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Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme

Mª Luz Couce *, Daisy E. Castiñeiras, Mª Dolores Bóveda, Ana Baña, José A. Cocho, Agustín J. Iglesias, Cristobal Colón, José R. Alonso-Fernández, José M. Fraga

Unidad de Diagnóstico y Tratamiento de Enfermedades Metabólicas Congénitas, Departamento de Pediatría, Hospital Clínico Universitario de Santiago, Spain

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ABSTRACT

Newborn screening (NBS) by tandem mass spectrometry started in Galicia (Spain) in 2000. We analyse the results of screening and clinical follow-up of inborn errors of metabolism (IEM) detected during 10 years.
Our programme basically includes the disorders recommended by the American College of Medical Genetics. Since 2002, blood and urine samples have been collected from every newborn on the 3rd day of life; before then, samples were collected between the 5th and 8th days. Newborns who show abnormal results are referred to the clinical unit for diagnosis and treatment.
In these 10 years, NBS has led directly to the identification of 137 IEM cases (one per 2060 newborns, if 35 cases of benign hyperphenylalaninemia are excluded). In addition, 33 false positive results and 10 cases of transitory elevation of biomarkers were identified (making the positive predictive rate 76.11%), and 4 false negative results. The use of urine samples contributed significantly to IEM detection in 44% of cases. Clinical symptoms appeared before positive screening results in nine patients (6.6%), four of them screened between days 5 and 8. The death rate was 2.92%; of the survivors, 95.5% were asymptomatic after a mean observation period of 54 months, and only two had an intellectual/psychomotor development score less than 85. Compared to other studies, a high incidence of type I glutaric aciduria was detected, one in 35,027 newborns.
This report highlights the benefits of urine sample collection during screening, and it is the first study on expanded newborn screening results in Spain.

1. Introduction

Most cases of inborn errors of metabolism (IEMs) occurring with clinical symptoms result in serious consequences to the affected infants, including mild to severe irreversible mental retardation, lifelong disability, physical handicaps, coma, and early death [1]. These effects can be prevented or significantly reduced in many cases if the IEM is diagnosed early: as a public health measure, newborn screening (NBS) has proved to be diagnostically effective and economically efficient.

As new technologies have emerged, public health authorities have incorporated them in expanded NBS programmes. In particular, tandem mass spectrometry (MS/MS) has gradually been adopted by many countries following the work of Millington et al. [2]. In a single 2–3 minute test, MS/MS can determine over 30 analytes in blood samples impregnated in paper, including amino acids and acylcarnitines. Consequently, it allows the detection of many inborn errors of intermediary metabolism, such as aminoacidopathies, galactosaemia (by measurement of hexose monophosphate [3]), organic acidurias, and disorders related to the oxidation of free fatty acids (FFAs). Otherwise, Pitt et al. reported the first study of urine profiling by MS/MS including a great range of metabolites [4]. Metabolic disorders for which other analytical techniques have been introduced include biotinidase deficiency (BD), screened for by a colorimetric assay [5].

Several studies have examined the long-term outcome and effects of expanded NBS for specific disorders, including classical organic acidurias [6], type I glutaric aciduria (GA-1) [7], maple syrup urine disease (MSUD) [8], and medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD) [9,10]. Others, most of them very recent, have

Abbreviations: BD, biotinidase deficiency; CIT 1, type I citrullinaemia; FFA, free fatty acid; GA-1, type I glutaric aciduria; GALE, classical galactosaemia; GALK, galactokinase deficiency; HPA, hyperphenylalaninaemia; IEM, inborn errors of metabolism; IQ, Intellectual Quotient; MADD, multiple acyl CoA dehydrogenase deficiency; MAT I/III, methionine S adenosyltransferase deficiency; MCADD, medium chain acyl coenzyme A dehydrogenase deficiency; 3 MCC, 3 methylcrotonyl CoA carboxylase; MMA, methylmalonic aciduria; MSCA, McCarthy Scales of Psychomotor Skills; MS/MS, tandem mass spectrometry; MSUD, maple syrup urine disease; MVA, mevalonic aciduria; NBS, newborn screening; PROP, propionic aciduria; PDI, Psychomotor Development Index; PGA, pyroglutamic aciduria; SCADD, short chain acyl CoA dehydrogenase deficiency; TYR 1, type I tyrosinaemia; WISC R, Wechsler Intelligence Scale for Children Revised.

* Corresponding author at: Unidad de Diagnóstico y Tratamiento de Enfermedades Metabólicas Congénitas, Departamento de Pediatría, Hospital Clínico Universitario de Santiago, A Choupana s/n, 15706 Santiago de Compostela, Spain.
E-mail address: maria.luz.couce.pico@sergas.es (M.L. Couce).

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investigated the overall impact of expanded NBS programmes, including long-term outcomes [1,11–14].

In Galicia (N.W. Spain), where NBS is performed on 99.9% of newborns and has long used both blood and urine samples, an expanded NBS programme incorporating MS/MS was begun in July 2000. In this paper we report follow-up findings of the patients that were referred to clinical unit for diagnosis and treatment from the NBS programme over a period of 10 years. Our programme has the peculiarity of receiving blood and urine samples simultaneously of all newborns. This is the first such study concerning an expanded screening programme in Spain.

2. Patients and methods

In our NBS programme, all newborns with abnormal NBS results suggestive of IEMs are referred for evaluation in the Diagnosis and Treatment of Congenital Metabolic Disease Unit of the University Clinical Hospital, Santiago de Compostela (henceforth "the clinical unit"), regardless of whether they present clinical symptoms. Included in this study were all those so referred from the NBS programme between July 1, 2000 and July 1, 2010. Only patients referred from the NBS programme were included.

2.1. Newborn screening

Samples of blood and urine impregnated on Whatman 903 paper were collected between the 5th and 8th days of life up to and including 2002, and on the 3rd day thereafter. (For readers unfamiliar with the use of urine for newborn screening, urine samples are collected by placing a paper slip between the diaper and the dry genitals – free of creams, talcs, etc. – and, once impregnated with urine, allowing it to dry at room temperature; care must be taken to avoid contamination by faeces.).

Amino acids, acylcarnitines and hexoses monophosphate in blood samples were determined by MS/MS in an Applied Biosystems Sciex API 2000 apparatus [3,15], and biotinidase activity by a colorimetric assay [5]. Urine samples were analysed for galactose [16] and cystine (by the Brand test) When a positive result was found, repeat or additional analyses were performed on the urine samples: MS/MS for amino acids, acylcarnitines, organic acids and acylglycines [4,17], and thin layer chromatography for galactose [16]. Thus all newborns were screened for amino acid disorders, fatty acid disorders, organic acidurias, galactosaemia, and BD (hypothyroidism and cystic fibrosis were also screened for, but are not the object of this study). Percentiles of MS/MS-measured analyte concentrations in the blood of healthy and diseased newborns were periodically reported to the Region 4 Genetics Collaborative Project [18] achieving clinical validation of our cutoffs.

If the results of the above analyses were clearly aberrant and suggestive of a severe disorder, the patient was immediately referred to the clinical unit. If the results lay outside the reference range but were not so aberrant as to constitute clear proof of severe disease, a second sample was requested by the NBS laboratory; if this second sample also tested positive, the patient was referred to the clinical unit.

2.2. Diagnosis and follow-up

For the present study the following variables concerning the situation of each patient at diagnosis were analysed: presence or absence of clinical symptoms, biochemical markers suggesting diagnosis, diagnosis, diagnosis confirmation methods, and whether dialysis techniques or other detoxification measures were employed. The variables considered in regard to the patient's situation at follow-up examinations were age, the use of dietary and/or pharmacological treatment, follow-up time, and progress.

Cases in which biochemical markers were abnormal at NBS but normal upon diagnostic testing were considered as false positives. Cases in which biochemical markers remained altered at first post-NBS evaluation but later became normal spontaneously were considered transitory.

The Psychomotor Development Index (PDI) or Intellectual Quotient (IQ) of survivors were evaluated using the Brunet Lézine Scale for infants, the Mc.Carthy Scales of Psychomotor Skills (MSCA) for preschool children, and the revised Wechsler Intelligence Scale for Children (WISC-R) for children over the age of 6 years. PDIs and IQs above 85 were considered normal.

The study was approved by our hospital's ethics committee.

3. Results

In Galicia, NBS programme is carried out in 99.9% of all newborns; this represents 4.44% coverage for newborns in Spain. During the study period we identified 137 cases of IEM as a direct result of screening 210,165 newborns, i.e. one case per 2060 newborns if the 35 cases of benign HPA are ignored. For each IEM detected, Table 1 lists the proportion of newborns in which it was detected together with data on the corresponding biochemical marker and on the patients' situation at diagnosis and at the last or latest follow-up examination.

The IEMs detected most often as the result of screening were the hyperphenylalaninaemias (benign HPA, 1/6005; PKU, 1/12,363), followed by MCADD, cystinuria and galactosaemia (comprising GALE, GALK and galactose-4 epimerase deficiency) (1/19,106 in each case). As we have reported previously [19], methionine S-adenosyltransferase (MAT I/III) deficiency was also detected relatively often (1/26,271 newborns), in all cases in the course of differential diagnosis for suspected homocystinuria. The cases of alkaptonuria and mevalonic aciduria (MVA), which are not regularly screened for by NBS programmes, were likewise detected in the course of differential diagnosis for other entities using urine tests. The most frequently diagnosed organic aciduria was GA-1 (1/35,027). In 44% of the 137 cases (all except the cases of HPA, PKU, MSUD, BD, and FFA disorders other than short-chain acyl-CoA dehydrogenase deficiency (SCADD)), urinary biochemical markers were of significant value in enabling or completing positive detection. Except for cystinuria, diagnoses were confirmed by enzyme and/or molecular studies (in one case of SCADD these results are currently pending).

Clinical symptoms were already shown at screening time by nine patients (6.6%), four of whom (two with MSUD, one with PROP and one with MMA) required extracorporeal removal therapy at this time. Three of these latter four cases occurred before 2003, when screening samples were collected at age 5–8 days; these three, and a further case of PROP (likewise pre-2003), were diagnosed at screening time. In the rest of the nine cases, the screening results were not conclusive but nevertheless expedited diagnosis.

Of the 137 IEM patients, four (2.9%) had died by the time the study data were analysed. Three of these four had severe infantile-onset organic acidurias (one MMA and two PROP), requiring extracorporeal removal therapy at diagnosis; these three died from acute infections at the ages of 2, 4 and 12 months. The fourth deceased patient, a case of MCADD, had remained asymptomatic thanks to dietary treatment until an acute respiratory infection caused metabolic decompensation and death.

At the time of data analysis, after a mean observation time of 54 months, 127 of the surviving 133 patients (95.5%) remained asymptomatic. Only two, both of whom already showed symptoms at diagnosis, had PDI/IQs below 85. Dietary and/or pharmacological treatment was being received by 89 of the 102 patients with IEMs other than benign HPA (87%).

According to the criteria specified in the Patients and methods section, there were 33 cases of false positive NBS results (Table 2)