Universal Screening Program in Pregnant Women and Newborns at-Risk for Sickle Cell Disease: First Report from Northern Italy

Mariachiara Lodî, Elena Bigi, Giovanni Palazzi, Lara Vecchi, Riccardo Morandi, Monica Setti, Silvana Borsari, Giuliano Bergonzini, Lorenzo Iughetti, and Donatella Venturelli

*Post Graduate School of Pediatrics, Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy; Oncology and Hematology Pediatric Unit, Department of Medical and Surgical Science for Mothers Children and Adults, University Hospital of Modena, Modena, Italy; Transfusion Medicine Department, University Hospital of Modena, Modena, Italy; Clinical Engineering, Local Primary Health Care of Modena, Modena, Italy; Community Women Health Clinic, Local Primary Health Care of Modena, Modena, Italy; Laboratory Medicine Department, University Hospital of Modena, Modena, Italy

**ABSTRACT**
The implementation of screening programs for early detection of patients with sickle cell disease has become necessary in Italy as a result of the high rate of migration from areas with a high prevalence of the disease (Sub-Saharan Africa, Middle East and the Balkans). Following a pilot study performed in the province of Modena, Italy in 2011–2013, an official screening program was established on May 31 2014 for all pregnant women, free-of-charge for the family according to the National Guidelines for Physiological Pregnancy. Hemoglobin (Hb) profiles of pregnant women within 10 weeks of pregnancy, of new mothers at delivery and of the newborns of mothers with variant Hb profiles (newborns at-risk), were evaluated by high performance liquid chromatography (HPLC). Samples from 17,077 new mothers were analyzed and 993 showed alteration of Hb patterns (5.8%) (1.0% Hb AS carriers); of the 1011 at-risk newborns, four (0.4%) carried sickle cell disease and 90 (8.9%) were Hb AS carriers. These data show that early diagnosis of sickle cell disease or carrier status can be obtained in high-risk newborns, providing valuable information on the frequency of these conditions in geographic areas in which the disease is historically rare.

**Introduction**
Screening programs for early detection of sickle cell disease patients are now necessary due to the high rate of migration to Europe from areas in which carriers of the sickle cell allele are 19.0 to 27.0% [1,2]. In Italy, more than 72,000 immigrants are estimated to carry sickle cell trait [3]. In Emilia Romagna region (4,488,146 inhabitants) immigrants represent about 12.0% of the whole population. Approximately 92,400 (13.2%) immigrants, mainly from Sub-Saharan Africa, Eastern Europe and the Middle East [4] live in the province of Modena (701,642 inhabitants, 2688 km², 261 inhabitants/km²). Almost 80.0% of African foreign residents in the province of Modena came from Ghana and Nigeria, where the percentage of Hb AS carriers ranges from 3.0 to 24.0% [2]. The prevalence of immigrants from these countries over the last 15 years correlates directly with a steady increase in the number of sickle cell disease patients (from five in 1995 to 86 in 2016). The growing number of new diagnoses in children after the first years of life prompted us to design the first selective neonatal screening program in the province of Modena, Italy from 2011 to 2013. This pilot study allowed us to identify patients with sickle cell disease early in life and to provide appropriate antibiotic prophylaxis, immunizations and comprehensive care as early as possible [5]. These encouraging results led us to establish a wider screening program, free-of-charge for the family and supported by the National Health System. A first level screening for sickle cell disease was offered to all pregnant women according to National Guidelines for Physiological Pregnancy [6]. We describe the results of the first 34 months of this sickle cell disease newborn screening as a first example in Italy.

**Materials and methods**
A newborn screening program for sickle cell disease started on May 31 2014 at the University Hospital of Modena, Modena, Italy for the detection of at-risk newborns of mothers carrying clinically significant hemoglobin (Hb) variants (Figure 1). Screening test for hemoglobinopathies was offered to all women within 10 weeks of pregnancy by gynecologists, according to National Guidelines for Physiological Pregnancy and performed after informed consent was obtained [6]. If a woman was found to be a carrier of a variant Hb pattern, the screening test was also offered to the partner along with the possibility of prenatal diagnosis performed on the fetus. If the test was not performed during

**CONTACT**
Dr. Donatella Venturelli, venturelli.donatella@policlinico.mo.it
Transfusion Medicine Department, Azienda University Hospital of Modena, via del Pozzo 71, 41124 Modena, Italy

© 2017 Informa UK Limited, trading as Taylor & Francis Group
pregnancy, screening was proposed at the time of delivery and, after informed consent was obtained, peripheral blood of new mothers, already collected at the Maternity Units of the Province of Modena and sent to Transfusion Medicine Department for the study of the hemolytic disease of the newborn (HDN), was evaluated for the Hb profile. The cord blood of the newborn of all mothers carrying clinically significant Hb, also stored at the Transfusion Medicine Department as required for assessment of HDN [7], was tested for Hb variants. In the event that no consent was received from the mothers, screening was not performed.

Blood samples from mothers and newborns were collected in EDTA and stored for 7 days at 5°C in accordance with the Italian legislation [8]. Primary screening for clinically significant Hb variants was performed by high performance liquid chromatography (HPLC) (VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) [9]. The screening laboratory participates in a quality control program that includes proficiency testing (National External Quality Assessment Scheme: UK NEQAS Abnormal Hemoglobin).

If the mother and/or the baby were found to be carrier of Hb AS or both carried the same clinically significant Hb alteration, a report with information regarding the status of carriers and difference between carrier state and the disease was sent by priority mail to the mother. The report explained the genetic transmission of the Hb variant and the importance of testing the partner in order to know the potential risk of having an affected child in future pregnancies. It was also suggested to analyze the Hb profile of all family members and to test the newborn again at 6 months of age to avoid false negative/positive results. The mother was also invited to show the report to the family doctor and to the pediatrician.

If the mother and her baby carried a different Hb variant, counseling with the family was scheduled at the Transfusion Medicine Department, University Hospital of Modena, Modena, Italy, with a trained hematologist. If the Hb profile of the partner was unknown, a blood sample was collected and analyzed by HPLC. If both parents were positive, specific clinical and genetic counseling for future pregnancies was offered together with HPLC analysis of newborn siblings.

If the newborn carried a homozygous or compound heterozygous Hb S (β²; HBB: c.20A>T) (i.e. affected by sickle cell disease), an appointment was scheduled with the parents and the hospital reference hematologist pediatrician for sickle cell disease diagnosis communication. Doctors and nurses provided specific education about inheritance patterns, description of main symptoms of the disease, the importance of preventive therapy (antibiotic prophylaxis and vaccination), the management of acute illness and on how to recognize and to prevent life-threatening complications. Educational supporting material (in English, Italian and French) was provided to each family to help educate the other family members at home. Confirmatory tests on the baby’s samples (gel electrophoresis, genotyping methods) were scheduled within 2 months of life. All affected sickle cell disease newborns referred to the Pediatrics Department of the University Hospital of Modena, Modena, Italy, were they started on a prophylactic antibiotic treatment and vaccination program. Clinical and laboratory follow-up for each patient was scheduled every 3–6 months depending on the

Figure 1. Screening protocol.
severity of the disease and on the treatment performed [hydroxyurea (HU) or chronic transfusion]. The patients were enrolled in a specific comprehensive care sickle cell disease program [10].

Results

From May 31 2014 to March 31 2017, we enrolled 17,077 pregnant women: 12,017 screening tests were performed within 10 weeks of pregnancy, and 5060 at delivery. An abnormal Hb pattern was found in 993 women. Four carried sickle cell disease and 90 Hb AS carriers were identified out of 1011 at-risk newborns examined. As shown in Table 1, of the 993 women 179 (18.02%) were heterozygous for Hb AS trait, one (0.1%) was homozygous for Hb SS, one (0.1%) was homozygous for Hb C (βC; HBB: c.19G>A), 95 (9.56%) resulted positive for the presence of other Hb variants. We also detected 316 (31.82%) heterozygous β-thalassemia (β-thal), 29 (2.92%) heterozygous δ-thalassemia (δ-thal), seven (0.7%) heterozygous hereditary persistence of fetal Hb (HPFH) (or δβ-thal). Altered levels of Hb A2 and Hb F were also detected: 124 women (12.48%) showed decreased values of Hb A2 (<2.0%), 40 (4.02%) were identified to be carriers of borderline Hb A2 (3.2–3.8%) levels and 190 (19.13%) had increased Hb F levels.

Cord blood samples of 1011 at-risk newborns were tested within a week of birth (median time 5.6 days). As shown in Table 1, four (0.4%) newborns carried sickle cell disease [one homozygous Hb S, three compound heterozygotes for Hb SC (βS/βC)], 90 (8.9%) carried Hb AS trait, 44 (4.35%) presented other clinically significant Hb alterations [Hb AC (βA/βS)], Hb AE (βA/βE); HBB: c.79G>A), heterozygous Hb O-Arab [β121(GH4)Glu→Lys; HBB: c.364G>A], possible heterozygous α-thalassemia (α-thal), borderline Hb A2 levels. The results were confirmed in all the newborns tested after 6 month of age.

Table 1. Hemoglobin variants in pregnant women/new mothers and at-risk newborns: frequency of clinically significant hemoglobin variants.

| Parameters          | Mothers with Hb Variants n (%) | At-Risk Newborns n (%) |
|---------------------|--------------------------------|------------------------|
| Normal              | –                              | 862 (85.26)            |
| Heterozygous Hb AS  | 179 (18.02)                    | 90 (8.90)              |
| Heterozygous Hb SC  | –                              | 3 (0.29)               |
| Homozygous Hb SS    | 1 (0.10)                       | 1 (0.10)               |
| Homozygous Hb CC    | 1 (0.10)                       | –                      |
| Other variants*     | 95 (9.56)                      | 42 (4.15)              |
| Heterozygous β-thal | 316 (31.82)                    | –                      |
| Heterozygous δ-thal | 29 (2.92)                      | –                      |
| Heterozygous α-thal | 1 (0.10)                       | –                      |
| Increased Hb F      | 190 (19.13)                    | –                      |
| Borderline Hb A2    | 40 (4.02)                      | 1 (0.10)               |
| Decreased Hb F      | 124 (12.48)                    | –                      |
| Heterozygous HPFH (δβ-thal) | 7 (0.70) | – |
| Others*             | 11 (1.10)                      | 11 (1.08)              |
| Total               | 993                            | 1011                   |

Hb: hemoglobin; Hb AS: βS/βC; Hb SC: βS/βC; Hb CC: βC/βC.

*Hb AC: βA/βC; Hb AE: Leperore: HBDx.330G>T; Hb G-Philadelphia: HBA2: c.207C>G (or HBA1) or 207C>A; Hb D-Punjab: HBB: c.346G>C; Hb AC: βA/βC; Hb O-Arab: HBB: c.346G>A; Hb G-San Jose: HBB: c.234A>G; Hb Camperdown: HBB: c.315G>C or 315G>T.

Discussion

This is the first report from Italy of a universal screening for sickle cell disease of pregnant women and newborns at risk with the aim of identifying sickle cell disease patients, start antibiotic prophylaxis within 2 months of birth, enroll affected children in a comprehensive care program, and determine of the incidence of Hb AS trait in the Emilia Romagna region. In this screening program about 1.04% of all pregnant women tested were Hb AS carriers, while 0.4% of the at-risk newborns carried sickle cell disease and 8.9% were Hb AS carriers. In Italy, there is no official national screening program for sickle cell disease. Only limited data are available from a few pilot studies in northern Italy. Rolla et al. [11] reported data on screening for newborns at-risk for sickle cell disease from December 2012 to January 2014 in Novara, Emilia Romagna. In this study, no affected sickle cell disease patients were identified, but 5.9% at-risk newborns were Hb AS carriers [11]. No patients were detected in a universal newborn screening performed in Ferrara, Emilia Romagna, from 2007 to 2009, but Hb AS carrier incidence was 0.8% [12]. Universal neonatal screenings for sickle cell disease are active in a few European countries: the UK [13], The Netherlands [14] and in some areas of Belgium [15], Spain [16] and Germany [17]. In 2010, Streetly et al. [13] reported the incidence of 0.05% sickle cell disease newborns and of 1.0% Hb AS carriers in the UK. Since 2000, a national screening program for at-risk newborns has been active in France [18]. Bardakjian et al. [18] evaluated data on-at-risk newborns tested for sickle cell disease from 1996 to 2007. In this report 3890/2,622,870 (0.14%) newborns were found to be affected with sickle cell disease and 64269/ 2,622,870 (2.45%) were heterozygous for Hb AS [18].

In our screening program, we also detected other types of hemoglobinopathies based on the method used for the test and the extension of screening to all pregnant women. Unlike other countries such as France [18], where β- and α-thalassemias are rare, about 1.8% of pregnant women in the province of Modena were β-thal carriers. Approximately 1.1% of all pregnant women also showed increased values of Hb F (>5.0%). Although this condition could be correlated with pregnancy, we addressed its significance by repeating the test a few months after delivery to rule out genetic conditions associated with HPFH. Altered Hb A2 values (Hb A2 <2.0% and Hb A2 between 3.2–3.8%) were detected in 0.96% of women. Further investigation at the molecular level is required due to the potential association with thalassemia carrier status.

This first level screening is free-of-charge and is supported by the National Health System. It represents an important example of collaboration between the local Obstetrics Units and the University Hospital where a network of trained healthcare providers can deliver the comprehensive care needed by sickle cell disease patients [19]. Our approach does not identify neonates of normal mothers and carrier fathers for whom universal screening would be required using HPLC on cord blood of all neonates. However, this type of screening could be easily implemented in other areas of Italy or in other countries with significant
migration flow because it is not expensive and no additional neonatal blood samples are required.

In addition, screening provides valuable epidemiological information on the frequency, geographic and ethnic distribution of sickle cell disease. The results of this screening demonstrate how migration flows have changed hemoglobinopathy frequencies in the geographic area under study due to the presence of new Hb variants whose association with β-globin gene mutations or with Hb S could result in the birth of patients with hemoglobinopathies of an unpredictable phenotype. The efficacy of this screening program on morbidity and mortality of patients with sickle cell disease will be evaluated in the future.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Piel FB, Tatem AJ, Huang Z, et al. Global migration and the changing distribution of sickle haemoglobin: a quantitative study of temporal trends between 1960 and 2000. Lancet Glob Health. 2014;2(2):e80–e89.

[2] Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704–712.

[3] Angastiniotis M, Vives Corrons JL, Soteriades ES, et al. The impact of migrations on the health services for rare diseases in Europe: the example of haemoglobin disorders. Sci World J. 2013;2013:527905.

[4] Osservatorio sull’immigrazione della provincia di Modena. Available from: www.provincia.modena.it/sociale.

[5] Venturelli D, Lodi M, Palazzi G, et al. Sickle cell disease in areas of immigration of high-risk populations: a low cost and reproducible method of screening in northern Italy. Blood Transfus. 2014;12(3):346–351.

[6] National guidelines for physiological pregnancy. Italian National Institute of Health. 2011 update (http://www.salute.gov.it/imgs/C_17_pubblicazioni_1436_allegato.pdf).

[7] New Legistalitve Decree of October 21 2005, no. 219. New transfusion discipline of transfusion and national production of blood products. Official Gazette of the Italian Republic; General Series no. 251 of October 27 2005 (http://www.camera.it/parlam/leggi/052191.htm).

[8] Decree of the Minister of Health, March 3 2005, article 14, paragraph 3. Characteristics and methods for the donation of blood and blood components. Official Gazette of the Italian Republic; General Series no. 85 of April 13 2005 (http://www.gazzettaufficiale.it/atto/serie_generale/carcaDettaglioAtto/originarioatto.dataPubblicazioneGazzetta=205-0413&att90.codiceRedazione=05A03442&elenco30giorni=false).

[9] Eastman JW, Wong R, Liao CL, et al. Automated HPLC screening of newborns for sickle cell anemia and other hemoglobinopathies. Clin Chem. 1996;42(5):704–710.

[10] Pass KA, Lane PA, Fernhoff PM, et al. US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). J Pediatr. 2000;137(4 Suppl):51–546.

[11] Rolla R, Castagno M, Zaffaroni M, et al. Neonatal screening for sickle cell disease and other hemoglobinopathies in the changing Europe. Clin Lab. 2014;60(12):2089–2093.

[12] Ballardini E, Tarocco A, Marsella M, et al. Universal neonatal screening for sickle cell disease and other hemoglobinopathies in Ferrara, Italy. Blood Transfus. 2013;11(2):245–249.

[13] Streetly A, Latinovic R, Henthorn J. Positive screening and carrier results for the England-wide universal newborn sickle cell screening programme by ethnicity and area for 2005-07. J Clin Pathol. 2010;63(7):626–629.

[14] Jans SM, van El CG, Houwaart ES, et al. A case study of haemoglobinopathy screening in the Netherlands: witnessing the past, lessons for the future. Ethi Health. 2012;17(3):217–239.

[15] Gulbis B, Cotton F, Ferster A, et al. Neonatal haemoglobinopathy screening in Belgium. J Clin Pathol. 2009;62(1):49–52.

[16] Cela de Julián El, Dulin Igüeze E, Guerrero Soler M, et al. Evaluation of systematic neonatal screening for sickle cell diseases in Madrid three years after its introduction. An Pediatr (Barc). 2007;66(4):382–386.

[17] Lobitz S, Frömmel C, Brose A. Incidence of sickle cell disease in an unscreened cohort of neonates born in Berlin, Germany. Eur J Hum Genet. 2014;22(8):1051–1053.

[18] Bardakdjian-Michau J, Bahauu M, Hurtel D, et al. Neonatal screening for sickle cell disease in France. J Clin Pathol. 2009;62(1):31–33.

[19] Quinn CT, Rogers ZR, McCavit TL, et al. Improved survival of children and adolescents with sickle cell disease. Blood. 2010;115(17):3447–3452.