

## Screening for sickle cell disease: focus on newborn investigations



Andrea Mosca<sup>1</sup>, Renata Paleari<sup>1</sup>, Giovanni Palazzi<sup>2</sup>, Alessia Pancaldi<sup>2</sup>, Lorenzo Iughetti<sup>2</sup>, Donatella Venturelli<sup>3</sup>, Roberta Rolla<sup>4</sup>, Enza Pavanello<sup>5</sup>, Ferruccio Ceriotti<sup>6</sup>, Massimiliano Ammirabile<sup>6</sup>, Stefano Capri<sup>7</sup>, Antonio Piga<sup>8</sup> and Giovanni Ivaldi<sup>9</sup> on behalf of the SIBioC Working Group on Diabetes and of the SIBioC-SIMMESN Working Group on Neonatal Clinical Biochemistry and Metabolic Diseases

<sup>1</sup>Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan (IT)

<sup>2</sup>Pediatric Unit, Azienda Ospedaliero-Universitaria Policlinico, Modena (IT)

<sup>3</sup>Department of Transfusion Medicine, Azienda Ospedaliero-Universitaria Policlinico, Modena (IT)

<sup>4</sup>Clinical Chemistry Laboratory, A.O.U. "Maggiore della carità", Università del Piemonte Orientale, Novara (IT)

<sup>5</sup>Department of Laboratory Medicine, Azienda Ospedaliera Universitaria Città della Salute e della Scienza, Torino (IT)

<sup>6</sup>Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, SC Patologia Clinica, Milan (IT)

<sup>7</sup>School of Economics and Management, Cattaneo-LIUC University, Castellanza (Varese) (IT)

<sup>8</sup>Department of Clinical and Biological Sciences, Università degli Studi di Torino, Torino (IT)

<sup>9</sup>formerly Laboratorio di Genetica Umana, Ospedali Galliera, Genova (IT)

### ABSTRACT

Drepanocytosis is a genetic disease relevant for its epidemiological, clinical and socio-economic aspects. In our country the prevalence is highly uneven with peaks in former malaria areas, but migration flows in recent years have led to significant changes. In this document we review the screening programs currently existing in Italy with particular emphasis on newborn screening, which in other countries around the world, including within Europe, is at most universal and mandatory. The essential laboratory issues are reviewed, from sampling aspects (cord blood or peripheral), to the analytical (analytical methods dedicated to neonatal screening and adult carrier detection) and post analytical (reporting, informative) ones. An economic analysis based on data collected in the province of Modena is also proposed, clearly showing that neonatal screening is also beneficial from an economic point of view.

**Key words:** hemoglobinopathies, screening, cord blood

### INTRODUCTION

Drepanocytosis is the most common structural hemoglobinopathy in the world. The disease is characterized by a point mutation with autosomal recessive transmission in the gene encoding for beta-globin [ $\beta(A3)6\text{Glu}\rightarrow\text{Val}$ ]. The amino acid substitution results in the production of a hemoglobin variant known as HbS (1-4).

Drepanocyte disease includes the homozygous HbSS form called sickle cell anemia (SCA) and the compound heterozygous forms with other structural defects of hemoglobin (mainly HbS-beta thalassemia and HbSC).

Because many clinical manifestations are common to the 15 different genotypes so far described (5), the terms sickle cell anemia (sickle cell disease, SCD) or drepanocytosis have been proposed to include them all.

The disease is very important, both clinically and epidemiologically, and a dedicated 2023 issue of *Lancet Haematology* was devoted entirely to this topic (6). In fact, individuals with SCD can face premature death and develop severe chronic complications that significantly affect their quality of life, work productivity, and economic stability. Furthermore, these patients often face cultural racism, which exacerbates their marginalization and undermines their mental well-being. Some typical

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Corresponding Author: Andrea Mosca, Department of Pathophysiology and Transplantation, Università degli Studi di Milano  
Email: [andrea.mosca@unimi.it](mailto:andrea.mosca@unimi.it)

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manifestations of the disease, related to vaso-occlusive crises and infections, are acute bone pain, respiratory distress, dactylitis, stroke, and in severe cases multi-organ damage and death. The organs most frequently affected are the spleen (hyposplenism), kidney, brain (cerebrovascular disease), bone and cartilage tissue, and liver. Finally, retinopathy and priapism (7) may appear. In children, it is common for pneumococcal infection to develop as a result of hyposplenism.

2006 data from the World Health Organization (8) indicate that at least 5% of the world's population carries variations in globin genes capable of causing qualitative or quantitative defects in hemoglobin synthesis, and that more than 330 000 affected children (83% drepanocytosis, 17% thalassemia) are born each year. Epidemiological data indicate that sickle cell anemia has a high prevalence in sub-Saharan and equatorial Africa, and to a lesser extent in the Middle East, India, and Mediterranean regions. The high incidence in these areas can be attributed to protection against malaria, a hypothesis initially proposed by Haldane JBS in the 1950s (9). Currently, SCD is a hemoglobinopathy affecting about 100 000 individuals in the United States and nearly 20 000-25 000 in Europe, mainly immigrants to European countries from endemic areas where more than 75% of affected individuals are born (10,11).

The incidence in Europe was fairly stable between 2000 and 2021, although globally the proportion of births with SCD increased to 13.7%, from 453 000 to 515 000 infants (12-14). In Italy, drepanocytosis is the most frequent structural hemoglobinopathy, with regions such as Sicily, and to a lesser extent Southern Italy, in which the gene frequency ranges from 0.5 to 13%. In Sicily, the presence of the  $\beta^s$  gene is mainly due to Arab domination in the historical period from 827 to 1072. The migratory flows recorded in the last 20 years from Africa, South America, and the Balkans have contributed significantly to the further spread of the disease throughout Italy, especially in the northern regions particularly affected by migration (15-17).

This opinion paper aims to assess the current status of screening for sickle cell disease and advocate for universal newborn screening for SCD in Italy. It particularly emphasizes the laboratory medicine aspects of tests essential for accurate diagnosis of this pathology.

## LABORATORY PROCEDURES

Regarding newborn screening, extensive reviews have been conducted on the various programs across Europe (18,19). In some countries (Netherlands, Spain, UK) screening is nationally organized and universal. In France, newborn screening is nationally organized in certain metropolitan regions, while it is universal in overseas territories. In Belgium and Germany, it is regionally organized with universal coverage. In Ireland the screening is organized nationally, but only for at-risk ethnic groups. In Italy, screening is carried out locally based on past experience and the prevalence of the HbS trait. In the provinces of Modena, Ferrara, Novara, and Pordenone, screening is carried out in a targeted manner because the significant prevalence of carriers is

related to immigration in past decades of particular ethnic groups from areas of high endemic prevalence (e.g., from Ghana, as far as Modena is concerned) (20). In the provinces of Padova and Monza, on the other hand, universal screening of women of childbearing age has been initiated, as well as in Sicily, based on a specific regional decree (21,22).

The flow chart we are proposing is shown in Figure 1. In accordance with the consensus document earlier mentioned (19) it is agreed that the goal of the newborn screening program is the detection of drepanocytosis, including all possible genotypes previously mentioned.

### Pre-analytical phase

In newborn screening, several factors contribute to variability, including the type of sample collection instrument, sample quality, transportation methods, and the duration before analysis. Assessing process performance may involve indicators such as the percentage of inadequate samples received and the percentage of samples arriving too late for processing.

The starting point involves selecting the type of collection, which may entail obtaining cord blood at the time of delivery, or capillary blood taken through a heel stick from a newborn within 36-72 hours of birth. In many situations, cord blood is chosen due to the woman typically being discharged on the second day after giving birth. This would also allow this collection to be queued up with the others scheduled for metabolic disease screening. However, ideally, blood from the newborn's capillary collection should be used, as cord blood carries the risk of contamination with maternal blood.

In the case of blood examination, it should be collected in EDTA and processed using the techniques outlined below. For collection on filter paper (DBS), a drop of blood (approximately 50  $\mu$ L) is applied to Guthrie cards, allowed to dry thoroughly, and then sent to the laboratory. It must be processed within two days upon arrival. Alternatively, capillary collection systems in EDTA can be used. In our experience, although the ability to elute hemoglobins from DBSs remains feasible even weeks later, hemoglobins become oxidized and degraded, making interpretation of the result increasingly problematic as days pass. This results in a progressive rise in baseline with the presence of degradation peaks, with the result that major components present at birth tend to decrease over time, and that samples 3 weeks after collection provide quantifications of hemoglobin components that are no longer reliable (23,24).

### Analytical phase

This phase includes all factors related to laboratory analysis. However, an appropriate quality assurance program must be used, as with other laboratory tests. This program must include, at a minimum, the components listed below:

- a standard operating procedure describing the method, how to interpret the results, and how to solve potential problems;
- a flowchart illustrating the role of each person involved

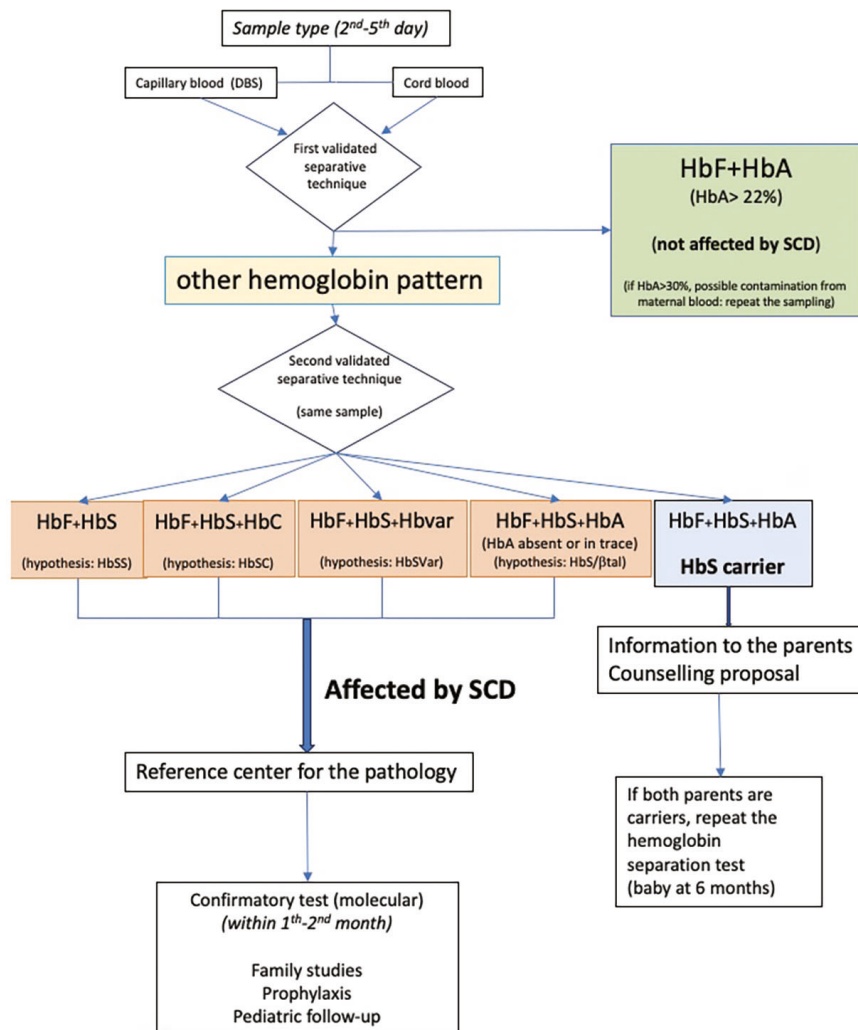
in the analytical process;

- internal quality control materials to oversee the method over time;
- documentation of any corrective action taken;
- participation in at least one external quality assessment program.

The preferred control materials are those with the same matrix as the samples being analyzed. These controls should include both physiological samples and carriers of hemoglobinopathies. Given the limited availability of cord blood, only two external quality assessment programs can be used, to our knowledge. In the United States, a program organized by the Centers for Disease Control

and Prevention (CDC, Atlanta, Georgia) is in operation. In Europe, a program organized by the United Kingdom National External Quality Assessment (UKNEQAS) is available.

In the initial examination, both HPLC and capillary electrophoresis (CE) are suitable for detecting the presence of HbS. Other methods, such as isoelectric focusing and mass spectrometry (MS/MS), are less commonly utilized due to limitations in availability and cost. Ideally, any method that can accurately detect HbS, quantify it alongside HbA<sub>2</sub> and HbF at the decision levels specified for the differential diagnosis of thalassemic forms (6), could be used.



**Figure 1**  
Flowchart for neonatal screening for sickle cell disease (SCD)

The Table 1 presents a concise list of validated separative methods for the separation and quantification of HbS, applicable not only to neonatal blood. The commonly used instrumentations, including both HPLCs

and CE, detect the presence of a peak potentially corresponding to HbS when it falls within a “window” or “zone” as defined by the instrumentation manufacturer, respectively. However, the various separation systems

**Table 1**

*Available methods for HbS separation and quantification.*

Principle	Manufacturer	Type	References	Note	Other
HPLC	ARKRAY, inc.	ADAMS A1c HA-8180T	41, 42	HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Bio-Rad	VARIANT nbs	43, 44	Hemoglobin % reported in windows as FAST (Bart's), F, A, E, D, S, C	Defined the procedure for DBS use. Applications to Hbpathies neonatal screening. Approved by FDA.
	Bio-Rad	VARIANT II beta-thal short	45	HbC, D, E and S are detected	Applications to Hbpathies neonatal screening (from cord blood). HbA <sub>2</sub> and HbF calibrated (2 points).
	Bio-Rad	Variant II dual kit		HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Bio-Rad	D-10 dual kit		HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Menarini (Lifotronic)	HbNEXT	46	HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Menarini (Trinity)	Premier Resolution	47, 48	HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Tosoh	G7	42, 49	HbC, D, E and S are detected	Defined the procedure for DBS use.
	Tosoh	G8 – beta thalassemia mode	41, 50	HbC, D, E and S are detected	Useful for Hbpathies screening on adults. HbA <sub>2</sub> and HbF are calibrated (2 points).
	Tosoh	G11– beta thalassemia mode	51, 52	HbC, D, E and S are detected	Useful for Hbpathies screening on adults. Reference intervals for HbA <sub>2</sub> and HbF defined for pediatric population. HbA <sub>2</sub> and HbF are calibrated (2 points).
CE	Sebia	CAPILLARYS 2 FP	53, 54	Hb C, D, E and S are detected	Useful for Hbpathies screening on adults.
	Sebia	MINICAP FP	55, 56	Hb C, D, E and S are detected	Useful for Hbpathies screening on adults.
	Sebia	CAPILLARYS NEONAT Fast	57, 58	Hb C, D, E, S and Bart's are detected	Defined the procedure for DBS use.
	Sebia	CAPILLARYS 3	47, 59	Hb C, D, E and S are detected	Useful for Hbpathies screening on adults.
	Sebia	CAPILLARYS 3 DBS	60	Hb C, D, E, S and Bart's are detected	Defined the procedure for DBS use.

may also identify certain variants as HbS, which may share similar chemical and physical characteristics with HbS but do not exhibit the sickling phenomenon, despite having the same or different amino acid substitutions. Dedicated databases (25) can be consulted for an overview of potentially interfering variants. Variants with such features are rarer than HbS, and even at birth could still be observed. It should also be noted that alpha variants possibly co-eluting or co-migrating with HbS are not a problem in analytical confirmation at birth, since they can be recognized as defects in the alpha chains by the unequivocal presence of HbF<sup>x</sup> ( $\alpha^x\gamma_2$ ).

The use of two separative methods with different principles could be a valuable aid in confirming some preliminary hypotheses. However, considering that at birth it is mainly important to recognize SCD conditions, whether homozygous or compound heterozygous, with varying degrees of clinical phenotypes, molecular methods should also be undertaken to confirm the diagnosis.

To this regard, dedicated kits in Reverse Dot Blot (RDB) or direct nucleotide sequencing (26) can be utilized. However, it is crucial to specify diagnostic limitations and nucleotide variations tested in the result report (e.g., *“the result does not, however, exclude the possible presence of mutations not included in the panel”*).

The need to confirm the HbS variant hypothesized at birth using methods different than those used in adults should also be considered. In the newborn, it is not possible to rely on the solubility test or the *in vitro* sickling test due to the presence of high concentrations of HbF. This may easily lead to false-positive results using the solubility test, and false-negative results with the Na metabisulfite sickling test. Some laboratories perform the sickling test on cord blood, aware that they may result in false negatives. Instead, a positive result confirms the diagnosis of SCD, obviating the need for molecular analysis. The molecular study will still be necessary for the exact definition of the defects contributing to SCD.

Confirmation of heterozygous conditions observed at birth can also be considered later, in adulthood or otherwise upon completion of the HbF switch. In a newborn screening setting, particularly when mandatory as in Italy, managing the diagnosis of heterozygosity is not straightforward. Ideally, this information should always be communicated to every mother who has been adequately informed about potential negative implications (such as non-paternity, iatrogenic effects, etc.) and has given consent (27).

Not all methods have established protocols for extracting hemoglobins from DBSs, but these can be easily clarified locally, often with guidance from manufacturers. The emergence of methods conducive to point-of-care testing has prompted pilot studies, particularly in countries with limited resources (28). A lateral-flow immunochemical method capable of detecting the presence of HbS and HbC should be noted (29).

### Post-analytical phase, results interpretation and reporting

Various pre-analytical information is important for the interpretation of neonatal analysis data. In particular, it is advisable to try to understand:

- gestational age;
- whether transfusions were performed at birth, or otherwise prior to collection;
- origin/ethnicity of both parents;
- whether there was neonatal jaundice;
- whether there were twins;
- test results for hemoglobinopathies in both parents;
- consanguinity status of the parents, if any.

The information in the first two points is considered essential, because hemoglobin status changes with the gestational period and because possible transfusion makes it impossible to characterize the phenotype of the newborn. From the experience of centers currently practicing neonatal screening on an ongoing basis, information regarding parents can often be difficult to find or may be deficient.

Table 2 displays the threshold values for various hemoglobins typically present at birth in individuals with non-pathologic hemoglobin status, as well as in the most common forms of SCD. The large variability in HbA levels in a subject without hemoglobinopathies can be explained by variability due to gestational age and the type of analytical technique used. HbA<sub>2</sub> values are not reported due to their very low expression in the first months of life, nor are other fractions of limited clinical significance (e.g., acetylated HbF). However, it should be kept in mind that the presence of HbA<sub>2</sub> at birth in concentrations >1% may be an indication of maternal blood contamination. Once the presence of HbS is confirmed in a compound defect condition, all down-stream procedures for identifying an affected individual should be initiated. The timing of definitive diagnosis for SCD (HbS/HbS, HbS/ $\beta$ thal, HbS/other hemoglobinopathy) should be extended in order to be able to study the parents and/or perform second level molecular investigations.

Once the analytical process is concluded, the data interpretation and the related report should highlight the following conditions:

- non-pathological hemoglobin status (AA);
- carrier subject (AS);
- affected subject (SCD).

It should be noted that the absence of hemoglobin A at birth, together with the appearance of non-physiological hemoglobin fractions, already points with certainty to some of the most frequent genotypes of drepanocytosis (HbSS, HbSC, HbSD, HbSE). Identification of any other variants will be flagged for later investigation, but is not the goal of screening.

Some possible formulations of the report, along with an interpretive overview of the laboratory results and accompanying comments, have been published (33). A facsimile parental information sheet for carrier cases, accompanying the report, can be downloaded from the website of the Italian Thalassemia and Hemoglobinopathy Society (SITE) (33) and could be used as a template to be

eventually adapted to various local needs. For affected patients, referral to the reference center should follow, as shown in the lower part of Figure 1.

## PRACTICAL OUTCOMES

### Benefits for infants with SCD

There is now well-established evidence that prophylactic treatment with penicillin from the third month of life and early vaccination for capsulated bacteria (*H. influenzae*, *S. pneumoniae*, *N. meningitidis*) can greatly reduce the risk of serious infections during the first 5 years of life (30-33). In addition, education work within the family, especially aimed at noticing early signs of anemia and infection in time, can save many lives (34), as well as being ready to use blood transfusions if needed. Finally, early detection of the disease always allows the family environment to implement informed choices for disease management and awareness of future critical issues in the reproductive phase (35).

When it comes to therapeutic options, the landscape has evolved significantly in recent years. While hydroxyurea used to be the primary treatment, there has been notable progress with the emergence of new drugs (crizanlizumab, voxelotor, and L-glutamine). This development reflects the growing interest of both academia and industry in addressing rare diseases. Although these products are not a cure for the disease, but primarily serve

to prevent and manage sickle cell crises, there have been significant therapeutic developments with the application of stem cells and gene therapy. For all these aspects, see the paper by Piel et al. (3) and the more recent paper by Ceglie et al. (36). In December 2023 European Medicine Agency (EMA) approved the first gene therapy for SCD and  $\beta$ -thalassemia, with a gene editing approach using CRISPR/CAS9 on the BCL11A gene and reactivation of fetal hemoglobin. Finally, an ongoing clinical trial has shown that continuous treatment with hydroxyurea significantly reduces the need for transfusions (37).

### Benefits for carriers

It is generally believed that HbS carriers can lead normal lives, and have no health problems except under very special conditions (e.g., high altitude). In fact, upon closer observation, skeletal muscle capillary structures are different in HbS carriers than in controls. Furthermore, observations on military personnel and athletes have revealed a 30-fold increased risk of sudden death. Abnormalities in the kidney are indeed among the most frequent clinical manifestations observed in individuals carrying HbS. This is because hyperosmolality and low pH in the renal medulla predispose to red blood cell sickling.

For all these reasons, awareness of the condition and timely medical information are crucial for improving the quality of life for individuals affected by this condition.

**Table 2**

*Cut-off values for hemoglobins at birth (adapted from reference No. 40).*

SCD condition	HbF %	HbA %	HbS %	HbC %	HbD Punjab %	HbO Arab %	Hb Lepore %	HbE %	Threshold values
Not carrier of hemoglobinopathies	<75	14-30*	-	-	-	-	-	-	Definitive when HbA >22 %
HbS/HbS	>80	0	<20	-	-	-	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/ $\beta^0$ -Tal	>80	0	<20	-	-	-	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/ $\beta^+$ -Tal	>80	< 5	<15	-	-	-	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/HbC	>72	0	<13	<15	-	-	-	-	Confirmatory test for the diagnosis of HbS/HbC is required
HbS/HbD Punjab	>74	0	<14	-	<12	-	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/HbO Arab	>74	0	<13	-	-	<13	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/Hb Lepore	>80	0	<20	-	-	-	0	-	Confirmatory test for the diagnosis of SCD is required
HbS/ $\delta\beta$ -Tal	>80	0	<20	-	-	-	-	-	Confirmatory test for the diagnosis of SCD is required
HbS/HbE	>75	0	<15	-	-	-	-	<10	Confirmatory test for the diagnosis of HbS/HbE is required
HbS carrier	>70	$\geq$ 5	<10	-	-	-	-	-	Confirmatory test for the diagnosis of HbS carrier is required

\* *min-max range*

For an in-depth discussion of the medical aspects of the HbS carrier, please refer to the recent SITE consensus (38). It is important that SCD Referral Centers reserve adequate space for counseling, either directly or through caregivers, on the health aspects of HbS carriers.

### Economic analysis

The introduction of a new Diagnostic/Therapeutic program cannot ignore a cost-effectiveness or cost-benefit analysis. Because the data available to date do not allow a full assessment of quality of life and disease complications in the long term, let alone mortality, this document limits the analysis to the first 5 years of life, using data from the province of Modena. This is a robust model, given the accuracy of epidemiological and clinical data derived from newborn screening initiated since 2014 and consolidated since 2015. This analysis does not consider costs related to hydroxyurea therapy,

possible stem cell transplantation or antibiotic treatment, focusing on the more relevant and fully available data of hospitalizations.

The salient epidemiological data are summarized in Table 3a. We estimated a reference population of 200 000, a number 72% lower than the total of the regional tables, but a reasonable value counting that immigrants from Nigeria and Ghana (carriers of HbS and HbC, as well as various forms of thalassemia) reside in Modena and the close surrounding area (mainly in Sassuolo and Nonantola, ceramic manufacturing areas). The collected data clearly show that the rate of hospitalizations is significantly lower in patients identified by neonatal screening. Consider also the figure, not easily quantifiable from an economic point of view, that 26 patients (from the pre-screening group) developed episodes of respiratory distress during monitoring, whereas only 5 patients in the post-screening group had similar events.

Data for the cost analysis are summarized in Table 3b.

**Table 3a**  
*Epidemiological data regarding the province of Modena.*

Parameter	Data
Study population	200 000 inhabitants
Birth rate	7.3/1 000 inhabitants
SCD prevalence (immigrants from Nigeria and Ghana)	1.2 %
Number of patients after screening introduction (2015) (group A)	50
Number of patients before 2015 (group B)	91
Total hospitalizations, mean per year (group A)	0.21 hospitalizations/year
Total hospitalizations, mean per year (group B)	0.66 hospitalizations/year
Mean hospitalization rate per person in group A (5 years follow-up)	0.73 hospitalizations/person
Mean hospitalization rate per person in group B (5 years follow-up)	3.73 hospitalizations/person

**Table 3b**  
*Economic analysis for neonatal screening in the province of Modena.*

Line No.	Parameter	Screening yes (born after 2015)	Screening no (born before 2015)
1	No. of years of observation	9	20 <sup>a</sup>
2	No. of patients with sickle cell disease	50	91
3	No. of evaluable patients with 5 years follow-up	34	78
4	Mean hospitalization rate per person (5 years follow-up)	0.73	3.37
5	Hospitalization (total number)	37	307
6	HPLC/CE analysis costs (€ 3.10/test) <sup>b</sup>	€ 4 569.40	-
7	Molecular analysis costs (€ 150.00/test) <sup>c</sup>	€ 5 100.00	€ 11 700.00
8	Hospitalization total costs (€ 400.00/day)	€ 14 800.00	€ 122 800.00
9	Total costs	€ 24 469.40	€ 134 500.00
10	Laboratory costs per sickle cell disease patient <sup>d</sup>	€ 284.39	€ 150.00
11	Hospitalization costs per patient <sup>e</sup>	€ 292.00	€ 1 348.00
12	Total cost per patient <sup>f</sup>	€ 576.39	€ 1 498.00

<sup>a</sup>The oldest patient in this group was born in 1994.

<sup>b</sup>Calculated on the base of an estimate of 160 births/year and considering that two HPLC/CE analyses are carried out on SCD patients (see reference 21).

<sup>c</sup>Calculated by accounting one analysis per diagnosed patient (line 3\*€ 150.00).

<sup>d</sup>Calculated as the total analysis costs/number of evaluable patients (sum of lines 6+7/line 3).

<sup>e</sup>Calculated as the product of the daily cost of hospitalization and the hospitalization mean per patient (line 8\*line4).

<sup>f</sup>Sum of cost amounts per patient (line 10+line11).

Because the two groups do not have the same numerosity, the data, where possible, have been normalized by patient and year. The choice of analytical method does not significantly impact costs, as both HPLC and CE analysis have comparable expenses. A flat-rate cost was estimated for molecular confirmatory analysis.

Overall, the data clearly indicate that performing newborn screening for SCD is also cost-effective.

## RECOMMENDATION SUMMARY

The document is aimed to offer an updated guide to the literature available at the present time for newborn screening for drepanocytosis, while anticipating the implementation of universal screening throughout Italy. Included in the document are also references to investigations involving the family, of great importance for proper neonatal diagnostics. However, in certain contexts, it is not always possible to carry out information for a variety of reasons (non-traceability of one of the parents, communication problems, etc.).

The box presents a summary of the main indications aimed at laboratory professionals and all those involved in this topic:

- *The laboratory can provide valuable information for the diagnosis of drepanocytosis by combining the validated analytical result with an appropriate interpretive key.*
- *Cord blood testing can highlight different forms of SCD.*
- *Analysis of blood spots should be carried out with a procedure validated by the diagnostic manufacturer and should not be performed more than two days after collection.*
- *The report should be given and kept in its complete form since it could be a reference point for second-level examinations and family counseling.*
- *When testing for HbS in parents, it is strongly recommended that the report of the presence of HbS be made only after a confirmatory test, such as the sickle cell or solubility test (39,40). The presence of a peak "in the S position" is not enough to make a safe report. The sickling test cannot be performed on blood on filter paper, since in the elution process red cells become lysed and can therefore no longer be visualized under a light microscope to assess their possible sickling.*
- *It is strongly recommended that procedures for internal and external quality assessment (EQAS) be performed regularly. For the latter, it would be helpful if national providers of EQAS programs would work to implement a program based on samples collected on Guthrie cards.*

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## CONFLICT OF INTEREST

None.

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