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Newborn screening using tandem mass spectrometry: A systematic review

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Abstract

Objectives To evaluate the evidence for the clinical effectiveness of neonatal screening for phenylketonuria (PKU) and medium-chain acyl-coA dehydrogenase (MCAD) deficiency using tandem mass spectrometry (tandem MS).

Study design Systematic review of published research

Data sources Studies were identified by searching 12 electronic bibliographic databases; conference proceedings and experts consulted.

Results Six studies were selected for inclusion in the review. The evidence of neonatal screening for PKU and MCAD deficiency using tandem MS was primarily from observational data of large-scale prospective newborn screening programmes and systematic screening studies from Australia, Germany and the USA. Tandem MS based newborn screening of dried blood spots for PKU and/or MCAD deficiency was shown to be highly sensitive (>93.220%) and highly specific (>99.971%). The false positive rate for PKU screening was less than 0.046% and for MCAD deficiency the false positive rate was less than 0.023%. The positive predictive values ranged from 20 to 32% and 19 to 100%, respectively.

Conclusions This review suggests that neonatal screening of dried blood spots using a single analytical technique (tandem MS) is highly sensitive and specific for detecting cases of PKU and MCAD deficiency, and provides a basis for modelling of the clinical benefits and potential cost-effectiveness.

Introduction

Inborn errors of metabolism (IEM) are a rare group of genetic disorders that can have serious clinical consequences for an affected neonate or young infant. If undiagnosed and untreated, these disorders can cause irreversible mental retardation, physical disability, neurological damage and even fatality.[1] Detection and accurate diagnosis soon after birth are important for achieving a rapid and favourable patient outcome. Whilst the incidence of each specific metabolic disorder is rare, their collective importance is deemed to be of considerable public health significance.[2] The most common disorders of IEM are phenylketonuria (PKU) and medium chain acyl-coA dehydrogenase (MCAD) deficiency.[2;3] In the UK, PKU and congenital hypothyroidism are the only disorders screened for routinely. Evidence indicates that the screening programme is very effective with few cases having been missed.[4] The UK screening programme for PKU is based on the application of three standard methods: the Guthrie bacterial inhibition assay, fluorometry, and chromatography. Neonatal screening for MCAD deficiency has not yet been introduced in the UK, primarily because this disorder is not detectable with current screening methods.[5] There has also been uncertainty about the natural history of MCAD deficiency and concerns about the specificity of the screening test.[6]

Tandem mass spectrometry (tandem MS) has the capability to detect a much wider range of metabolic disorders than conventional methods.[2;3;7] Analysis for these additional conditions can be undertaken using the same blood spot sample provided for PKU: no additional specimen collection or sample preparation is required. Analysis of samples by tandem MS is rapid, can be performed in large batches and, with automatic sample introduction, processed in 24 hours.[8] This technology has been demonstrated to be suitable for the reliable detection of PKU[9] and some other IEM's.[10-12] Of the many metabolic disorders that can be detected, MCAD deficiency is the most comprehensively studied.[13]

In 1997, two reports were published[2;3] by the UK NHS R&D Health Technology Assessment (HTA) Programme, examining the case for extending the neonatal screening programme. These reports were generally favourable to the introduction of some screening for selected disorders but with caveats. They placed a high priority on evaluating MCAD deficiency and recommended further studies on the application of tandem MS to neonatal screening. The failure to fund these studies left many stakeholders disappointed and frustrated.[14;15] However, with the subsequent widespread, international development and adoption of newborn-screening programmes using tandem MS,[16] the HTA Diagnostic Technologies & Screening Panel commissioned an updated review with economic modelling. We conducted a systematic review of the evidence to assess the clinical effectiveness of neonatal screening for IEM's using tandem MS.[17] This paper summarises and updates the key findings of the HTA review[17] in respect of PKU and MCAD deficiency only.

Methods

Twelve electronic bibliographic databases were searched in June 2003 (including the Cochrane Library, Medline, EMBASE and CINAHL) covering the biomedical, scientific, and grey literature.[17] The search combined free text and thesaurus terms relating to neonatal screening for IEM using tandem MS. No date or publication type restrictions were applied. The full search strategy is available from the authors. Searches were supplemented by hand searching relevant journals and conference proceedings.

Diagnostic study types that provided data on the sensitivity, specificity or positive predictive value of neonatal screening using tandem MS for PKU and/or MCAD deficiency between June 1996 (the date of the previous systematic literature search)[2;3] and June 2003 were included in the review. No language restrictions were applied to searches, though only, English language papers were considered for inclusion.

Selected papers were read and critically appraised by a single reviewer. Relevant information from included studies was abstracted directly into an evidence table. Uncertainties were resolved by discussion with another reviewer and clinical advisers. The quality of evidence for diagnostic and screening studies was assessed using established guidelines.[18;19] [20]Summary results were tabulated with detailed descriptive qualitative analyses and were considered for quantitative meta-analysis.

Results

We identified 68 potential studies, published after June 1996 (data prior to this date incorporated in included studies), on neonatal screening for IEM using tandem MS, of which six were included in the review (Figure 1). Table 1 lists study characteristics.

Six studies assessed newborn screening for PKU and/or MCAD deficiency using tandem MS. These studies provided data from newborn screening programmes in Australia[21] and the USA[22;23] and from systematic screening studies (non-newborn screening programmes) from the UK,[6] Germany[25] and the USA.[24] Five used a prospective cohort design with study durations from approximately two[21;23] to seven[24] years. The UK study, of approximately three years, used a retrospective cohort design.[6] All newborn dried blood spot specimens from Australia,[21] Germany[25] and the USA[22-24] were obtained within seven days after birth.[23;24;26] The UK study did not report the timings, though standard UK practice is between six and 14 days of age. Diagnosis of PKU or non-PKU hyperphenylalaninaemia was generally established using repeat analysis and/or repeat blood spot specimens.[23;25] Various thresholds were used to identify infants with MCAD deficiency (Table 1).

Table 2 summarises the diagnostic sensitivity, specificity and positive predictive value of tandem MS. Quantitative meta-analysis was not undertaken because of clinical heterogeneity of the studies.

The false positive rate of PKU screening was less than 0.046%.[23;25] In one program,[23] 92 infants were flagged for phenylalanine. Of these, seven were identified with PKU and 11 with non-PKU-hyperphenylalaninaemia (i.e. the positive predictive value of 19.565%). If the phenylalanine concentration and the phenylalanine to tyrosine ratio were both considered, then the positive predictive value increased to 28.125%. In a systematic screening study of 250,000 newborns,[25] the sensitivity for PKU screening was 93.220%. However, if the milder form of the disease (non-PKU-hyperphenylalaninaemia) was excluded, the diagnostic sensitivity increased to 100%.

Results obtained from prospective newborn screening programmes for MCAD deficiency showed that the false positive rate was less than 0.023%,[21-23] whereas in systematic screening studies the false positive rate was less than 0.018%.[6;24;25] In one study [21] eleven infants were identified as false positives, although, four died before a second sample could be collected. MCAD deficiency was eliminated by enzyme analysis in cultured skin fibroblasts in one patient, whereas MCAD deficiency in the other three infants was eliminated on the basis of information obtained from clinicians and post-mortem findings.

Only one programme reported the presence or absence of false negative results: no known false negative results were found.[22] None of the prospective studies included a rigorous method to identify those who might have been missed by the screening process. The UK retrospective study[6] did not identify any false negative results after examination of the regional registers for metabolic diseases and deaths. In this study,[6] the sensitivity of the screening process was also

found to be 100%, however, the authors reported that the sensitivity of the test was difficult to ascertain, because many occurrences of MCAD deficiency were not diagnosed on clinical grounds.

Discussion

A systematic review of the published literature shows that neonatal screening of dried blood spots for PKU and MCAD deficiency is highly sensitive and highly specific using a single analytical technique (tandem MS).

The evidence of neonatal screening for PKU and MCAD deficiency using tandem MS is primarily from observational data of large-scale prospective newborn screening programmes and systematic screening studies.[21-25] Randomised controlled trials of screening for rare disorders are difficult because of the enormous numbers that would be needed for adequate power.[21] Observational data from large-scale prospective collaborative studies can provide information on test and programme performance and clinical outcome to guide policy decisions.[3;14;27]

Despite the high sensitivities[6;22;25] and high specificities[6;21-25] of tandem MS based neonatal screening for both PKU and MCAD deficiency, the diversity in the preferences of metabolites together with the cut-off limits used to define a positive outcome restricts direct comparison of the diagnostic performance between studies.

The positive predictive value for identification of PKU and MCAD deficiency was found to be higher than that for the disorders screened by the traditional, non tandem MS methods (0.5 to 6.0%).[28] Compared with previously used diagnostic methods (bacterial inhibition assay, fluorometric assay), tandem MS based screening can be performed earlier with lower false positive rates.[7;9;29;30] Detection of milder variants of a disorder can be addressed by adjusting the

cut-off levels closer to normal limits.[31-33] Octanoylcarnitine is the predominant marker for MCAD deficiency, however it is not specific for MCAD deficiency and is expected to be elevated for other disorders and in neonates treated with valproate or fed a diet high in medium-chain triglycerides.[34] Most of the included studies used different criteria's to confirm a diagnosis. In two studies from the USA,[22;24] infants were considered to have MCAD deficiency solely on the basis of diagnostic acylcarnitine profiles whereas Carpenter et al.,[21] Schulze et al.[25] and Zytkovicz et al[23] applied explicit criteria for the diagnosis of MCAD deficiency. In the UK retrospective study,[6] which used explicit criteria for diagnosis of MCAD deficiency, the authors reported that in most cases of MCAD deficiency, diagnosis was not based on clinical grounds but developed symptoms in early childhood.

Worldwide, there is an increasing trend to discharge mother and baby within the first day or two of life.[26] In this review, most dried blood spot samples obtained in prospective newborn screening studies were collected less than 72 hours after birth,[22-24;26] which is considerably earlier than in the UK, where neonatal screening samples are normally collected between six and 14 days of life.[2;3] The age at which screening is undertaken will affect the sensitivity and specificity of the screening process as concentrations of metabolites change over time. For example, in the UK, specimen collection is delayed until the sixth day of life or later in order to maximise sensitivity for the PKU screen.[3] The earlier collection of newborn blood spots in the UK might facilitate quicker and more clinically effective therapy. However, it might also influence test performance, underlining the need for a better infrastructure to support clinical follow-up, management and high quality clinical services for identified cases and their families.[2;14] Clayton and co-workers,[12] who reported their experience in diagnosing MCAD deficiency in a UK population concluded that if neonatal screening were undertaken at seven to ten days, the number of false positive and false negative results should be negligible.

The UK newborn screening programme for PKU is well established and there is universal agreement that neonatal screening for PKU is justified.[2;3;35;36] The mainstay of treatment for MCAD deficiency is a high carbohydrate intake, orally or intravenously during fasting and/or intercurrent illness.[37] The key concern regarding screening for this disorder is that the presentation varies widely with some individuals not presenting until they are adults, and an unknown number remaining undiagnosed or asymptomatic.[14] Potential consequences of diagnosis for this group include anxiety about the risk of hypoglycaemia during early childhood and adverse effects of clinically unwarranted treatment.[27] However, it has been suggested that such individuals are at as much risk as the symptomatic cases but are fortunate not to have encountered a sufficient metabolic stress to trigger a crisis. As a result, all babies with MCAD deficiency detected by newborn screening are at risk and treatment is required in all.[38]

Tandem MS equipment is now in use in at least five major centres in the UK, resulting in a centralised 'core' of knowledge and experience in this country.[8] This review suggests that tandem MS is highly sensitive and specific for detecting PKU and MCAD deficiency. The evidence base provides a basis for a review of clinical benefit and the economic attractiveness of using tandem MS for PKU and MCAD deficiency screening.

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Figure 1. Study flow chart

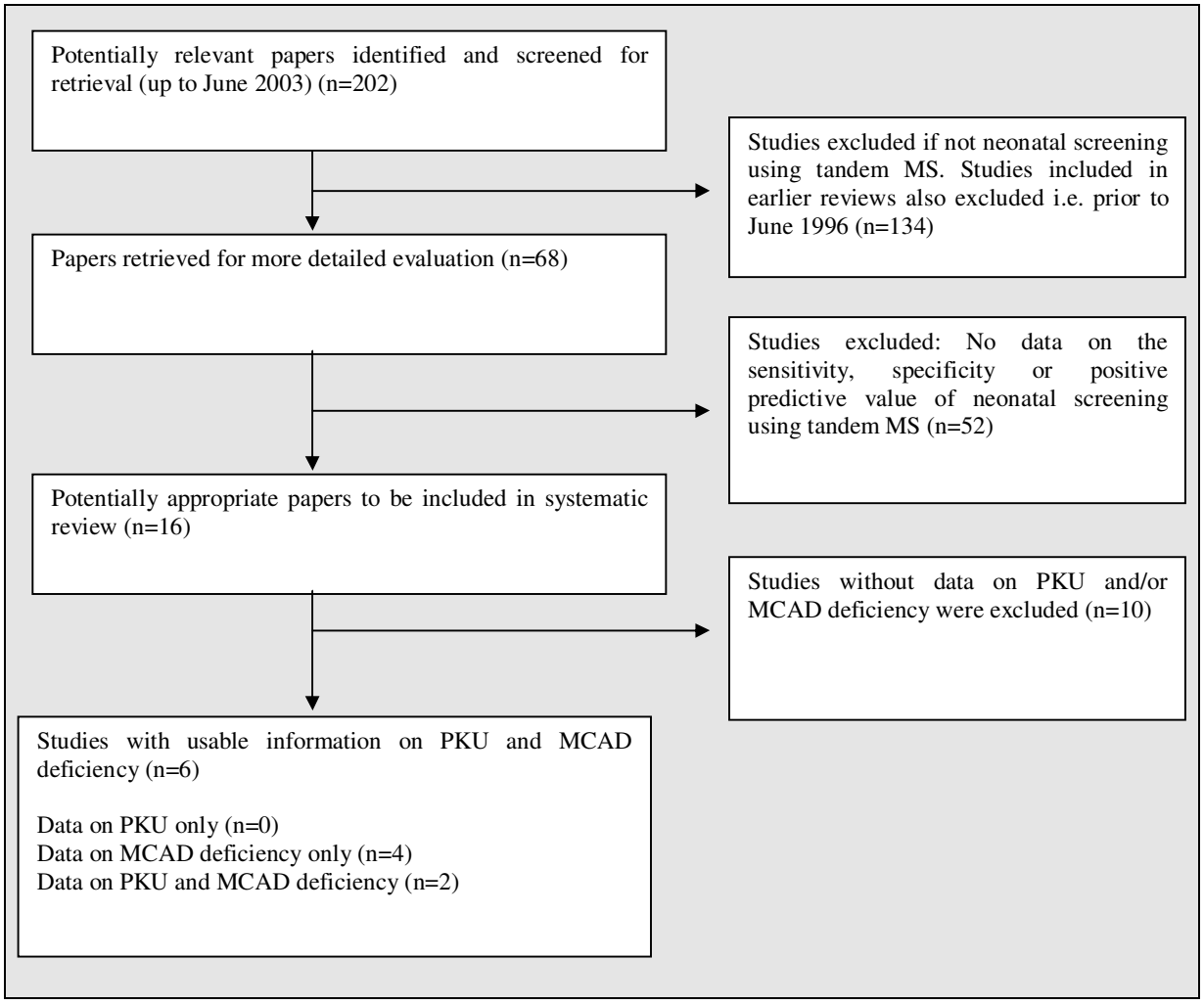

Table 1. Study and population characteristics

Study	Study type	Location	Population	Sample type and Age at sampling	Target condition(s)	Threshold for disease identification	Confirmatory test
Newborn screening programmes							
Carpenter et al. 2001[21]	Prospective cohort study	New South Wales Newborn Screening Programme, Australia	Consecutive neonates undergoing routine newborn screening (>99%) in New South Wales and Australian Capital Territory between April 1998 and March 2001. Ethnicity not reported	Analysis of acylcarnitines as their butyl esters from dried blood spot samples taken at 3 days (median). Over 99% of babies were sampled before day 6	MCAD deficiency	Octanoylcarnitine concentration ≥1 µmol/L	Polymerase chain reaction assay for 985A→G mutation, analysis of plasma, repeat blood spot acylcarnitines and urinary organic acids and fibroblast fatty acid oxidation Patients were diagnosed with MCAD deficiency if one or more of the following criteria were met: homozygous for 985A→G mutation, raised hexanoylglycine and suberylglycine in urine, increased hexanoylcarnitine, octanoylcarnitine or decenoylcarnitine in plasma; studies of fibroblast fatty acid oxidation rate or acylcarnitine studies
Chace et al. 1997[22]	Prospective cohort study	Pennsylvania & North Carolina Newborn Screening Program, USA	Newborn infants screened between September 1992 and January 1997. Ethnicity not reported	Analysis of acylcarnitines as their butyl esters from dried blood spot samples taken <72 hours after birth	MCAD deficiency	Octanoylcarnitine concentration ≥0.3 µmol/L	DNA analysis for 985A→G mutation and repeat blood spot analysis of octanoylcarnitine
Zytkovicz et al. 2001[23]	Prospective cohort study	New England Newborn Screening Program, USA	Newborn infants screened over a two-year period from February 1999. Ethnicity not reported	Analysis of acylcarnitines and amino acids as their butyl esters from dried blood spot samples taken between 1 and 3 days after birth	PKU and MCAD deficiency	*PKU* Phenylalanine concentration >139 µmol/L; phenylalanine to tyrosine ratio >1.5 *MCAD deficiency* Octanoylcarnitine concentration >0.5 µmol/L	Re-tested original samples and confirmation of disorders according to standard metabolic procedures. For MCAD deficiency, DNA analysis for 985A→G mutation and raised hexanoylglycine and suberylglycine in urine

Table 1. Study and population characteristics-*continued from previous page*

Study	Study type	Location	Participants	Sample type and Age at sampling	Target condition(s)	Threshold for disease identification	Confirmation test
Systematic screening studies (non-newborn screening programmes)							
Andresen et al. 2001[24]	Prospective cohort study	Pennsylvania, Ohio, New Jersey, Illinois, Florida, North Carolina, USA	Newborn infants born between December 1992 and January 2001. Ethnicity not reported	Analysis of butylated acylcarnitines from dried blood spot samples taken <72 hours after birth	MCAD deficiency	Mild profile defined as octanoylcarnitine concentration of 0.5 to 2.0 µmol/L with octanoylcarnitine to decanoylcarnitine ratio of 2 to 4; Severe profile defined as octanoylcarnitine concentration >2 µmol/L and octanoylcarnitine to decanoylcarnitine ratio of >4	Repeat analysis, repeat blood spot specimen (if possible) and mutations verified by DNA assay
Pourfarzam et al. 2001[6]	Retrospective cohort study	Northern region of the National Health Service, UK	Newborn infants born between January 1991 and July 1993. Ethnicity not reported	Analysis of butylated acylcarnitines from stored (4˚C for up to 4 years) dried blood spot samples. Age of sampling not reported	MCAD deficiency	Octanoylcarnitine concentration >0.3 µmol/L with octanoylcarnitine to hexanoylcarnitine ratio >4.0	Analysis of acylcarnitine in blood, organic acids in urine (suberylglycine, phenylpropionyl-glycine, and hexanoylglycine), free and total carnitine in plasma, molecular genetic test (DNA analysis for 985A→G mutation) and fibroblast fatty oxidation
Schulze et al. 2003[25]	Prospective cohort study	Baden-Wűrttemberg, Germany	Newborn infants born between April 1998 and September 2001. >98% white	Analysis of acylcarnitines and amino acids as their butyl esters from dried blood spot samples taken between 3 to 7 days (median 5 days)	PKU and MCAD deficiency	*PKU* Phenylalanine concentration >150 µmol/L; phenylalanine to tyrosine ratio >1.7 *MCAD deficiency* Hexanoylcarnitine concentration >0.21 µmol/L and /or octanoylcarnitine >0.32 µmol/L; decenoylcarnitine >0.28 µmol/L; decanoylcarnitine >0.48 µmol/L or molar ratio octanoylcarnitine to acetylcarnitine >0.02; octanoylcarnitine to decanoylcarnitine >1.6; octanoylcarnitine to dodecanoylcarnitine >1.6	PKU: phenylalanine >600 µM; non-PKU-hyperphenylalaninaemia, phenylalanine >150 and <600 µM. MCAD deficiency: enzyme activity in fibroblasts/ lymphocytes; mutational analysis; phenyl propionate loading; hexanoyl and suberylglycine in urine and enzyme residual activities.

Table 2. Effectiveness of neonatal screening for phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency using tandem MS

Disorder	Author	Total screened	True Positives	False positive (Specificity %)	False negatives (Sensitivity %)	Positive predictive value (%)
PKU	Schulze et al. 2003[25]	250,000	55*	115 (99.954)	4 (93.220)‡	32.353
	Zytkovicz et al. 2001[23]	257,000	18†	74 (99.971)	Not reported	19.565
MCAD	Carpenter et al. 2001[21]	275,653	12	11 (99.996)	Not reported	52.174
	Chace et al. 1997[22]	283,803	16	0 (100.000)	0 (100.000)	100.000
	Zytkovicz et al. 2001[23]	184,000	10	42 (99.977)	Not reported	19.231
	Andresen et al. 2001[24]	930,078	62	0 (100.000)	Not reported	100.000
	Pourfarzam et al. 2001[6]	100,600	8	0 (100.000)	0 (100.000)	100.000
	Schulze et al. 2003[25]	250,000	16	46 (99.982)	0 (100.000)	25.806

PKU, phenylketonuria; MCAD, medium-chain acyl-CoA dehydrogenase deficiency; HPA, hyperphenylalaninaemia
* 24 Classic PKU, 31 non-PKU-hyperphenylalaninaemia
† 7 Classic PKU, 11 non-PKU-hyperphenylalaninaemia
‡ All 4 false negative cases were non-PKU-hyperphenylalaninaemia