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NEWBORN SCREENING FOR SUCCINYLACETONE, A PATHOGNOMONIC MARKER FOR TYROSINEMIA TYPE I

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ABSTRACT AND INTRODUCTION

Fumarylacetoacetate hydrolase (FAH) is the terminal enzyme involved in the tyrosine catabolic pathway [1,2]. Individuals with hereditary tyrosinemia type I (HT 1) are homozygous for one of several possible FAH mutations and are gradually deficient for this enzyme activity. As a result, the immediate precursors of FAH accumulate and are metabolised to succinylacetone (SUAC) [1, 3]. These precursors and SUAC have toxic effects on different metabolic pathways [4, 5]. Affected individuals show hepatic and renal disease as well as neurologic manifestations. Liver dysfunction might be diagnosed in young infants (< 6 months). Untreated, the trait might lead to premature death. A timely treatment combining nitisinone and a low-tyrosine diet has significant corrective impact and greatly increases survival rate. HT 1 has a general incidence of 1:100,000 with a much higher occurrence in particular ethnic groups such as French-Canadian (1:16,000) [6], Norwegian and Finnish (both 1:60,000) [7]. Using elevated tyrosine concentration as a marker for this disease has limited value because it is common for tyrosinemia type II and III and other liver diseases. However, an increase in SUAC is pathognomonic for HT 1.
The objective of this study was to evaluate a validated commercial LC-MS/MS test assaying newborn blood spots for SUAC among other markers for hereditary metabolic disorders (Table 1). Dried blood spots of 2,500 newborns were screened using the method described based on extraction and derivatisation steps to determinate the cut-off as the 99.5th percentile of amino acids, acylcarnitines and SUAC. One patient sample with a confirmed HT 1 was randomly included into the sample pool to clarify the diagnostic sensitivity.

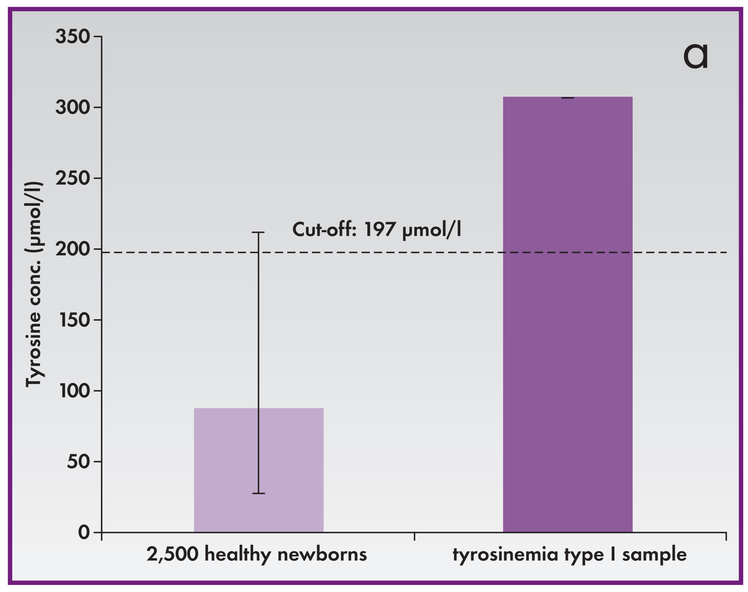
MATERIAL AND METHODS

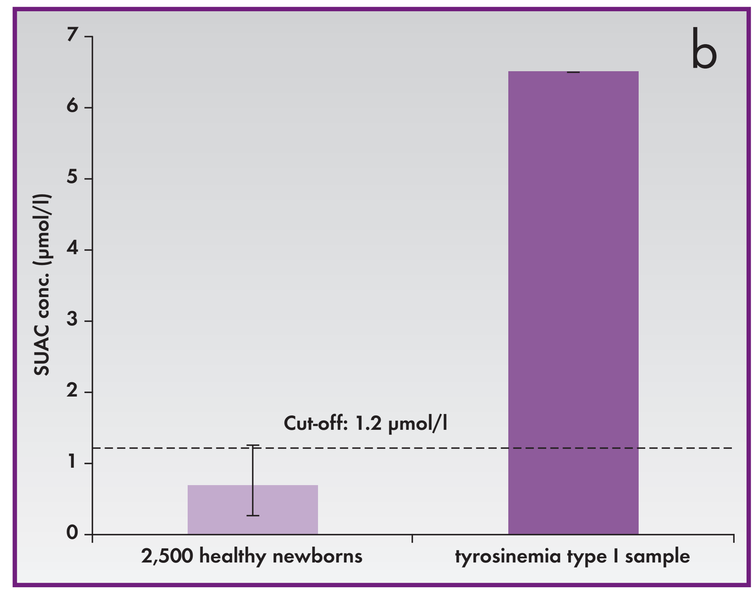
2,500 blood samples were collected routinely in the Austrian Newborn Screening Program for Inborn Errors of Metabolism. The kit ***MassChrom***® Amino Acids and Acylcarnitines from Dried Blood (Chromsystems, Munich/Germany) was used for the semi-quantitative determination of SUAC by tandem mass spectrometry [8, 9]. For cut-off determination we defined three exclusion criteria: (i) values of confirmed positive samples, (ii) samples of premature babies (born before the 32th week of gestation), and (iii) samples which were obtained within 36 h after birth.
The measurements were performed on a TQD mass spectrometer (Waters, Milford/MA USA) at the Department of Paediatrics and Adolescent Medicine, Medical University of Vienna, Austria.
Sample preparation was done following the manufacturer's instructions based on analytes' extraction out of the filter paper following by derivatisation of the analytes to butyric esters. For the determination of SUAC the blood spot was extracted a second time using the internal standard for SUAC. To ensure reproducible quantification of the analytes, isotopically-labelled (deuterated) internal standards were used for calibration and measurement.

RESULTS

Analyte	Cut-off (µmol/l)	Analyte	Cut-off (µmol/l)
Succinylacetone	1.2	C0-Carnitine	50
Alanine	561	C2-Carnitine	57
Arginine	47	C3-Carnitine	4.9
Aspartic acid	147	C4-Carnitine	0.62
Citrulline	34	C5-Carnitine	0.46
Glutamic acid	800	C5DC-Carnitine	0.22
Glycine	834	C6-Carnitine	0.13
Leucine	226	C8-Carnitine	0.11
Methionine	45	C10-Carnitine	0.21
Ornithine	258	C12-Carnitine	0.56
Phenylalanine	92	C14-Carnitine	0.38
Tyrosine	197	C16-Carnitine	5.8
Valine	191	C18-Carnitine	1.6

Table 1: **Defining cut-offs for SUAC, 12 amino acids and 13 acylcarnitines.** Cut-off values were determined for all listed analytes. The commercial LC-MS/MS-test embraces 12 amino acids and 13 acylcarnitines for the newborn screening program, besides the new added analyte SUAC.





Determination of tyrosine and SUAC amounts in 2,500 neonatal blood samples to define cut-offs. Tyrosine (a) and SUAC (b) amounts in all specimens were compared and the relevant kit was evaluated for its safe diagnostic value. The randomly included positive control was correctly recovered. The cut-off for tyrosine was set at 197 µmol/l and for SUAC at 1.2 µmol/l.

CONCLUSION:

- The patient sample with the known target disease HT 1 was identified according to the significant elevated HT 1 specific marker SUAC. Evaluation of the ***MassChrom***® Amino Acids and Acylcarnitines from Dried Blood kit showed a convincing performance for SUAC.
- Tyrosine as the only marker is not sufficient enough to verify HT 1. Inclusion of SUAC in newborn screening programs should increase the safe identification of HT 1 in neonates and minimize false positive or false negative results.

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