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Results of a multicenter universal newborn screening program for sickle cell disease in Italy: A call to action

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Abstract

Background: Sickle cell disease (SCD) is a chronic multisystem disorder requiring comprehensive care that includes newborn screening (NBS) as the first step of care. Italy still lacks a national SCD NBS program and policy on blood disorders. Pilot single-center screening programs and a regional targeted screening have been implemented so far, but more evidence is needed in order to impact health policies.

Population and methods: NBS was offered to parents of newborns in gynecology clinics in Padova and Monza, tertiary care university hospitals in northern Italy. High-performance liquid chromatography (HPLC) was performed as the first test on samples collected on Guthrie cards. Molecular analysis of the beta-globin gene was performed on positive samples.

Results: A total of 5466 newborns were enrolled; for 5439, informed consents were obtained. A similar family origin was seen in the two centers (65% Italians, 9% mixed couples, 26% immigrants). Compared with SCD NBS programs in the United States and Europe, our results show a similar incidence of SCD patients and carriers. All SCD patients had a Sub-Saharan family background; HbS carriers were 15% Caucasians (Italian, Albanians) and 10% from other areas (North Africa–India–South America); carriers of other hemoglobin variants were mainly (47%) from other areas.

Conclusions: Our results demonstrate the feasibility of a multicentric NBS program for SCD, give information on HbS epidemiology in two Northern Italian Areas, and support previous European recommendation for a universal NBS program for SCD in Italy: a high incidence of patients and carriers has been detected, with a high percentage of Caucasian carriers, impossible to identify in a targeted NBS.

KEYWORDS
Italy, newborn screening, sickle cell disease

1 | INTRODUCTION

Sickle cell disease (SCD) is a chronic and complex multisystem disorder requiring comprehensive care that includes newborn screening (NBS), health education, and management of acute and chronic complications.[1,2] Early diagnosis through NBS programs allows the timely implementation of preventive measures such as penicillin prophylaxis, vaccination against capsulated bacteria and influenza virus, adequate healthcare measures (i.e., spleen palpation), stroke prevention programs, and hydroxyurea treatment, thereby reducing morbidity and mortality.[3–6] Despite the above-mentioned advantages of an

Abbreviations: ACS, acute chest syndrome; Hb, hemoglobin; NBS, newborn screening program; SCD, sickle cell disease; VOC, painful vaso-occlusive crisis

early diagnosis and the inclusion of NBS as the first step for comprehensive care in all international guidelines, including the Italian National guidelines,[7–12] Italy still lacks a national SCD NBS program and a national policy on blood disorders, regardless of the recent increase in the number of SCD patients due to globalization and population movements.[13] Pilot single-center screening programs and even a regional targeted screening have been implemented so far,[14–17] but more evidence is needed in order to impact national health policies. In fact, a National NBS Plan is in place in Italy since the 1990s, and it includes mainly metabolic disorders, but the National Health System is organized on a regional basis and every region can organize the NBS with certain independence. Therefore, there are significant differences in the regional legislations regulating NBS and in the development of the single regional NBS programs in terms of the diseases to be included, funding allocation, and program organization.

In order to obtain more evidence to support the need of NBS for SCD in our Italian setting, we performed a multicenter universal newborn screening for SCD in North-East Italy, to evaluate the feasibility and efficacy of universal NBS and to determine SCD epidemiology in the areas of Padova and Monza (North Italy). The project was conceived in the frame work of a public–private partnership in order to enhance present and future sustainability in times of financial constraints for universal healthcare systems in Europe.

2 | METHODS

2.1 | Study design

Two public tertiary care university hospitals, both Reference Centers for Pediatric Hematologic Disorders—including SCD—at the national (Italian Association of Pediatric Hematology Oncology-AIEOP) and international levels (European Reference Network for Rare Hematological Disorders-EuroBloodNet), in two North Italian regions (Lombardia–Monza and Veneto–Padova), partnered with local charities to organize the NBS program, relying partly on resources already available in the two centers (i.e., staff).

Guthrie cards for high-performance liquid chromatography (HPLC) analysis were collected for newborns in both centers, after a specific SCD-informed consent was signed by the mothers. Information on family origin was recorded on the Guthrie card. The study outline and sample flow was slightly different in the two centers due to a different organization of the local teams.

In Padova, from May 2, 2016, until November 30, 2017, SCD NBS was performed but not in the framework of the metabolic NBS program. Therefore, a dedicated SCD NBS research nurse performed NBS counseling to the mothers of the Maternity Unit and collected the samples from infants; infants from the neonatal intensive care unit (NICU) were excluded as well as infants born on Thursdays and Fridays due to a lack of funding to cover the discharges occurring during weekends. The heel prick sample was collected immediately after the sample for the metabolic screening. In Monza, from September 1, 2016, until August 31, 2017, SCD NBS was performed in the framework of the metabolic NBS; therefore, counseling and sample collection were not performed by a dedicated SCD nurse but by the staff nurses in both the maternity ward and the NICU seven days a week. Premature babies were also screened for SCD at the moment of the metabolic screening. Guthrie cards from Monza were sent to Padova through courier once a week, utilizing the already-established network for pediatric oncology samples' centralization.

TABLE 1 Origin of foreign parents, according to areas at risk for SCD or other hemoglobinopathies[18]

	High	Medium	Low	NN	Total
Monza (MZ)	47 (7.7%)	294 (48.4%)	263 (43.1%)	5 (0.8)	609
Padova (PD)	141 (16.8%)	226 (27%)	459 (54.6%)	14 (1.6%)	840
MZ + PD	188 (13%)	520 (35.9%)	722 (49.8%)	19 (1.3%)	1449

Analyses of Guthrie cards from both centers were done in Padova's Clinic of Pediatric Hematology-Oncology Laboratory with HPLC as the first test (Bio-Rad NBS Variant). A confirmation test with molecular analysis of the beta-globin gene was performed in HPLC samples in which hemoglobin S or other hemoglobin (Hb) variants were detected or if the level of hemoglobin A1 was < 5%, both in premature and at-term babies. Results were sent to referring pediatric hematologists in both centers. SCD patients were enrolled in the comprehensive care programs in Padova and Monza; hemoglobin S carriers and carriers of other hemoglobin variants were offered a genetic counseling extended to the family.

Approval by the Ethics Committee of both institutions was obtained before the start of the project.

3 | RESULTS

In Padova, 4320 babies were born during the study period, and 2826 (65%) were approached during the five days per week of the study; in Monza 2640, babies were born, and all (100%) were approached for the study. A total of 5466 newborns were enrolled, and informed consents were obtained for 5439 of 5466 (99.5%): 2821/2826 (99.8%) in Padova and 2618/2640 (99.1%) in Monza. For 27 of 5466 (0.5%) children, consent was not obtained due to the following reasons: not requested because the mother was in the operating room or in the intensive care unit or child not recognized ($n = 4$, all in Padova); consent was refused by family ($n = 23$ refusal, 22 in Monza and one in Padova). In Monza 221 children were screened while in the NICU because they were born premature.

A total of 5439 newborns were effectively screened in Padova and Monza; all the samples were adequate for analysis and had HPLC performed. All HPLC-positive cases have been confirmed by molecular biology.

3.1 | Demographics of the study population

Of the 5439 cases, 1449 (26.6%) were immigrants. Family origin was similar in the two centers (65% Italians, 9% mixed couples, 26% immigrants), but Padova had a higher percentage of immigrants (16.7% vs 7.7%) coming from areas at high risk of hemoglobinopathies, according to the classification of countries in high, medium, or low, depending on the prevalence of the S mutation (Table 1).[18]

TABLE 2 The incidence of SCD patients, hemoglobin S traits, and other hemoglobin variants in Padova (PD) and Monza (MZ)

	Newborn	Positive test	Newborn SCD	Newborn S carriers	Other hemoglobin variants
Monza (MZ)	2618	24 (0.91%)	1 (0.038%)	9 (0.34%)	14 (0.53%)
Padova (PD)	2821	39 (1.38%)	3 (0.10%)	28 (0.99%)	8 (0.28%)
MZ + PD	5439	63 (1.16%)	4 (0.07%)	37 (0.68%)	22 (0.40%)

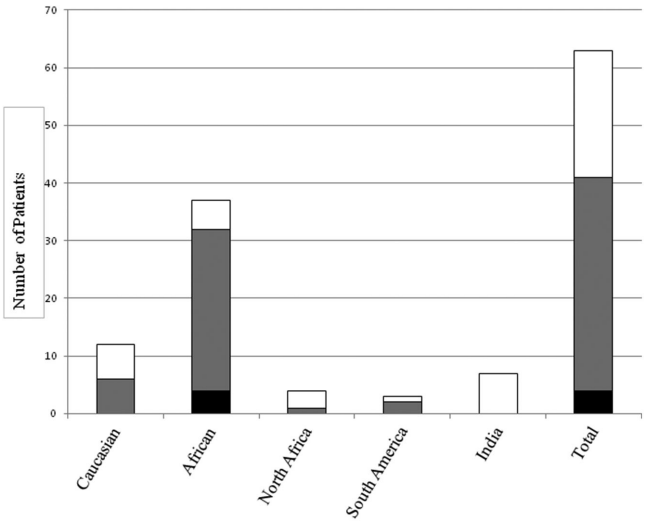

FIGURE 1 Distribution of carriers of HbS and patients affected by SCD, according to ethnicity. White: carriers of other hemoglobin anomalies; gray: hemoglobin S carriers; black: sickle cell disease patients

3.2 | Incidence of HbS and other hemoglobinopathies

The incidence of SCD patients, Hb S trait, and other hemoglobin abnormalities are shown in Table 2. Twenty-two patients were carriers of other hemoglobin variants (nine HbAD, nine HbAC, and 4 HbAE).

All SCD patients had a Sub-Saharan family background; hemoglobin S carriers were 28 of 37 from Sub-Saharan Africa (76%), 6 of 37 (16%) Caucasians (Italian, Albanians), and 3 of 37 (8%) from other areas (North Africa–India–South America), while carriers of other hemoglobin variants were 5 of 22 (23%) from Sub-Saharan Africa, 6 of 22 (27%) Caucasians, and 11 of 22 (50%) from other areas. The distribution of hemoglobinopathies and hemoglobin variants according to ethnicity is shown in Figure 1.

All families of HbS-positive newborns welcomed genetic counseling and accepted to perform HPLC analysis to the siblings and parents.

4 | DISCUSSION

Our results demonstrate the feasibility and efficacy of a multicenter, multiregional NBS program for SCD in Italy, preformed through a public–private partnership and utilizing the facilities and the sample shipment system of the well-established AIEOP pediatric hematology-oncology network.

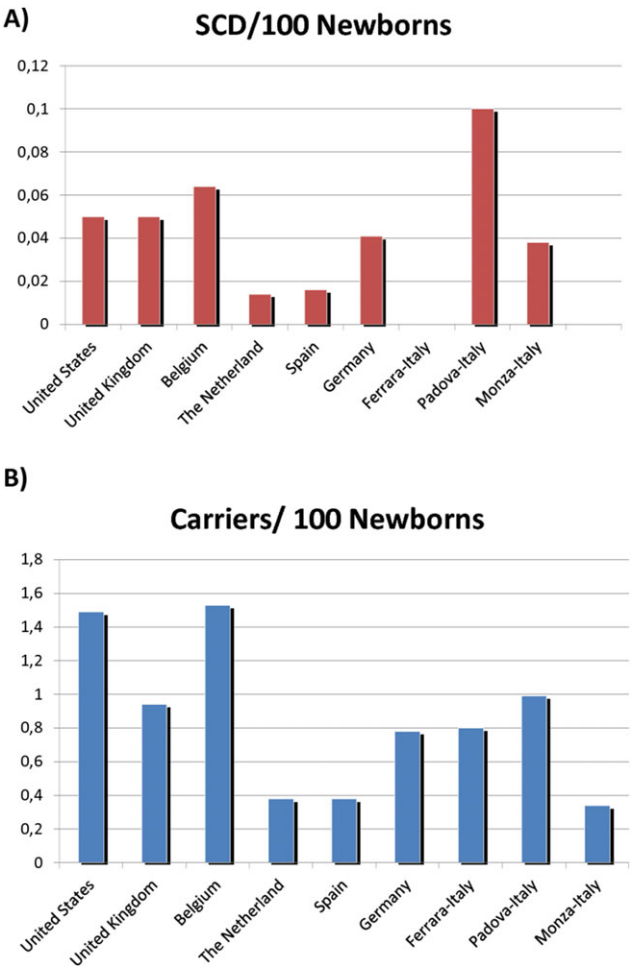

FIGURE 2 Incidence of SCD patients (A) and hemoglobin S carriers (B) in other Universal Newborn Screening Programs in North America[19] and in Europe.[20–23] Confidence intervals (CI) for SCD patients in Padova and Monza are CI 0.04–0.31 and 0.01–0.22, respectively; CI for hemoglobin S carriers are 0.69–1.43 and 0.18–0.65, respectively

Notwithstanding the limited number of samples included in our study compared with other SCD universal NBS programs in the United States and Europe, our results show similar incidence of SCD patients and carriers (Figure 2A and B),[19–23] confirming the need to perform universal NBS for SCD in the two regions involved (Lombardia and Veneto). Nevertheless, compared to other countries, in the two project areas, there is a higher frequency of carriers of hemoglobin variants among individuals of Caucasian origin, both Italians and Albanians, impossible to identify in a targeted screening program. These data reinforce the need of a universal NBS for SCD in our regions in North-East Italy. Further larger studies need to be conducted in the entire country to support a national universal NBS in Italy.

The other ongoing pilot projects in Italy are targeted (either in newborn or in pregnant women)[12,14]; therefore, it is difficult to draw comparison with them in terms of the incidence of affected newborns; only one previous universal NBS was conducted in Ferrara but with a limited number of newborns and no SCD affected newborn was detected.[17]

A slight difference could be observed between the two centers in terms of the incidence of carriers and SCD newborns, in spite

of being both the Veneto and Lombardia regions among the areas with the highest immigrant population in Italy.[24] Nevertheless, upon a closer look, Monza has a lower prevalence of immigrants compared with other areas of Lombardia, while Padova has a higher prevalence of immigrants, especially those coming from high-risk areas of hemoglobinopathies.

The two centers seem to have a slightly different rate of acceptance of SCD NBS: 22 parents refused screening in Monza and only one in Padova. Although the overall rate of acceptance was above 99%, we believe that the presence of a dedicated nurse to perform counseling, consent collection, and screening might have had a positive effect on the parents, increasing the willingness to be screened.

All families accepted the genetic counseling. This is in line with internationally recognized guidelines and patients' perspectives.[25,26]

HPLC and molecular analysis for this pilot program were adequate and cost effective in our setting.

Our project had two limitations: due to budget constraints, it was not possible to perform the NBS in Padova for seven days a week and it would be important to ensure adequate coverage for the entire week in order to enroll all newborn babies; we did not perform a cost-effectiveness analysis and therefore our results cannot give a real estimate of the costs of a universal NBS in our setting. Future larger studies in our regions and in other areas should include detailed cost-effectiveness data in order to support health policy decision-making regarding national NBS for SCD.

5 | CONCLUSION

Our results demonstrate the feasibility of a multicenter NBS program for SCD in our regions in North-East Italy; they also give information on HbS epidemiology in two Northern Italian Areas and support previous European recommendation for a universal NBS program for SCD, at least in our areas of the country: a high incidence of patients and carriers has been detected, with a high percentage of carriers of non–Sub-Saharan African and Caucasian origin, impossible to identify in a targeted NBS. Therefore, pilot regional universal NBS in Veneto and Lombardia are now being planned. Moreover, advocacy actions will now be undertaken with more strength by SCD reference centers belonging to the Italian Association of Pediatric Hematology Oncology (AIEOP) Network and the European EuroBloodNet Reference Network to involve public health authorities and promote further larger studies in other areas of the country to determine the need of a national universal NBS program.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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