is not ideal in these cases. In this regard, Nanjela [24] emphasizes that venous blood collection for neonatal screening is not perfect, especially for the testing of patients with SCD. The concept that capillary blood, obtained by stinging on the heel or finger and transferred to a filter paper, is the best method to use for detecting metabolic diseases in large populations of newborns, was introduced in Scotland by Guthrie and Susie since 1963 [12]. As a result, newborn blood samples have been regularly collected in many countries to detect many disorders, including SCD, which makes HemoTypeSC™ the best "point of care" test for neonatal screening in poor populations.

Currently, HemoTypeSC™ is an inexpensive test than other known POC tests, making it the cheapest known test nowadays. Both the study conducted by Cindy Steele *et al.* [11] and by Nankanja R *et al.* [28] confirmed the POC HemoTypeSC™ as inexpensive, competitive lateral-flow immunoassay test because the straightforward design of this test leads to an end-user cost less than $2 per test [11].

The reading of the test by the newborn's mother was consistent with that of the test performer. This conformity of the result as well by the mother as by the investigator denotes the ease of realization of the test, even by an inexperienced person. Given the diagnostic accuracy of HemoTypeSC™, this test also remains the cheapest.

The limitations of the study are the sample size and the fact that there was only one case of HbSS. That could not allow performing rigorous statistics.

5. Conclusion

HemoTypeSC™ is a sensitive and specific point of care test which would open the neonatal screening program of sickle cell disease for resource-poor countries. This test is adapted to the tropical climatic conditions and doesn't need the use of electricity; that could be a solution for Kisangani because it is rapid, cheap, and reliable. For these reasons, we aim to widespread this pilot study to all of the

hospitals in Kisangani for systematic newborn screening in the short future time.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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