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Newborn screening: what pediatricians should know

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Abstract

Objective: To review the literature on the current situation of neonatal screening worldwide and in Brazil. To define the role of pediatricians in neonatal screening programs.

Sources: Scientific articles selected by means of searches run on the medical websites MEDLINE, Cochrane, PubMed (MeSH) and MD Consult, using the keywords newborn screening, neonatal, pediatrics, diagnosis, primary care, ethics and their equivalents in Portuguese, in isolation and in combination, in addition to medical textbooks on genetics and inborn errors of metabolism, published between January 1998 and December 2007, the National Neonatal Screening Program technical standards and routines manual, and Ministry of Health decree 822/2001.

Summary of the findings: Published data demonstrate a great diversity in the number of diseases included in the neonatal screening programs of different countries. In Brazil, the National Neonatal Screening Program was set up in 2001, to screen for phenylketonuria, congenital hypothyroidism, sickle-cell anemia and cystic fibrosis. Screening for a wider range of conditions using mass spectrometry is currently the subject of disagreement and discussion of financial and ethical issues.

Conclusions: Neonatal screening is one of the most important advances for the prevention of pediatric diseases. Nevertheless, implementation is complex, multidisciplinary and dependent on public health policies and, to date, there is no consensus on which diseases should be included. A large number of scientific and ethical questions need to be discussed in order to better define the screening panels to be implemented. Pediatricians have important roles to play in all stages of neonatal screening programs.

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Introduction

Neonatal screening was proposed by Dr. Robert Guthrie in 1963.[1] The method proposed, and later adopted widely all over the world, was a bacterial inhibition assay performed with dry blood samples, collected on filter paper, to detect phenylalanine concentrations. The treatment for phenylketonuria based on restricting phenylalanine in the diet has already been discovered a decade earlier, but if initiated after onset of the disease's symptoms, it did not reverse the neurological damage. Guthrie's objective was to identify individuals with phenylketonuria during the presymptomatic phase in order to initiate treatment earlier.

Later, several other metabolic, endocrinal, hematological and infectious diseases were added to the screening panel. The diseases most often screened for worldwide are: phenylketonuria, congenital hypothyroidism, sickle-cell anemia and other hemoglobinopathies, cystic fibrosis, galactosemia, biotinidase deficiency, congenital adrenal hyperplasia, maple syrup urine disease, medium-chain acyl-coenzyme A dehydrogenase deficiency (MCAD) and tyrosinemia.[2]

The diseases tested for vary greatly in different countries, or even in the different regions or states of a single country, depending on health policy decisions that are taken based on epidemiological, ethnic, social, economic and ethical factors.

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Pediatricians can find information on the panels of diseases tested for in many different parts of the world on the website of the International Society for Neonatal Screening (ISNS): http://www.isns-neoscreening.org/. This site provides useful information, protocols and recommendations on neonatal screening and links to a large number of neonatal screening societies worldwide.[3]

A major change took place during the 1990s, when tandem mass spectrometry (MS/MS) was introduced for the quantitative analysis of amino acids and acylcarnitines on filter paper. Nowadays diseases of amino acid metabolism and fatty acid oxidation and the organic acidemias can also be diagnosed. It is possible to screen for more than 40 metabolic diseases using a single blood sample in approximately 2 minutes.[2,4-9]

Today, neonatal screening is the best known and most widely used genetics-related preventative pediatric public health initiative in the world.[10-12] In Brazil, it is also the largest initiative carried out by the Brazilian National Health Service (SUS - Sistema Único de Saúde) in the area of genetics. Although pediatricians and family doctors have a preeminent role to play in the success of screening, the participation of both specialists in the programs that exist worldwide remains low.[2,12-15] In this article we will review the literature and provide an update on the aspects of neonatal screening that are most important to pediatricians, with the intention to form a critical opinion and to increase their participation in the Ministry of Health's National Neonatal Screening Program (NNSP) and intervention in the private screening tests that exist in Brazil.

The concept of neonatal screening

Screening is a process of filtration, of choosing. The neonatal screening tests are not diagnostic, they separate a population of newborn infants into two groups: one made up of those who may have a given disease, the other by those who probably do not have it.[6] In order to perform neonatal screening, it is, therefore, necessary that there be an adequate test with high enough sensitivity (the capacity to correctly identify those who have the disease, i.e., with no or very few false negatives) and reasonable specificity (the capacity to correctly identify those who do not have the disease, i.e., with few false-positives).[11,16]

Criteria for neonatal screening programs

The criteria habitually used for screening programs generally follow those proposed by James Wilson and Gunnar Jungner in 1968, in a document published by the World Health Organization (WHO). These criteria are as follows: the condition to be screened must be an important health problem; the natural history of the disease must be well-known; there must be an identifiable early stage; early treatment must provide greater benefits than at later stages; an appropriate test must be developed for the early stage; the test must be acceptable to the population; intervals must be defined for repeating the test; healthcare service provision must be adequate for the extra clinical work resulting from screening; the risks, both physical and psychological, must be less than the benefits.[17]

Neonatal screening does not always manage to meet all of these criteria, since the group of diseases is highly heterogenous and controlled randomized trials are difficult to carry out, as some of them are very rare.[5,18]

The characteristics of neonatal screening

Neonatal screening is not simply carrying out tests to identify the concentrations of certain substances in the blood. It is also more than a public system to ensure that each result is linked to a given newborn child who will then undergo diagnostic testing and, if indicated, be referred for appropriate treatment. Neonatal screening is a system of five stages that is habitually organized and carried out by the public health system, which has the resources and authority necessary to carry out universal screening, and in which pediatricians play an important role.[2,13,14,17,19-21]

The first step is to carry out the screening tests themselves. The objective is to achieve universal screening, i.e., all newborn infants should be screened. During this phase obstetricians and pediatricians are of fundamental importance. Parents need to be aware that neonatal screening exists and receive explanations in advance about: the benefits of early detection of the diseases being screened and which diseases these are; the risks for newborns who do not undergo testing; the correct age for testing; the need for subsequent tests to confirm diagnosis when screening is positive; the possibility of false positives; and the follow-up process and the mechanism by which results are received.[2,21]

Pediatricians must be aware of the factors that can have an effect on results, such as incorrect age (in Brazil it is recommended that samples be taken between 3 and 7 days of life), prematurity, diet, transfusions and total parenteral nutrition.[2]

The second stage, which in Brazil is called the active search, is following up on results and locating infants and their families, primarily when results are positive, since the time at which treatment is started is crucial to preventing morbidity, mortality and sequelae. Families must be informed of abnormal results as quickly as possible so that confirmatory diagnostic tests can be carried out. The family should be supported by a pediatrician who should explain the significance of positive screening results and the possibility of false positives and arrange referral for confirmatory testing.[22,23] On receiving abnormal screening results, the first action a pediatrician should take is to confirm whether the child is well and asymptomatic. Any child who is not well should be urgently assessed and may need to be admitted to hospital for support or specific treatment.[24]

Negative results must also be provided as quickly as possible. The policy that no news is good news must not be adopted.[20] Performing screening arouses expectations in families and they have the right to know the results as quickly as possible.

The third stage is carrying out the diagnostic tests, which will vary depending on the disease and which, often, require specialized laboratories. In this stage true positive results are differentiated from false positives.[20] Pediatricians will need to be guided by specialist disease centers and, very often, will refer their patients for treatment over the long term.

The fourth stage is treatment, which is very often lifelong. In a large proportion of these diseases multidisciplinary follow-up is needed in addition to regular care by the referring pediatrician, who has more opportunities for contact with the patient and their family. Therefore, the pediatrician will need to keep informed of pathophysiologic, clinical and psychosocial features of the disease. Genetic counseling is also part of this stage, including the detection of other carriers in the family, the recurrence risk, and the possibilities for prenatal diagnosis in couple´s future pregnancies.[2,21,25]

The fifth stage is the periodical assessment of all stages and components of the system: validation of the tests employed, verification of efficiency of the active search and of interventions, confirming the benefits for patients, their families and society. This is the critical evaluation of the system which must be constant and ongoing. In this stage population coverage is investigated and the time taken for each stage is calculated, and an analysis made of obstacles to early diagnosis and treatment. The efficacy of treatment is determined and problems with execution and maintenance identified. The impact of diagnoses on families is also investigated as are the effects of screening on the population.[2,21,25]

The American College of Medical Genetics (ACMG) also recommends a sixth stage in which health professionals and the public are educated.[2,26]

These characteristics demonstrate that neonatal screening is more than merely performing laboratory tests. It is a complex system and, for it to be successful, it is indispensable that the health system participates.

Free and informed consent

With the addition of DNA testing to neonatal screening, consumers, health professionals and healthcare policy makers began to propose that a free and informed consent form be introduced, both for the screening tests and for the destination of the samples. Consensus has not yet been reached, but specialists recognize the benefits of the free and informed consent form as an instrument for educating parents.[2,27] With the capacity to extract DNA from the filter paper and carry out DNA testing has come the need for parents to know what will happen to the material collected from their children.

Benefits and risks of neonatal screening

Neonatal screening involves both benefits and risks. Among the benefits is the detection of serious, but treatable diseases before the symptoms emerge, preventing problems such as mental retardation or even death. Another benefit is the identification of carriers of certain diseases, making it possible to offer genetic counseling and achieve conscious reproduction.

The most important risks are: failure to identify some (few) affected newborn infants (false negatives), causing anxiety to parents in false positive cases, detection of false paternity, detection of diseases for which there is no effective treatment.

It should also be borne in mind that, in some diseases, there are variants that only manifest later in life and lead to false-negative diagnoses.

Screening with tandem mass spectrometry

Tandem mass spectrometry is a system where two mass spectrometers are placed in sequence and separated by a collision cell. The blood collected on filter paper is eluted and ionized by eletrospray. Ions are separated by charge in the first spectrometer, selected by a computer program, and pass into the collision cell where they are fragmented. The fragments are then analyzed and identified according to their mass by the second spectrometer.[4,8,19,24]

The entire analysis process takes around 2 minutes and, at the end, more than 40 metabolic diseases can be identified from a single sample.[8] These include aminoacidopathies, fatty acid oxidation disorders and organic acidemias. This makes possible the early diagnosis and presymptomatic treatment of many metabolic diseases. However, there are doubts about the efficacy of treatment in some diseases, about the natural history of others and about their cost-benefit ratio and the ethics involved.[17,28]

In common with other neonatal screening programs, the expanded screening using MS/MS was not implemented on the basis of controlled trials documenting its efficacy, but on the basis that without early diagnosis, the clinical course of metabolic diseases is uniformly bad. Furthermore, the technology has become available and economically feasible, facilitating its application.[7,24]

The detection of rare diseases with which pediatricians are unfamiliar, the need for an immediate follow-up, complicated logistics, fast execution of confirmatory tests, specialized treatment and the need to avoid family anxiety all demand the development of a well-organized network, linking the screening system to a service with appropriate infrastructure for the treatment of metabolic diseases.[29]

Nowadays there is a great deal of disagreement about how many and which diseases should be screened for using this method. Some authors defend the position that the greatest

possible number of diseases should be diagnosed, considering that, for pediatricians, the essence of the specialty is preventative medicine and that, even for diseases for which there is no treatment, it is important that families know the diagnosis and are given appropriate genetic counseling.[4,30,31] Other authors consider that there is not sufficient information about the efficacy of expanded screening and that there is a lack of properly conducted research into treatment for these diseases. They question the strategy of offering results about a large number of diseases for which there is little or no evidence of benefit for those affected.[32,33]

Certain unfavorable aspects that have been discussed include the level of stress triggered in the families of children given false positive results[34] and increase in long-term costs without, to date, knowing whether the benefits are worth it.

Some of the possible causes of false-positive screening results with MS/MS are prematurity, dietary supplementation with medium-chain triglycerides or carnitine,[35,29] physiological variations in analyte levels, parenteral nutrition and antibiotics that contain pivalic acid , which can masquerade as isovaleric acidemia.[19,36] Tyrosine is often elevated in preterm infants and even in full-term children, without there being a defect in the metabolism of this amino acid. Maternal vitamin B12 deficiency changes the profile of infants' acylcarnitines, posing as propionic acidemia. These changes disappear if the vitamin is replaced.[24]

False negatives can occur depending on the age at which the sample was collected, because the analytes exhibit differences in the postnatal profile.[37] Normal levels may occur during the neonatal period, for example, in homocystinuria. Diagnosis of glutaric acidemia type I is difficult because the metabolites of interest for analysis may remain normal when patients are not in acute crises.[24]

One disease that practically all countries with access to MS/MS technology consider appropriate for screening is MCAD, because of its potential lethality, high frequency in the population and the simple and safe treatment once diagnosis has been made.[17,30] Nevertheless, we now know that many of the cases of MCAD that are diagnosed are mild and that, even if left undiagnosed, they would not progress to decompensation.[18] Some diseases cannot be easily identified by MS/MS screening, including: lysosomal storage diseases (for example: mucopolysaccharidosis), the porphyries, carbohydrate metabolism diseases (for example: fructosemia), congenital lactic acidemias, peroxisomal diseases and the majority of the oxidative phosphorylation disorders. Urea cycle diseases that do not lead to increased citrullin levels are also not detected.[24]

Addition of tandem mass spectrometry tests to screening programs

The response to neonatal screening by MS/MS was different in the United States, Europe and in some other countries in other continents.

In the United States, the ACMG[17,26,38] convened a multidisciplinary group (the Newborn Screening Expert Group) which reviewed the entire neonatal screening structure in the different states and defined a panel of 29 diseases for neonatal screening. They listed nine organic acid metabolism diseases, five fatty acid metabolism disorders, and six amino acid metabolism diseases, screened for with MS/MS, in addition to sickle-cell anemia and two other hematological diseases, congenital hypothyroidism, galactosemia, congenital adrenal hyperplasia, cystic fibrosis, biotinidase deficiency and deafness, assessed by other methods.[39]

After analyzing neonatal screening in the United States, this group made some very important statements, including the following principles:

1 - Screening should be universal and should be a public responsibility.

2 - It should be primarily focused on affected infants with a secondary focus on all other newborn infants, their families, health professionals and the general public.

3 - Neonatal screening is not restricted to tests. It is a coordinated and inclusive system that consists of education, screening, follow-up, diagnosis, treatment, and management and periodic program assessments.

4 - Physicians and other public and private components of the program should be in close communication to guarantee confirmatory tests and appropriate follow-up and care of the newborns identified.

5 - The diseases recommended should be based on scientific evidence and expert opinion.

6 - To be included in the screening program a disease must fulfill the following minimum.conditions: it must be identifiable during a phase in which it would not ordinarily be clinically detected, there must be a test with appropriate sensitivity and specificity, and there must be benefits from early detection, timely intervention and effective treatment.

7 - Health data must be centralized to allow longitudinal monitoring of the specific diseases of the program.

8 - The program must have policies in place to ensure confidential storage and appropriate use of specimens.

9 - Public awareness, professional training and family education are responsibility of the program.[26]

In Europe, MS/MS implementation has been slower. In January of 2007, seven countries had expanded their screening, the majority of which did so after 2004. In some of these countries, MS/MS screening does not cover the entire country. The number of diseases screened for with MS/MS was much lower and varied from two (phenylketonuria and MCAD), in Great Britain and Switzerland to 20 in Austria.[40] These differences with relation to the United States are the result of different risk-benefit assessments.

Two European countries, Germany and Great Britain, merit special consideration. In Germany, expanded screening began in 1999 with an unrestricted approach. In 2002, the health authorities decided to limit the number of metabolic diseases detected by MS/MS to 10 and ruled that positive findings of other diseases, discovered accidentally, would be ignored and not passed on.[5] Furthermore, all samples are disposed of 3 months after collection.

In Australia and Japan, expanded screening is carried out without restrictions.

In Great Britain, two technology assessment groups were set up and reached different conclusions: one group found strong reasons for introducing a pilot MS/MS screening study for a larger number of diseases;[41] while the other group recommended a pilot study of screening for MCAD, glutaric aciduria type 2 and phenylketonuria[42] and was against including any other diseases.

Physicians' knowledge about expanded screening by tandem mass spectrometry

After the introduction of expanded screening by MS/MS in the United States, a great deal of research was carried out with pediatricians and family doctors to evaluate their roles. These investigations found that, although these specialist demonstrated interest and many of them were actually involved in expanded screening, their knowledge about the diseases involved was scant and they were not prepared for treatment and management of children found to be positive by screening.[13,14,22,23] The Newborn Screening Expert Group found a clear disparity between the information available and the information needed by the primary care physician (pediatricians and family doctors) to ensure an immediate response to positive screening tests and so recommended that professional training should be the responsibility of the screening system. They also developed a list of actions (ACT sheets) to be taken in the event of a positive diagnosis for each of the diseases proposed for testing.[24,43] These are available on the National Newborn Screening and Genetics Resource Center's website : http://genes-r-us.uthscsa.edu/.

Neonatal screening in Brazil

The first experiments with neonatal screening in Brazil began during the 1960s and were introduced by the pediatrician Prof. Benjamim Schmidt.[44] He was director of the Associação dos Pais e Amigos dos Excepcionais (APAE – literally the "association of the parents and friends of the special" – it is a not-for-profit social organization dedicated to the prevention of diseases that cause disability and the inclusion of people with disabilities) in São Paulo when it started neonatal screening for phenylketonuria in 1976. In 1980, APAE added congenital hypothyroidism to their screening.[15,45]

After this, many other private laboratories, primarily located in the Southeast and South administrative regions of Brazil, made tests available for neonatal screening for a range of diseases.[44,45]

In 1990, the Children's and Adolescents' Statute (Law 8069/1990) made neonatal screening obligatory. In 1992, Ministry of Health decree 22 reaffirmed the obligatory nature of neonatal screening and added testing for phenylketonuria and congenital hypothyroidism. These procedures were then added to the SUS table of tests to be offered by any laboratory, which gave greater impetus to neonatal screening in the different states of Brazil and resulted in the first state-wide programs.[44,45]

In 2001, Ministry of Health decree 822, of the 6th of June, created the NNSP.[15,44,45] This expanded the neonatal screening program in Brazil to four diseases (phenylketonuria, congenital hypothyroidism, sickle-cell anemia and cystic fibrosis) and the objective was to achieve 100% coverage of live births. This is a public program, coordinated by the Ministry of Health, although it does involve laboratories and other private institutions in its structure. It defines the neonatal screening as a five-stage process, similar to what was recommended by the ACMG (2005), i.e., laboratory testing, active search for suspected cases, diagnostic confirmation, treatment and follow-up by a multidisciplinary team. A protocol was compiled with clear standards that guide all phases of the screening program. Control is maintained by means of monthly reports that each specialist center must send to the Ministry of Health.

The program should be implemented in three phases, depending on the level of organization and coverage in each state. During phase I, the diseases screened for are phenylketonuria and congenital hypothyroidism. In phase II, screening for sickle-cell anemia and other hemoglobinopathies is added to the phase I diseases. In phase III, screening for cystic fibrosis is added to the panel of diseases.[44]

By May of 2006, all of the states in Brazil had already introduced neonatal screening for phenylketonuria and congenital hypothyroidism, all functioning according to the NNSP protocols. Ten states were in phase II and three had reached phase III. Coverage for congenital hypothyroidism and phenylketonuria was approximately 80%, which corresponds to 2,497,291 newborn infants/year.[44] Notwithstanding, some states already had better than 95% coverage for all four diseases.[46,47]

When compared with screening in the United States, it might seem that the panel of diseases screened by the NNSP is a timid intervention. In fact this is exactly the same panel that is offered in Great Britain, since it is only after April of 2009 that screening for MCAD will be offered to 100% of the population in those countries.[48]

Diseases included in the National Neonatal Screening Program

Phenylketonuria. This is an inborn error of metabolism, of autosomal recessive etiology, resulting from an absence or

almost total deficiency of the enzyme phenylalanine hydroxylase, which leads to increased plasma phenylalanine concentrations and increased urinary excretion of phenylpyruvic acid. The level of phenylalanine in blood is greater than 10 mg/dL or 600 µmol/L. Cases are classified according to phenylalanine levels as mild phenylketonuria between 10 (600 µmol/L) and 20 mg/dL (1,200 µmol/L), and as classical phenylketonuria above 20 mg/dL (1,200 µmol/L). Values between 4 mg/dL (240 µmol/L) and 10 mg (600 µmol/L) are observed in transitory or permanent hyperphenylalaninemia patients, depending on clinical course, and these people will not require dietary treatment. It is, however, necessary to be alert to increased phenylalanine in females since permanent hyperphenylalaninemia, in common with phenylketonuria itself, can result in their offspring suffering from a condition known as maternal phenylketonuria (microcephaly, mental retardation and congenital heart disease). Differential diagnosis should also involve ruling out a deficiency of BH4, which is a coenzyme of phenylalanine hydroxylase.[2,15,45] The incidence of phenylketonuria varies across the different states and regions of Brazil, from 1:21,000 to 1:13,500 live births. If not treated as soon as possible, those affected frequently develop mental retardation and behavior disorders. Treatment consists of restricting phenylalanine in the diet and monitoring serum phenylalanine levels.[15]

Congenital hypothyroidism. This is a deficiency of thyroid hormone at birth and is one of the principal treatable causes of mental retardation, with an incidence of 1:4,000 to 1:3,000 live births. In general it is the result of some type of thyroid dysgenesis, 85% of cases are sporadic and there are a range of etiologies. Screening is made by assaying tireoyd stimulating hormone TSH and free thyroxin. Treatment is by oral levothyroxine replacement, with clinical monitoring of growth and development, and blood hormone levels. In a small percentage of cases, congenital hypothyroidism can be the result of hormonal synthesis defects, of an autosomal recessive nature.[15,45]

Sickle-cell anemia. This is a group of diseases characterized by abnormal hemoglobin β chains, resulting in anemia due to chronic hemolysis and intermittent episodes of vessel oclusion accompanied by intense pain and other complications. Its incidence is 1:2,500 to 1:1,000 live births, and its etiology is autosomal recessive. Neonatal screening can identify individuals with other hemoglobinopathies and also carriers. Prophylaxis against infections, immunization and education of families to identify the principal complications and seek treatment rapidly can reduce morbidity and mortality.[15,45]

Cystic fibrosis. This is a disorder of exocrine function associated with the cystic fibrosis transmembrane conductance regulator (CFTR) protein which regulates the flow of ions in epithelial surfaces. The principal findings are in the exocrine pancreas, lungs, intestine, liver, sweat glands and the male reproductive system. There are severe repercussions for nutrition and growth, and many of those affected die from pulmonary problems. Incidence varies with ethnicity, being more common in Caucasians, among whom incidence is 1:3,500 live births. In Brazil, its incidence is around 1:10,000 live births.[47] Its etiology is autosomal recessive.

Screening is made by assaying immunoreactive trypsin (IRT), and diagnosis is confirmed by the sweat test (Ministry of Health). In Brazil, the prevalence of the delta F 508 mutation is much lower than in the United States and Europe, and the profile of the most frequent mutations is also different, making the use of a mutation panel difficult.

Treatment requires nutritional support, supplementation of liposoluble vitamins (A, D, E, K), pancreatic enzyme replacement, bronchodilators, respiratory physiotherapy and prevention of pulmonary infections. Early diagnosis reduces its morbidity, and some studies have reported increased survival.[15,47,49-52]

One problem with neonatal screening for cystic fibrosis is the rate of false positives results, which create an expectation of severe disease and are numerous when IRT assays are used. False negatives are associated with meconium ileus and late screening, since IRT levels fall off after 3 weeks (Table 1).[45]

Other screening tests in Brazil

Private laboratories carry out diagnostic tests for other diseases for which neonatal screening is possible, but which have not yet been added to the NNSP. These laboratories do not have universal screening as their objective and neither do they participate in a screening program with the five characteristic stages of neonatal screening, but they do offer screening tests and the initial guidance for obtaining diagnosis and treatment. Since they are offered at many private maternity units, it is necessary that pediatricians inform themselves about the tests and the diseases they identify.

Biotinidase deficiency is an autosomal recessive disease that affects biotin recycling. Biotin is a water soluble vitamin of the B complex which acts as a cofactor for carboxylase complex enzymes. The disease may manifest from a few weeks after birth up to 10 years of age. Secondary biotin deficiency results in neurological abnormalities, dermatological disorders, hearing loss and optical atrophy and, later, may cause mental retardation.[45,54,55] Although incidence in the United States is approximately 1:126,000 to 1:62,000, treatment is simple and inexpensive, by oral biotin replacement.

In the Brazilian state of Paraná, screening for biotinidase deficiency is already offered as part of their neonatal screening program, which is affiliated to the NNSP and, in the state of Minas Gerais, the state neonatal screening program has an ongoing pilot project screening for this disease (personal experience).

Table 1 - Laboratory procedures used for neonatal screening in Brasil [2,15,47,53]

Disease	Filter -paper screening (method)	Confirmation	False positives	False negatives
Phenylketonuria	- Phenylalanine - Fluorometric, enzymatic or MS/MS	- Repeat with fresh sample or phenylalanine and tyrosine by HPLC or MS/MS	- Mother with phenylketonuria - High protein intake	- Premature sampling - Low protein intake - Prematurity - Transfusion - Dialysis
Hypothyroidism	- TSH or T4 and TSH - RIA, fluorometric or enzymatic	- T4 and TSH venous blood by RIA	- Sample taken during first 24 hours of life - Prematurity	- Transfusion
Sickle - cell anemia	- Hemoglobin - IEF or HPLC	- IEF or HPLC - If there has been a transfusion, wait 3 months	- Transfusion - Prematurity	- Transfusion
Cystic fibrosis	- IRT - Immunofluorometry or TRF	- Repeat IRT on filter paper after 15 days, sweat test and/or DNA analysis	- Low Apgar score - Agenesis of pancreatic ducts - Intestinal obstruction	- Meconium ileus - Patients without pancreatic insufficiency - Sample taken after 30 days

HPLC = high performance liquid chromatography; IEF = isoelectric focusing; IRT = immunoreactive trypsin ; MS/MS = tandem mass spectrometry; RIA = radioimmunoassay; TRF = time resolved fluorescence.

Congenital adrenal hyperplasia is a cluster of enzymatic defects of genetic origin which interfere in cortisol biosynthesis, and, frequently, aldosterone biosynthesis. Incidence is approximately 1:15,000. The salt-wasting forms can cause death and may lead to virilization in women. The most common defect is 21-hydroxylase deficiency, which can be identified during neonatal screening by 17-OH-progesterone assay. There are problems with relation to defining cutoff points for the test, and if samples are taken after 7 days the benefits of screening are reduced.[2,56] Treatment is with glucocorticoids and with mineral corticoids for salt-wasting forms. There is disagreement about the cost-benefit ratio of the test and whether further studies are needed to justify neonatal screening for this disease.[57] Congenital adrenal hyperplasia is also the subject of a pilot project in the state of Minas Gerais (personal experience).

Galactosemia is an increase in the concentration of galactose in the blood, caused by a variety of autosomal recessive conditions. The most common of these is 1-phosphate uridyltransferase deficiency, which has an incidence of 1:40,000 in the United States. The clinical manifestations are vomiting, jaundice, hepatomegaly, cataracts, delayed development and septicemia, among others. Treatment consist in exclusion of galactose from the diet and, despite improvements in survival, efficacy is limited with relation to long term complications.[2,58]

Medium-chain acyl-coenzyme A dehydrogenase deficiency is the principal disease among the fatty acid oxidation defects. Its most common manifestations are episodes of hypoglycemia, which can be triggered by infections or prolonged fasting. These are symptoms with early onset that may appear during the first days of life. A significant percentage of patients die from the first episode.[45]

In Brazil, laboratories screen for this disease using polymerase chain reaction (PCR) to test for the A985G mutation, which is responsible for this disease in the great majority of cases (98%) in developed countries, or by MS/MS. In the United States MS/MS is used to screen for this disease.[45]

Infectious diseases and congenital infections, such as toxoplasmosis, cytomegalovirus, syphilis, rubella, Acquired Immunodeficiency Syndrome and Chagas disease, can also be included in neonatal screening. There is no systematic view of what value screening for these conditions might be for newborns.[26,30] Another important factor is that, in a large proportion of pregnancies, diagnostic investigation of the mother is carried as part of prenatal care.

In the state of Minas Gerais, a pilot project concluded that including Chagas disease in neonatal screening could not be justified. Another pilot project with congenital toxoplasmosis found elevated incidence, especially in regions with lower

socioeconomic status, and detected that severe ocular damage had already occurred by the time of diagnosis (personal experience).

Congenital deafness has an estimated incidence of 1:1,000 live births and a range of etiologies. At least half of these cases can be attributed to genetic causes (both syndromic and non-syndromic); while the other half are related to environmental factors (for example: exposure to ototoxic drugs, rubella or cytomegalovirus).[10] The objective of neonatal screening is to identify hearing loss early to enable faster intervention. Hearing deficiencies that are not identified and are left untreated affect speech and other cognitive abilities.

Screening is carried out using computerized equipment that measures the automatic auditory response of the brainstem or distortion evoked otoacoustic emissions. The second method is more widely used in Brazil, for economic reasons and because of the simplicity of the technique; however, it produces higher rates of false-positives, particularly if there is some type of obstructive process in the auditory canal.[10]

Tandem mass spectrometry in Brazil

Some laboratories in Brazil have already acquired mass spectrometers and are offering tests using this technology. Before requesting these tests pediatricians should check with the laboratory which diseases are being offered. They should also find out how diagnosis and treatment in positive cases are conducted, and verify which specialist metabolic disease centers will monitor or supervise treatment, since Brazilian experience in the management of the various diseases diagnosed by these tests is still very scarce.

Some ethical questions relating to neonatal screening

The debate about neonatal screening, especially about expanded screening by MS/MS, involves a range of ethical considerations about which pediatricians need to know.

Traditionally, neonatal screening programs were restricted to diseases for which early detection and treatment offered medical benefits to those affected. One of the WHO criteria is *treatability*. One of the objections to screening using MS/MS is that it is used to screen for diseases which may have clinical significance but which are not treatable or which have a treatment whose health benefits have not yet been well-established. However, this principle of treatability has been partially flouted before, when screening for sickle cell anemia and other hemoglobinopathies and also cystic fibrosis were introduced. Furthermore, those that defend this method of screening argue that patients benefit from receiving information in advance about the symptoms that they may develop and that families benefit from genetic counseling, being able to take reproductive decisions in a conscious manner. A secondary consideration is that previous cases of unexplained deaths of siblings may, retrospectively, have their causes explained.[30,59]

When it started, neonatal screening, in recognition of the benefits for affected newborn infants, was made compulsory. Nowadays, it has evolved to recognize the right of parents to refuse it entirely or to refuse information about a specific disease. Cultural and religious motives justify this decision. Therefore, it is also recognized that parents must be informed prior to samples being taken and receive explanations about the benefits and risks, and the recommendation is to use free and informed consent forms.[2,60] However, this is not yet a universal practice.

Patients have the ethical right to have the results of neonatal screening, diagnosis and all phases of follow-up treated as confidential.[2,60]

Another ethical question is related to the final destination of the specimens collected, how to store them and preserve them to protect patient privacy and what precautions to take with relation to posterior use. The capacity to extract DNA from the filter paper samples has made them a precious bank of DNA and any use must follow rigorous ethical strictures. It must be remembered that, in Iceland, a commercial company was legally able to acquire access to extensive health records held by the country and related to banks of biological material.[61]

Questions have also been asked about diagnosing diseases with unknown incidence and natural history, and where there are uncertainties about the reliability of diagnostic tests and effectiveness of treatment.[62] The majority of the diseases screened for using MS/MS have incidence rates well below 1:50,000.[63] A negative test result does not rule out some of these diseases since they have variant forms that will only manifest later in life, leading to false negatives.[2]

One argument in favor of expanded screening is the low cost of adding a significant number of diseases.[2,4-6,8,9,63] In contrast, others argue that to continue to expand the panel of diseases simply because to do so is easy and cheap is not a prudent approach to public policy.[32]

The identification of carriers (in the genetic sense of the word, i.e., otherwise healthy people who are heterozygotes for a mutation to a gene for an autosomal recessive disease) has been a problem in neonatal screening since the introduction of tests for sickle cell anemia and cystic fibrosis. This can help with family planning, but can also lead to discrimination and bad feeling. Since, for each affected person the number of carriers is far greater, the use of technologies which increase the number of people identified as carriers of little-known diseases will demand ever greater genetic counseling resources. There are no clinical studies of the impact of identifying carriers in neonatal screening.[2,7,12]

Another delicate issue, which was accentuated by expanded screening with MS/MS in the United States, is the loss of the State's power over public health policies, resulting in unorganized spending and diverting resources from other

areas. Privatization reduced the State's jurisdiction over public health policies. Voluntary use of private screening services by parents created a secondary screening system that was not universal, depending on parents' selective knowledge and capacity to pay.[64]

Private screening companies are progressively involving themselves in neonatal screening in practically all of the states in the United States and compete with the public system to offer a paid service for the diagnosis of MCAD and another 50 metabolic diseases.

This issue touches on another fundamental ethical aspect, equality, i.e. equal rights for all. According to this principle, neonatal screening should be universal, with the supply of different screening panels within the same country being unacceptable.[2,26,59,60]

Aspects of expanded screening considered to be negative include the level of stress caused to the families of children with false-positive results[34] and increased costs over the long term, with no guarantee, so far, that the benefits are worth it.

These are just some of the ethical questions related to neonatal screening that are currently being discussed. It is important that pediatricians know about them and discuss them in order to be prepared to respond to the challenges that undoubtedly will emerge along with advances in genetics. It is difficult to predict all of the future possibilities of DNA technology, since microarray techniques are already bringing up much more complex questions. Greater ethical problems will probably emerge as the capacity to detect adult onset diseases increases, or to detect susceptibility to diseases by means of tests carried out on newborn infants. Under what circumstances would this be ethical?

It is necessary to begin the debate on these subjects.[63]

Conclusions

Neonatal screening is the largest genetics-related public health program in the world. It is a 5-stage system, generally conducted by the public health service. Although pediatricians have a preeminent role to play, they have little knowledge about the diseases screened for, their treatment and prognosis.

Brazil has a neonatal screening program that adheres to international guidelines. The panel of diseases screened for is the same as in some developed countries. To the extent that the program is consolidated more diseases will probably be added.

Expanded screening by MS/MS appears to be irreversible, since it is a considerable technical advance. However, for it to become established, certain medical and ethical questions need to be answered.

Neonatal screening brings up ethical problems which need to be discussed, because they only begin to trace the outline of the major ethical challenges that screening based on molecular biology will undoubtedly bring with it.

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