CLINICAL EFFECTIVENESS OF NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM USING MASS SPECTROMETRY. PART II:

- **Methylmalonic acidemia**
- **Propionic acidemia**
- **Tyrosinemia type I**

Spanish Full text

Summary

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 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 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 diseases, i.e., methylmalonic acidemia, propionic acidemia and tyrosinaemia type 1.

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). Two search strategies were used, one centred on epidemiology, natural history, morbidity, mortality, diagnosis and treatment, and the other centred on the screening of the disease. To retrieve all existing systematic reviews and assessment reports on inborns errors of metabolism screening programmes, we updated the bibliographic search of the *avalia-t* report from 1 January 2006 to June 2013. After reading the abstracts of the resulting papers, studies were selected on the basis of a series of inclusion/exclusion criteria. Subsequently this procedure was completed by a manual review of the bibliographic references cited in the papers selected.

Results and discussion: inborns 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 high degree of disability. 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 technique and the establishment of a screening protocol defining the analytes to be used, specific cut-off points for each population and laboratory, and in cases where second-tier tests are called for, the corresponding analytes and cut-off point. The screening protocol will determine the test's sensitivity and specificity.

Methylmalonic acidemia (MMA) is essentially an autosomal recessive genetic disorder caused by a complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (mut^0 and mut^-) or by a defect in the transport or synthesis of its cofactor, adenosyl-cobalamin: included in this group are the subtypes CblA and CblB (without homocystinuria) and CblC, CblD, CblF, CblJ and CblX (with homocystinuria). CblX is transmitted through X-linked inheritance and is due to a defect in the transcriptional coregulator HCFC1. Another form of MMA is caused by a deficiency of the enzyme methylmalonyl-CoA epimerase or by depletion of mitochondrial DNA. Approximately, 60% of cases of methylmalonic acidemia (MMA) are due to alterations in the MUT gene (isolated form). The remaining cases are due to alterations of MMAA and MMAB genes, associated with the enzymatic subtypes CblA and CblB respectively. Unlike other countries, in the case of Spain the most frequent type of MMA is CblC. Incidence of MMA ranges from 1:50 000-1:100 000 births. MMA may be responsive or resistant to vitamin B12 treatment. In Europe, the estimated mean prevalence of vitamin-B12-resistant methylmalonic acidemia is 1,9 cases/100 000 population. Its clinical course is characterised by the risk of episodes of potentially mortal metabolic decompensation. It is the most common clinical form, in which, after a symptom-free period ranging from hours to the first weeks of life, patients rapidly go on to develop lethargy, vomiting and dehydration, with weak muscle tone (hypotonia) and encephalopathy. Partial deficiency, sensitive to vitamin B12, commences with clinical signs and symptoms in the first months or years of life and its subsequent progress can be characterised by eating problems, failure to gain weight and grow at the expected rate (failure to thrive), hypotonia and delayed development. MMA continues to be associated with appreciable morbidity and mortality, and its principal complications are intellectual disability, tubulointerstitial nephritis with progressive renal failure, basal ganglia involvement, pancreatitis, growth failure, immunodeficiency and optic nerve atrophy. The mortality rate and neurological complications are greater in the vitamin-B12-resistant form. Diagnosis in symptomatic patients is based on analysis of organic acids in urine by means of gas chromatography/mass spectrometry, analysis of aminoacids in plasma and/or urine, blood spot acylcarnitine profiling by means of mass spectrometry and a series of tests to establish the enzymatic subtype. The treatment goal is to ensure good metabolic control by avoiding decompensations and preventing complications, reduce toxic metabolites, and achieve good nutritional status accompanied by optimal height-weight and neurocognitive development. Treatment is based on: a high-protein, high-calorie diet, low in propionate amino-acid precursors (valine, isoleucine, methionine and threonine); administration of carnitine as a detoxicant of propionyl and metronidazole groups which reduces the production of propionate, by acting on the intestinal bacteria that metabolise non-absorbed proteins; and in vitamin-B12-sensitive cases, administration of hydroxicobalamin. Screening is performed by mass spectrometry (MS/MS) using samples of dried blood spot, in which elevated concentrations of propionylcarnitine (C3) are detected, giving rise to a considerable number of false positives (FPs). The use of different ratios between acylcarnitines and other additional metabolites, such as glycine and methylmalonyl-carnitine, enables the false positive rate to be reduced. Screening programme sensitivity attained values of 100% in all studies but one, in which 3 false negatives (FNs) were detected and sensitivity was rated as 50%. Specificity in all cases was very close on 100%. PPV was above 20%, with the exception of one study where the appearance of a positive result led to the screening test being repeated using the same dried blood spot sample. To reduce the false positive rate a second-tier test has been developed, which is performed on the same blood spot tested in the primary screened, and consists of determining methylmalonic, methylcitric and homocysteine acid levels by means of another MS/MS methodology. In Spain second-tier testing is not performed: it is only in Catalonia where this methodology is used in the final validation stage. Diagnosis is confirmed by enzymatic determinations and mutational analysis of specific genes. No increase in the frequency of MMA has been detected dating from the implementation of MS/MS screening programmes, which indicates that screening does not entail an overdiagnosis of mild forms which would otherwise not be diagnosed. A considerable percentage of MMA cases became manifest before screening was performed or before the results became available. Although screening appears to decrease mortality, there is no direct evidence that would allow for conclusions to be drawn about its effects on short- and long-term morbidity and mortality.

Propionic acidemia (PA) is an autosomal recessive genetic disorder caused by a deficiency of the enzyme propionyl-CoA carboxilase. World-wide incidence is estimated to range from 1:50 000-1:100 000 births. The neonatal form is the most frequent, and the acute form is manifested by vomiting, loss of appetite and somnolence in the first days of life in a previously healthy infant; without treatment, patients progress to lethargy, convulsions, coma and death. Late-onset propionic acidemia is characterised by delayed development, chronic vomiting, protein intolerance, growth failure, hypotonia and, occasionally, basal ganglia infarction and cardiomyopathy; affected children may suffer from acute decompensations with encephalopathy, convulsions and/or coma in situations of catabolic stress, such as infections or surgical interventions. The main complications of propionic acidemia are neurological and cognitive disorders, pancreatitis and cardiac complications. Diagnosis is based on determination of urine organic acids by chromatography/mass spectrometry and plasma acylcarnitines by tandem mass spectrometry. Diagnosis is confirmed by determination of enzymatic activity or analysis of the PCCA and PCCB genes. Chronic treatment is based on restriction of protein intake and administration of carnitine and laxative drugs. Screening is performed by tandem mass spectrometry (MS/MS) using dried blood spot samples, in which elevated concentrations of propionylcarnitine (C3) are detected. As in the case of methylmalonic acidemia screening, the use of C3/C2, C3/C0, C3/C16, C3/C4, C5/C3, C3/Met ratios and other metabolites, such as glycine and methylmalonyl carnitine, enables the false positive rate to be reduced. Sensitivity was 100% in all the studies. Specificity in all cases was very close on 100%. PPV was above 20%, with the exception of one study where the appearance of a positive result led to the screening test being repeated using the same dried blood spot. To reduce the false positive rate a second-tier test has been developed, which is performed on the same dried blood spot sample in the event of a positive result in the initial screening test. No increase in the frequency of PA has been detected dating from the implementation of MS/MS screening programmes. One study which compared 35 cases of PA diagnosed after the appearance of clinical signs and symptoms to 20 cases diagnosed through newborn screening, showed that the latter allowed for statistically significantly earlier diagnosis of the disease. The age of symptom onset was similar in both groups, as was the number of metabolic decompensations and hospital admissions, a finding which indicates that, even though screening allows for early diagnosis, it does not prevent the appearance of metabolic crises. There were no differences in cognitive development between the patients in the two groups but there was a statistically significant negative linear correlation between the number of metabolic crises and the intelligent quotient. There were somewhat fewer non-neurological clinical complications in the newborn screening group, though the age of the patients included means that conclusions cannot be drawn about the long-term effects. Overall mortality was 8%, with a tendency to be lower in the newborn screening group (0% vs. 12%), though without this reaching statistical significance.

Tyrosinaemia type 1 (HT1) is an autosomal recessive genetic disorder caused by a deficiency of fumarylacetoacetate hydrolase (FAH), the enzyme implicated in the final step in tyrosine (Tyr) metabolism. The overall world-wide detection rate is estimated at 1:249 016 births and prevalence at <1 case per 100 000 births. The acute neonatal form is the most frequent and is characterised by a rapid deterioration of hepatic and renal function, which frequently appears in the first days of life. It is manifested by delayed development, jaundice, diarrhoea, vomiting, enlarged liver (hepatomegaly), cabbagelike odour, dysnea and progressive liver dysfunction which, without treatment, leads to death due to liver failure in the first year of life. The chronic form generally appears at 6 months of age and is similar but with milder characteristics of liver disease and renal tubular dysfunction, leading to hypophosphatemic rickets, cirrhosis and development of hepatocellular carcinoma. Mortality in these cases occurs during the first 10 years of life. Both forms can present with severe neurological crises that include painful paresthesia, hypertension, tachycardia, paralytic ileus and muscle weakness. Moreover, accumulation of succinylacetone (SUAC) can give rise to acute intermittent porphyria, since it inhibits biosynthesis of the heme group. Diagnosis is based on the study of Tyr and SUAC levels by MS/MS and CG-MS. The elevation of SUAC levels is pathognomonic. Diagnosis is confirmed by determination of urinary and plasma levels of SUAC, determination of porphobilinogen synthase (PBG-

S) activity in heparinised whole blood or erythrocytes, demonstration of the deficiency of FAH activity, and genotyping. Treatment is based on a low-protein diet (Tyr and Phe) in combination with the administration of nitisinone (NTBC or Orfadin®). Liver transplantation is reserved as an alternative for critically ill patients in whom drug treatment has failed and in those who present with hepatocellular carcinoma. Screening is performed by MS/MS and thin-layer chromatography, methods used to quantify blood tyrosine values. It has been shown that, used alone, quantification of Tyr levels as a marker for TH1 is of little value due to the high number of FPs. Plasma SUAC accumulation in affected newborns is pathognomonic, so that this analyte's inclusion in the screening panels increases specificity and decreases the risk of FPs and FNs. Sensitivity and PNV were 100% except in two studies, due to detection of FNs. Specificity exceeded 97% and rose as high as 100%, and PPV was extremely variable, ranging from 0,04% to 100%. Three FNs were observed in cases where the respective protocols exclusively relied on determination of Tyr values. To reduce the number of FPs, methods have been implemented which quantify SUAC values as the primary marker or use a second-tier test, first determining Tyr and then SUAC in the same dried blood spot using another MS/MS methodology. The results of this review indicate that the test's sensitivity was 66.7% when Tyr quantification alone was used, and that this rose to 100% when SUAC concentration was used.

Conclusions:

- The evidence of the effectiveness of screening programmes of inborn errors of metabolism evaluated in this review is of low quality and is based on observational studies –fundamentally longitudinal or comparative case series and cross-sectional studies without a control group- with direct evidence being furnished in only some cases.
- Neither methylmalonic nor propionic acidemia would meet the requirement of having a sufficiently long latency period to ensure that the screening programme could yield the expected benefit, if there were no way of guaranteeing that results could be obtained before symptom onset. Although screening seems to reduce immediate mortality in MMA, there is no direct evidence that would enable conclusions to be drawn about its effects on short- and long-term morbidity and mortality. In the case of PA, screening does not seem to prevent the number of metabolic decompensations or alterations in the cognitive development of such patients. A comparative study reported that mortality was lower among patients diagnosed through newborn screening than among those diagnosed on the basis of clinical signs and symptoms, though without this proving statistically significant.
- Tyrosinaemia type 1 has a sufficiently long latency period to ensure that the screening programme could achieve the benefit expected from the intervention, since newborns do not present with the disease until the first weeks or months of life. With respect to the screening test, exclusive quantification of tyrosine levels has been shown to have negligible value as a marker, displaying little sensitivity and specificity. Accumulation of plasma SUAC is pathognomonic, so that the development of MS/MS screening methods for determination of its concentration increases specificity and reduces the risk of FPs, FNs and the recall rate. One of the chief contributions of screening is the early detection of a cause of acute liver failure, leading to the prevention of liver disease mortality and the possible development of hepatocellular carcinoma in chronic forms.
- Thanks to the efficacy of the treatment and the existence of a specific test for detection of tyrosinaemia type 1 in a dried blood spot sample (SUAC), this inborn error of metabolism meets the criteria for benefiting from the advantages of newborn screening.
- Before implementing a screening programme, a suitable protocol must be drawn up to maximise the test's sensitivity and specificity, stipulating the analytes to be used, the specific cut-off points for each population and laboratory and, where applicable, the need for second-tier testing.
- Lastly, it is essential to set up information systems based on pertinent, relevant and reliable results, which would make it possible to assess whether the activities or processes developed

within the context of a given screening programme were in line with health needs, from the standpoint of both the population and the health-care system. Furthermore, such information would serve as an aid for measuring goal achievement, establishing priorities and making decisions.