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Neonatal cystic fibrosis screening program in the state of Paraná: evaluation 30 months after implementation

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Abstract

Objectives: To present and analyze the results of the National Neonatal Cystic Fibrosis Screening Program in Paraná, 30 months after its implementation.

Methods: This is a descriptive study, with an analysis of the data from the screening of around 98% of all neonates in the period from September 2001 to April 2004, undertaken at the Neonatal Screening Program laboratory of the *Fundação Ecumênica de Proteção ao Excepcional do Paraná*. Blood samples for the Guthrie test were collected on hospital discharge, ideally between the second and sixth days postpartum, and filter papers were sent for immunoreactive trypsin assay by the immunofluorometric method. Children whose immunoreactive trypsin assay results were ≥ 70 ng/ml for two distinct samples during the first 30 days of life, were referred for sweat conductivity testing by the Wescor method. In cases when the result was greater than 50 mMol/l quantitative chlorine and/or sodium in sweat was assayed (iontophoresis with pilocarpine).

Results: From a total of 456,982 tests, 4,028 (0.9%) children presented a first immunoreactive trypsin assay above the cutoff point set. Four hundred and seventy-eight of these (12.5%) also had a second blood sample assayed with immunoreactive trypsin above 70 ng/ml and 56 (11.7%) of these were referred to specialized clinics after their sweat conductivity test results were above 50 mMol/l and 48 (0.01% of the total number of children screened) had a diagnosis of cystic fibrosis confirmed. The incidence for the state of Paraná was 1:9,520, although some children have not yet been fully investigated.

Conclusions: Neonatal screening for cystic fibrosis in the State of Paraná, in accordance with Health Ministry directives, was a pioneering initiative for Brazil. Many patients were diagnosed early, even asymptomatic ones, which is a challenge to improving prognosis with this fatal disease.

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Introduction

Cystic fibrosis (CF), or mucoviscidosis, is an autosomal recessive disease. More than 1,000 mutations have been described that can be responsible for the disease, with incidence rates that vary from 1:2,000 to 1:10,000 among populations of Caucasian origin, among whom the disease is manifest with greater frequency. In Brazil, it is estimated that the incidence of the disease is 1:10,000 live births, although the frequencies of mutations vary from geographical region to region, which is possibly also reflected in varying disease prevalence rates.[1] In the USA

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and countries in Europe, diagnosis is performed early, before the first year of life is complete, which allows these children to be treated and monitored with respect of variables that have a direct influence on disease prognosis, such as, for example, monitoring of stature and weight curves and the presence of upper airway colonization by pathogens which have an intimate relationship with worse disease prognosis.[2]

From the year 2001 onwards, with the approval of the National Neonatal Cystic Fibrosis Screening Program, the implementation of the program by the laboratory at the *Fundação Ecumênica de Proteção ao Excepcional do Paraná* (FEPE-PR) saw Cystic Fibrosis screening become a reality in Paraná. Figures from before the National Neonatal Cystic Fibrosis Screening Program was instituted tell a sad story, with the mean age at diagnosis of the disease being around 1.6 years.[3] These children, at the point of diagnosis, already exhibited significant malnutrition and early colonization by the germs habitual to CF, not to mention all the morbidity involved before diagnosis. The mean number of hospital admissions per year was four per child wit durations of approximately 10 to 60 days.[3]

When implanting neonatal screening for a given disease, the cost-benefit relationship for the population should principally be taken into account, although this is not a necessary condition according to United Nations (UN) criteria.

The primary objectives of the study were to publish the results of the National Neonatal Cystic Fibrosis Screening Program in Paraná and to perform an analysis of the results obtained 30 months after it started.

Methods and patients

This was a descriptive study, analyzing data provided by the FEPE/PR and from the medical records of patients in outpatients treatment at the *Hospital de Clínicas* during the period September 2001 to April 2004. The protocol was approved by the Committee for Ethics in Research on Human Beings at the *Hospital de Clínicas* of the *Universidade Federal do Paraná*.

The study population includes all children screened (around 98% of live births) during this period in the state of Paraná.

Blood samples were collected according to an already-established protocol for neonatal screening for diseases such as phenylcetonuria, hypothyroidism congenital and hemoglobinopathies, preferably performed after the first 48 hours of life, by heel-prick with a sterile lancet and collection of the drop of blood on filter paper. After drying, samples were sent by post to the FEPE based in the city of Curitiba.

At the FEPE, these filter papers were punched with a standard instrument to provide samples with 3 mm diameter and, from these samples, quantitative readings of immunoreactive trypsin (IRT) in whole blood total were taken in duplicate by an automated system by means of the immunofluorometric method (autoDELFIA^{TM} kit). The cutoff point set for the first IRT assay was 70 ng/ml. Children were recalled for a second blood test if their first IRT assay, for both samples, were above the established cutoff point. Immunoreactive trypsin assays were performed during the first month of life.

If the second IRT assay returned a value greater than or equal to 70 ng/ml then sweat conductivity testing was performed.

The cutoff point for the sweat conductivity tests was set at 50 mMol/l.[4] The sweat test (conductivity) was performed passing an iontophoretic current along the child's forearm, sweat was collected with the Macroduct Sweat Collection System® and readings taken with Wescor's Sweat Check Conductivity Analyser.

All children who presented conductivity values above the cutoff point were referred for sweat testing with stimulation by iontophoresis with pilocarpine and sodium (Na^+) and/or chloride (Cl^-) assays by flame photometry, which is the gold standard for diagnosis of the disease. Children with values for electrolytes in sweat above 60 mEq/l, on two occasions, were diagnosed with CF and explanations about the disease and genetic counseling provided, in addition to treatment.

Results

From the total number of children screened in the State of Paraná during the period August 2001 to April 2004 (456,982), 4,028 presented a first IRT assay result above the cutoff point, approximately 0.9 % of the total sample.

All of these children were recalled, although just 3,815 attended for the second test. Of these recalled children, 478 (12.5%) returned a second IRTresult over the cutoff point, which equates to 87.5% of false positives at the first assay. The sweat conductivity test identified 63 children with values over 50 mMol/l. Fifty-six of these were referred to specialist centers (a further seven are still in the process of being recalled). Quantitative determination of electrolytes in sweat on two occasions confirmed a diagnosis of CF in 48 (0.01% of the total) children who are now in outpatients follow-up, a mean of 18 cases/year. These values reveal an incidence of the disease of 1:9,520 births in the State of Paraná, but it should be remembered that some children have not yet completed the investigation process, which may increase this incidence.

The mean IRTassay value for these 48 children was 172.4±79.6 ng/dl and their blood samples were collected after a mean of 5.8 days of life. The second IRTassay, performed for 40 children, returned a mean value of 170.3±82.5 ng/dl. These blood samples were collected after a mean of 22.4 days of life, achieving the objective of performing screening within the first 30 days of life. Cystic fibrosis diagnoses were confirmed after testing electrolytes in sweat.

The mean weight at birth was 3,074 g, but at the point of diagnosis 16 (33.3%) had weight and/or stature below the third percentile of that expected for their age.

There was a predominance of the white race (89%) and the ratio of males:females was 1:1. Five patients had a positive family history and 10% presented meconium ileus.

Discussion

There is no consensus on neonatal screening for CF due to doubts as to whether the benefits outweigh the costs and undesirable effects. To date, few countries, such as Australia, New Zealand, France, Austria, Poland, United States and England have national neonatal screening programs, however these programs are not all uniformly established across the entire national territory.[5] In the majority of these countries there is no specific legislation or guideline criteria on how neonatal CF screening should be undertaken.

Criteria for implementing a neonatal screening program have been established by the WHO[6] and CF fulfills these criteria. In Brazil, neonatal screening for was instituted with publication in the *Diário Oficial da União* on 07/06/2001 Ministerial Decree 822 which regulates standards for neonatal screening in Brazil. In Paraná, it is the responsibility of the FEPE to implement and apply the program. This decree states that, in addition to detecting suspected cases, diagnostic confirmation, follow-up and treatment of the patients must be performed.

Arguments in favor of neonatal CF screening are the undeniable gains in terms of early diagnosis, the repercussions of this for nutritional status and preventing malnutrition with the implications this has for the respiratory and cognitive systema.[7-13] In Paraná, the mean age at diagnosis of CF used to be 1.6 years.[3] With the advent of neonatal screening it is possible to confirm diagnosis during the second month of life. Furthermore, the mean number of cases diagnosed at the center yearly has increased from eight to 18 cases.[3] There are yet more benefits, such as early treatment at centers of excellence, prevention of complications[14] and genetic counseling, in addition to allowing incidence to be calculated, avoiding late diagnosis and contributing to greater knowledge of the natural history of the disease.[12,13]

There are arguments against neonatal screening based on the fear and anxiety generated from when the tests are started up to when false positive children are excluded, [15] and discovery of heterozygotes carrying the gene, in those programs that involve testing for CF mutations.[16]

Diagnosis can be made at several different opportunities: antenatal, when there are already cases in the family (by chorionic villous biopsy followed by genetic analysis) which could reduce the prevalence of CF after genetic counseling,[17] during the first year of life, due to early manifestations such as meconium ileus; by neonatal screening or, finally, when there are clinical manifestations of the disease.

The children identified by neonatal screening in Paraná, with confirmed CF diagnoses were well-nourished at birth, the majority of the white race and the frequency of meconium ileus was similar to observations made by other authors.[18]

Trypsinogen is an enzyme produced by the pancreas which normally reaches the intestinal lumen. In patients with CF, an obstruction of the pancreatic ducts impedes the secretion of the enzyme and there is activation to trypsin. This explains the elevated levels of the enzyme in the blood of children who have the disease,[19] which are even observed among patients without pancreatic insufficiency.[20]

Ideally, blood should be collected between the second and sixth days of life, since there is a progressive drop in levels of the enzyme as the days pass.[21] The screening system in Paraná has managed to adhere to this recommendation with high rates of coverage in performing the test and rapidity in forwarding samples.

Many different protocols are employed for neonatal cystic fibrosis screening.[22] There are countries which only perform IRTassays on two separate occasions followed by the sweat test and there are others that add to this tests for the principal mutations involved in the pathogenesis of the disease during the screening investigation. In Paraná, the option of two IRTassays on separate occasions during the first 30 days of life was chosen on the basis that the DF508 mutation is homozygous in just 26.3% of patients with CF.[23] this means that if that method had been chosen then diagnoses would have been made in just 1/4 of cases.

One problem with IRTassays is the lack of consensus on what is the normal value. The number of false positives and false negatives depends on the cutoff value chosen. The higher the cutoff point, the greater the chance of false negatives and the lower the cutoff point the greater the number of false positives. The figure of 70 ng/ml corresponds to percentile 99.8, as established by Hammond et al.,[24] and gives the test a sensitivity of around 90% and a specificity of 98%. The experience with the IRT/IRT followed by electrolytes in sweat testing protocol in Paraná is that the number of diagnoses increased and there was just a single report of one child with the disease who went undetected. This child did not present meconium ileus and values from IRTassays were 70.8 ng/ml on the first occasion and 48.2 ng/ml from the second test, performed after 23 days of life.

Certain factors such as low Apgar scores, intestinal obstruction and agenesis of the pancreatic ducts can also elevate IRTvalues, causing difficulties with interpretation of the results.[24] Repeating the test within the first 30 days of children's lives reduces the number of false positives by around 90% and also avoids the progressive reduction in values causing problems with interpretation. For Rock et al.,[21] persistently elevated IRT values should not be expected before performing the sweat test. There was a high number of false positives at the first IRTassay, reducing significantly after the second assay and further

still after the sweat test. Figures for sensitivity, specificity, positive and negative predictive values for the test could not be calculated because the number of children with CF who were not identified by screening is unknown.

Conductivity analysis is a screening test that correlates well with quantitative electrolyte assay.[4] All of the children diagnosed with CF exhibited high values for sweat conductivity.

The percentage of children diagnosed (based on the total number of children screened) after almost 3 years of the Neonatal Cystic Fibrosis Screening Program in Paraná is satisfactory, bearing in mind that the number of diagnoses per year has doubled, although it remains below the number that had previously been estimated based on the frequency of the mutation in our State.[23]

The main difficulties were with standardization of sample collections, with forwarding the material to the center of excellence and, primarily, with recalling those patients with abnormal test results (to a great extent because of difficulties in locating them and communication difficulties with some of the more distant locations), which are similar factors to those described by Hammond et al.[24]

Several different screening protocols have been proposed since the disease has different mutations regionally, with varying frequencies of each gene's occurrence. There is not yet uniformity between countries or even, in many cases, between different regions of the same country.

Studies relating the costs of neonatal screening with the diagnoses achieved are rare. It is conjectured that perhaps training pediatricians in early recognition of the disease might have similar effects and results as neonatal screening. Since, here in Brazil, the average diagnosis is made after 4 years,[25] and since this can be a catastrophe for the patient in terms of nutritional development, bacterial colonization and delayed cognitive development, the benefits of neonatal screening appear to be undeniable in our country. There is no way of comparing costs since there is no way of measuring the direct and indirect costs generated by an undiagnosed child with CF for the health service.

Early diagnosis has allowed the healthcare professionals who care for these children to accrue a better understanding of the disease and its natural history and has provided the opportunity for parents to understand the situation and the care required during the first year of life and offers them the opportunity for family planning. Furthermore, neonatal screening is a means publicizing CF, since it has now been added to the Guthrie test battery for more than 98% of children born in the State of Paraná.

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