Enhancing Newborn Screening for Tyrosinemia Type I

Hepatorenal tyrosinemia, also known as tyrosinemia type I (Tyr-I) is an autosomal recessive inborn error of metabolism. The primary enzyme defect has been attributed to a deficiency of fumarylacetoacetase (EC 3.7.1.2) (1). Tyr-I is usually asymptomatic in newborns, but if left untreated it affects liver, kidney, bone, and peripheral nerves; in its most severe form, affected infants may die from liver failure in the first months of life (2). The majority of Tyr-I patients can be treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) with promising outcomes (3, 4). The incidence of this condition is estimated to be 1:100 000 but may be much higher in certain populations such as the province of Quebec, where it occurs once in every 20 000 live births (5). Given the effectiveness of treatment and a test for its marker in the Guthrie specimen, Tyr-I fulfills the criteria for newborn screening and is included in the American College of Medical Genetics’ core panel of newborn screening recommendations (6).

Most newborn screening programs detect Tyr-I by measurement of tyrosine in Guthrie specimens (dried blood spot) (7–9). This method is convenient because tyrosine can be extracted and monitored along with other amino acids and acylcarnitines present in newborn screening panels (7–9). It has become clear, however, that tyrosine alone is an inadequate marker for screening of Tyr-I because tyrosine measurement lacks specificity and cannot distinguish Tyr-I from other disorders in tyrosine catabolism and transient hyperglycinemia (10, 11). Even more disconcerting are reports of missed cases of Tyr-I in newborns with either normal or only modestly increased concentrations of tyrosine (9, 12–14). A number of recent publications have reported newborn screening results for tyrosine at or near the population average for confirmed cases of Tyr-I (9, 12–14). Cases have been reported with tyrosine concentrations of approximately 120 μmol/L, and one case was reported with a tyrosine concentration of approximately 50 μmol/L. Data from New York State Newborn Screening show the average concentration of tyrosine in Guthrie specimens in the New York State population is approximately 85 μmol/L. If tyrosine is used as the only marker for Tyr-I, it is very likely that cases will be missed.

Increased concentrations of succinylacetone (SUAC) are characteristic of Tyr-I (2). A method for the analysis of SUAC in Guthrie specimens has been reported (13, 14). This assay was demonstrated to be suitable for newborn screening in that it is sensitive, selective, simple, and amenable to processing high numbers of samples. SUAC can be extracted from residual specimen punches that were extracted previously for amino acids and acylcarnitines, and the SUAC is then quantified by tandem mass spectrometry. Because this SUAC measurement requires a separate extraction and analysis from the amino acid/acylcarnitines analysis, however, this method would essentially double the number of samples to be run daily on a laboratory’s mass spectrometer. It may be possible to implement the extraction and analysis of SUAC as a second-tier test of only samples with increased tyrosine, but the cutoff concentration for tyrosine would have to be set low, so a significant number of samples would need to be analyzed. In New York State, we estimate that a cutoff of 180 μmol/L for tyrosine would result in SUAC analysis of approximately 20 samples per day, and a cutoff of 120 μmol/L would result in 125 samples per day. Even then, it is still likely that cases of Tyr-I would be missed. Since December 2007, this program has tested all specimens for SUAC using the method described by Allard et al. (13). To date, SUAC analyses have not identified a Tyr-I specimen.

In this issue of Clinical Chemistry, Turgeon et al. (15) report a combined assay for SUAC, amino acids, and acylcarnitines that does not require any additional mass spectrometer instruments. In this assay, punches from the Guthrie specimen are first extracted using a methanol solution containing amino acid, carnitine, and acylcarnitine internal standards (ISs) (7–9). The methanol extract is removed and transferred to another microtiter plate for butylation of the amino acids and acylcarnitines, and the residual blood spots are left in place. SUAC is extracted from these spots in an acetonitrile:water IS solution containing hydrazine. After extraction of SUAC from the leftover spots, the eluates are transferred to another round-bottom 96-well plate. The butylated sample extract derived from each sample is added to the corresponding well in the plate containing the SUAC-hydrazone/IS residue, thereby combining the amino acid/acylcarnitine and SUAC extracts into a single well. Tandem mass spectrometry analysis is performed for SUAC by selected reaction monitoring (SRM) experiments added to the precursor, neutral loss, and SRM scans for amino acids, carnitine, and acylcarnitines (7–9). In this and other
reports, extraction and analysis of residual punch samples for SUAC have been demonstrated to be linear, accurate, and reproducible (13–15). In addition, Turgeon et al. (15) show that the combined method has little or no effect on the results observed for amino acids and acylcarnitines when analyzed separately. A comparison of the results from more than 10,000 samples with and without the extraction and analysis of SUAC showed only small increases in the recoveries of amino acids and acylcarnitines from the additional (SUAC) extraction. The only significant difference is a higher reported concentration of C12 acylcarnitine. Because C12 is by itself not indicative of any particular disorder, this is not a serious interference.

The method presented by Turgeon et al. (15) requires no substantive new technology or instruments for a laboratory to implement analysis of SUAC, although the laboratory must be set up to deal with hydrazine, a suspected carcinogen. Because the extracts of amino acids and SUAC are combined, they may be analyzed in the same tandem mass spectrometry analysis. Standards and reagents are available and relatively inexpensive. Quality control samples for the SUAC analysis can be made easily as separate samples or incorporated into amino acid/acylcarnitine QC samples. Because residual punched samples are extracted and analyzed, no additional sample punching is required; the major investment required is that of time and labor for the sample preparation, which is relatively straightforward, requiring only pipetting and drying. The SUAC may be evaluated with the same software and templates that already exist for the evaluation of amino acids and acylcarnitines. Because the concentrations of SUAC have been shown to be much higher in Tyr-I patients than in the normal population, it is expected that there should be few positive samples to retest and report (12–15). This can have a significant impact on laboratory workload, and, more importantly, reduce the number of screen-positive results requiring follow-up and/or diagnostic evaluation.

There are two issues not addressed by the authors of this combined assay. First, systems must be put in place to be sure there is no mix-up in the sample extracts during recombination of SUAC and amino acid/acylcarnitine extracts. Second, in our experience, samples extracted in acetonitrile:water are much dirtier than samples extracted with methanol. Over the long term, these samples may affect the performance and shorten the life of the mass spectrometers, although these effects may be mitigated with proper cleaning and maintenance.

The combined result of SUAC and tyrosine appears to be definitive for Tyr-I. Of 51 programs listed in the National Newborn Screening and Genetics Resource Center website (www.genes-r-us.uthsc.edu, accessed January 17, 2008), 45 report testing for Tyr-I. In 2006, 577 screen-positive results for Tyr-I were reported by these US programs, with only 1 diagnosis confirmed. Those programs that are not now measuring SUAC as a first-tier marker for Tyr-I should consider it.

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