Critical analysis of the neonatal screening program for hemoglobinopathies

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There are about 270 million people worldwide who are carriers of abnormal hemoglobins with clinical outcomes ranging from asymptomatic to death. In Brazil, due to the composition of the population, with influence of Caucasians, Blacks and Asians, there is a considerable number of individuals with genes related to sickle cell disease and thalassemia.[1]

The first neonatal screening program was developed for hemoglobinopathies in Brazil in the 1990s.[2] In 2001, the Ministry of Health established the National Program for Neonatal Screening (NPNS) within the Brazilian National Health System (SUS). This program is implemented in three phases and aims to nationally screen for four diseases; each state is classified based on its coverage and infrastructure: Phase I – congenital hypothyroidism and phenylketonuria, phase II – congenital hypothyroidism, phenylketonuria and hemoglobinopathies; Phase III – congenital hypothyroidism, phenylketonuria, hemoglobinopathies and cystic fibrosis.[3]

In the city of São Carlos, State of São Paulo, neonatal screening for hemoglobinopathies started in 1999; thus when the NPNS was implanted, the city was already in phase II. São Carlos currently has a population of 221,936 inhabitants[4] and is in phase III of the NPNS. This study is a critical analysis of this program in the city of São Carlos. Its aims were: (1) to evaluate the effectiveness of neonatal screening and (2) to describe the incidence of abnormal hemoglobins in the population.

This is a descriptive study of the population of newborn babies in São Carlos submitted to neonatal screening tests between 2007 and 2010 in the entire government healthcare network. The neonatal screening tests performed in the private healthcare system were not included as they were not included in the screening results database of the Municipal Center for Epidemiological Surveillance. The initial phase of this study consisted of surveying the number of live newborns in São Carlos between January 1, 2007 and December 31, 2010. For this, data was used from the Live Birth Information System (Sistema de Informação sobre Nascidos Vivos, SINASC)[5] of the Health Ministry. Data on live newborns whose mothers resided in São Carlos at that time were included in this study as was information from the Epidemiological Surveillance Center itself; the data for 2009 and 2010 have not been published by SINASC yet.

The data were input on Excel 7.0 spreadsheets (Microsoft Corp., United States). The following formula was used to calculate NPNS coverage: coverage = number of screened cases x 100 / total number of live newborns. The chi-square test was used to compare differences in the NPNS coverage rates over the years with the aid of the Statistical Package for Social Sciences (SPSS) version 17.0. Significance was set for an alpha error of 5% (p-value < 0.05).

Blood for screening was collected on filter paper by the heel prick technique in primary healthcare services. The samples were sent to a clinical pathology laboratory where isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) were used to classify hemoglobin. In IEF, hemoglobins are identified by the migration of negatively charged proteins on agarose gel, which become stable when the hemoglobin pH equals the pH of the gel. In HPLC, hemoglobins are separated by chromatography in an ion exchange column and the results are displayed in chromatographs with retention time on the x-axis and percentages on the y-axis. The results were sent to government healthcare clinics to be communicated to the parents of the newborn babies. This study was approved by the Human Research Ethics Committee of UFSCar. (# 300/2010).

A total of 11,318 children were born during the study period and 10,589 newborn screening tests were performed by government healthcare clinics giving an average coverage of 93.56%. Table 1 shows the NPNS coverage rate over the four years of the study. There was a statistically significant drop in the results in 2009 and 2010 compared to 2007 and 2008.

Table 1 - Coverage of neonatal screening performed by the National Program for Neonatal Screening in São Carlos

Year	Births	Screened population	Coverage (%)	p-value
2007	2880	2736	95.0	
2008	2835	2668	94.11	
2009	2812	2597	92.35	
2010	2791	2588	92.73	< 0.0001
2007-2010	11,318	10,589	93.56	

Chi-square = 21.331; Degree of freedom = 3

Abnormal results were found in 360 (3.40%) tests and 302 (2.85%) children were identified as having hemoglobinopathies (Table 2). Among the results considered abnormal, 3 cases (0.03%) had only hemoglobin F suggesting a diagnosis of beta-thalassemia major, in 7 cases (0.07%) the level of hemoglobin A was very low suggesting beta-thalassemia minor or intermediate

Table 2 - Abnormal results and abnormal hemoglobins detected by the National Program for Neonatal Screening

	2007	2008	2009	2010	2007-2010
Screened population	2736	2668	2597	2588	10,589
Sickle cell trait	50 (1.62%; 1:62)	53 (1.99%; 1:50)	42 (1.62%; 1:62)	51 (1.97%; 1:51)	196 (1.85%)
Sickle cell anemia	0	0	1 (0.04%. 1:2,597)	0	1 (0.009%)
Hemoglobin C trait	12 (0.44%; 1:228)	10 (0.07%; 1:1,368)	13 (0.50%; 1:200)	13 (0.50%; 1:199)	48 (0.45%)
Alpha-thalassemia (hemoglobin Bart's)	11 (0.40%; 1:249)	11 (0.41%; 1:242)	6 (0.23%;1:433)	11 (0.42%; 1:235)	39 (0.37%)
Sickle cell trait + alpha-thalassemia	0	1 (0.04%. 1:2,668)	0	0	1 (0.009%)
Hemoglobin C trait + alpha-thalassemia	0	2 (0.07%; 1:1,334)	0	0	2 (0.019%)
Indeterminate hemoglobins variants	2 (0.07%; 1:1,368)	6 (0.22%; 1:445)	1 (0.04%; 1:2,597)	6 (0.23%; 1:431)	15 (0.14%)
Suspected beta-thalassemia major (only hemoglobin F)	0	0	2 (0.08%; 1:1,298)	1 (0.04%; 1:2,588)	3 (0.03%)
Suspected beta-thalassemia minor or intermediate (very low hemoglobin)	0	0	2 (0.08%; 1:1,298)	5 (0.19%; 1:518)	7 (0.07%)
Inconclusive results	20 (0.73%; 1:137)	13 (0.49%; 1:205)	5 (0.19%; 1:519)	10 (0.39%; 1:259)	48 (0.45%)

and in 48 cases (0.45%) the tests were inconclusive and should have been repeated; however new samples were not sent to the laboratory. Among children with anomalous hemoglobin, 15 (4.97%) showed indeterminate hemoglobin, i.e., variant hemoglobins different to the most commonly identified hemoglobins (Hb F, Hb A, Hb S, Hb C, Hb Bart), which were not characterized adequately by the tests and required further investigations using other laboratory methods.

Neonatal screening is currently the best-known preventive pediatric measure in genetics and is used worldwide. In Brazil, it is the largest government program in the field of medical genetics.[6] The most recent NPNS data published by the Health Ministry show a significant heterogeneity in the coverage of Brazilian states which reflects the different economic, social, political, cultural and health conditions. The average NPNS coverage in São Carlos in the period was higher than the national average at 78.92% in 2007. The coverage of some Brazilian states and the phase are listed in Table 3. It should be noted that the state of São Paulo, where São Carlos is situated, only entered phase III of the NPNS program in 2010.

Table 3 - State, phase of National Program for Neonatal Screening and coverage in 2007

State	Phase	Coverage[7]
Amapá	I	52.41%
Piauí	I	61.62%
Ceará	I	76.21%
Tocantins	I	77.67%
Rio de Janeiro	II	79.05%
São Paulo	II	82.24%
Mato Grosso do Sul	II	84.07%
Minas Gerais	III	88.57%
Santa Catarina	III	88.73%
Paraná	III	100%

A study carried out between 1992 and 2004, involving 30 cities in São Paulo state, showed that 17 cities had above 90% coverage, 8 cities had coverage between 80% and 90%, 2 cities had coverage between 70% and 80% and 3 had coverage below 70%.[8] Therefore, São Carlos has higher coverage rates for neonatal screening compared to the national average but is in line with most of the cities surveyed in São Paulo state. The city of São Carlos is located at the geographic center of the state. It is an important regional center, with an economy based on industry and agriculture and has trade and provides services (including health services) to the populations of the surrounding small towns. Two major public universities and two research centers of the Brazilian Agricultural Research Corporation are located in São Carlos, which reinforces its character of a regional center for scientific and technological development. The city has a high Human Development Index (HDI) that was 0.841 in 2000, ranking it 17th in São Paulo State and 67th in Brazil.[9] Therefore, a high coverage rate for neonatal screening should be expected for São Carlos. The drop in coverage in 2009 and 2010 may reflect restructuring of the primary healthcare services in the city at that time, which were gradually reduced so that the Basic Health Clinic model could be implanted in accordance with the Family Health Program and with family healthcare clinics. As a consequence of this change, there was relocation of healthcare professionals and reassignment of functions, which may have affected the coverage results. In order to increase local coverage, it is important to train healthcare professionals. Testing should be offered whenever the newborn or its mother visits a government healthcare clinic for any other reason. Moreover, it is important that clinics are built in better locations and more visits are made to the newborn's family to facilitate sample collection in cases that need to be tested a second time to clarify inconclusive results.

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 laboratory diagnosis of the newborns and provide appropriate counseling and treatment. Without this, the benefits achieved by early identification of diseases are lost.[10] Some Brazilian physicians believe that genetic counseling of patients with hemoglobinopathies and their parents is a secondary procedure, optional or the sole responsibility of the geneticist. However, given the hereditary nature of hemoglobinopathies, genetic counseling is an essential part of newborn screening and its omission is a serious flaw and an obstacle to improving the NPNS.[11]

In São Carlos, the Epidemiological Surveillance Service refers thalassemia patients and those who are homozygous for sickle cell and hemoglobinopathy C for specialized treatment and genetic counseling in the Municipal Hematology Service. Genetic counseling of heterozygous individuals (carriers of the sickle cell trait or hemoglobin C trait) is performed by trained nurses who work in primary healthcare services. When a child with the sickle cell trait or hemoglobin C trait is identified, the mother is offered testing (electrophoresis) and, if she has any abnormality, the test is also performed on the patient's siblings. If the mother has no abnormalities according to the results of electrophoresis, it is assumed that the heterozygous state is inherited from the father; however, because of ethical issues related to the possible identification of false paternity, no diagnostic test is performed on the alleged father of the child.

Hemoglobins S and C originated in Africa and spread throughout the Americas due to the slave trade. Thus, in Brazilian regions where the population of African descendants is numerically smaller, such as in the south, the prevalences of hemoglobin S and C are also lower.[10] In the state of São Paulo, the estimated incidences of sickle cell trait, sickle cell anemia and of the hemoglobin C trait are 2.49%, 0.045% and 0.78%, respectively.[12] In São Carlos, the incidences of sickle cell trait (1.85%), sickle cell anemia (0.009%) and hemoglobin C trait (0.45%) were lower which must reflect the ethnic background of the population. According to the 2010 Census, 78.74% of the São Carlos population declared itself Caucasian[4] with a strong influence of German and Italian immigration,[13] populations for which the prevalence of these hemoglobins is not as high as in those of African descent.

In South and Southeast Brazil, where European colonization was more prominent, a higher incidence of thalassemia[10] was expected. In a study of 1,565,439 children from 295 cities in São Paulo state, Hb Bart was detected in 0.59%, thus diagnosing alpha-thalassemia and in 0.0009% of the cases the screening identified only hemoglobin F, suggesting the diagnosis of beta-thalassemia major.[14] In São Carlos, the incidence of alpha-thalassemia diagnosed by newborn screening was 0.37%, but this number may be underestimated because low levels of Hb Bart, in general less than 1%, are difficult to detect with the laboratory techniques used.[15] Beta-thalassemia major was suspected in 0.03% of the cases when only hemoglobin F was present, but we should take into account that premature infants may only have hemoglobin F in the neonatal screening test and, hence, there is a need of reassessment. Beta-thalassemia minor and intermediate forms are not identified in newborn screening as they are usually diagnosed at around six months of age. Notwithstanding, infants who have low hemoglobin in neonatal screening should be reassessed to exclude this diagnosis. Therefore, it is important to introduce new laboratory methodologies in the NPNS to enable the diagnosis of thalassemia, in particular in the Brazilian southern and southeastern regions. In situations where indeterminate hemoglobin variants are also identified there is a need to further characterize the samples using other laboratory techniques, in particular molecular biology methods.

The neonatal screening program for hemoglobinopathies in São Carlos showed a reasonable coverage rate (93.56%) albeit still lower than the goal of 100%. The results of this study reinforce the need for a consolidation of universal coverage by the government healthcare system in order to achieve a more efficient network of comprehensive care for patients with hemoglobinopathies.

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