XUNTA DE GALICIA
CONSELLERÍA DE SANIDADE
Dirección Xeral de Innovación e Xestión da Saúde Pública

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Axencia de Avaliación de Tecnoloxías Sanitarias de Galicia

CLINICAL EFFECTIVENESS OF NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM USING MASS SPECTROMETRY. PART III: Carnitine uptake deficiency (CUD), Short-chain Acyl-CoA dehydrogenase deficiency (SCADD), Very long-chain Acyl-CoA dehydrogenase deficiency (VLCADD)

Spanish full text

SUMMARY

Introduction: the goal of newborn screening programmes is to ensure presymptomatic identification and early treatment of treatable congenital disorders, in order to reduce morbidity-mortality and possible disabilities associated with these diseases. Such programmes should 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 tandem mass spectrometry (MS/MS) marks a radical change in the screening of metabolic diseases because, in contrast to conventional methods, a high number of analytes associated with metabolic diseases can be detected by just one analytical procedure. Even so, newborn 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 disorders: carnitine uptake deficiency (CUD), short-chain acyl-CoA dehydrogenase deficiency (SCADD) and very-long chain acyl-CoA dehydrogenase deficiency (VLCADD).

Methods: systematic literature review of the principal biomedical databases (Medline, Embase, Cochrane Library Plus, HTA (Health Technology Assessment), DARE (Database of Abstracts of Reviews of Effectiveness), NHS EED (NHS Economic Evaluation Database), ISI Web of Science and *Índice Médico Español* (*IME*), among others). Two search strategies were used, one -with no time limit- centred on epidemiology, natural history, morbidity, mortality, diagnosis and treatment, and the other centred on the screening of each disease. To retrieve all existing systematic reviews and assessment reports on inborn errors of metabolism screening programmes, we updated the bibliographic search of the *avalia-t* reports until April 2014. After reading the abstracts of the resulting papers, studies that met the pre-established inclusion/exclusion criteria were selected. Subsequently, this procedure was completed by a manual review of the bibliographic references cited in the papers selected.

Results and discussion: inborn errors of metabolism are disorders 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 high degree of impairment. The screening test of these three diseases consists of the collection and analysis of a blood sample obtained by heel prick (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 methodology and the establishment of a screening protocol defining, among others, the analytes to be used and the specific cut-off points for each population and laboratory. The screening protocol will determine the test's sensitivity and specificity.

Carnitine uptake deficiency (CUD) is an autosomal recessive inherited disorder caused by a mutation in the *SLC22A5* gene which encodes the carnitine transporter *OCTN2* and whose alteration affects energy metabolism. Estimated world-wide incidence is 1:100 000 births, with Spain registering an

incidence of one case per 79 507 newborns (NBs) across the period 2001-2012. The clinical manifestations can vary widely from asymptomatic patients to others with cardiomyopathy linked to muscle weakness, liver disease and hypoglycaemia, typical in acute episodes, with encephalopathy, coma and death. The most common forms of presentation are seen during infancy (metabolic/hepatic) and early childhood (myopathic/cardiac). The most severe presentation takes place in the breastfeeding stage, often associated with fasting or febrile processes, with hypoketotic hypoglycaemia and metabolic crises that can cause brain damage or mental retardation. Without treatment, the disease progresses to convulsions, coma and death. The early childhood presentation is principally characterised by cardiac manifestations which can prove fatal if not adequately treated. Adults may be asymptomatic or present with mild symptoms, such as weakness or fatigue. CUD can be detected in newborns by measuring free carnitine levels (C0) in dried blood spot by MS/MS. Diagnosis is confirmed by quantitation of C0 and acylcarnitines (AC) with MS/MS, enzymatic study of carnitine transport in lymphocytes or cultured fibroblasts and molecular analysis, which allow for genetic counselling and prenatal diagnosis. The goal of treatment is to maintain plasma carnitine levels normal, and prevent hypoglycaemia and periods of fasting. Treatment is based on a fractional carbohydrate-rich diet and restriction of fats and tryglicerides, in combination with the administration of oral L-carnitine supplements of proven efficacy. Long-term prognosis is very favourable, with normal development as long as patient adherence to the regimen is good. Sensitivity and negative predictive value (NPV) were 100% in all studies but one, owing to the presence a false negative (FN) result. Specificity was close on 100% in all cases and, though the global positive predictive value (PPV) was 12%, it varied widely, ranging from 1.15% to 64.7% due to the presence of false positive (FP) results. During the intrauterine life, carnitine is transferred across the placenta to the fetus, so shortly after birth, the infant´s carnitine level may actually be a reflection of the mother´s. Indeed, 4.6% of all FPs were due to transitory decreases in C0 concentration, which were really a reflection of maternal deficit. To reduce the number of FPs, some screening programmes, in addition to determining plasma C0 concentrations, regard it as highly useful to include the decrease in global acylcarnitine profiles and their low urinary reabsorption as part of the initial screening test or as an additional marker in the same dried blood spot. Owing, however, to the absence of data, the effect of this could not be assessed. Only one FN was observed, reflecting the test's high sensitivity and NPV. Despite the fact that evidence of the benefits of screening was of low quality, practically all the cases detected in the studies and screening programmes included in this review were asymptomatic and presented with no metabolic decompensations or cardiac alterations after the start of treatment, with normal growth being reported. Only one case with onset after performance of the test was detected, and it was characterised by major developmental delay. However, the absence of a clear phenotype-genotype relationship means that it is extremely difficult to predict which patients will really develop the disease. Despite the fact that many screening programmes initiate treatment once diagnosis of CUD has been confirmed, there is no way of knowing the outcome that these NBs would have had, if they had not been treated. The benefits of screening primarily include a reduction in morbidity and, by way of an indirect benefit, the complementary screening of relatives in general and maternal cases in particular.

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a genetic disorder inherited in an autosomal recessive manner, caused by deficiency of short-chain acyl-CoA dehydrogenase, which intervenes in mitochondrial fatty acid β-oxidation by catalysing the dehydrogenation of short-chain fatty acids (4-6 atoms of carbon). SCAD deficiency is considered to be a multifactorial, polygenic disease, and the presence of two polymorphisms that are very frequent in the general population, namely 511C>T and 625G>A, is thought to be a susceptibility factor which requires the presence of other genetic and environmental factors. While the incidence of SCAD deficiency is unknown,

screening programmes have shown that it might affect one per 40 000-100 000 NBs, with an estimated world-wide incidence of one case per 58 035 NBs. There are no reliable data on this disorder's prevalence. SCAD deficiency can present in the first weeks of life (generalised neonatal), with muscle tone abnormalities, hypoglycaemia and vomiting, though the majority of cases appear progressively before 5 years of age, with hypotonia and developmental delay. There is also an adulthood presentation (localised) which mainly affects the muscles after intense physical effort, causing muscular problems such as pain, weakness or chronic myopathy. In most cases, individuals are asymptomatic and display a good health status, thus raising questions about the test's clinical relevance. Countries such as Germany, the United Kingdom, Holland, Denmark and Australia exclude SCAD deficiency from their screening panels, due to its negligible clinical relevance and the fact that its early detection is not regarded as clinically useful. Diagnosis is based on elevation, during metabolic decompensations, of butyrylcarnitine (C4) concentrations in plasma and/or ethylmalonic acid (EMA) and derivatives in urine, whereas plasma carnitine (C0) concentration is generally normal. Diagnosis is confirmed by urinary organic acid analysis, quantitation of acylcarnitines in plasma, determination of enzymatic activity in muscle biopsies or cultured skin fibroblasts, and genetic studies, which allow for genetic counselling and prenatal diagnosis. Elevation of plasma and urine C4 and/or EMA concentrations respectively are characteristics of but not diagnostic criteria for SCAD deficiency, and so differential diagnosis is necessary. The need for treatment is questionable, since most of the cases diagnosed are asymptomatic and uncertainty surrounds the treatment's effectiveness in terms of preventing disease manifestations. Similarly, there is no clear consensus on the recommendations for dietary treatment or supplementation with carnitine and/or riboflavin. In the long term, treatment does not significantly improve clinical evolution, and symptoms generally tend to improve with age. Disease screening is performed by MS/MS using dried blood spot, in which elevated concentrations of C4 are detected. Since exclusive determination of C4 concentration by MS/MS does not discriminate between SCADD and isobutyryl-CoA dehydrogenase deficiency, discriminatory capacity is enhanced by inclusion of EMA concentrations, MS and/or different C4-based ratios. Sensitivity and NPV were 100% in all studies but one, due to the presence of 3 FNs. Specificity was close on 100%, and the overall PPV was 15.8%, though it proved very variable owing to the presence of FPs. The percentage of false positives was 0.013% for all studies as a whole, due mainly to exclusive determination of C4 concentration. There were 4 FNs in two studies, which might have been due to the high C4 cut-point, delay in taking the specimen, or modifications made to the analytical method over the course of the pilot project. Those studies which furnished pertinent data indicated that 98% of cases were asymptomatic at date of diagnosis and that, while not all of these received treatment, they nevertheless displayed normal development. The benefits of screening are essentially of a social and family nature, with the most important taking the form of reduced family anxiety and genetic counselling.

Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is a genetic disorder inherited in an autosomal recessive manner, caused by deficiency or dysfunction of very long-chain acyl-CoA dehydrogenase, which intervenes in the first of the four stages that make up β-oxidation of mitochondrial fatty acids and is specific to those having 14 to 20 carbon atoms. Estimated world-wide incidence is 1:50 000-120 000 births, with Spain registering an incidence of 1:147 655 and 7 confirmed cases across the period 2001-2012. The disorder's exact prevalence is not known. Clinical signs and symptoms generally appear during the neonatal period (50%) or childhood (30%), with this form of presentation being more severe and lethal than that of adolescence or adulthood (20%). Three phenotypes of differing severity and age of onset have been described. Firstly, there is the neonatal form (myopathic with multiple-organ failure), which is characterised by early onset and has a disease course characterised by cardiomyopathy, pericardial effusion and arrhythmias, hypotonia,

hepatomegaly and intermittent hypoglycaemia; without treatment, mortality is high and usually occurs before the first year of life. Secondly, there is the infantile form (hepatic with hypoketotic hypoglycaemia), which is more moderate, does not cause cardiac alterations and displays a lower mortality; it is characterised by development of hypoketotic hypoglycaemia induced by fasting or infectious processes, severe brain dysfunction (encephalopathy) and hepatomegaly. Lastly, there is the adolescent or adulthood form (late-onset myopathic), which appears progressively from 10 years of life onwards and is induced by exercise, fasting or stress; the disease course is marked by muscle fatigue, intermittent rhabdomyolysis, muscle cramps and/or pain and intolerance to exercise. Diagnosis is based on the study of plasma levels of free fatty acids and specific acylcarnitines (C14:1, C14:2 and C14), and of urinary organic acids which show a characteristic profile of dicarboxylic and 3-hydroxicarboxilic acids, absence of or decrease in ketones, and presence of myoglobin. Diagnosis is confirmed by urinary organic acid analysis, quantitation of acylcarnitines in plasma, determination of VLCAD activity in fibroblasts, lymphocytes or tissue, and molecular analysis. The treatment is common to other defects of fatty acid β-oxidation and focuses on preventing and controlling acute episodes. Preventive measures consist of forestalling hypoglycaemia by avoidance of prolonged fasting in situations of stress or infectious processes, and ensuring a fractional carbohydrate-rich diet and restriction of long-chain triglycerides. Additional recommendations include medium-chain triglyceride and L-carnitine supplementation. Disease screening is performed by MS/MS, using dried blood spot in which elevated concentrations of C14:1, C14 and/or C14:2 are detected. Some screening programmes use C12:1, C16 or C16:1, C18:1 levels or C14:1-based ratios (C14:1/C12:1 or C14:1/C16) as additional markers. Sensitivity was 100% in all studies but one, in which it fell to 75% due to the presence of 1 FN. Specificity and NPV were very close to 100% and the PPV was extremely variable, ranging from 3% to 84% due to the presence of false positives. For all studies as a whole, the percentage of FPs was 0.003%: the programmes which obtained the highest FP percentages and thus the lowest PPVs proved to be those that solely used C14:1 or C14 concentrations as markers, while joint assessment with specific combinations of markers was observed to improve the test's effectiveness. Only one FN was observed, reflecting the test's high sensitivity and NPV. Despite the fact that evidence of the benefits of screening was of low quality, practically all the cases detected in the studies and screening programmes included in this review were asymptomatic and presented with no metabolic decompensations or cardiac alterations after initiation of treatment, with normal growth being reported. Three cases with onset after performance of the test were detected. Two of these were successfully treated and displayed normal development, and one died due to acute metabolic decompensation at two days of life. While treatment appears to reverse the symptoms in screened and unscreened patients alike, it should be noted that, in comparison with patients who are clinically diagnosed, early initiation of treatment in patients detected by newborn screening reduces morbidity and mortality. Nevertheless, it cannot be stated with any degree of assurance that preventive treatment would impede the appearance of symptoms for life, and there is no way of knowing the possible evolution that the screened asymptomatic patients would have had, if they had not been treated.

Conclusions:

- The evidence of the effectiveness of screening programmes of inborn errors of metabolism assessed in this review was of low methodological quality, and was based on observational studies and, in some instances, on pilot programmes, furnishing direct evidence in some cases only.

- Carnitine uptake deficiency (CUD) has a sufficiently long latency period to ensure that a screening programme could achieve the expected benefit. However, the absence of a clear genotype-phenotype relationship means that the disease's natural history is not properly known, thus

rendering it extremely difficult to predict precisely what percentage of patients will really develop the disease. Furthermore, despite having an effective treatment available, there is no clear consensus as to whether it would be more effective to introduce treatment in the latency or in the symptomatic stage.

- Short-chain acyl-CoA dehydrogenase deficiency (SCADD) does not meet some of the main requirements for implementation of screening programme, namely, severe disease or important health problem, clear definition of diagnostic criteria or clear knowledge of the natural history of the disease. Moreover, there is insufficient evidence of the usefulness of newborn MS/MS screening for SCAD deficiency.

- Although very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) has a sufficiently long latency period to ensure that the screening programme could yield the expected benefit, there are doubts about the screening test's reliability and whether or not the therapeutic intervention would be more effective if applied in the latency rather than in the symptomatic stage.

- Prior to implementing a screening programme, however, an appropriate protocol would have to be drawn up which maximised the test' sensitivity and specificity and which defined, among others, the primary and secondary markers to be used and the specific cut-points for each population and laboratory.

- Lastly, information systems would have to be set up, which were based on pertinent, relevant and reliable results and made it possible to assess whether the activities or processes undertaken within the context of the screening programme were tailored to health needs, not only from a population standpoint, but also from that of the health system. Such information would serve as an aid when it came to measuring the attainment of goals, setting of priorities and taking of decisions.