Sickle cell disease in areas of immigration of high-risk populations: a low cost and reproducible method of screening in northern Italy

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Background. From 2005 to 2010, we observed a 10-fold increase of newly diagnosed sickle cell disease in children in the province of Modena (northern Italy). The median age at diagnosis was 24 months. Since these children are too old for optimal disease management, earlier detection of the disease is needed for prophylaxis and comprehensive care before the occurrence of clinical manifestations.

Materials and methods. In each Maternity Unit of the province of Modena, blood samples are collected daily for assessment of haemolytic disease of the newborn. We designed a selective, low-cost haemoglobin screening for sickle cell disease in high-risk immigrants. We enrolled 469 mothers from sub-Saharan countries and their neonates for a primary screening of peripheral blood haemoglobin variants using high-performance liquid chromatography.

Results. Of the 469 women approached, 330 (70.36%) agreed to undergo the test. Ninety-two (27.88%) were carriers of variant haemoglobin, 48 newborns (51%) of these carriers had the carrier trait and 9 (9.6%) were affected (haemoglobin SC compound heterozigote - HbSC, haemoglobin S homozygote - HbSS).

Discussion. These results support the feasibility and usefulness of a selective screening for the detection of haemoglobin variants in high-risk subjects in an area in which sickle cells disease is not endogenous. We achieved the goal of detecting subjects with carrier trait/disease in order to implement preventive measures that reduce the clinical manifestations of sickle cell disease. We are, however, aware that it will be necessary to extend this screening to the overall population in the near future.

Keywords: haemoglobinopathies screening, sickle cell disease and trait, immigrants.

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Introduction

Sickle cell anaemia has recently been recognised as a global health problem by the United Nations and the World Health Organization[1]. Most children born with the disease die in their first years of life[2]. In developing countries with high disease burden, treatment is rarely available and screening programmes for identifying, at birth, affected neonates or those with the trait are not universally established. The identification of affected children, before the onset of clinical symptoms, would allow the implementation of measures that may decrease mortality during early childhood, such as timely administration of prophylactic penicillin by 2 months of age, as well as vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae*[3-6]. A recent study that included children with haemoglobin S homozygote (HbSS) diagnosed only by neonatal screening (1983-2007) showed that, based on this approach, the estimated survival at 18 years of age increased up to 94%[7].

Recent immigration from Africa into the northern regions of Italy has changed the frequency and distribution of sickle cell disease (SCD)[8] in the area, with an increase of newly diagnosed SCD patients. In Emilia Romagna, a region in the north of Italy with an official population of 4,432,439 inhabitants, immigrants represent 11.2% of the total population[9]. The area of Modena, a province of Emilia Romagna with a total population of 700,914 inhabitants, is home to approximately 92,400 (13.2%) immigrants, mainly from sub-Saharan countries (Ghana, Nigeria, Senegal) in which SCD is endemic[10-13]. The increased flow of immigrants from these countries over the last 15 years correlates directly with a steady increase of SCD patients (from 5 in 1995 to 55 in 2010), accounting for more than 50% of the cases in the region of Emilia Romagna[14,15]. The average age at diagnosis of 24 months of this group is clearly not ideal for starting appropriate prophylaxis[3,4,16,17]. Since no official neonatal screening for SCD is performed in Italy, we designed a selective,

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low-cost screening for SCD in new mothers and neonates involving all maternity units in the province of Modena. The proposed screening differs from those involving only neonates[18] carried out in other countries and takes advantage of the organisation of the Transfusion Medicine Departments whose mission is to diagnose the haemolytic disease of the newborn (HDN) and to develop preventive measures in the area of their jurisdiction[19].

This model is also well suited for the characterisation of new mothers in the absence of epidemiological data on haemoglobinopathies frequency in any area of immigration from high-risk countries. Finally, the opportunity to inform parents and infants of future reproductive risks is among the most valued additional benefits of our screening programme.

Materials and methods

A pilot study was started in September 2011 at the University Hospital of Modena for the detection of clinically significant haemoglobin (Hb) variants in the blood of new mothers from sub-Saharan Africa in accordance with already existing guidelines on newborn screening[20]. The geographical origin of new mothers, mainly from Ghana and Nigeria, was determined by identification of nationality and/or country of birth. The local ethical committee approved the study.

Five Maternity Units in the province of Modena collected cord blood from newborns and peripheral blood from their mothers. The blood samples were taken immediately after delivery and sent to the Transfusion Medicine Department as required for assessment of HDN (blood group, antiglobulin Coombs' test). A high prevalence of endemic SCD in the country of origin of the mother was the criterion for enrolment in this study. A three-language (Italian, English, French) informed consent form together with the blood group report were then sent to the Maternity Units. The mothers' blood samples, stored at 5 °C at the Transfusion Medicine Departments, were tested for the presence of clinically significant Hb variants. Blood samples from the mothers and neonates were collected into EDTA and stored for 7 days in accordance with Italian legislation[21].

Primary screening for clinically significant Hb variants was performed by high-performance liquid chromatography (HPLC) (Variant 2 Biorad Laboratories, Hercules, CA, USA)[22]. The screening laboratory participates in a quality control programme that includes proficiency testing (National External Quality Assessment Scheme: UK NEQAS Abnormal Haemoglobin).

In the event of no consent from mothers, no test was performed.

The subsequent steps depended on the result of the maternal blood test (Figure 1). If no clinically significant Hb variant was detected, a report indicating that no additional tests were necessary was sent to the mother and the family doctor. If, however, a clinically significant Hb variant was detected, the cord blood was tested for Hb variants and, thereafter, an appointment was scheduled with the mother, her partner and the baby. A blood sample was collected from the partner and subjected to HPLC analysis.

When the baby was found to be a carrier of a clinically significant Hb variant information regarding the status of carriers of the common Hb variants was given to parents with specific supporting material (in English, Italian and French), also explaining the need to study all family members. Counselling was offered to make sure that parents understood the difference between carrier state and disease, the distinction between S carrier status, which is clinically important[23-25], and other carrier states, which are unlikely to be clinically important but may have genetic relevance. A blood sample was collected from the partner and analysed by HPLC.

If the neonate was a S homozygote or compound heterozygote (i.e. affected by SCD), counselling was planned by an English/French/Italian-speaking haematologist, using illustrations to explain the genetics of SCD, its main symptoms, the reasons for prophylaxis, and management of acute illness. Confirmatory tests on the baby's samples (gel electrophoresis, genotyping methods) were scheduled.

Affected SCD newborns are referred to the Paediatrics Department of the University Hospital of Modena to be enrolled in the clinical protocol for newly-diagnosed SCD patients and start prophylactic antibiotic treatment immediately. Comprehensive care, a multidisciplinary approach to the disease and 24-hour care are also provided.

If both parents were positive, specific clinical and genetic counselling for future pregnancies was offered together with HPLC analysis of newborn siblings.

All babies born of a mother or father who was a carrier of a clinically significant Hb variant were tested again at 6 months of age to avoid false negative/positive results.

Each individual (affected, carrier or normal) as well as his/her family doctor or pediatrician was informed by priority mail of the results of their blood tests.

Results

From September 2011 to May 2013, this pilot study was proposed to 469 women from sub-Saharan Africa, selected among new mothers at five Maternity Units in the province of Modena. Three hundred and thirty (70.36%) gave consent to enrolment in the study. As reported in Table I, 51.4% were from Ghana, 32.4% from Nigeria, 3.2% from Guinea, 2.8% from Senegal, 2.1% from the Ivory Coast and 5.5.% from other countries.

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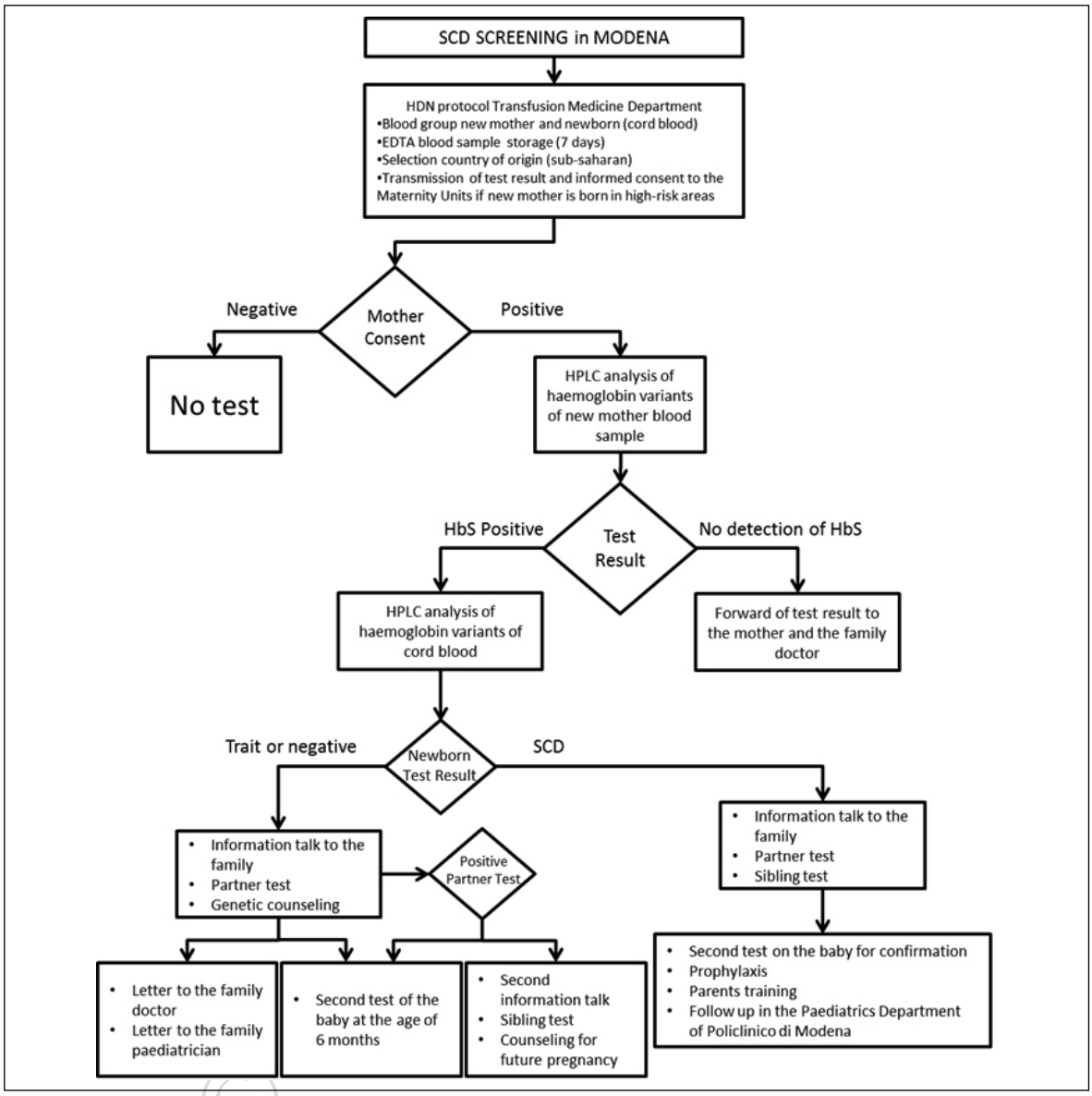

Figure 1 - Screening protocol.
HDN: haemolytic disease of the newborn; EDTA: ethylendiaminetetra-acetic acid anticoagulant; HPLC: high performance liquid chromatography; HbS: haemoglobin S heterozygous profile/sickle cell trait.

Table I - Country of origin of selected new mothers and frequency of normal and variant Hb in enrolled (n=330) new mothers. Other: countries with less than 10 new mothers selected.

	Selected mothers	Enrolled mothers	
		Negative	Hb variant
Ghana	241 (51.4%)	112 (47.1%)	53 (57.6%)
Nigeria	152 (32.4%)	77 (32.4%)	29 (31.5%)
Guinea	15 (3.2%)	8 (3.4%)	4 (4.3%)
Cameroon	13 (2.8%)	10 (4.2%)	1 (1.1%)
Senegal	12 (2.6%)	8 (3.4%)	1 (1.1%)
Ivory Coast	10 (2.1%)	9 (3.8%)	1 (1.1%)
Other	26 (5.5%)	14 (5.7%)	3 (3.3%)
Total	469 (100%)	238 (100%)	92 (100%)

Hb: haemoglobin.

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The Hb profile was normal in 238 samples (72%) and variant in 92 cases (28%).

As reported in Table II, 55 (58.8%) were HbAS heterozygous, 1 (1.1%) was an HbSC compound heterozygote, 19 (20.7%) were HbAC heterozygous, 1 was HbCC homozygous (1.1%), 5 (5.4%) were Hb Lepore heterozygous, 3 (3.3%) were β-thalassemia heterozygous, 5 (5.4%) had persistent HbF heterozygosity and 3 (3.2%) had other variants.

Cord blood samples from all the neonates (n=94) of the 92 carrier mothers (2 twin deliveries) were tested (Table II) within a week (median time 5.6.days): 46 (48.9) had normal Hb, 24 were HbAS heterozygous (25.5%), 1 was HbCC homozygous (1.1%), 12 were HbAC heterozygous (12.8%), 6 were HbSC compound heterozygotes (6.4%), 2 were β-thalassemia heterozygous (2.1%) and 3 were HbSS homozygous (3.2%).

After confirmatory electrophoresis, the nine affected neonates started antibiotic prophylaxis within 2 months of birth and were enrolled in the follow-up programme for SCD patients of the Department of Paediatrics. An additional diagnosis of HbSC compound heterozygosity was made through analysis of the newborns' siblings.

Forty-one partners were tested (Table II): 10 others were out of the country and 38 declined to be tested. The Hb profile was normal in 27 cases (66.1%); in the remaining 14, one HbSC compound heterozygote (2.4%), six HbAS heterozygotes (14.6%), one HbCC homozygote (2.4%), three HbAC heterozygotes (7.3%), two β-thalassemia heterozygotes (4.8%) and one Hb Lepore heterozygotes (2.4%) were identified.

Table II - Frequency of clinically significant haemoglobin variants in new mothers and Hb profile of newborns and partners.

	Hb variant new mothers	Hb profile	
		Newborns	Partners
Normal	-	46 (48.9%)	27 (66.1%)
HbAS heterozygous	55 (58.8%)	24 (25.5%)	6 (14.6%)
HbAC heterozygous	19 (20.7%)	12 (12.8%)	3 (7.3%)
Lepore heterozygous	5 (5.4%)	-	1 (2.4%)
HbF	5 (5.4%)	-	-
β-thalassemia heterozygous	3 (3.3%)	2 (2.1%)	2 (4.8%)
HbSC heterozygous	1 (1.1%)	6 (6.4%)	1 (2.4%)
HbCC homozygous	1 (1.1%)	1 (1.1%)	1 (2.4%)
HbSS homozygous	-	3 (3.2%)	-
Other	3 (3.2%)	-	-
Total	92 (100%)	94 (100%)	41 (100%)

Hb: haemoglobin; HbAS: haemoglobin AS; HbAC: haemoglobin AC; HbF: haemoglobin F; HbSC: haemoglobin SC; HbCC: haemoglobin CC; HbSS: haemoglobin SS.

Discussion

We report here the results of a pilot SCD screening programme carried out in immigrants from countries of high incidence of the disease, mainly Ghana and Nigeria[12], who now live in the province of Modena in north Italy. The screening was designed to detect clinically significant Hb variants in new mothers and their neonates and was made possible by the availability of their blood samples collected daily for the study of HDN at local Transfusion Medicine Department. Accordingly, a database of Hb profiles of sub-Saharan immigrants was set up in the province of Modena, an area in which SCD is not present in the indigenous population.

The study shows that early diagnosis of SCD or carrier status is feasible and may be achieved in subjects at risk (92 out of 330 new mothers) providing valuable epidemiological information on the frequency of these conditions and their geographic and ethnic distribution (higher in women coming from high-risk countries, e.g. Ghana and Nigeria) as detailed in Table I. As shown in Table II, the same applies to the 48 out of 94 neonates who were identified as subjects at risk/affected by SCD. Not all the partners were willing to provide blood samples so we cannot make any further comment on them.

Of greater importance, the screening we performed allowed prophylaxis (penicillin and vaccination) to be started within 2 months of birth which is much earlier than the previous time of diagnosis (average of 24 months of age), clearly too late to have beneficial effects. Additional benefits of the screening were a more appropriate specific management of the disease in the post-natal period, and the opportunity of counselling for clinical complications potentially associated with carrier status.

In addition, the detection of carrier status in infants and families allowed: (i) education about future reproductive choices; (ii) genetic information on clinical implications of the carrier status; (iii) informed reproductive choices for the child. The set up of a specific database for carriers will clarify the importance of identifying this condition in the future.

This study was made possible by the establishment of a cooperative network involving physicians, nurses of the Maternity Units and the new mothers, which enabled an unusually high compliance of the participants (70.36% of the mothers). Moreover, the costs of the screening were limited as the HPLC test is not expensive and no additional blood was drawn.

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The medical benefit of obtaining an early diagnosis of SCD and the limited cost of the procedure support the project of extending this reliable, simple and cost-effective screening protocol routinely to all the subjects of a high-risk population.

The screening programme described here differs from screening carried out elsewhere as it examines both neonates and new mothers and is designed for a specific area where the disease was not present before immigration from high-risk countries. This screening represents the first step to promote a socially conscious approach for diagnosis and treatment of a disease hitherto uncommon in northern Italy. Although migration flows are unpredictable, our model can be adapted to any area of first immigration to improve family awareness on education, prevention and appropriate prophylaxis for neonatal SCD.

Our approach does not identify neonates of normal mothers and carrier fathers for whom universal screening would be required. A universal, expensive newborn screening may be considered if the aim is to identify as many as possible newborn SCD patients and carriers through the most ethically appropriate protocol. This powerful strategy will be essential in the future as high-risk immigrants and indigenous populations intermingle increasingly. However, the present social situation of controlled immigration, with the percentage of immigrants in the province of Modena being 13.2%, does not justify the immediate establishment of universal screening.

Nevertheless, the preliminary results obtained with selective screening indicate that there was a need for such screening and that SCD children and carriers can be identified even when resources are limited.

In conclusion, we think that our selective screening may be a well-suited approach for areas of recent immigration in which immigrants are still reproductively segregated although we are aware that the future goal will be to implement universal screening.

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