Newborn Screening by Tandem Mass Spectrometry (MS-MS)

Newborn screening for selected inherited metabolic disorders is a well-established public health measure in most developed countries [1]. The purpose is to identify and properly diagnose individuals in the neonatal period to enable early medical intervention to prevent or significantly reduce clinical symptoms such as mental retardation. Some of the basic criteria for determining which inherited disorders are suitable for newborn screening include: (a) the disorder has a relatively high incidence so that the cost per diagnosed individual is reasonable, (b) an effective and not overly expensive medical treatment is available, (c) a relatively inexpensive screening test is available that is suitable for high volume testing (i.e., preferably automatable), and (d) the screening test has a very high sensitivity (i.e., a very low rate of false negatives) and high specificity (i.e., a low rate of false positives, all of which require expensive follow-up).

The prototype for newborn screening based on these criteria is that for phenylketonuria (PKU), developed >30 years ago and now used for screening >10 million infants a year worldwide [2]. PKU is a relatively common inherited disorder, with a frequency of ~1 in 12,000. About 97% of patients with PKU have hyperphenylalaninemia due to an inherited deficiency of hepatic phenylalanine hydroxylase, which metabolizes the amino acid phenylalanine to tyrosine. Because phenylalanine is an essential amino acid derived entirely from the diet, treatment by restriction of phenylalanine intake with special formulas and diets makes it possible to lower plasma phenylalanine concentrations sufficiently to prevent mental retardation and to prevent damage to the fetus in maternal PKU [2]. The initial technique for PKU newborn screening involved obtaining blood just before discharge from the hospital by heelstick with the blood absorbed on filter paper, dried, and sent at room temperature to screening laboratories. The approximate phenylalanine concentration in discs cut from the dried blood spots was determined by the Guthrie bacterial inhibition assay, in which phenylalanine overcomes the inhibition of bacterial growth so that the diameter of the bacterial growth around the spot is proportional to the concentration of phenylalanine in the blood. This method has been replaced in many centers by a fluorometric assay for phenylalanine. Effective infrastructures have been established by each state to ensure that the screening is comprehensive and that proper follow-up of positives will both identify false positives and lead to appropriate treatment of the diagnosed PKU patients.

In addition to PKU, newborn screening for galactosemia due to a deficiency of galactose-1-phosphate uridylyltransferase is performed by most states, which either use a direct bacterial inhibition assay for galactose or measure the activity of the enzyme and also screen for hypothyroidism (largely nongenetic) [1]. Bacterial inhibition assays were established to determine the amino acid leucine for the neonatal screening for maple syrup urine disease due to a deficiency of branched chain a-ketoacid dehydrogenase and also the amino acid methionine for the neonatal screening for homocystinuria due to a deficiency of cystathionine-β-synthase [1]. These bacterial inhibition assays are still used by the ~20 states to screen for maple syrup urine disease and homocystinuria. Maple syrup urine disease is relatively rare, with a frequency of ~1 in 185,000 [3]. Treatment with special diets to restrict the intake of the essential branched chain amino acids leucine, isoleucine, and valine significantly improves the poor prognosis of neurological deterioration and death in the untreated severe classic forms of maple syrup urine disease. Homocystinuria is even rarer, with a frequency between 1 in 200,000 and 1 in 335,000 [4]. About half of the affected patients respond to treatment with pyridoxine, and nonresponders benefit from a diet restricted in methionine.

Despite the benefits of newborn screening in reducing the morbidity, mortality, or mental retardation for the above disorders, newborn screening remains restricted to these disorders and to small programs for a few other inherited disorders, so that most states screen for only three to five disorders. Among the reasons for this is that the frequency of many genetic disorders is unknown but is thought to be very low, given an incomplete knowledge of the number of diagnosed cases in the literature (which almost certainly underestimates the frequency). Inclusion of more disorders in newborn screening would add to the cost because until now a different test (with its added costs) had to be performed for each disorder. Also, the limited amount of blood and blood spots available for screening limits the number of disorders that can be screened when each disorder requires a blood spot for different assay techniques. Another significant increase in cost for additional disorders is the expense of the added follow-up of positive screening results, where the false-positive rate can be as high as 0.1%. In addition, treatments for many genetic disorders have not been as effective as those for PKU. However, the recent availability of commercial formulas for several additional disorders involving amino acid catabolism (the organic acidurias) and the use of detoxifying agents such as carnitine are improving clinical management. Finally, the state screening programs have been fairly conservative in applying technological advances for newborn screening. Changes in healthcare, especially the trend toward earlier discharge after delivery, shorten the time available for the concentrations of amino acids in blood of affected patients to rise as they normally do with protein intake over the first days postpartum [2]. For comprehensive screening, it is best to obtain the newborn blood spots at the time of discharge, but early discharge (as soon as 12 h instead of days after birth) is placing higher demands on the accuracy and sensitivity of the tests, which must detect smaller increases.

A significant technological advance for accurately measuring amino acids and other compounds of importance in diagnosing a larger group of genetic metabolic disorders has occurred recently that is likely to have a very significant impact on the sensitivity, specificity, and scope of newborn screening: tandem mass spectrometry (MS-MS) with stable isotopically labeled internal standards [5]. Stable isotope dilution MS coupled with a separation technique to isolate the compounds of interest is considered a definitive method for quantifying analytes with specificity, sensitivity, and accuracy. MS-MS, a further advance, greatly reduces sample preparation requirements and eliminates time-consuming separation techniques before mass spectrometry by separating closely as within the MS-MS, giving very high specificity even with complex mixtures. Changing the mass spectrometer settings by computer control enables the precise analysis of multiple analytes of different chemical classes in minutes, greatly shortening the time of the analysis. The basic principle is that the analytes are ionized by a soft ionization technique—static liquid secondary ionization (LS), fast atom
bombardment, or electrospray—that minimizes fragmentation of the molecules. The molecular ions are selected in the first mass spectrometer quadrupole and undergo collision-induced fragmentation in a second quadrupole, after which the daughter ions are selected in the second mass spectrometer (third quadrupole). By selecting appropriate scanning functions, specific daughter ions of a specific molecular ion are detected and quantified.

In a series of articles in Clinical Chemistry from 1993 to this issue, Chace and Millington and their colleagues have developed extremely accurate methods for the newborn screening of PKU and tyrosinemia [6], maple syrup urine disease [7] and homocystinuria and other hypermethioninemias [8]. Semiautomated methods are used to extract the amino acids from dried blood spots with added stable isotopically labeled internal standards and to form butyl esters (60 specimens in ~2.5 h). Using LS MS-MS, most α-amino acids are quantified by scanning the daughter ions at a mass difference of 102 (loss of butyloximate) from the simultaneous parent ion scan. Very high analytical specificity is obtained because the loss of mass 102 appears to be unique to alpha amino acids. The methods are very sensitive, with limits of detection well below the normal blood concentrations of the amino acids. The recoveries of the amino acids are very good, and the precision is excellent (CVs = 5–10%). The specificities are much better than current screening methods and are enhanced by the use of ratios of concentrations of amino acids (e.g., the ratio of phenylalanine to tyrosine for PKU). Moreover, most false-positive blood spots by fluorescent or bacterial inhibition methods are not positive by MS-MS.

An added capability of MS-MS is that analytes other than amino acids can be determined in the same derivatized sample by using different scan functions. Increased concentrations of acylcarnitines, which are diagnostic for seven inherited fatty acid oxidation disorders and seven organic acidurias, can be detected by scanning the parent ions and detecting a common single daughter ion at m/z 85 [5]. This technique has been used to screen for medium-chain acyl-CoA dehydrogenase deficiency, a relatively frequent fatty acid oxidation disorder that can be fatal in infancy [9]; screening 80,371 neonates in Pennsylvania by this method gave a frequency of 1 in 8930 for this disorder. The combined experience of Naylor in Pennsylvania and Chace in North Carolina in screening 168,793 newborns for amino acid, organic acid, and fatty acid oxidation disorders by MS-MS was presented recently (September 1995) at the 33rd Symposium of the Society for the Study of Inborn Errors of Metabolism (unpublished). Frequent disorders were classical PKU (12 patients), hyperphenylalaninemia (7 patients), and medium-chain acyl-CoA dehydrogenase deficiency (12 patients). Additional disorders were maple syrup urine disease (3), glutaric acidemia type I (4), 3-methylcrotonyl-CoA carboxylase deficiency (4), propionic acidemia (2), methylmalonic acidemia (1), and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (1). Because of the large number of disorders detected by this single technique, 49 affected patients were detected, yielding an aggregate frequency of 1 in 3445.

These results suggest that widespread use of MS-MS for newborn screening would be efficient in detecting a large number of amino acid, organic acid, and fatty acid oxidation disorders. The cost of follow-up of false positives for the diseases that are screened would be decreased because of the high specificity of MS-MS, and the added follow-up cost for additional disorders would not be large because of the very low rate of false positives. Although the capital cost of the instrumentation for MS-MS is high, it is decreasing as new instruments are developed. The extraction, derivatization, and introduction of the samples into the mass spectrometer can be largely automated, as can the computer analysis of the results. With these continued developments and the expansion of the number of inherited disorders that can be screened, the cost per patient diagnosed by MS-MS should be very reasonable.

References

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