# Twenty Years of Neonatal Screening for Sickle Cell Disease in Brazil: The Challenges of a Continental Country with High Genetic Heterogeneity

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**Abstract**

Sickle cell disease (SCD) is the most common inherited hematological disease worldwide. The benefits of diagnosis and early intervention have led to the wide dissemination of public health programs worldwide. Through neonatal screening programs, it is possible to reduce morbidity and mortality in the first 5 years of life. The prophylactic use of penicillin, the anti-pneumococcal vaccine and other intensive care, increase the survival and quality of life of people with SCD. The aim of this study is to present the 20-year history of screening for hemoglobinopathies in Brazil and its challenges. From 2001 to 2019, an average of 2,400,000 children were screened per year nationwide, with the coverage being of 82,16%. The screening of 54,9% of newborns is collected up to their 5$^{th}$ day of life. The prevalence of SCD was 1:2,263 newborns; therefore, it was the second most-common disease detected by the program of Brazil, being only after hypothyroidism (1/2,175 live births). The healthcare system should provide the necessary infrastructure to confirm the diagnosis of newborns and to provide appropriate counseling and treatment. The early diagnosis and treatment, as well as the follow-up with a multidisciplinary team, are fundamental to the survival rate and the quality of life of patients.

**Keywords:** Newborn screening, Sickle Cell Disorder, Brazil, National Program.

## Introduction

Hemoglobins are genetically determined, therefore changes to their genes, a disorder that can lead to the production of proteins with characteristics different from the ones expected and can result in the formation of a pathological hemoglobin, in several cases, it offers pathophysiological consequences to its carrier. Hemoglobinopathies are recognized as one of the most common genetic pathologies around the world. Amongst them, the sickle cell disorder (SCD) and the β thalassemia have a greater impact on morbidity and mortality, affecting millions of individuals worldwide.[1]

In the scientific world, the SCD has been discovered more than 100 years ago; however, it was mainly in the past decades that the world has advanced towards its prognosis, certainly in virtue of the results provided by Neonatal Screening Programs, its diagnostic benefits and early prophylaxis, which can reduce morbidity and mortality. This early diagnosis has provided a greater knowledge on the early complications of SCD, leading to the creation of prophylactic measures, such as the use of penicillin, the administration of pneumococcal vaccines and other precautions, including the use of hydroxyurea.[2] These actions significantly increase survival rates and improve the quality of life of patients with SCD, reducing sequelae and complications, such as painful crises and splenic sequestration, thus resulting in a better quality of life.[3] In neonatal screening,

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in addition to the early diagnosis, it is also possible to detect heterozygous newborns and, in these cases, to offer tests for couples, by identifying which couples may be at risk of having children with SCD. According to Pan-European Consensus Conference there is an orientation that the carrier status (all mutations that might cause SCD) should be reported and couselling offered to carriers, but the identification of carriers is still debated in several European countries.[4]

Hemoglobinopathies, unlike most other inborn errors of metabolism, are human genetic mutations; that is, they are genetic alterations highly frequent in the global population. Since the population is broadly miscegenated and the severity of complications of SCD can be prevented from adequate actions if they are implemented early in life (penicillin prophylaxis, vaccinations, spleen palpation, parents' education, etc), all national programs must be universalized, instead of oriented to a specific portion of its citizens. Consequently, any program that offers screening for these anomalies on a national scale must have the necessary infrastructure, not only to confirm the laboratory diagnosis of newborns, but also to provide a complete treatment for patients and a specialized genetic counseling for newborns and families.

### *Arrival and allocation of immigrants in Brazil*

The Brazilian population is characterized by its vast genetic heterogeneity, caused by the diversity of racial groups forming it and by the different degrees of miscegenation in all regions of the country.[5]

The mutation in the beta globin gene first appeared in Africa, having then spread to other parts of the world, including Brazil. The arrival of Africans in Brazilian territory began almost 400 years old. Up to its discovery in 1500, Brazil was inhabited by Amerindians, encompassing a population of around two million people. From 1,500 onwards, the immigration of Portuguese people began and, between the 16$^{th}$ and 19$^{th}$ centuries, the arrival of African people took place. Therefore, the hemoglobin S (Hb S), responsible for SCD, was introduced by the slave trade, which enslaved people from Africa and brought them to work in Brazil, first in sugarcane plantations, on the Northeast, and later in the extraction of precious metals, in Minas Gerais. This continued miscegenatory activity is believed to have facilitated the spread of variant hemoglobins in different regions of Brazil. There is a strong association between the clinical presentation of the SCD and the different regions of origin of the slaves. Slaves came from different regions of Africa, featuring important association between different haplotypes and clinical features of the disease. HBB*S haplotypes have been studied in different Brazilian populations, as tools to clarify different population origins. The CAR haplotype (Central Africa) was the most frequent one, followed by the BEM (Benin) haplotype, demonstrating the main regions that slaves came from to Brazil.[6]

In addition to Portugueses and Africans, other immigration flows occurred between the 19th and 20th centuries, primarily of individuals from Italy, Germany and Spain.[7] They arrived mainly in the State of São Paulo and in the Southern region of Brazil.[8] Currently, the proportion of races varies according to the region of the country. For instance, the percentage of the African component is lower in the South (11%), whilest the highest values are found in the Northeast and Southeast (18%-20%).[5] Brazilian studies have demonstrated great regional differences regarding the prevalence of HbS and HbC, which range from 0.2% (Southern region) to 9.8% (Northern region).[9] This portays hemoglobinopathies as a public health problem, even though with distinct incidences in the different states. The aim of this study is to present the 20-year history of screening for hemoglobinopathies in Brazil and its challenges.

## Methods and Results

### *Incorporation of hemoglobinopathies in Brazilian neonatal screening programs*

Due to the extensive arrival of the African population, resulted from the slave trade in the 16th-18th centuries, the World Health Organization recommends the implementation of programs for hemoglobinopathies prevention and control in Latin America.[10]

The greatest challenge for developing preventive programs is that they require support from official health agencies, training of the personnel for the diagnosis, and genetic and clinical counseling for patients. Currently, only four countries in Latin America have universal screening programs for hemoglobinopathies; they are Brazil, Costa Rica, Panama and Uruguay.[11–14] Other countries are conducting pilot studies, in an attempt to demonstrate the importance of implementing national programs.[15]

Nowadays, aproximately 10 million people have abnormal hemoglobins in Brazil, and around three thousand people are annually born with the homozygous form.[16]. Therefore, a universal coverage is important because the distribution of sickle cell diseases is also uneven across Brazilian territory. Brazil, with its gigantic territorial area and its geographic, cultural and economic differences, represents a special challenge for any national public health policy, especially neonatal screening. The first neonatal screening program for hemoglobinopathies was developed in the 1990s, in the State of São Paulo.[17] Independent actions, without any coordination or standardization, were incorporating screening for hemoglobinopathies as a mandatory test in all live births in a particular region of the country. In 1998, the State of Minas Gerais was the first to introduce a Universal Neonatal Screening Program for SCD, inside the already existing Program for phenylketonuria and congenital hypothyroidism.[11]

The Brazilian National Neonatal Screening Program (NNSP) was implemented as an official program of the federal government in 2001.The Program establishes the mandatory diagnosis of abnormalities in the newborn metabolism, including screening for hemoglobinopathies.[16,18] The NNSP has the role of detecting, confirming, monitoring and treating suspected cases of six

important diseases: phenylketonuria, congenital hypothyroidism, SCD and other hemoglobinopathies, cystic fibrosis, biotinidase deficiency and congenital adrenal hyperplasia. The NNSP was the first federal action to create a universal public health program for the early diagnosis and treatment of selected congenital diseases. Within the scope of the Unified Health System (SUS), the NNSP must be articulated between the Ministry of Health, State and Municipal Health Departments. Its implementation took place gradually in each state, mainly due to regional differences and to the installed capacity to incorporate, from the detection of the disease, confirmatory exams, evaluation by a multidisciplinary team and treatment in high complexity specialized centers.[19] As of 2013, all 26 Brazilian states have been qualified to screen and treat hemoglobinopathies; up to 2018, the country had 30 Reference Services distributed along its states, according to report from the Ministry of Health, and 24,300 collection units distributed in all regions.

The Figure 1 presents studies with the different prevalence of SCD and sickle cell traits in newborns from different regions of Brazil.

In a historic series of 2014–2016, an average of 2.425.392 children were screened per year nationwide, with the coverage being of 82,16% newborns.[31] Although the Brazilian Program aims at universal, integral and equitable access, there is a variation in coverage rates found throughout the national territory and is associated in differents health care network capacity, to variations in the population's access to it and also the existence of a private access health network that does not report its data, leaving a gap in the population data of the Ministry of Health.

The collection takes place at the primary care collection points, free of charge through the NNSP, organized in each state. In some states, this collection is carried out at maternity wards, depending on the guidelines of local managers. The guidance for the ideal collection period is from the 3rd to the 5th day, according to the greater sensitivity of the available technologies inherent to diseases within the scope of the national program. The screening of 54,9% of newborns is collected up to their 5$^{th}$ day of life. Collections in non-ideal periods can be considered as neglect, cultural issues or difficulties in access.[31]

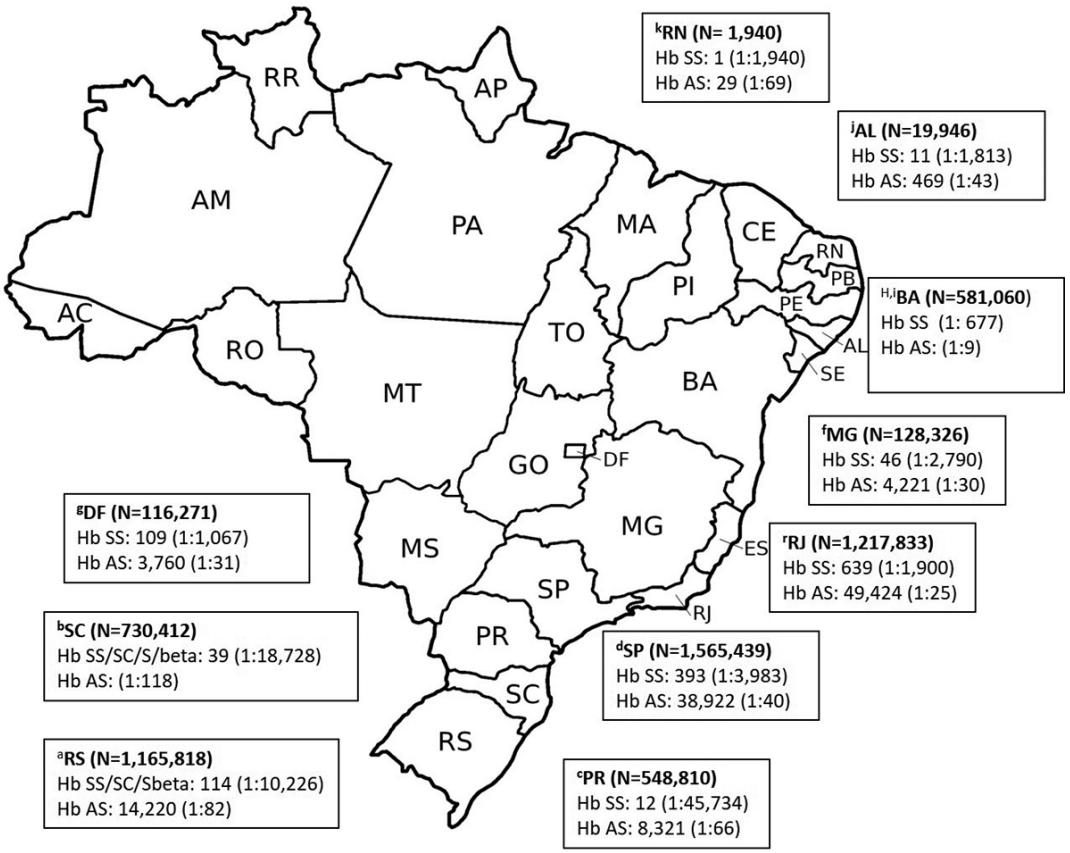


**Figure 1.** Presents studies with the different prevalence of SCD and sickle cell traits in newborns from different regions of Brazil.

[a]RS (Rio Grande do Sul – Cardoso *et al*., 2017 [20]); [b]SC (Santa Catarina – Eller *et al*., 2016 [21]); [c]PA (Paraná – Watanabe *et al*., 2008 [22]); [d]SP (São Paulo – Hadachi *et al*., 2009 [23]); [e]RJ (Rio de Janeiro - Lobo *et al*., 2014 [24]); [f]DF (Distrito Federal – Diniz *et al*., 2009 [25]); [g]MG (Minas Gerais – Paixão *et al*., 2001 [15, 26]); [h,i]BA (Bahia – Adorno *et al*., 2005 [27]; Amorim *et al*., 2010 [28]); [j]AL (Alagoas – Lipinski-Figueiredo *et al*., 2009 [29]); [k]RN (Rio Grande do Norte – de Araujo *et al*., 2004 [30])

The birth prevalence of SCD was 1: 2,263 newborns (0.44 per 1,000 live births); therefore, it was the second most-common disease detected by the NNSP program within the Federative Republic of Brazil, being only after hypothyroidism (1/2,175 live births).[32]

The median indicator of the newborn's age on the date of the first consultation marks the time that all stages of the screening were performed, culminating in the first consultation of the newborn's specialty diagnosed with any of the diseases in the program. According to the ministry of health, the median age of the first consultation for SCD was 47 days (18 – 172 days).[31] After diagnostic confirmation, the child initiated penicillin in the first month of life, immunobiological approaches, and then referral to the regional Specialized Care Service. In most states, the reference center is the Blood Center, which is normaly integrated with the local health network to meet other health needs, such as the use of hydroxyurea, transcraneal Doppler and other procedures.

## *Methods Used in NBS for Hemoglobinopathies*

Around the world more than 1,500 molecular defects have been reported in different globin genes. (http://globin.cse.psu.edu/globin/hbvar). The variability of the hemoglobin profiles found in Brazilian studies reflects the heterogeneity of the country's population.[9]

The correct diagnosis of rare variant hemoglobins prevents the application of wrong procedures and therapies, also providing materials for studying structural, functional and anthropological aspects. For the identification of hemoglobin fractions with pathophysiological potential in the neonatal stage, it is also important to highlight another characteristic of human hemoglobins: their gene regulation, which is specific to the development period and in which we find typical proteins of the embryonic, fetal and adult periods.[33] Thus, in neonatal screening tests aimed at the early laboratory diagnosis of hemoglobinopathies, we must consider some elements: the stage in which samples are collected (at birth or during the first week after), because the percentage values of adult hemoglobins are still not stabilized, therefore expressing the profile of the fetal period; the amount of variant hemoglobin, which has low percentages in most cases; and the method for collecting samples: on filter paper, the sample oxidizes more easily due to the contact with air; besides, fractions of more fragile or unstable hemoglobins can be lost, degraded. Thus, diagnostic methods for this purpose go beyond what is expected for protein separation, once they need to be accurate for the correct identification of fractions in less quantities.[34]

Based on the biochemical principle of protein separation, with the goal of identifying fractions with a low percentage and often under oxidation conditions, methodologies have been developed and improved, both nation and worldwide. However, the comparison between them is always present, drawing attention to the characteristics of hemoglobins, the type of collection and the age of the person.[35] High-performance chromatography (HPLC), capillary electrophoresis (CE), isoelectric focusing (IEF) and Mass spectrometry (MS/MS) are appropriate methods for NBS for SCD as the first methods.

In Brazil, initially, and still in some centers, IEF is used for screening. This method is perfectly accurate because it separates hemoglobin fractions by their isoelectric point, a characteristic of each protein. However, the manual demand, the delay in obtaining results and the number of samples for each collectionled to the implementation of innovations, leaving the system more active and automated. Nonetheless, a few hours were still necessary for its processing and for the diagnostic completion. With the HPLC on automated equipment, the use of specific diagnostic kits to the types of collection and the period of sample collection, it was possible to streamline the entire process. Moreover, the automation in the perforation and identification of samples allowed the reduction of errors in their exchange and an interfacing with the whole laboratory, speeding even more the finalization of tests and delivery of results. The systems were improved as the needs for screening were presented worldwide and, nowadays, specific systems for the neonatal diagnosis of hemoglobinopathies are guaranteed to be used in laboratories around the world.[36]

Due to the often need of confirming the profile found in a methodology by another method, CE was created to mainly assist in the identification of the associated hemoglobins variant forms. Since it is automated, fast and accurate, it has been widely used in laboratories for both the screening and confirmation of the phenotypes obtained.[37]

Mass spectrometry employs a specific methodology and requires specific training for its execution and interpretation, but it allows a safe correlation of the phenotype/genotype. This methodology is not yet a reality in most services worldwide, even in Brazil. CE and MS / MS are not routinely used in Brazilian laboratories.

However, analyses of the mutations were necessary to carry out genetic counseling for family cases and diagnostic clarification for interactions of thalassemia forms and hemoglobin variants. The confirmation of genotypic profiles was based on the principle of gene amplification by PCR and mutations search by RFLP, specific allele, and sometimes sequencing of genes to identify less frequent mutations.[38] Knowing the population profile assists in the search for these mutants, and in the cases of miscegenated populations, such as the Brazilian, the molecular profile has been frequently requested. Thus, it is important to have specialized laboratories to support the elucidation of clinically relevant rarer hemoglobin profiles.[39] The high cost of molecular studies does not allow all services to have access to this technology in the day-to-day care of patients with SCD in Brazil.

One of the major challenges of implementing population studies is the cost for the implementation of national programs. Although not a reality in Brazil yet, recent studies using rapid point-of-care (POC) have demonstrated excellent specificity and sensitivity to detect SCD during neonatal screening, shortening health care access for children positive for SCD. The cost for national SCD NBS programs may decrease significantly if efficient,

accurate and inexpensive point-of-care (POC) devices become available; a number of these POC testing devices have recently been developed.[40]

### *The challenges of neonatal screening for hemoglobinopathies in different services and evaluation of the Brazilian National Program*

The target disease of a NBS programme for haemoglobinopathies in Brazil is SCD, including all genotypes as in other consensus worldwide.[4] And one of the main goals of the NNSP is to ensure that 100% of the country's live births have access to the tests.[41]

In Brazil, the coverage is highly variable.The deficit in the collection coverage is associated to the lack of priority by municipal managers, to the lack of information about the diseases screened in the program, to the exams being carried out in a supplementary network, to the cultural traits not considered relevant for neonatal screening, to the lack of structure and supplies, and to the difficulty in accessing health centers and transporting samples.

According to the great genetic, cultural and economic diversity in the different regions of Brazil, we can cite the profile of the NNSP in two geographically distinct states as an example:

### *Screening for Hemoglobinopathies in the State of Bahia*

In Brazil, SCD is considered a public health problem and the state with the highest occurrence is Bahia, with 1:650 live births and incidence of 1:17 live births HbS mutation.[27]

The State of Bahia, located in the northeast region of Brazil, has a population of 14,930 million inhabitants, a birth rate of 18.8 live births per 1,000 inhabitants and a Human Development Index (HDI) of 0.660. According to IBGE data (PNAD continues), 81.1% of the population claims to haveblack or brown skin.[42] This data reflects the Afro-descendants miscegenation of the Bahia population.

Neonatal screening for sickle cell disease and other hemoglobinopathies began in July 2000, by means of a contract signed with the State Health Department. In 2001, the APAE-Salvador Neonatal Screening Reference Service was accredited by the Ministry of Health, which was followingthe requirements of Ministerial Decree No. 822/GM, dated June 6, 2001.[19] At that moment, the screening was expanded both in coverage and in health care of newborns. Since then, we have confirmed the high incidence of SCD in the population of the State, which has the largest Afro-descendant population in the country.

After these 20 years of neonatal screening, more than 3 million and 300 thousand newborns were screened in the State of Bahia, including the screening for sickle cell disease, with an average coverage varying from 85% to 89% in the recent years.

Thus, in the NNSP more than 5,000 newborns have already been diagnosed with some form of SCD, which requires an organization of the service network to meet the demands of these families, including basic and specialized care.

### *Screening for Hemoglobinopathies in the State of Rio Grande do Sul*

The State of Rio Grande do Sul (RS), located in the Souther most region of Brazil, has a population of 11,400 million inhabitants and a birth rate of 11.6 live births per 1,000 inhabitants, with an HDI of 0.746. Regarding race, 80% of the population declares to have white skin, which is explained by the great European immigration to the region.

The annual average coverage is of 75.88%.[20] When comparing it to the data from other Southern states, the coverage percentage in RS is below Santa Catarina's (90%) [43] and Paraná's (100%) [22] and it is also below the national data. By assing the tests carried out in private network laboratories of the state, one can believe that there is a coverage of 99% of newborns. However, there is no concrete data regarding the number and results of the private network, making it difficult to know the real scope of the NNSP in the State of RS.

In accordance with the diversity amongst the different Southern states of the country, RS presents results for sickle cell disease (1:10,226 or 0.0098%) higher than the ones from the States of Santa Catarina [21] and Paraná [22], whose population have a similar ethnic composition, differing from other regions of the country where there was a greater African immigration, such as the Southeast and the Northeast.

Although coverage is an essential parameter, a neonatal screening program should not be evaluated without analyzing the consultations of children with abnormal results. While developing neonatal screening, the healthcare system should provide the necessary infrastructure to confirm the laboratory diagnosis of newborns and to provide appropriate counseling and treatment. Without this, the benefits achieved by the early identification of diseases can be lost.[44] Despite the advances made in the diagnosis and treatment of SCD, the survival rates have not yet managed to surpass the fifth decade of life, with an overall life expectancy estimated at around 30 years. It is known that socio-environmental conditions, personal care, antibiotic prophylaxis, vaccination, access to health services, hydration and adequate nutrition have a strong influence on the clinical evolution of the individual with SCD.[45] This occurs especially in regions where it is more difficult to have an early diagnosis, access to prophylaxis using penicillin and pneumococcal immunization, as well as treatment with Hydroxyurea.[46] Treatment and management of the disease remain costly, making full access to care available only for the most privileged; otherwise, access is very limited because of increasing pressures on public health services.[47]

In Brazil, one of the challenges of treating and monitoring patients can be justified by the lack of standardization in the issue of results. There are no national standardized procedures to guide the active search process. There are several problems with identifying cards for locating patients, with inadequate records of names and addresses, and with changes of address hindering active search processes. An alternative, as it exists in

some countries in Europe (Belgium, Cyprus, Germany, Greece, Spain and UK) would be a national registriers for SCD could enhance monitoring of changing demographics, service delivery, and patients outcomes, and improve patient access to care with standardized data collection and coordinated follow-up. [4] In 2006, the Ministry of Health established a Technical Advisory Council for Sickle Cell Disease to help states and municipalities organize their programs of care for people with SCD in Brazil. The implementation of the SCD network in all the states is intended to guarantee decentralized care beginning with diagnosis, with the assistance of a multi-professional and multidisciplinary team, providing health education with a focus on self–management and access to specialized and high complexity care in all states of Brazil. Nonetheless, there are still few published studies on follow up and mortality that characterize the different aspects of this hemoglobinopathy in the Brazilian population and the results differ from region to region according to the different levels of organization of the assistance networks existing in the states.

Financial support, specific research, training programs, early diagnosis and intervention programs has made it possible to know the survival rates and the main causes of death amongst people with SCD. In addition, it allows preventive prophylaxis for patients with sickle cell anemia, such as vaccination and the administration of antibiotics (penicillin) up to the 5$^{th}$ year of age, to prevent from infections, which is its major mortality cause. The results of Minas Gerais showed that neonatal screening for SCD was not sufficient to significantly reduce child mortality. Economic and social development and increase of the knowledge about SCD among healthcare professionals and family are needed to overcome the high mortality rate. Several actions of care for patients with SCD need to be reviewed.[48] The studies conducted in the states of Rio de Janeiro and Mato Grosso do Sul reported that hydroxycarbamide therapy decreases mortality among patients with SCD. [49–50]

There is a lack of planning, monitoring of actions and federal funding for specific neonatal screening actions, which would qualify the comprehensive care processes for diagnosed patients, and a lack of relationship and integration to the Health Care Network, for the assembeling of programs for care regionalization. The great movement of populations, and their settlement in countries different from their homeland, is resulting in the emergence of diseases in geographic areas where they historically did not exist.

In the recent years, major advances have been made as to the diagnosis and treatment of these diseases, and more will come. However, in poor countries, such as the ones from Africa and Asia, the number of patients tends to increase. Brazil is also included on the list, because the resources for its public health care system not only are not increasing, but they also seem to be reducing. The country is at risk of going backwards in all the progressit has made until today, from the diagnosis to the treatment stage.

## Conclusion

The variability of hemoglobin profiles identified in the different regions of Brazil reflects the heterogeneity of its population. Complications of SCD can be delayed and mitigated by early detection, reinforcing the importance of neonatal screening programs. Consequently, neonatal screening for SCD is being introduced progressively in different regions.

Overthese 20 years of a Brazilian neonatal screening program for hemoglobinopathies, the correct diagnosis of hemoglobin variants prevented the application of inappropriate therapies and procedures. Together with notification, these are essential for the planning of public health policies.

SCD population programs will only have social and ethical impacts if they incorporate their actions: to the early diagnosis, which optimizes the effectiveness of preventive and prophylactic actions in reducing morbidity and mortality, through the monitoring carried out by a multidisciplinary team; to the social care that each person with the disease requires; to the access to genetic information/guidance for family members; and to the access to essential medications. Prophylactic care is the essence of the treatment up to the fifth year of life, period with the highest occurrence of deaths and serious complications.

The early diagnosis and treatment, as well as the follow-up with a multidisciplinary team, are fundamental influences to the survival rate and the quality of life of patients with SCD.

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