

SUMMARY

THE RELEVANCE OF THE NEONATAL URINE SCREENING FOR INBORN ERRORS OF METABOLISM PERFORMED IN QUÉBEC

Introduction

Screening newborns for a number of hereditary diseases is common practice throughout the world. In Québec, a neonatal screening program, or, more precisely, screening activities, has been in operation for more than 30 years through the initiative of the Réseau de médecine génétique du Québec. These screening activities target all Québec newborns. The overall objective is to reduce the morbidity and mortality associated with certain hereditary genetic diseases, specifically, inborn errors of metabolism (IEMs), whose prognosis can be improved with early diagnosis and treatment. The Québec Newborn Urine Screening Program (*Programme québécois de dépistage néonatal urinaire* : PQDNU), which operates at the Centre hospitalier de l'Université de Sherbrooke (CHUS), presently screens for more than 25 inborn errors of metabolism.

Individually, IEMs are rare diseases, but when considered together, they can involve a large number of individuals. These hereditary diseases are, for the most part, of autosomal recessive transmission and can manifest at all stages of life, from the neonatal period to adulthood. Usually, IEMs manifest clinically as nonspecific symptoms, and the clinical diagnosis is made by exclusion. The most severe cases can lead to death within the first week of life. Some patients may also present with metabolic decompensation, which can lead to irreversible sequelae (mental retardation, debilitating neurological disorder, failure to thrive). Early diagnosis, that is, before the onset of clinical symptoms, permits targeted interventions and averts long hospital stays for the purpose of making a diagnosis. When treatment is available, early diagnosis permits early intervention and improves the prognosis. In the case of very rare inborn errors of metabolism (or those whose etiology has been known for only about 15 years), there is no

evidence regarding the long-term prognosis or the quality of life of those affected.

Description of the Québec Newborn Urine Screening Program

Participation in the program is voluntary, and the average participation rate has been 90% since the 1990s. It is the parents who collect a sample of the newborn's urine on a paper blotter (provided in the kit) at 21 days of age and who mail the blotter to CHUS's neonatal urine screening laboratory. In Québec, urine samples are presently analyzed by means of a colorimetric technique, multiplex thin-layer chromatography (TLC), which those in charge of the program find simple, rapid, reproducible and inexpensive.

After a metabolic disorder is detected, confirmatory tests have to be performed to quantify the amino acids (by ion exchange chromatography) and the organic acids (by gas chromatography/mass spectrometry). Upon confirmation of a positive result, the infant is immediately referred to one of Québec's four referral centres for diagnostic confirmation and a clinical follow-up, and the parents are given genetic counselling.

Assessment objectives

The objective of this report is, at the requestor's demand, to assess the scientific relevance of the neonatal urine screening performed in Québec. It will be noted that this assessment was carried out in a particular context where the analytical techniques developed, since 1971, at the province's neonatal urine screening laboratory have guided the choice of IEMs targeted by the PQDNU.

The purpose of the assessment is threefold: 1) to assess the clinical relevance of screening for each of the 18 diseases for which at least one affected child has been detected in Québec since 1973 in the PQDNU; 2) to evaluate the efficacy and efficiency

of the screening techniques; and 3) to weigh the benefits of neonatal urine screening against its drawbacks.

Methodology

In screening, relevance is defined as an improvement in morbidity and mortality in a given population and can be assessed by means of a list of public health criteria. The research team at the Institut national de santé publique du Québec drew up a list of 14 criteria when preparing its evaluation report on Québec's neonatal blood screening program for genetic diseases. The list served as a basis for our assessment. These criteria, which are inspired by those proposed by Wilson and Jungner in 1968, are a Québec adaptation of those used by the United Kingdom's National Screening Committee and are used to justify the implementation of a population screening program. The criteria are grouped by theme: the health problem (its seriousness, incidence and natural history, and the possibility of primary prevention), the screening test (its validity, efficacy, clinical and therapeutic efficacy, and acceptability by the population), the treatments (the efficacy of the available treatments and the clinical guidelines, the organizational aspects of management) and the program (its effectiveness in reducing mortality and morbidity, its social and ethical acceptability, and its benefits and opportunity costs).

The clinical relevance of screening for each of the 18 diseases selected for this report was assessed from information in specialized manuals, recent literature reviews, and evidence-based assessment reports from 1995 to August 2008. An update based on certain observational or primary-intervention studies led to the addition of new information to that from the above-mentioned reviews.

The performance of neonatal urine screening by TLC, alone or compared with tandem mass spectrometry (MS/MS) using a blood or urine sample, was the subject of a systematic literature review with no time limit. The quality of the primary studies was assessed with the QUADAS analytical checklist.

In addition, we carried out a systematic review of the economic studies on urine screening with TLC published since 1975. As well, we updated the systematic review of the economic studies

presented in AETMIS's report on MS/MS. As for study quality, it was assessed with the Drummond checklist.

The ethical, psychosocial and organizational issues were examined within the analytical framework proposed in AETMIS's report on MS/MS. Chapter 7 of that report was first updated on the basis of data published since 2003. This was followed by a systematic review, with no backward time limit, up to August 2008 with regard to the issues specific to TLC and urine screening programs.

In addition, interviews were conducted with pediatricians to gain a better understanding of the flow of events at the referral centres, where patients are followed in the wake of a positive screening test result.

Clinical relevance of screening for the 18 inborn errors of metabolism detected by the PQDNU

Urea cycle disorders, triple H syndrome, and methylmalonic aciduria, propionic aciduria and glutaric aciduria I are serious or even fatal conditions that must be managed and treated quickly to ensure the affected infants' survival and to prevent complications. 3-methylcrotonylglycinuria I is a disorder for which the literature on the course of the untreated disease is somewhat controversial.

Cystathioninuria, hyperhistidinemia, hypersarcosinemia and Hartnup disorder are considered benign or as having few serious consequences on the patient's health. Therefore, no particular intervention is required in such cases.

The evidence concerning IEMs associated with metabolite transport is quite limited. First, dicarboxylicamino aciduria is a very rare condition for which no particular clinical manifestation profile has been reported. As for cystinuria, it can cause urinary calculi, which can sometimes result in significant kidney damage if the disorder is not treated. Preventive measures avert the occurrence or decrease the frequency of urinary calculi. It will be noted that a high urine cystine concentration before the age of 1 year may be due to renal immaturity and that few infants develop urinary calculi before this age. Fanconi-Bickel syndrome

is a disorder whose complications can be relatively serious, but not fatal, and which, if treated, usually do not leave any permanent sequelae. The only available treatments are palliative in nature.

Prolidase deficiency may lead to permanent central nervous system damage and can be fatal. Little is known about the efficacy of the treatments, but the preliminary data seem encouraging.

Pyroglutamic aciduria can, if not treated, lead to mental retardation, and it can be fatal. Although the literature on the subject is sparse, it is reported that treatments, which are now available, improve the long-term outcomes by protecting against central nervous system damage.

National newborn screening programs

Screening programs throughout the world are very heterogeneous, and most of them use a blood sample. On the one hand, Australian and American organizations recommend screening all newborns for more than 29 hereditary diseases (20 of which are IEMs detectable by tandem mass spectrometry). On the other hand, in the United Kingdom, population screening is recommended only for two IEMs (PKU and MCADD), in addition to congenital hypothyroidism and other hereditary diseases (sickle cell anemia, deafness and cystic fibrosis).

In Canada, the number of neonatal diseases systematically screened for varies from province to province, but most provinces have, in the past few years, adopted MS/MS as the technology of choice for screening for a number of these diseases. For example, Ontario chose MS/MS in 2005 and opted for the disease profile inspired by the recommendations issued by an expert group in the United States. The number of diseases targeted by the Ontario neonatal screening program therefore increased, between 2006 and 2008, from 3 to about 29, 20 of which are IEMs.

Performance of multiplex thin-layer chromatography for neonatal urine screening for inborn errors of metabolism

There are no studies on population-based urine screening with TLC or that compare

this technology to other population screening technologies, such as MS/MS. A few diagnostic studies have shown sensitivity results ranging from 54.4 to 100%, depending on the IEM studied. We cannot, on the basis of these studies, draw any conclusions regarding the performance of TLC in the context of an IEM screening program. We do, however, note that several factors can influence its performance, namely, age at sampling time, sample quality, pharmacologic and dietetic interferences, and the reproducibility of chromatography plate interpretations. Given the absence of clinical studies, we are unable to quantify the consequences of these technical limitations in terms of the proportion of true-positive and false-positive results. The studies on MS/MS indicate that the sensitivity of this technique generally exceeds 90% when screening for several of the diseases targeted in the QNUSP. Although technical factors may influence these results, MS/MS has the undeniable advantage of being able to detect, earlier than TLC performed on a urine sample, disorders such as propionic aciduria (PA) and methylmalonic aciduria (MMA), whose prognosis, especially the mortality associated with them, can improve if treatment is administered promptly.

Economic aspects of multiplex thin-layer chromatography

There are no studies specifically concerning the efficiency of TLC used in a urine screening program for inborn errors of metabolism. One recent publication that discusses MS/MS-based screening of groups of diseases and certain studies examined in AETMIS's report on this technology conclude that screening for several diseases at the same time using the same blood sample may yield efficiency benefits. When the quantitative tests necessary for confirming positive results on TLC are taken into account, the unit cost is probably less than that of urine screening with TLC.

Ethical, psychosocial and organizational issues associated with neonatal urine screening

An array of ethical, psychosocial and organizational issues raised by neonatal screening in the literature, regardless of the technology used, are discussed in one of AETMIS's previous reports

and are summarized in the present assessment. However, urine screening with TLC screening raises different issues than blood screening with MS/MS:

- **Sample timing:** Neonatal urine screening performed on the 21st day of life (the result of the screening test is obtained only at day 30) raises a major problem. In many cases, the symptoms of the disease manifest before the result of the screening test is available. For some IEMs, this situation can be avoided by using a blood sample obtained 48 hours after birth.
- **Consent:** The fact that the urine sample is sent by the parents is considered implicit consent as such (voluntary consent, but not necessarily informed), unlike in neonatal blood screening, for which the current practices usually tend to dispense with explicit parental consent. Whatever the case, in both instances, parental consent raises an important ethical issue.
- **The sampling method:** The collecting of a urine specimen is considered noninvasive, unlike the obtaining of a blood sample, which, because of the invasive nature of the procedure, may lead to parental refusal. As for a urine sample, it poses the inconvenience of having to be obtained by the parents and may, therefore, result in oversights or delays in sending the sample. On the other hand, it does have the advantage of permitting the detection of IEMs associated with metabolite transport.

Conclusions and recommendations

This report examines the relevance of screening for IEMs, rare diseases for which the evidence is often insufficient (or for which the published studies are of poor methodological quality) and does not, therefore, permit any definitive conclusions. Since we assessed the relevance of screening for IEMs based on those screened for in the QNUSP, it is difficult, based on the present analysis, to determine all the IEMs that should be screened. Thus, the choice of IEMs to be introduced or not to be introduced into the neonatal screening program is only a proposal for establishing priorities.

Despite these limitations, we did observe that for urea cycle disorders, triple H syndrome,

methylmalonic aciduria, propionic aciduria, 3-methylcrotonylglycinuria I, and glutaric aciduria I, the balance between the benefits (for example, a reduction in morbidity and mortality with early treatment) and the drawbacks (for example, the parental anxiety generated by false-positive or false-negative results) tilts in favour of mass neonatal screening.

Other disorders presently screened for in the QNUSP, such as cystathioninuria, hypersarcosinemia, hyperhistidinemia and Hartnup disorder, generally seem benign. Population screening for these diseases does not, therefore, seem warranted, given that the drawbacks for the patient and his/her family and for the health-care system, mainly in terms of diagnostic investigations, medical follow-ups and patient treatments, outweigh the benefits.

Between these two extremes is a gray area in which we find diseases for which the benefits of early screening are debated because, among other things, of a lack of evidence. They are cystinuria, dicarboxylic amino aciduria, Fanconi-Bickel syndrome, prolidase deficiency and pyroglutamic aciduria.

Because of a lack of comparative data on the different screening techniques and on the performance of the current TLC screening in Québec, we are unable to draw any conclusions about the actual performance of TLC. However, we do observe that waiting until the 21st day of life to obtain a sample can considerably affect screening performance. First, for some IEMs, a large percentage of severe cases manifest clinically before that day. Next, the very nature of the diseases and the false-positive results may wrongly label certain newborns who will not develop any symptoms associated with these disorders, in addition to having drawbacks in terms of diagnostic investigations, medical follow-ups and unnecessary stress. Furthermore, TLC, whose results are qualitatively interpreted, requires a high level of clinical expertise in recognizing abnormalities and additional analyses using other techniques to quantify the amino and organic acids. Given these considerations, MS/MS seems advantageous in relation to TLC. It can be performed during the first few days of life, it has an excellent level of diagnostic accuracy, and it is a quantitative method

as such that does not depend as much on interpreter expertise.

In light of these observations, AETMIS recommends that:

- 1) neonatal screening be maintained for the following IEMs and that it be performed by MS/MS on a blood sample: classic citrullinemia, argininosuccinic aciduria, hyperargininemia, citrullinemia type II, triple H syndrome, methylmalonic aciduria, propionic aciduria, 3-methylcrotonylglycinuria I, and glutaric aciduria I;
- 2) the four disorders considered benign (cystathioninuria, hypersarcosinemia, hyperhistidinemia and Hartnup disorder) be eliminated from the neonatal screening program;
- 3) the relevance of screening for cystinuria, Fanconi-Bickel syndrome, dicarboxylic amino aciduria, prolidase deficiency and pyroglutamic aciduria (conditions for which the benefits of early screening are debated because of a lack of evidence) be assessed by a consensus among the clinical specialists and the other health professionals concerned and with the affected individuals and their families before deciding to include these disorders in a neonatal screening program;
- 4) the relevance of adding other IEMs for neonatal screening be assessed in a planned manner based on the available scientific evidence and irrespectively of the IEMs presently screened for;
- 5) the performance and viability of the QNUSP be evaluated with regard to metabolite transport disorders: cystinuria, Fanconi-Bickel syndrome and dicarboxylicamino aciduria. A more thorough evaluation including the program's clinical results should be considered in order to support any decision concerning the maintaining of urine screening for these disorders in its current structure and to decide, among other things, when the urine sample should be obtained, taking into account the process of renal function maturation in infants;
- 6) insofar as possible, a retrospective study be carried out using existing Québec epidemiological and clinical data in order to determine more accurately the incidence of IEMs and their clinical course as a function of when therapeutic interventions are initiated;
- 7) the MSSS develop a complete frame of reference for a possible provincial neonatal screening program, along with the creation of a computerized registry for compiling all the diagnostic, epidemiological and clinical data, including case follow-up data, in order to evaluate the program on an ongoing basis.