Tandem mass spectrometric determination of succinylacetone in dried blood spots enables presymptomatic detection in a case of hepatorenal tyrosinaemia

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Summary Tyrosinaemia type I, or fumarylacetoacetase deficiency, causes hepatorenal damage by accumulation of fumarylacetoacetate. Patients are generally in good condition at birth, but are at risk of developing serious metabolic crises with liver failure and hepatic coma. An early start of treatment with NTBC and a tyrosine-balanced diet can prevent harm to the patients. The application of tandem mass spectrometry to newborn screening allows for easy determination of tyrosine to detect the presence of hypertyrosinaemia in the neonate, but most patients with tyrosinaemia type I do not present with high tyrosine levels at the time of newborn screening. We report on a 7-week-old girl presenting with acute hepatopathy and severe coagulopathy due to tyrosinaemia type I. The metabolic screening, which was performed by tandem mass spectrometry at the age of 48 h, had revealed normal values for tyrosine and methionine that were well within ranges observed in the general population and equally normal ratios of methionine/tyrosine and tyrosine/serine. In this patient even lowering the cut-off levels for tyrosine and methionine would not have provided better sensitivity. Residual blood spots from the newborn screening filter paper were retrospectively analysed using a specific mass-spectrometric method for the detection of succinylacetone and revealed a 5-fold elevated succinylacetone concentration. This indicates that identification of all newborns with hepatorenal tyrosinaemia is only possible by determination of succinylacetone as part of the newborn screening process.

Abbreviations
AT3 antithrombin 3
FAH fumarylacetoacetase
HT I tyrosinaemia type I
NTBC 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione
PCV packed cell volume/haematocrit
TCT thrombin clotting time

Introduction

Tyrosinaemia type I (hepatorenal tyrosinaemia, HT I) is a metabolic disease of autosomal recessive inheritance (OMIM 276700). HT I is caused by deficiency of the enzyme fumarylacetoacetase (FAH), an enzyme that catalyses the terminal step in tyrosine degradation. FAH deficiency results in the accumulation of fumarylacetoacetate, a strong alkylating agent which causes hepatorenal damage. Succinylacetone is the metabolic degradation product of fumarylacetoacetate...
and is the only specific biochemical marker of HT I. Patients with HT I are generally in good condition at birth. However, within the next weeks to months they are at high risk of developing serious metabolic crises with liver failure which, if untreated, may lead to death from hepatic coma (Mitchell et al 2001). In the long term the accumulation of maleylacetoacetate and fumarylacetoacetate and their saturated derivatives increases the risk for liver cirrhosis, hepatocellular carcinoma and renal failure. This serious condition is potentially treatable with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), a drug which was initially used as a herbicide. First experiences showed that an early start of therapy together with a tyrosine-balanced diet can prevent harm and maintain a good quality of life for the patients (Lindstedt et al 1992; Lock et al 1998).

Therefore, it is essential to establish a reliable tool for early diagnosis of the disease. With the development of tandem mass spectrometry and its application in newborn screening we are now able to assess levels of tyrosine to detect the presence of hypertyrosinaemia. However, in most cases elevated levels are not related to tyrosinaemia but to a nonpathological condition named benign transient hypertyrosinaemia of the newborn (Mitchell et al 2001). The high proportion of false-positive results has led to the decision to exclude this method from German neonatal screening programmes (Harms et al 2002). Neither the reduction of cut-off levels nor the addition of other metabolites such as methionine, serine and certain ratios (Fischer ratio or tyrosine/serine ratio) has helped to improve the specificity of screening or early detection (Chace and Kalas 2005; Goulden et al 1987). In 2004 a novel tandem mass-spectrometric method for the direct determination of succinylacetone was created to detect hepatorenal tyrosinaemia (Allard et al 2004). First studies with this or other advanced methods indicated their potential use for presymptomatic detection of hepatorenal tyrosinaemia and for avoidance of false-positive results (Magera et al 2006; Sander et al 2006).

### Case report

We report on a patient with tyrosinaemia type I who showed extremely low values for tyrosine and methionine by newborn screening at the age of 48 h. The female patient was the second child of healthy unrelated parents. Pregnancy was uneventful except for diet-controlled gestational diabetes. The mother noticed normal limb movements during pregnancy and the girl was born at 40 weeks of gestation by Caesarean section owing to cessation of labour. At birth, weight was 4100 g (94th centile), length was 51 cm (40th centile) and head circumference was 35.5 cm (70th centile). Apgar scores and pH of umbilical venous blood were normal. At the age of 7 weeks the patient was admitted to the hospital with an acutely increasing abdominal girth and sub-febrile temperatures as an emergency. Abdominal ultrasound showed massive hepatomegaly with ascites. Laboratory investigations were consistent with liver failure (clotting time TCT 171 s (normal range <40 s), Quick 8% (normal range >50%), AT3 10% (normal range 80–100%), PCV 0.2 and platelet count $84 \times 10^9/L$). Measurement of amino acids in dried blood spots showed highly elevated methionine and moderately elevated tyrosine levels (see Table 1, week 7). Urinary analysis for organic acids confirmed the diagnosis of tyrosinaemia type I by the presence of succinylacetone. With the start of NTBC therapy, tyrosine levels increased and returned to lower levels (400 µmol/L) after restricting tyrosine intake (Fig. 1). Under treatment with NTBC and a tyrosine-balanced diet, the patient started thriving well. The course of admission was complicated by myoclonic seizures with hypsarrhythmias that required anticonvulsive treatment. Unfortunately, now at the age of 3 years, the girl shows signs of severe mental retardation and is suffering from recurrent seizures. While the aetiology of this seizure disorder could not be delineated, we assume that it is related to the patient’s initial presentation with liver failure.

This case gives further evidence that neonates with HT I may be detected in the newborn screening

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Tyrosine and methionine concentrations in dried blood after birth and at start of therapy (newborn screening normal ranges: tyrosine 30–350 µmol/L; methionine 6–50 µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Normal range</td>
</tr>
<tr>
<td>2 days</td>
<td>7 weeks</td>
</tr>
<tr>
<td>Methionine (µmol/L)</td>
<td>28</td>
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</tbody>
</table>
programme by additional determination of succinylacetone rather than by amino acids in dried blood spots alone. To identify HT I patients such as the one described here using tyrosine as a marker would require lowering the cut-off for tyrosine to a level which would result in a false-positive rate of close to 2–3%. Residual blood spots from the newborn screening filter paper were retrospectively analysed in 2006 using a specific mass-spectrometric method for the detection of succinylacetone (Sander et al 2006). The succinylacetone level in the dried blood of the 3-year-old screening card was 28.4 µmol/L (cut-off <10 µmol/L). This indicates that the use of the tandem mass spectrometric method for the determination of succinylacetone enables a presymptomatic detection of hepatorenal tyrosinaemia.

Fig. 1 Levels of tyrosine and methionine in dried blood after start of NTBC therapy at the age of 2 months and restricting tyrosine intake at the age of 9 months (arrows)

References


