Newborn Screening for Lysosomal Storage Disorders

The concept of screening newborns for inherited metabolic disorders was the brainchild of Robert Guthrie, an upstate New York microbiologist with a passion to prevent the devastating and irreversible neurologic damage sustained by victims of untreated phenylketonuria (PKU). The solution he developed was a simple and inexpensive bacterial inhibition assay for phenylalanine in blood *(1)*. He also invented a unique method of specimen collection, in which peripheral blood was collected from a newborn's pricked heel onto a special cotton fiber filter-paper known as a "PKU card" or "Guthrie card". After the blood had dried, the specimen was mailed to a laboratory that would identify any child at risk for PKU from a concentration of phenylalanine above an age-matched control limit. Further diagnostic testing was then required to determine whether the disease was present. Treating affected children with a phenylalanine-depleted diet, started within the first month of life, was effective in preventing mental retardation. Unable to persuade the state of New York to conduct newborn screening with the test, Guthrie convinced Massachusetts that the cost of screening the entire population of newborns and treating affected children with the special diet was less than the cost to society of untreated PKU cases. Thus, in 1963 began state-mandated newborn screening for PKU *(2)*. The test was gradually adopted by other states and eventually by countries all over the world.

The success of PKU screening in dried blood spots (DBS), which identifies ~200 new cases annually in the United States alone, prompted the addition of tests for other disorders that fit the PKU paradigm. By the mid-1990s, most newborn screening programs were screening for only three to six metabolic disorders, and many were also screening for hemoglobinopathies. By that time, a new test based on tandem mass spectrometry (MS/MS) was shown to be effective for the identification of up to 20 disorders in several different metabolic pathways [e.g., see Refs. *(3–6)* from this Journal]. The range of detectable disorders includes medium-chain acyl-CoA dehydrogenase deficiency (MCAD), one of several related fatty acid oxidation disorders. The basic method was developed by a team at Duke University in collaboration with the North Carolina Newborn Screening Laboratory *(7)*. Subsequently, the method was automated *(8)* and brought into limited private and public service in several locations, including Australia, South America, Saudi Arabia, Bavaria, Pennsylvania, North Carolina, and New England.

The fact that the tandem mass spectrometer is expensive and that MS/MS can recognize some disorders that are arguably unresponsive to treatment is troublesome to many in the field of newborn screening. Nevertheless, most of the United States and many other countries have expanded or are in the process of expanding their newborn screening programs by adding MS/MS. The incidence of individual metabolic disorders ranges from ~1 in 10^4 to <1 in 10^6, and the experience of programs reporting results is that the overall incidence of disease detection by MS/MS is ~1 in 4.5×10^4, which represents a significant increase in diagnosed cases *(9)*.

Having just attained a reasonably broad-based level of acceptance, the new paradigm of expanded newborn screening using MS/MS is already under pressure to add yet more conditions, chief among them being the lysosomal storage disorders (LSDs). This group of more than 40 conditions has an overall incidence in the United States of ~1 in 4×10^4. The fact that new treatments, including bone marrow transplantation and enzyme replacement therapy, are available or are becoming available for several of them suggests that many children could benefit from early diagnosis and intervention *(10)*. Biochemical testing is of limited value for this group of disorders because of a lack of both general and specific biomarkers in the blood and the chemical heterogeneity of the storage material *(11)*.

By adapting standard enzymology methods to a reduced scale, Chamoles et al. *(12)* could determine α-L-iduronidase activity in DBS and thus identify patients with mucopolysaccharidosis type I (MPS-I), representing all three clinical subtypes of Hurler, Scheie, and Hurler–Scheie syndromes. Subsequent reports showed detection of several additional LSDs in DBS by this method, including Pompe, Fabry, Gaucher, Sandhoff, Nieman–Pick, and Tay–Sachs disease *(13–16)*. One limitation of the technology is that it does not readily lend itself to multiplexing.

In the meantime, a MS/MS-based method for determination of enzyme activities in cell lysates was developed by Gerber et al. *(17)*. An adaptation of this method enabled direct analysis of galactocerebroside β-galactosidase activity in DBS for the diagnosis of Krabbe disease *(18)*. The elegance of this method is that a synthetic enzyme substrate is exposed to the activity, and the rate of appearance of the reaction product is accurately quantified by MS/MS using an isotope-labeled or closely related structural homolog as an internal standard. In a landmark study, this group developed a multiplex MS/MS-based assay that can detect at least five different enzyme activities in a single specimen *(19)*. In principle, the extract from a 5-mm disk cut from a single DBS is divided into five portions, each of which is incubated for 24 h with a cocktail of reagents specific to a particular enzyme activity. The solutions are then combined and purified by a combination of solvent–solvent and solid-phase extraction. Final concentrations of the enzymatic products can be determined by electrospray ionization MS/MS in a single analysis because each enzyme of interest is targeted with a substrate that yields a product having a different mass. All steps are performed in 96-well microtiter plates, the preferred platform for high-throughput screening.

In this issue of *Clinical Chemistry*, Wang et al. *(20)* describe a MS/MS method for detection of MPS-I in DBS. The authors claim that their method is now capable of measuring the activities of at least six lysosomal enzymes

on a single platform. Moreover, the compatibility of the assay with microtiter plates and multichannel (or robotic) pipetting techniques is attractive for high-throughput screening. It seems that we are poised on the brink of another expansion of newborn screening into the arena of LSDs, and the group state that pilot studies are being initiated to develop this method for full-scale neonatal screening. This is a truly exciting prospect to those having the interests of MPS patients at heart.

Considerable challenges remain to be overcome before the wholesale adoption of this method. In addition to the complexity of the reagents and the lack of commercial availability of the enzyme substrates and internal standards, the method involves numerous steps, several of which have not previously been performed in newborn screening laboratories. From an organizational standpoint, the time frame to complete these assays is considerably longer than that of other newborn screening methods and could delay reporting of results by at least 24 h. Moreover, it is not practicable simply to add these new assays to the MS/MS platforms in newborn screening programs fortunate enough to have them already in place; they will require additional MS/MS systems, with the associated costs and logistic problems.

The history of developments in newborn screening suggests that it could take several years to fully realize the potential of this new method. For example, from the first report of the potential of MS/MS to screen for inborn errors of fatty acid and amino acid catabolism *(7)* to the first report of a full-scale pilot program in a state newborn screening laboratory *(9)* took more than 10 years. A circumscribed prospective pilot study ideally should target a limited number of conditions, preferably those that manifest in early infancy, such as Pompe disease and Hurler syndrome, and for which an effective therapy is currently available. Such a pilot program should also involve more than one center. This effort will require the assistance and goodwill of the inventors and the support of the LSD community. The rewards will surely be worth the patience.

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