CLINICAL EFECTIVENESS OF NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM USING MASS SPECTROMETRY. PART I: Maple Syrup Urine Disease, Homocystinuria, Glutaric Aciduria Type I, Isovaleric Acidaemia, Long-chain 3-Hydroxyacyl CoA Dehydrogenase Deficiency

Spanish full text

ABSTRACT

Introduction: The goal of newborn screening programmes is to ensure the presymptomatic identification and early treatment of treatable congenital disorders, in order to reduce morbidity-mortality and possible disabilities associated with these diseases. Such programmes must guarantee equitable and universal access for all newborns in the target population, and provide accurate information to parents so as to help them with their decision-making. The introduction of mass spectrometry marks a radical change in the screening of metabolic diseases (or congenital metabolic diseases) because, as compared to conventional methods, a high number of analytes associated with metabolic diseases can be detected by just one analytical procedure. Nevertheless, newborn disease screening should not be initiated, unless the advantages to the newborn of early detection have been clearly defined and guarantees are in place to ensure appropriate diagnosis, follow-up and treatment of all children detected by the health-care system.

Objectives: To assess the clinical effectiveness of newborn screening of the following congenital errors of metabolism: maple syrup urine disease; homocystinuria; glutaric aciduria type I; isovaleric aciduria; and long-chain 3-hydroxyacyl CoA dehydrogenase deficiency.

Methods: Systematic literature review of the principal biomedical databases (Medline, Embase, Cochrane Library Plus, Health Technology Assessment, Database of Abstracts of Reviews of Effects, National Health Service Economic Evaluation Database, ISI Web of Science and *Índice Médico Español*, among others). To retrieve all existing systematic reviews and assessment reports on congenital errors of metabolism screening programmes, we updated the bibliographic search of the avalia-t report from 1 January 2006 to September 2012. We also conducted specific searches targeting the natural history, epidemiology, analytical validity and clinical utility of the screening of each disease assessed, in order to update the *PHG Foundation* report from 1 January 2009 to September 2012. After perusal of the abstracts of the resulting papers, studies were selected on the basis of a series of inclusion/exclusion criteria.

Results and discussion: The bibliographic searches retrieved 811 studies, 60 of which were included. These were all observational, fundamentally longitudinal or compared cases series and cross-sectional studies with no

control group, which furnished direct evidence in some cases only, with a high risk of bias due to the use of historical series for comparison purposes.
Congenital errors of metabolism are diseases of great aetiological, diagnostic and prognostic complexity, which are generally of a chronic and progressive nature and often present with an elevated morbidity-mortality and a high degree of disability. The screening test of these five diseases consists of the collection and analysis of a heel blood sample (and, sometimes, also of urine). Whereas obtaining the sample is safe and simple, the analytical process is complex and entails the previous fine tuning of the technique and the establishment of a screening protocol defining the analytes to be used, specific cut-off points for each population and laboratory and, where applicable, second-tier tests. The screening protocol will determine the test's sensitivity and specificity.

Maple syrup urine disease (MSUD): Incidence at birth is very variable, with an estimated prevalence of 15.6 cases per 100,000 population. Classical form (75%-80% of cases) has a short latency period and symptoms and signs tend to appear in the first days of life, with ketoacidosis, lethargy and hypotonia, which can lead to death. In the intermediate form, progressive neurological symptoms of psychomotor retardation and development commence between 5-6 months and 6-7 years of age. In the intermittent form, patients experience normal psychomotor growth and development during childhood, with metabolic crises at any age. In the thiamine-responsive form, presentation is similar to that of the intermediate form, with psychomotor retardation predominating and good response to thiamine. In these forms in which the clinical disease begins much later, the screening programme could achieve the expected benefit. Subunit E3 deficiency in the branched-chain alpha-keto acid dehydrogenase (BCKDH) enzymatic complex is very infrequent, and symptom onset tends to take place around the second month of life, with progressive neurological deterioration. Without treatment, mortality in newborns who present with the classic form of the disease is high. Diagnosis is made by analysis of amino acids in plasma and urine using different techniques and of organic acids in urine using gas chromatography-mass spectrometry. Plasma alloisoleucine elevation is pathognomonic. Confirmation is made by sequencing the genes that code for the various subunits of the BCKDH enzymatic complex. Disease screening is performed by quantifying leucine, isoleucine and valine values with tandem mass spectrometry (MS/MS). Intermittent forms of MSUD may not be detected. Treatment consists of dietary restriction of branched-chain amino acids and supplementation with low-protein-content foods, such as greens, vegetables and fruit. Thiamine supplements are usually given and stress is laid on the prevention and urgent management of metabolic decompensations. Sensitivity showed elevated values (except in three studies), while specificity was close on 100% in all cases. The PPV was very low (1.79%) and lower than recommended. Both sensitivity and PPV clearly depend on the screening protocol. In order to reduce the number of false positives, some laboratories are using a binary liquid chromatography-mass spectrometry (LC-MS/MS) screening method to measure leucine, isoleucine, alloisoleucine and

hydroxyproline in a single blood sample. Disease prognosis depends principally on the time of exposure to high concentrations of branched-chain amino acids particularly leucine, in blood. No evidence has been found to indicate that MSUD screening reduces mortality. There is direct evidence to show that, among patients who undergo newborn screening, leucine levels at the time of diagnosis are lower than when diagnosis is based on clinical symptoms. This means that detoxification treatment can be introduced early, thus preventing the appearance of neonatal encephalopathy crisis and improving patients' clinical outcomes, and their intellectual development in particular. With respect to fulfilment of the requirements for the disease's implementation in a screening programme, MSUD would not comply with the need to have a sufficiently long detectable latency period in over 80% of cases, in programmes in which screening results are not obtainable prior to symptom onset. Similarly, it would not fulfil the criterion of having a valid, reliable and efficient screening test, since sensitivity in the different studies was very variable and the PPV of the screening programmes assessed was very low (1.79%), depending clearly on the protocol used. A screening method that measured leucine, isoleucine, alloisoleucine and hydroxyproline in a single blood sample could improve the analytical results.

Homocystinuria: This has a world-wide incidence of approximately 1: 250 000 newborns, and is slightly lower in Spain where the most frequent mutation is p.T191M. There are two main disease phenotypes, pyridoxine-sensitive or pyridoxine -insensitive cases. The disease has a long disease latency period and the clinical disease becomes evident at 2-3 years, with myopia, lens dislocation, osteoporosis, long limbs, vascular system disorders which can cause thromboembolisms and involvement of the central nervous system with frequent intellectual disability. The diagnosis is made by quantification of total plasma homocysteine levels in the absence of pyridoxine supplement for 2 weeks. Similarly, an increase in plasma homocystine and methionine is observable, and homocystine is detectable in urine. The molecular study of the CBS gene and measurement of CBS enzyme activity are also confirmatory. Early detection allows for initiation of the treatment, which consists of dietary restriction of methionine and administration of pyridoxine, betaine, folic acid and vitamin B12 supplements. In screening, blood methionine levels are quantified by MS/MS and, because methionine levels are not usually sufficiently elevated in pyridoxine-sensitive patients at the date of screening, it is the pyridoxine-insensitive patients that would benefit most from a screening programme. The cut-off points used are very variable. In some laboratories, ratios such as Met/Phe or the Leu\Ile values are additionally used. The test's specificity and NPV are close on 100%. In the two studies in which sensitivity could be quantified, this was 50% and 100%. The PPV of screening was very low (0.80%) and the percentage of false positives was 0.08%. An alternative two-step or binary screening method has been described, in which measurement of methionine by MS/MS is followed by measurement of homocysteine with LC-MS/MS in the same blood sample, with a sensitivity and specificity of almost 100% (with homocysteine also being quantified). Without treatment, the prognosis of patients with classical

homocystinuria is bleak, with progressive morbidity and mortality. Newborn disease screening enables early diagnosis and early introduction of the treatment. This in turn reduces blood homocysteine levels and prevents intellectual disability and the appearance of thromboembolic episodes, the leading cause of mortality in such patients. Screening of homocystinuria, through quantification of methionine, would not meet the requirement for its implementation, i.e., the need for a valid, reliable and efficient screening test, since in pyridoxine-sensitive patients, methionine levels would not be sufficiently elevated at the date of screening. In addition, the PPV of the screening programmes assessed was very low (0.80%). A screening method that measured methionine with MS/MS and then homocysteine with LC-MS/MS in the same blood sample, could improve the analytical results.

Glutaric aciduria type I (GA-1): GA-1 has a world-wide incidence of 1: 107 000 births, with the most prevalent mutation in Europe being p.R402W. In the Spanish population, the most prevalent mutation is c.293A>T, followed by mutations p.R402W and p.V400M. MSUD has an extensive disease latency period because, even though macrocephaly is usually already present from birth (74% of cases), it is not accompanied by any other sign or symptom. The most frequent form of disease presentation is an acute episode of encephalopathy after an episode of metabolic stress, which generally occurs at around 9 months of age (90% during the first or second year of life). The sequelae are bilateral striatal lesions with movement disorders (generally dystonia). Normal growth is maintained by early lysine-free treatment, supplemented with L-carnitine and, sometimes, with riboflavin. Screening is performed by quantifying blood glutarylcarnitine (C5DC) levels by MS/MS, though some laboratories also quantify proportions of other acylcarnitines, such as C5CD/C3, C5DC/C2, C5DC/C8 or C5DC/C16 ratios. Although the test's sensitivity and specificity are close on 100%, some patients with low-excretor variant may go undetected. The percentage of false positives was 0.02% and the PPV was 3.27%. Early diagnosis of the disease through screening and the introduction of a suitable treatment can lead to a modification of the natural history of GA-I. As compared to unscreened children, screening reduces mortality and the presentation of the encephalopathy crisis, with this being the most relevant event regarding the prognosis of the disease. Moreover, screening enables the attainment of adequate motor development (or only a slight delay), in contrast to unscreened patients, the majority of whom develop dystonia, as well as eating and other severe movement disorders. GA-I would fulfil all the requirements for its implementation in a screening programme.

Isovaleric aciduria (IVA): IVA's world-wide incidence is 1 case per 114,000 births, though higher incidences have been reported in Spain, and where the most predominant mutation is c.932C>T (p.A282V). The most frequent clinical presentation is the acute neonatal form, with acidosis and encephalopathy which can progress to coma and death. Half of all symptomatic cases manifest in the first week of life and up to 84% in the second week of life, so that for the benefits of screening to be effective the results must, if possible, be

obtained before the first week of life. Nevertheless, the intermittent chronic form has a lengthy latency period. Mortality is higher if the metabolic crisis appears before the fifth week of life. Treatment consists of diet (protein restriction and supplementation with carnitine and glycine) and specific measures aimed at preventing and managing metabolic decompensations. Isovalerylcarnitine (C5) is used for disease screening but some laboratories also calculate other ratios, such as C5/C8, C5/C4, C5/C3 or C5/C2. The sensitivity and specificity values obtained by different IVA screening programmes are close on 100%, albeit with a very low positive predictive value (0.82%). In addition, screening could favour the identification of mild or disease-free patients. There is no evidence to show that newborn isovaleric aciduria screening reduces early mortality in patients with acute forms of the disease. In the chronic intermittent form, however, it would enable most of the cases to be detected prior to symptom onset. There is indirect evidence to indicate that early detection of the disease improves the neurocognitive outcome of these patients. With respect to fulfilment of the requirements for the disease's implementation in a screening programme, IVA would not meet the need to have a sufficiently long detectable latency period in over 80% of cases, unless the availability of the screening results before the first week of life could be ensured. Although the variety of protocols used in the various studies makes it is difficult to assess the existence of a valid, reliable and efficient screening test, it would nonetheless seem that the inclusion of other analytes or ratios could improve the test's PPV.

Long-chain 3-Hydroxyacyl CoA Dehydrogenase Deficiency (LCHADD): The incidence of LCHADD is 1 case per 144,000 births. The most frequent mutation is c.1528G>C, which appears in 65%-90% of patients. The clinical profile is characterised by acute metabolic decompensations, liver dysfunction, heart disease and non-specific chronic symptoms. Rhabdomyolysis, progressive retinitis pigmentosa and peripheral neuropathy can also be observed. Although the most common age of clinical appearance is from 6 weeks to 6 months, a small group of patients (15%) presents with hypoglucaemia in the first month of life. Treatment consists of preventing fasting by ensuring regular feeding, with intake of carbohydrates, restriction of long-chain fatty acids and supplementation with other medium-chain fatty acids, in order to prevent the appearance of metabolic crises. Early treatment is effective in preventing episodes of hypoglucaemia and sudden infant death, and could prevent the appearance of heart disease and delay the progression of retinopathy and polyneuropathy. Screening is based on the analysis of C16-OH-acylcarnitine levels. Other long-chain hydroxyacylcarnitines, such as 3-hydroxypalmitoylcarnitine (C16OH) and 3-hydroxyoctadecanoylcarnitine (C18OH), are also determined. The screening test's sensitivity and specificity are close on 100%, with a PPV of 16%. Screening must, however, be conducted within a narrow time window, prior to 72 hours of life, because from this point onwards the acylcarnitine profile may be normal in anabolic conditions. LCHADD screening allows for early diagnosis of the disease and the introduction of early treatment, which could be effective in preventing the appearance of heart disease, episodes of hypoglucaemia and sudden infant

death and delaying the progression of retinopathy and polyneuropathy, and so enhance long-term outcomes. LCHADD would meet all the requirements for being implemented in a screening programme.
Currently, no primary prevention measures have been implemented for these five diseases. Since all are autosomal recessive in nature, one possible measure would be to detect carriers in high-risk communities, though those that are known are very much in the minority. Another measure to be implemented would provide for the follow-up and genetic counselling of relatives of persons affected by the disease.
The balance between benefit and harm of any screening programme is difficult to ascertain. On the one hand, there are the direct benefits for newborns detected and true positives, among whom presymptomatic detection may reduce morbidity-mortality and possible disease-related disabilities, and so succeed in improving their vital prognosis. In view of the low prevalence of the diseases being assessed here, the number of newborns benefited would be very small. On the other hand, evidence as to benefits is of low quality and in some instances only indirect evidence is available. Furthermore, the lack of studies with a sufficiently long follow-up period means that there is no information on the long-term results of early detection. Other benefits to be assessed would be familial or social, though there is widespread agreement about screening programmes, in the sense that their justification should be based solely on the direct benefit for the newborn. On the other hand, harms from a screening programme tend to be concentrated in the false positives yielded by the tests, inasmuch as these generate anxiety and concern among parents which can last until definitive results are forthcoming and which may persist even though the disease is not confirmed and, in particular, in possible overdiagnosis and overtreatment deriving from detection of mild or asymptomatic forms of the disease being screened. These possibilities of causing harm affect a very high number of newborns and their families.

Conclusions:

- Evidence as to the effectiveness of the screening programmes of congenital errors of metabolism assessed in this review was of low quality and was based on observational studies, fundamentally longitudinal or compared case series and cross-sectional studies with no control group, which furnished direct evidence in some cases only.
- Two congenital errors of metabolism, GA-I and LCHADD, would fulfil all the requisites for implementation in screening programmes. In two cases, MSUD and IVA, the requirement of having a sufficiently long detectable latency period would not be met, unless the availability of the screening results before symptom onset could be ensured. In three diseases, no valid, reliable and efficient screening test can be claimed to exist, due to low sensitivity (using methionine levels in the case of homocystinuria) or irregular sensitivity (in the case of MSUD) or the diversity of screening protocols used (IVA). In all three cases, the PPV of the commonly used screening tests was very low.

- Before any screening programme can be implemented, an appropriate protocol that maximises the test's sensitivity and specificity must be drawn up, defining the analytes to be used, specific cut-off points for each population and laboratory, and, where applicable, second-tier tests.
- Lastly, information systems must be set up, based on pertinent, relevant and reliable results that make it possible to assess whether the activities or processes developed within a screening programme are tailored to health needs, both from the standpoint of the population and from that of the health-care system. Such information will be of aid when it comes to measuring attainment of goals, setting priorities and making decisions.