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Succinylacetone as primary marker to detect tyrosinemia type I in newborns and its measurement by newborn screening programs

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ABSTRACT

Tyrosinemia type I (TYR I) is caused by autosomal recessive fumarylacetoacetate hydrolase deficiency and is characterized by development of severe liver disease in infancy and neurologic crises. If left untreated, most patients die of liver failure in the first years of life. Intervention with medication is effective when initiated during the first month of life. This improvement in the treatment of TYR I patients influenced the decision to include TYR I in the US Secretary of the Department of Health and Human Services' (HHS) Recommended Uniform Screening Panel. However, while tyrosine is routinely measured in newborn screening (NBS) by tandem mass spectrometry (MS/MS), elevated tyrosine levels are not specific to TYR I. To improve the specificity of NBS for TYR I, several assays were developed to measure succinylacetone (SUAC) in dried blood spots (DBS). SUAC is a pathognomonic marker of TYR I, and its detection by NBS MS/MS is possible. This review of the current status of NBS for TYR I in the US is the result of discussions at the HHS Secretary's (Discretionary) Advisory Committee on Heritable Disorders in Newborns and Children about the inconsistent implementation of effective NBS for TYR I in the US. We sought to understand the different TYR I screening practices in US NBS programs. Results indicate that 50 out of 51 NBS programs in the US screen for TYR I, and a successful SUAC performance evaluation scheme is available from the Centers for Disease Control and Prevention. Programmatic and methodological barriers were identified that prevent widespread adoption of SUAC measurements in NBS laboratories. However, since SUAC detection is currently the best approach to NBS for TYR I, a further delay of the addition of SUAC measurement into NBS procedures is discouraged. SUAC measurement should improve both the false positive and false negative rate in NBS for TYR I thereby yielding the desired benefits for affected patients at no expense to the overall population served.
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1. Introduction

Tyrosinemia type I (TYR I, hepatorenal tyrosinemia; OMIM #276700) is caused by autosomal recessive fumarylacetoacetate hydrolase (FAH, EC 3.7.1.2) deficiency and is characterized by development of severe liver disease in infancy, renal impairment leading to hypophosphatemic rickets, and neurologic crises. Due to a founder effect among the French–Canadian population, TYR I is common in Quebec (1:17,000 newborns) while it is less frequent elsewhere (ca. 1:100,000) [1]. At least 78 FAH gene mutations are known [2]. If left untreated most patients die of liver failure in the first years of life [1]. Dietary restriction of phenylalanine and tyrosine was the first attempt at treating this condition [3]. The goal was to prevent the accumulation of toxic metabolites by reducing the availability of tyrosine and its immediate precursor, the essential amino acid phenylalanine. While dietary treatment slows disease progression, long-term outcome with respect to complications, in particular the development of liver cancer, remained poor. As of the late 1970s into the 1990s liver transplantation became the treatment of choice as experience in pediatric organ transplantation increased and overall transplantation outcomes improved. Liver transplantation was considered curative of TYR I as long as treatment was initiated early and before significant kidney disease developed [4,5]. Nevertheless, initial mortality of this invasive procedure remains relatively high (10–15%), long-term immunosuppression is required, and eventual organ failure often occurs [6].

In 1992, treatment with the herbicide 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) was proposed because

Abbreviations: SUAC, Succinylacetone; NBS, Newborn screening; DBS, Dried blood spot; TYR I, Tyrosinemia type I; MS/MS, Tandem mass spectrometry; HHS, US Department of Health and Human Services; S(D)ACHDNC, US Health and Human Services Secretary's (Discretionary) Advisory Committee on Heritable Disorders in Newborns and Children; CDC, Centers for Disease Control and Prevention; NSQAP, Newborn Screening Quality Assurance Program; MPP, 3-(5-Methyl-1H-pyrazol-3-yl) propanoic acid; PT, Proficiency testing; QC, Quality control; CL, Confidence limits; RUSP, US Recommended Uniform Screening Panel; LDT, Laboratory-developed test.

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it is an effective inhibitor of 4-hydroxyphenylpyruvic acid dioxygenase which is an enzyme in the tyrosine degradation pathway upstream of FAH [7]. This pharmacologic intervention prevents the formation of maleyl- and fumarylacetoacetate as well as relevant derivatives all of which are known to cause the pathologies observed in TYR I [8]. Along with a phenylalanine and tyrosine reduced diet, oral NTBC has become the mainstay of treatment for TYR I patients. NTBC is commercially available as Orfadin (Nitisinone) since 2002, and a report by Larochelle et al. describing the outcome of TYR I patients born between 1984 and 2004 in Quebec demonstrates that oral NTBC administration is not only better than dietary treatment alone and safer than organ transplantation, but particularly effective when initiated during the first month of life [9]. This dramatic improvement in the treatment of patients with a previously deleterious disease was the primary argument for TYR I inclusion in the US Secretary of Health and Human Services' (HHS) Recommended Uniform Screening Panel (RUSP) [10].

However, while tyrosine, along with other amino acids, is routinely measured in newborn screening (NBS) dried blood spot (DBS) specimens by tandem mass spectrometry (MS/MS), the finding of hypertyrosinemia is not a specific marker for TYR I and it is most often associated with common and benign transient tyrosinemia of the neonate (TTN). To further complicate matters, tyrosine concentrations in patients with TYR I overlap significantly with tyrosine concentrations in the unaffected population (Fig. 1). To improve the specificity of NBS for TYR I, several assays were developed for the measurement of succinylacetone (SUAC) in DBS. SUAC is a pathognomonic marker of TYR I as it is only generated from maleyl- and fumarylacetoacetic acids when FAH is deficient. The initial approaches to its measurement were indirect by determination of the activity of delta-aminolevulonic acid dehydratase which is inhibited by SUAC. Routine delta-aminolevulonic acid dehydratase activity measurements by a colorimetric method [11] were first implemented in Quebec's NBS program. This assay was later modified by Holme et al. [12]. Schulze et al. further improved the analysis through application of spectrophotometry which allowed for quantitative measurements and more objective result interpretation [13]. Allard et al. at the New England NBS program [14] developed an MS/MS based assay to measure SUAC directly. However, because SUAC extraction from DBS requires an acidic solution, simultaneous extraction of SUAC, amino acids and acylcarnitines with methanol is not possible. Allard therefore used the left-over DBS punch following the extraction of amino acids and acylcarnitines to extract and derivatize SUAC using hydrazine for subsequent analysis by MS/MS. The disadvantage of this approach is the need for additional equipment and personnel required to measure SUAC in each DBS; accordingly, this method was never implemented in a screening laboratory as a primary screening test. However, by mid-2008 the New England NBS program had modified this method to measure SUAC in multiple samples at a time and re-analyze only individual samples of a batch that revealed an elevated concentration of SUAC in the pooled samples [15]. Others opted to measure the isoxazole propionate derivative of SUAC in a two-tier approach where SUAC was measured only in samples with tyrosine concentrations above a lowered cut off [16]. But this approach was also deemed insufficiently sensitive given the significant overlap of tyrosine concentrations in TYR I patients and the normal population (Fig. 1) [17]. Further work achieved the ability to measure SUAC, amino acids and acylcarnitines simultaneously. One method was designed to first extract and derivatize amino acids and acylcarnitines using butyl esterification (BE) and then extract and derivatize SUAC from the leftover DBS punch using hydrazine. The derivatized samples were then combined and amino acids, acylcarnitines and SUAC were measured together by MS/MS. The concentration of SUAC was determined by comparison to isotopically labeled SUAC ($^{13}C_5$-SUAC) as internal standard [18,19]. Another method achieved extraction of all analytes from the same sample by subsequent addition of methanol and hydrazine-containing solutions [20].

Because the methods described above are considered laboratory developed tests (LDT) they cannot be implemented by those NBS laboratories that are required by state law to use commercial, kit-based assays.

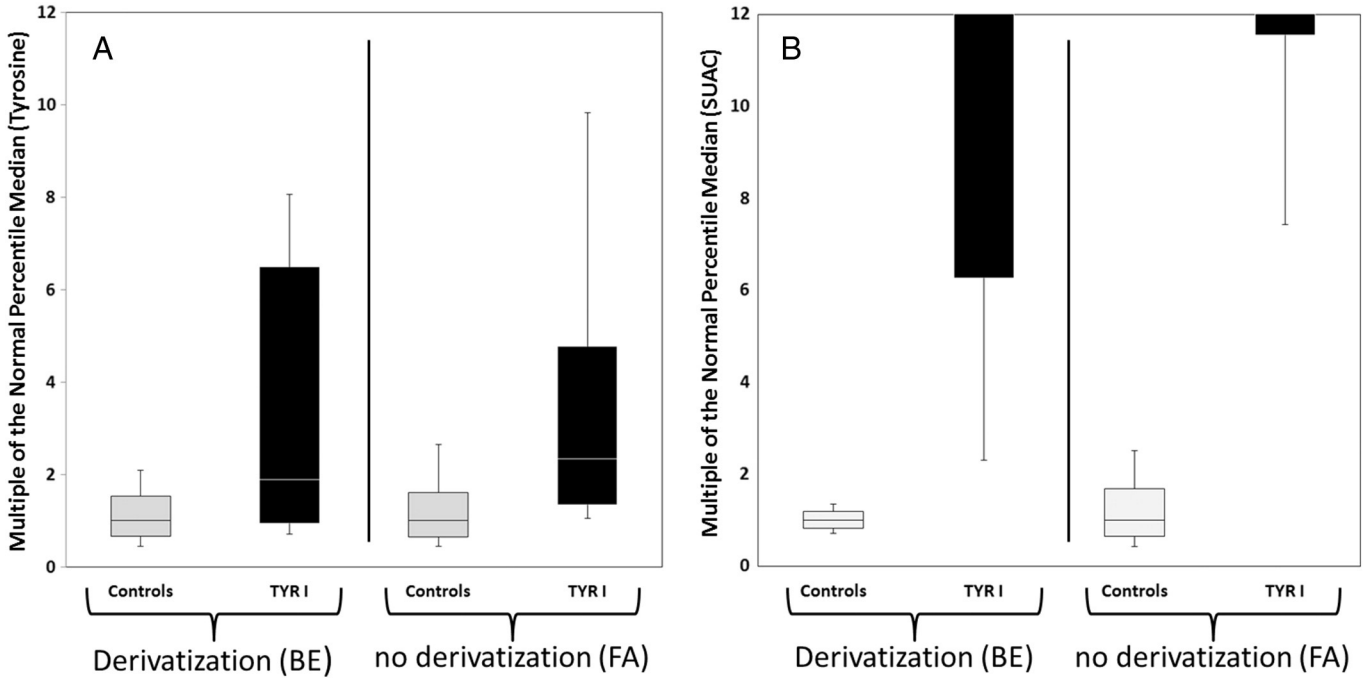

Fig. 1. Plots by condition from the region 4 MS/MS collaborative project [17]. Plots compare tyrosine (panel A) and SUAC (panel B) values observed in NBS samples of controls and TYR I patients based on sample preparation including derivatization (butyl esterification, BE) or not (free acid, FA). Black boxes indicate values observed in newborns with TYR I; grey boxes indicate values observed in control populations (upper whisker end: 99th percentile; top of box: 90th percentile; line in box: median; bottom of box: 10th percentile; lower whisker end: 1st percentile). Data are based on information provided by 15 NBS programs (including 9 US programs) using derivatization (BE) and 14 programs (including 6 US programs) that do not derivatize (FA).

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