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METABOLIC SCREENING IN THE NEWBORN

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INTRODUCTION

The concept of metabolic screening for the recognition, diagnosis and treatment of inborn errors of metabolism has evolved as new methodology for detection and improved treatment have become available.¹ The diagnosis of metabolic disorders is challenging because of (1) the episodic nature of metabolic illness, (2) the wide range of clinical symptoms that are also associated with more common conditions, (3) the low incidence of these disorders, (4) the consequent lack of experience among the pediatric sub-specialties, and (5) the need for specialty testing. Although the incidence of each disorder is in the range of 1:10⁴ to 1:10⁷, there are thousands of known patients with metabolic disorders. It is probable that collectively, the total incidence exceeds 1:4000. Consequently they certainly account for significant morbidity and mortality in the newborn population.

Without doubt, the most opportune time to diagnose an inborn error of metabolism is at birth. Early recognition

and correct diagnosis enables appropriate treatment, without which tragic outcomes are all too common. Public awareness of metabolic diseases was all but unknown in the United States until 1964; at that time widespread neonatal testing was introduced for phenylketonuria (PKU), a disease resulting from lack of phenylalanine hydroxylase activity and affecting about 1:23,000 newborns. Since then, most states have expanded screening to a handful of additional diseases that fit the "PKU paradigm" – a treatable disease for which an inexpensive screening test is available and that has dire consequences if left untreated.² Currently, most states are screening for at least four disorders: PKU, congenital adrenal hyperplasia of the 21-hydroxylase type, galactosemia because of galactose-1-phosphate uridylyltransferase deficiency, and congenital hypothyroidism due to defects of thyroxine synthesis.

The case of PKU screening exemplifies the benefits of early diagnosis of a metabolic disease to patients, their families and society as a whole. The benefits of finding and treating these patients far outweigh the costs of screening the entire population.

Expanded newborn screening is a very recent development that utilizes tandem mass spectrometry (MS/MS) to screen for more than 20 inborn disorders of metabolism from a single blood spot.¹⁻³ This review explores the development and application of MS/MS as a clinical diagnostic testing method and its impact on newborn screening.^{2,4}

ACYLCARNITINES AND DISORDERS OF FATTY ACID AND AMINO ACID CATABOLISM

The driving force for applying MS/MS in clinical diagnostics was the need to analyze a class of compounds called the acylcarnitines which can accumulate from the defective catabolism of fatty acids and certain amino acids, especially leucine, isoleucine and valine.¹⁻³ These normal metabolic pathways are located in the mitochondria, and are mediated by coenzyme A (CoA) leading to metabolic end-products, such as acetyl-CoA. When there is a metabolic block, abnormal acyl-CoA species accumulate inside the

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mitochondria, and can only escape by biochemical transformation using alternate pathways. One of the most important detoxification pathways is an exchange reaction to form a corresponding acylcarnitine – a biochemical end-product that can cross mitochondrial membranes and exit the cell (Figure 1).

A patient with a defect of fatty acid oxidation typically develops symptoms after several hours of fasting, as may occur during an intercurrent illness. Reserves of glucose are exhausted and the cell switches to the fatty acid and gluconeogenic amino acid oxidative pathways as the primary energy sources. In a defect of fatty acid oxidation, abnormal metabolites can accumulate very rapidly and result in overwhelming cellular dysfunction – causing the symptoms of metabolic decompensation. Depending on the pathway affected, these symptoms can include vomiting, lethargy, respiratory distress, apnea, coma, cardiac arrhythmias, often accompanied by acidosis, ketosis, hypoglycemia and hyperammonemia. It is during such episodes that patients are at high risk for permanent neurological damage. A delay in emergency treatment of a few hours can be fatal. If intravenous glucose is administered on time, the symptoms and the biochemical abnormalities are rapidly ameliorated. The most common defect of fatty acid oxidation is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. It may present with Reye-like symptoms, or sudden death, yet there can be affected asymptomatic siblings within the family. Severe outcomes are entirely preventable by appropriate treatment.

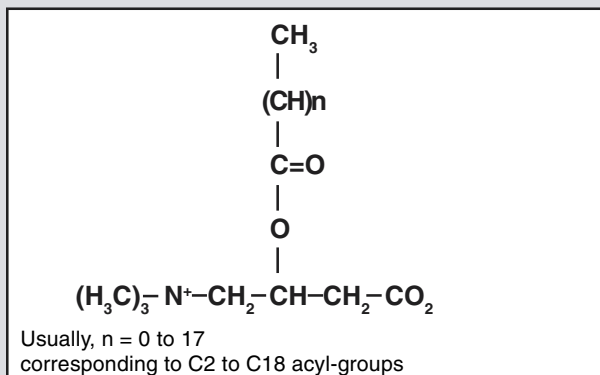
The acylcarnitines in blood reflect the primary accumulating mitochondrial acyl-CoA metabolites in

disorders of fatty acid and amino acid catabolism. Thus, an acylcarnitine “profile” will recognize almost all of the defects in these pathways. While older methods cannot detect acylcarnitines, these metabolites are readily amenable to MS/MS coupled with a “soft” ionization technique such as electrospray (ESI) or fast atom bombardment (FAB).^{1-3,5}

TANDEM MASS SPECTROMETRY AND THE ANALYSIS OF MIXTURES

The tandem mass spectrometer, MS/MS, usually consists of a pair of analytical quadrupole mass analyzers separated by a reaction chamber or collision cell. The triple quadrupole MS/MS is a modern system for analyzing complex mixtures. The mixture to be analyzed undergoes a “soft” ionization to create predominantly quasi-molecular ions, and is injected into the first quadrupole, which separates the molecular ions from each other. The ions then pass in order of mass/charge (m/z , ratio) into the reaction chamber or collision cell, where they are subjected to controlled fragmentation by collisions with an inert gas such as argon or helium. These fragments of the molecular ions then pass into the second analytical quadrupole where they are analyzed according to their m/z ratio. Electrospray ionisation is a ‘soft ionisation’ technique which enables the direct analysis of polar or high molecular weight biological substances like amino acids, acylcarnitines and proteins. These compounds can be detected and quantified directly from the solution without need to volatilize the sample. It offers excellent sensitivity (sub-picomole detection limits). Because separation of compounds in the mixture is by differences in mass spectral behavior instead of by column

Figure 1
Acylcarnitine



Structure of acylcarnitine intermediates in fatty acid oxidation inside the mitochondria. For example, in MCAD deficiency the accumulated acylcarnitine has a side chain containing 8 carbons, such that $n = 7$ as depicted here.

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chromatography, the entire process from sample injection and ionization to mixture analysis and data acquisition by computer takes only seconds.

The acylcarnitine “profile”, generated from a small amount of blood either spotted into filter paper or after coagulation as plasma or serum, can identify more than 20 metabolic defects of fatty acid oxidation and organic acid metabolism, including MCAD deficiency (Table 1). A specimen can be sent to a diagnostic facility by overnight courier and the MS/MS analysis be completed by lunchtime on the day of arrival. MCAD gives a clear diagnostic acylcarnitine pattern as compared with normal controls (Figure 2). This is also true for most of the other disorders of fatty acid and amino acid catabolism. Thus, acylcarnitine analysis has become a valuable front-line diagnostic test for these disorders.

TANDEM MASS SPECTROMETRY AND EXPANDED NEONATAL SCREENING

Five steps are critical to effective newborn screening: screening, follow-up, diagnosis, management, and evaluation.⁴ The following sections discuss the experience with each of these steps in respect to MS/MS newborn screening.

Screening. Table 1 summarizes 2 years of initial experience by the North Carolina State Laboratory of Public Health, when 237,774 babies were screened.

In accordance with other newborn screening programs, MCAD deficiency was detected with the highest

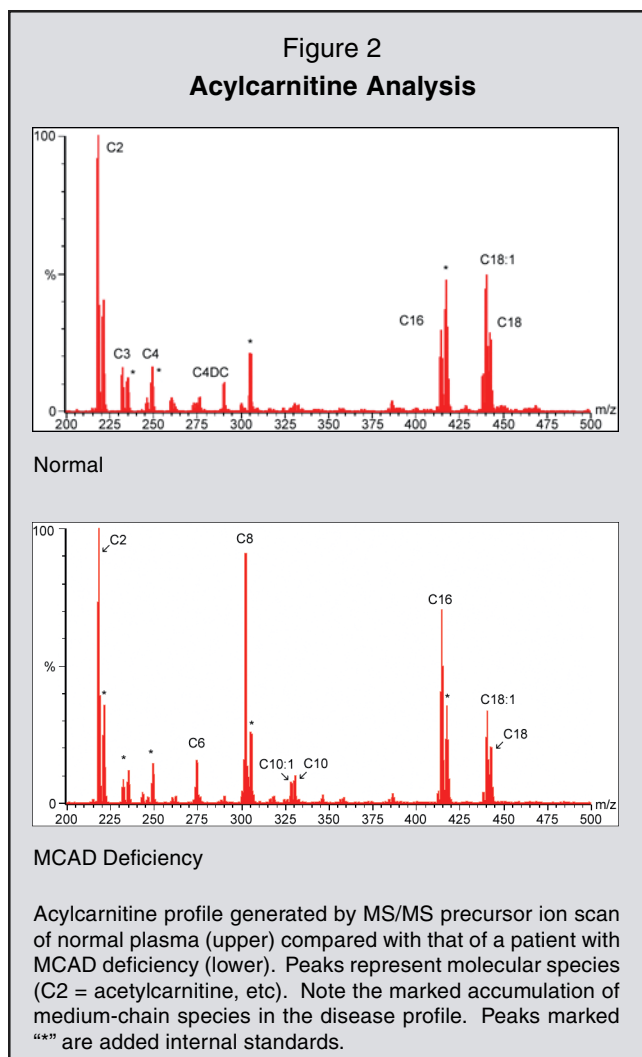


Table 1
Disorders of metabolism detected by MS/MS newborn screening (4/20/99 until 4/15/01)^{6,7}

Fatty acid oxidation	Organic acid metabolism	Amino acid metabolism
<ul style="list-style-type: none"> • MCAD (medium chain acyl-CoA dehydrogenase) deficiency (21) • VLCAD (very long chain acyl-CoA dehydrogenase) deficiency (1) • SCAD (short chain acyl-CoA dehydrogenase) deficiency (3) • GA (glutaric acidemia) type II* • CPT II (carnitine palmitoyl transferase II) Deficiency* • LCHAD/TFP (long chain 3-hydroxyacyl-CoA dehydrogenase) deficiency* 	<ul style="list-style-type: none"> • 3-MCC (3-methyl crotonyl-CoA carboxylase) deficiency (7) • Propionic acidemia (1) • Methylmalonic acidemia (2) • Glutaric acidemia, type I (1) • β-ketothiolase (SKAT or mitochondrial acetoacetyl-CoA thiolase) deficiency (1) • Isobutyryl-CoA dehydrogenase deficiency (1) • 2-methylbutyryl-CoA dehydrogenase deficiency (1) • Isovaleryl-CoA dehydrogenase deficiency (3) • Malonic Acidemia* 	<ul style="list-style-type: none"> • Phenylketonuria (14) • Argininosuccinic acid lyase deficiency* • Citrullinemia (1) • MSUD* (Maple Syrup Urine Disease)

*Cases of these disorders, reported by other screening programs, had not yet been detected in North Carolina. (n)= number of patients. Total number of neonates screened 237,774.

frequency. The incidence of MCAD deficiency was estimated at 1 in 13,600 live births in North Carolina. The overall incidence of disorders of metabolism detected by MS/MS newborn screening was 1 in 4,400 live births.

Beyond implications for the affected infant, newborn screening can have implications for maternal health. An association between the risk of serious complications of pregnancy, especially in the HELPP syndrome (hemolysis, elevated liver function tests and low platelets) with the occurrence of acute fatty liver of pregnancy in the mother and a fetus affected with LCHAD deficiency, was first established 10 years ago. Since then there has been a growing awareness that the presence of other fatty acid oxidation disorders, including MCAD deficiency, can also cause pregnancy complications.

Follow-up. Initial follow-up was directed according to cut-off values for each metabolite, typically set at 4 standard deviations above the mean. In the case of an abnormal value, repeat screening samples were requested. If the initial sample had a higher "alert" value, or if the second sample remained above the cutoff, the infant's local physician was contacted immediately. The possibility of a metabolic disorder was discussed and recommendations for follow-up were made. Infants were referred directly to a metabolic genetics center. If the elevated metabolite(s) did not signal a specific or life-threatening disorder, blood and urine samples were sent to the centers from the local physicians for follow-up testing.

The importance of appropriate cut-off values and adequate follow-up testing was illustrated by an infant with glutaric acidemia, type I (GA-I), initially detected on the basis of elevated glutarylcarnitine in the bloodspot.⁶ Initial cut-off values for each metabolite are typically set by a statistical determination of 4 standard deviations above the normal mean, but must be adjusted up or down for some metabolites based on experience during newborn screening. Although the patient had an abnormal blood acylcarnitine profile at birth, the repeat specimen was normal; thus, newborn screening ultimately failed to indicate the diagnosis of GA-I. Newborn screening is a powerful tool to potentially diagnose presymptomatic infants; however, it should not be considered a diagnostic test. In order to allow a precise diagnosis and treatment of GA-I, we recommend a complete evaluation, including both a plasma acylcarnitine profile and a urine organic acid analysis of any patient with elevated glutarylcarnitine in a blood spot acylcarnitine profile. The North Carolina State Laboratory has adjusted the cut-off value for glutarylcarnitine to increase the sensitivity of the newborn screening test for GA-I and this is now

suggested as a general recommendation for laboratories screening for GA-I by MS/MS.

Diagnosis. The diagnoses of fatty acid oxidation disorders is established by testing urine organic acids and a plasma acylcarnitine profile; whereas, the diagnoses of organic acid metabolism disorders is confirmed by plasma amino acids +/- urine organic acids. Enzyme analysis is required to diagnose disorders where the elevations of metabolites in blood and urine do not provide a conclusive diagnosis.

Since the addition of MS/MS to the North Carolina Newborn Screening Program, 20 infants with elevated hydroxyl-isovalerylcarnitine (C5OH) levels were evaluated. Eight of these 20 infants had persistent elevations of both 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in their urine, highly suggestive of 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. Other enzyme deficiencies that could provoke elevated C5OH, including biotinidase and holocarboxylase synthetase deficiency, were eliminated from the differential diagnosis by confirmatory enzyme testing. In 4 of the remaining 12 infants, maternal 3-MCC deficiency was demonstrated. It is likely that the remaining 8 of these 12 infants for whom urine organic acids normalized also represented maternal 3-MCC deficiency; however, follow-up testing was not requested from the mother or she refused to provide her samples in each case. Infants and mothers with 3-MCC deficiency commonly have clinically significant carnitine deficiency, which motivated the detection and treatment of these individuals.

Management. The prompt referral of patients with confirmed or suspected life-threatening disorders of metabolism is critical to fulfill the mission of newborn screening. The successful treatment of inborn errors of metabolism provides justification for MS/MS newborn screening. For example, untreated MCAD deficiency presents as hypoketotic hypoglycemia and is commonly lethal, due to hepatic failure which often mimics Reye syndrome. Since the initiation of MS/MS newborn screening, there have been no deaths among confirmed MCAD deficiency and no cases of missed MCAD deficiency. Treatment consisted of early referral to a metabolic-genetics center, avoidance of fasting, L-carnitine supplementation, and prohibition of formulas containing medium-chain triglyceride (MCT oil). Likewise, nutritional and pharmacologic treatment is available for other disorders detected by MS/MS.

However, the treatment of other potentially detectable disorders of metabolism has been less than optimal, related to issues of detection or delays in detection. While tyrosinemia, type 1, can be effectively treated with

a life-saving enzyme inhibitor, tyrosine levels are not elevated during the newborn period to allow detection of that disorder. More frustrating has been the ineffectiveness of treatment in disorders with severe complications early in life, including glutaric acidemia, type II (GA-II) and maple syrup urine disease (MSUD). GA-II cannot be effectively treated when the presentation is severe, and MSUD can only be effectively treated when a formula lacking branched-chain amino acids is used prior to the onset of symptoms which usually occurs in the first 10 days of life. Although treatment is available for GA-I, MSUD and tyrosinemia, type I, these disorders are quite rare outside selected population isolates (eg. MSUD among the Amish). Consequently, aggressive, earlier detection by more specialized approaches to newborn screening is not practiced.

Evaluation. Newborn screening programs require periodic review and analysis of outcome measures to be successful. Adjustment of cut-off values is one important exercise in MS/MS newborn screening, since the cut-off values determine the likelihood of false positive or false negative results.⁷ False negative results should be assiduously avoided. False positive results can hamstring a program. Specific causes of false positives are listed in Table 2.

Ratios of metabolites are helpful in the interpretation of elevations unrelated to a metabolic disorder, such as the ratio of C8:C10, which is elevated in MCAD deficiency but not in MCT oil supplementation. Age-specific cut-off values could potentially reduce the frequency of false positive results because the majority of spurious elevations are related to prematurity.⁷ Until age-specific cut-off values are available, the newborn screening laboratory typically obtains serial specimens from premature infants until the postconceptual age approaches 40 weeks.

The effectiveness of modifying cut-off values was illustrated by the experience with C5OH. The initial cut-off for C5OH was determined statistically (4 standard

deviations above the mean); the cut-off was increased when the false positive rate was determined to be unacceptably high. Thereafter, the cut-off for C5OH was increased to 5 standard deviations. This adjustment of cut-off values for normal samples has reduced the number of initially elevated samples from 1 in 720 to 1 in 7,400 infants screened, and dramatically reduced the ratio of falsely positive initial screens to a truly positive test in affected infants from 65 to 1 to 3.3 to 1. There was no reduction in the rate of 3-MCC detection observed after the cut-off for C5OH was increased, and no infants with symptomatic 3-MCC deficiency have come to the attention of the North Carolina medical community since the MS/MS screening began.

CONCLUSION

The difference in newborn screening brought about by MS/MS is the ability to detect more than 20 inborn disorders of metabolism from a single blood dot with a single test. The method detects a confirmed disorder in about 1 in 4,000 cases screened. The most common diseases are MCAD deficiency, PKU, and 3-MCC deficiency. Early diagnosis and treatment of these cases is preventing adverse outcomes, and screening programs are reporting a very low incidence of false positives and false negatives. About half of the states are either screening newborns by MS/MS or have made a decision to do so soon. Even so, there is controversy and debate regarding what is perceived to be a paradigm shift, since the testing equipment is expensive and some of the disorders it detects have no effective treatment. However, once a state decides to implement this method it must accept the responsibility of performing the test properly and of treating diagnosed patients. To do so means providing adequate professional support to include dietitians, genetic counselors, biochemical geneticists and appropriate mechanisms in place for follow-up testing. Pediatric Endocrinologists are often called to consult with infants with emergencies due to inborn errors of metabolism, a good review of the subject should be kept at hand.⁸

Table 2

Causes for false positive results in MS/MS newborn screening

Condition	Metabolites affected	False positive
MCT oil supplementation	C8, C10	MCAD deficiency
Prematurity	C4, C5, C8	GA-II & MCAD deficiency
Prematurity	Tyrosine	Tyrosinemia
Carnitine supplementaion	C0, C2, C3, (+ others)	Propionic acidemia & others

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Letter to the Editor:

Dear Dr. Blizzard and Editors of GGH:

I've been reading GGH for years, and have found it so useful. This month's timely release of the intersex review really "hit the mark". I work in a state birth-defects surveillance department. The non-physicians have expressed tremendous interest in the management of ambiguous genitalia, either as an isolated finding, or related to exstrophy. This review will serve as the focal point for our next monthly teaching session to be supplemented by your review (*GGH* Vol. 19, No. 1).

Angela E. Lin, MD
Brigham-Women's Hospital
MA Center Birth Defects Prevention

Dear Dr. Linn: Thank you!

Dear Other Readers:

Please let us know your positive and negative comments – and your agreements or disagreements regarding the abstracts and their comments and the lead articles. Your input is absolutely necessary for us to maintain, upgrade, and disseminate your agreements and disagreements. We encourage you to respond quickly after your thoughts and criticisms come to mind.

Robert M. Blizzard, MD

Abstracts from the Literature

Factors Determining the Pattern of the Variant Creutzfeldt-Jakob Disease (vCJD) Epidemic in Great Britain

Editorial Preface: Growth hormone (GH) extracted from human pituitaries obtained at autopsy was first given to children in 1958. Twenty-seven years later (1985), the first cases of Creutzfeldt Jakob Disease (CJD) resulting from such injections were observed in individuals who had received GH injections 8 to 10 years prior to that time. The fact that no cases of CJD were reported reflects the long latent period between exposure and the onset of symptomatic disease.

The exact number of the pituitary injections that may have been contaminated with the CJD prion is unknown. GH from only one of three laboratories in the U.S. extracting pituitaries has been associated with CJD. All three of the laboratories extracting GH used different procedural techniques. In retrospect, the GH extraction procedure of two of the three laboratories eliminated the active prion from the final product. From 1985 until April 2003 only 26 cases of CJD were recorded among several thousand (7,700) recipients in the U.S. who had received native human growth hormone. All U.S. patients with CJD received GH prior to 1977; afterward a new purification step was added to the GH extraction procedure.

The early symptoms of CJD consist of degenerative neurological function. Death unfortunately follows within a period of 6 to 36 months. The number of catastrophes to date in the United States have been relatively small, particularly in light of the number anticipated in 1985 when the first two deaths were reported within a month of each other. Postulation, with reasonable justification, was that the incubation period and susceptibility to the disease were influenced by the dose of contaminated material, possibly the age of the recipient, and possibly by an individual's genetic susceptibility. The latter was suspected on the basis of a few studies using scrapie disease in sheep as a prototype since CJD, occurring primarily in humans, is similar to scrapie disease in sheep. These diseases produce degenerative neurological alterations; although the histology of the pathological findings in the central nervous system are different. They are known as spongiform cerebral encephalopathies.

Abstract: In 1985 and 1986 a similar but different spongiform encephalopathy manifested itself in England when humans were first diagnosed with "mad cow