Tandem Mass Spectrometric Determination of Malonyl-
carnitine: Diagnosis and Neonatal Screening of
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Malonic aciduria (OMIM 248360) is a rarely diagnosed
autosomal-recessive inborn error of metabolism caused by
genital deficiency of malonyl-CoA decarboxylase
(MCD; EC 4.1.1.9). Hypoglycemia, seizures, developmen-
tal delay, and cardiomyopathy are among the most com-
mon symptoms, but both clinical signs and the time of
presentation of patients with MCD deficiency can be
variable. To date, only eight cases have been reported
in the literature (1–8), and recently molecular defects within
the MCD gene (MLYCD) have been elucidated for the first
time in some of these cases (8–11). At least in part, clinical
heterogeneity might be caused by the fact that MCD is
expressed in different compartments of the cell and that
MLYCD mutations with different effects on the subcellu-
lar localization of the MCD protein may thus affect
different metabolic pathways (Wightman et al., submitted
for publication).

To date, only symptomatic patients with MCD defi-
cency have been detected, and many of them were
already severely handicapped at the time of diagnosis
because of residues of acute metabolic crises or from
episodes of cardiac decompensation, which may develop
as a consequence of secondary carnitine deficiency.
Typically, the key to diagnosis is the excessive amount of
malonic acid in the patient’s urine, which can be detected
by gas chromatography–mass spectrometry. This then
leads to a confirmatory test, such as the measurement of
MCD activity in cell extracts, or to molecular genetic
testing.

Detection of the carnitine ester of malonic acid has been
mentioned previously in single cases of malonic aciduria
(4,6,7). In the study reported here, we systematically
investigated the concentration of malonylcarnitine in the
blood of MCD-deficient patients by electrospray ioniza-
tion tandem mass spectrometry (ESI-MS/MS). Because
MCD deficiency may be amenable to dietary and medical
treatment, we also evaluated whether an increase in blood
malonylcarnitine is detectable in the neonatal period,
which would be mandatory for early presymptomatic
diagnosis.

Details of the method used by our group have been
reported previously (12). In summary, acylcarnitines
were analyzed by ESI-MS/MS with an API 365 ESI-
MS/MS system equipped with a Turbolon spray device,
Series 200 lp HPLC pump, and a Series 200 autosampler
(PE-SCIEX). For that purpose, 3.2-mm (1/8-inch) spots
were punched from Guthrie test cards by an automated
punching device and transferred to a 96-well microtiter
plate. After the addition of internal standard solution
(containing 7.5 pmol of l-[1H3]octanoylcarnitine), methan-
ol was added, and after elution, the methanolic extract
was evaporated to dryness. Acylcarnitines were derivat-
ized to the corresponding butyl esters with butanolic
hydrochloric acid. Samples were redissolved in acetoni-
trile–water–formic acid (50:50:0.025 by volume) and intro-
duced into the ionization chamber. Acylcarnitines were
measured in multiple-reaction monitoring (MRM) mode
with the ion pairs 347.4/85.0 Da and 360.4/85.0 Da for

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l-[3H]octanoylcarnitine and malonylcarnitine, respectively. Malonylcarnitine was quantified relative to l-[3H]octanoylcarnitine, assuming the same extraction and ionization efficacy for both carnitine esters.

Dried blood spots were obtained from two siblings with MCD deficiency at different time points. A detailed description of the clinical and biochemical data and the metabolic effect of their genotype (MLYCD 634insA/59insC) is to be published elsewhere (Wightman et al., submitted for publication). During the period of the study, both patients were in stable clinical condition without any signs of metabolic derangement. At the time of the first determination of malonylcarnitine, patient 1 was 4 years and 2 months of age, and at that time she was already on oral carnitine therapy; patient 2 was investigated for the first time at the age of 7 months before any treatment had been initiated. In addition, the neonatal Guthrie test cards of both patients were traced back, and the concentrations of acylcarnitines were also determined in these samples. To determine the reference interval for malonylcarnitine in dried blood samples, we analyzed 4333 unselected samples of healthy newborns.

At the time of the first determination, the blood malonylcarnitine concentration was 2.4 μmol/L in patient 1 and 3.6 μmol/L in patient 2. At the follow-up investigations, the malonylcarnitine concentration ranged between 1.66 and 3.78 μmol/L (n = 8) in both patients. For comparison, in dried blood samples from the healthy controls, malonylcarnitine ranged from “not detectable” to 0.30 μmol/L (99.5th centile; n = 4333). In the neonatal test card of patient 1, which had been stored for 4 years and 4 months, malonylcarnitine was not detectable. In contrast, in the test card of patient 2, stored for 9 months, the malonylcarnitine concentration was 0.40 μmol/L.

The linearity and precision of the assay and the recovery of malonylcarnitine could not be calculated because malonylcarnitine is not commercially available. The detection limit (signal + 3 SD of a sample free of analyte) for malonylcarnitine was 0.08 μmol/L, and the quantification limit (signal + 6 SD of a sample free of analyte) was 0.13 μmol/L. To determine the stability of malonylcarnitine in dried blood samples stored at room temperature, we reanalyzed eight samples from patients with MCD deficiency after various storage times at room temperature. All samples showed a decrease in malonylcarnitine concentration. Regression analysis with curve fitting revealed significant agreement (P <0.001) with a logarithmic model \( y = e^{0.0028t} \times 100\% \). In this equation, \( y \) is the concentration of malonylcarnitine relative to the first measurement, \( t \) is the time of storage between the first and the consecutive measurement, and the constant \( b \) was calculated to be −0.0028. With this model, the half-life \( (t_{1/2}) \) of malonylcarnitine in dried blood on filter paper stored at room temperature could be calculated to be 248 days (Fig. 1).

With this equation and the malonylcarnitine concentration measured in the screening card of patient 2 after 296 days of storage, the initial malonylcarnitine concentration was calculated to be 0.92 μmol/L. With a presumptive malonylcarnitine concentration of ~1 μmol/L in patients with MCD deficiency during the first days of life, the decrease in malonylcarnitine can be calculated. Concentrations should be expected to drop below the quantification limit after ~730 days. Even with a 10-fold higher initial concentration (10 μmol/L), malonylcarnitine would no longer be detectable after ~1500 days. These considerations make it plausible that we could not detect malonylcarnitine in the screening card of patient 1 (after storage for 1514 days).

The diagnostic specificity of the test cannot yet be finally evaluated, although our preliminary data from 4333 newborns and the two patients with MCD deficiency seem to show that ESI-MS/MS has a high specificity for the detection of MCD deficiency. The highest concentration of malonylcarnitine in the healthy newborns was 0.43 μmol/L, whereas the malonylcarnitine concentrations in dried blood samples from the two patients with MCD deficiency was 3.9- to 8.8-fold higher than the highest value of the reference population.

Several groups have reported that MCD-deficient patients benefit from a low-fat, high-carbohydrate diet, with the effects being improved urinary organic acid excretion and the avoidance of hypoglycemic episodes (2, 3). Both our patients and cases from the literature (4) also showed a dramatic effect of carnitine treatment, which demon-
strates that cardiac decompensation can be avoided in MCD-deficient patients. Finally, by analogy with fatty acid oxidation disorders such as medium-chain acyl-CoA dehydrogenase deficiency (13), one may conclude that knowledge of the diagnosis will improve outcome because early intervention is then possible in catabolic episodes that otherwise may lead to metabolic derangement. It therefore seems reasonable to suppose that early diagnosis and treatment can reduce the morbidity and mortality associated with MCD deficiency and to include MCD deficiency in neonatal metabolic screening programs. In this study, the malonylcarnitine concentration in the neonatal screening test card was increased in only one of the two patients investigated, but this was a problem of stability after sample storage for several years.

We conclude that (a) malonylcarnitine is detectable by ESI-MS/MS, (b) malonylcarnitine is increased in the blood of patients with malonic aciduria, and (c) ESI-MS/MS-based neonatal screening programs should be able to detect patients with MCD deficiency, a treatable metabolic disorder, before the development of symptoms.

**References**


**IFCC Method for Lactate Dehydrogenase Measurement in Heparin Plasma Is Unreliable, Andries J. Bakker, Bidjaiperkash Mirchi, Johannes T. Dijkstra, Freck Reitsma, Haye Syperda, and Appie Zijlstra** (Department of Clinical Chemistry, Klinisch Chemisch Laboratorium, PO Box 850, 8901 BR Leeuwarden, The Netherlands; * author for correspondence: fax 31-582882227, e-mail a.j.bakker@kcl.znb.nl)

Various societies for clinical chemistry have proposed recommendations for the measurement of the activity of lactate dehydrogenase (LDH; l-lactate:NAD+ oxidoreductase; EC 1.1.1.27) (1–5). Depending on the pH of the buffer, the activity of LDH can be measured by the increase as well as the decrease in NADH. The optimum pH for the pyruvate-to-lactate conversion is 7.4–7.8. The German (2) and French (5) Societies for Clinical Chemistry recommend this reaction. The IFCC (4) recommends the lactate-to-pyruvate conversion, for which the optimum pH is 8.8–9.8.

In our laboratory, we have measured LDH according to the recommendations of the French society (SBFC) for years. This measurement was routinely performed with a Modular analyzer (Roche GmbH). When this SBFC method for LDH measurement needed to be replaced because our supplier stopped producing the necessary reagents, we decided to introduce the method based on the recommendations of the IFCC. Because we did not want to change our reference values (<450 U/L), we introduced a conversion factor to make the results obtained with the IFCC method directly comparable to the results obtained with the SBFC method.

When we compared both methods using patient samples for which LDH was requested, we found numerous outliers, which forced us to do some additional investigations. To determine which method was responsible for the outliers, we began measuring duplicates for LDH with both methods. For these measurements we used lithium-heparin-plasma samples collected in plastic tubes with plasma separator (product no. 367994; Becton Dickinson). All measurements took place in the primary tubes, i.e., LDH measurements were performed directly in the lithium-heparin plasma after plasma was separated from the blood cells by centrifugation (1800 g for 5 min), but without pipetting the plasma to secondary tubes. In these experiments, we found a rather high frequency of duplicate errors for LDH measurements with the IFCC method (27 of 152; 17.8%), whereas we found no such excessive frequency of duplicate errors with the SBFC method (2 of 140; 1.4%; Fig. 1A). Duplicate errors were defined as those differences that exceeded the 95% confidence limits for the difference between duplicates.

To estimate the 95% confidence interval for the difference between duplicates, it is important to know the standard deviation of the assay (SDa) used. In our hands the CV for the LDH measurements according to the IFCC as well as the SBFC was ≤2%; for the IFCC method, control 1 gave a mean of 344 U/L with a CV of 1.3% (n = 198), and control 2 gave a mean of 813 U/L with a CV of 1.1% (n = 198); for the SBFC method, control 1 gave a