Tandem mass spectrometry in newborn screening

American College of Medical Genetics/American Society of Human Genetics Test and Technology Transfer Committee Working Group

Tandem mass spectrometry (MS/MS) has been used for several years to identify and measure carnitine esters in blood and urine of children suspected of having inborn errors of metabolism. Indeed, acylcarnitine analysis is a better diagnostic test for disorders of fatty acid oxidation than organic acid analysis because it can often detect these conditions when the patient is not acutely ill.[1] More recently, MS/MS has been used in pilot programs to screen newborns for these conditions and for disorders of amino and organic acid metabolism as well. The purpose of this article is to describe MS/MS and discuss its potential role in newborn screening programs.

The mass spectrometer is a device that separates and quantifies *ions* based on their mass/charge (m/z) ratios. In gas chromatography-mass spectrometry of organic acids, for example, organic acid derivatives are first subjected to gas chromatography and then enter the mass spectrometer, where each is ionized and fragmented and the abundance and m/z ratio of the various fragment ions are determined.

The modern tandem mass spectrometer usually consists of two quadrupole mass spectrometers separated by a reaction chamber or collision cell; the latter is often another quadrupole. The mixture to be analyzed is subjected to a *soft* ionization procedure (e.g., fast atom bombardment or electrospray) to create quasimolecular ions, and is injected into the first quadrupole, which separates these *parent ions* from each other. These ions then pass (in order of m/z ratio) into the reaction chamber, where they are fragmented; the m/z ratios of the fragments are then analyzed in the second quadrupole. Because separation of compounds in the mixture is by mass spectrometry instead of chromatography, the entire process, from ionization and sample injection to data acquisition by computer, takes only seconds.

The computer data can be analyzed in several ways. One can use a *parent ion* mode to obtain an array of all parent ions that fragment to produce a particular daughter ion, or a *neutral loss* mode to obtain an array of all parent ions that lose a common neutral fragment. Further, these *scan functions* can be changed many times during analysis, so that one can detect and measure butyl esters of acylcarnitines (by the signature ion at m/z 85) and the butyl esters of α-amino acids (by loss of a neutral 102 fragment) in the same sample.

MS/MS thus permits very rapid, sensitive and, with appropriate internal standards, accurate measurement of many different types of metabolites with minimal sample preparation and without prior chromatographic separation. Because many amino acidemias, organic acidemias, and disorders of fatty acid oxidation can be detected in 1 to 2 minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs. Some conditions that can be diagnosed by MS/MS are listed in Table 1, together with the compound(s) on which diagnosis is based.

Amino acid quantitation by MS/MS is more accurate than most methods now in use for newborn screening and would thus provide more specific and sensitive screening for phenylketonuria,[2] maple syrup urine disease,[3] and homocystinuria.[4] Analysis by MS/MS would also permit the screening menu to be expanded to include a number of disorders that are not currently covered (Table 1).[5–6] Among these are medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and glutaric acidemia type I (GA1), which are relatively common and difficult to detect before the onset of symptoms and whose outcome is substantially improved by early treatment.

Infants with MCAD deficiency seem healthy in early infancy but develop episodes of hypoketotic hypoglycemia during the first years of life; the first episode is fatal in 30% to 50% of patients. Most of these deaths could be prevented if dietary treatment and measures to prevent fasting were begun before the onset of symptoms. Infants with GA1 develop normally until they suddenly develop acute encephalopathy and irreversible striatal damage during the first 2 to 3 years of life. There is increasing evidence that striatal damage can usually be prevented by L-carnitine and vigorous treatment of catabolic episodes if begun before the onset of symptoms.

This guideline is designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed toward obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

Table 1
Some disorders detectable by tandem mass spectrometry

Disorder	Diagnostic metabolite
Amino acidemias	
Phenylketonuria	Phenylalanine & tyrosine
Maple syrup urine disease	Leucine + isoleucine
Homocystinuria (CBS deficiency)	Methionine
Citrullinemia	Citrulline
Hepatorenal tyrosinemia	Methionine & tyrosine
Organic acidemias	
Propionic acidemia	C_3 acylcarnitine
Methylmalonic acidemia(s)	C_3 acylcarnitine
Isovaleric acidemia	Isovalerylcarnitine
Isolated 3-methylcrotonylglycinemia	3-Hydroxyisovalerylcarnitine
Glutaric acidemia (type I)	Glutarylcarnitine
Hydroxymethylglutaric acidemia	Hydroxymethylglutarylcarnitine
Fatty acid oxidation disorders	
SCAD deficiency	$C_{4,6}$ acylcarnitines
MCAD deficiency	$C_{8,10:1}$ acylcarnitines
VLCAD deficiency	$C_{14,14:1,16,18}$ acylcarnitines
LCHAD and trifunctional protein deficiency	$C_{14,14:1,16,18}$ acyl- and 3-hydroxy acylcarnitines
Glutaric acidemia type II	Glutarylcarnitine
CPT-II deficiency	$C_{14,14:1,16,16:1}$ acylcarnitines

It is important to note that MS/MS cannot replace current programs to screen for biotinidase deficiency, hypothyroidism, hemoglobinopathies, virilizing adrenal hyperplasia, and galactosemia; these conditions cannot be identified by MS/MS at this time and must be detected by other means.

Several issues must be considered before MS/MS is added to ongoing newborn screening programs. The instrument itself, including the computer and autosampler, is expensive, access to alternate instruments is imperative in the event of breakdown, and laboratory personnel must be trained extensively to operate and maintain it. Nonetheless, if the cost of instrumentation is amortized over several years, MS/MS probably can be added to existing newborn screening systems for an incremental cost on the order of $10 per sample. It is important to note that the cost of screening itself would be the same regardless of the number of tests added to the screening menu. Costs for other screening components, however, e.g., patient retrieval, verification of diagnosis, treatment, etc., would vary.

The inclusion of additional disorders in the newborn screening menu could increase the number of patients identified each year by 50% to 100%, and more physicians, nutritionists, and genetic counselors will be needed to deal with their ongoing medical and nutritional care. Reimbursement for the medical foods needed to treat these disorders must also be addressed, because many third-party payers do not cover medical foods, and state laws and regulations regarding reimbursement vary.

It has been argued that MS/MS analysis should not be used in newborn screening until more is known about its sensitivity (false negatives) and specificity (false positives) for each of the diagnosable disorders. Extensive experience with MS/MS, albeit mostly with patients outside of the immediate newborn period, has shown that the number of false positives is very small. As was the case with all current screening methods, the number of false negatives will only be learned after newborn screening is implemented, and children that are not detected as newborns are diagnosed later in life. Thus, as with all newborn screening methods, screening should be accompanied by follow-up sufficient to ensure that data on false negatives and false positives is collected. These considerations argue for pilot demonstration programs with adequate resources to acquire and report technical and clinical results.

Many conditions that can be detected by MS/MS, such as citrullinemia, propionic acidemia, and methylmalonic acidemia, do not respond consistently to treatment. Nonetheless, some patients do better with early diagnosis and treatment, and early diagnosis can avoid trauma and expense to the family and allow options for family planning to be considered before other affected siblings are born.

The issue of informed consent for MS/MS screening is complicated, in part because uniformly effective therapies have not been developed for all the conditions the methodology can detect and because it may detect previously unrecognized metabolites and/or disorders. An example is the detection of asymptomatic maternal 3-methylcrotonyl-CoA carboxylase deficiency by acylcarnitine screening of newborn blood spots.[7] However, the computer parameters of the MS/MS can be set to ignore certain molecular ions if a decision is made not to screen for a particular disorder.

In summary, MS/MS can provide substantial benefits to patients and their families if thoughtfully integrated into newborn screening programs, provided that sufficient funding is made available to cover the costs of the additional and necessary personnel, medications, and medical foods. Indeed, the expense and complexity of the instrumentation and the need for trained metabolic physicians to care for the additional patients could make it very difficult for states with small populations and/or few trained personnel to implement MS/MS, and development of regional laboratories and services may well be necessary to address this need.

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