remains unclear whether cyclosporine directly affects homocysteine metabolism or whether increased tHcy may be attributable to impaired renal function or higher prevalence of other confounders in these individuals. Serum creatinine is the most important predictor of tHcy, whereas serum folate had only minor influence. Avoidable, unhealthy lifestyle factors (e.g., smoking and obesity) and associated diseases (e.g., diabetes, hypertension, and hypercholesterolemia) did not influence tHcy significantly in our study.

The authors are grateful to Alicia Muñoz, Marisol Silió, Blanca Navalón, Manuela Gómez and Trinidad Rodríguez for skillful technical assistance.

References

Rapid Diagnosis of Methylmalonic and Propionic Acidemia: Quantitative Tandem Mass Spectrometric Analysis of Propionylcarnitine in Filter-Paper Blood Specimens Obtained from Newborns, Donald H. Chace, James C. DiPerna, Theodore A. Kalas, Ronald W. Johnson, and Edwin W. Naylor (Neo Gen Screening, PO Box 219, Bridgeville, PA 15017; * author for correspondence: fax 412-220-0784, e-mail dhchace@neogenscreening.com)

Methylmalonic acidemias (MMAs) and propionic acidemias (PAs) comprise a group of congenital disorders of branched-chain amino acid metabolism (1). PA is caused by deficiency of propionyl-CoA carboxylase, whereas MMA results from deficiency of either methylmalonyl-CoA mutase or defects in the production of adenosylcobalamine. Deficiency of vitamin B(12), a cofactor for methylmalonyl-CoA mutase, will also produce signs and symptoms consistent with MMA (2–4). Other biochemically closely related disorders are the cobalamin (Cbl) defects, classified as forms A through G. (5). Clinical signs and symptoms include failure to thrive, metabolic acidosis, persistent ketotic episodes, hypoglycemia, hypotonia, hyperammonemia, and neurologic symptoms (6). Children with PA, MMA, or Cbl disorders often present with acute illness as neonates or infants. Treatment of MMA and PA includes careful monitoring and limitation of branched-chain amino acid intake (4) as well as possible
supplementation with biotin, vitamin B₁₂, and l-carnitine (7).

Tests for the diagnosis of MMA and PA are most often performed on urine, plasma, and whole-blood samples and involve gas chromatographic–mass spectrometric analysis of organic acids (8–11). Tandem mass spectrometry (MS/MS) has been used to semiquantitatively identify presymptomatic and affected infants (12–17). Confi-
Table 1. Quantitative results for groups of specimens in this study.

<table>
<thead>
<tr>
<th></th>
<th>C3, μmol/L</th>
<th>C2, μmol/L</th>
<th>C3/C2</th>
<th>C3/C16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, &lt;7 days (n = 1368)</td>
<td>1.5 (0.21–4.7)</td>
<td>13 (3.0–42)</td>
<td>0.11 (0.03–0.40)</td>
<td>0.46 (0.12–2.6)</td>
</tr>
<tr>
<td>True positive (n = 14)</td>
<td>8.7 (4.4–87)</td>
<td>10 (3.6–21)</td>
<td>0.85 (0.56–4.1)</td>
<td>3.5 (2.1–20)</td>
</tr>
<tr>
<td>False positive (n = 50)</td>
<td>5.4 (4.4–10)</td>
<td>27 (15–59)</td>
<td>0.20 (0.11–0.35)</td>
<td>1.9 (1.3–3.8)</td>
</tr>
<tr>
<td>False negative (n = 1)</td>
<td>2.7 (4.4–10)</td>
<td>14 (3.1–35)</td>
<td>0.20 (0.04–0.33)</td>
<td>0.79 (0.36–4.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C3, μmol/L</th>
<th>C2, μmol/L</th>
<th>C3/C2</th>
<th>C3/C16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, ≥7 days High risk (n = 255)</td>
<td>1.0 (0.20–4.3)</td>
<td>8.8 (2.8–33)</td>
<td>0.11 (0.03–0.47)</td>
<td>1.2 (0.36–4.6)</td>
</tr>
<tr>
<td>Presumptive positive repeats (n = 11)</td>
<td>8.8 (3.4–53)</td>
<td>11 (2.2–47)</td>
<td>1.1 (0.37–2.6)</td>
<td>13 (3.2–31)</td>
</tr>
<tr>
<td>False-positive repeats (n = 50)</td>
<td>1.1 (0.22–5.65)</td>
<td>8.6 (3.1–35)</td>
<td>0.13 (0.04–0.33)</td>
<td>1.0 (0.36–4.2)</td>
</tr>
</tbody>
</table>

The product ion spectra for the butyl esters of C3 and its internal standard, [2H3]C3, showed prominent ions at m/z 85, whereas the precursor ion spectra at m/z 85 showed a single ion at m/z 274 and m/z 277 for the protonated molecular ions of C3 and [2H3]C3, respectively (data not shown). These results define the basis for detection of C3 in an acylcarnitine profile. Acylcarnitine profiles were obtained as three scan functions: (a) free carnitine (FC) selected-reaction monitoring (SRM) analysis; (b) short-chain acylcarnitine SRM analysis; and (c) full-scan acylcarnitine profile (m/z 270–500). SRM acquisitions of FC or acetylcarnitine (C2) and C3 were performed separately from a full-scan acquisition because they enabled enhanced sensitivity via optimized instrument settings for specific acylcarnitines, direct quantitative analysis of the C3/C2 ratio, and internal instrumental quality assurance/quality control.

A control acylcarnitine profile obtained from an unaffected newborn is shown in Fig. 1A together with a SRM profile (inset) showing the C3/C2 ratio. Abnormal acylcarnitine profiles obtained from newborns with PA and MMA are shown in Fig. 1B and C, respectively. The molar ratios of C3 to C2 in both profiles were increased compared with the control. Fig. 1D shows the profile of a false-positive result. Interestingly, this profile shows an increase in the concentration of C3 with a C3/C2 ratio comparable to that of an unaffected infant.

Calibration curves for C3 were linear over a concentration range of 0–16 μmol/L with a slope of 0.73, a y-intercept of 1.41 μmol/L, and a r² of 0.99. The detection limit was ~0.05 μmol/L with a signal-to-noise ratio of 3. Analytical recoveries of C3 were 89%, 74%, and 79% for triplicate analyses of blood enriched with 1, 4, and 16 μmol/L C3, respectively. The analytical imprecision (CV) for 10 replicate injections of identical aliquots from a singly prepared blood sample was 5.8%. The intra- and interassay CVs were 14% and 26%, respectively (n = 30 and 26, respectively). This precision was sufficient for differentiation between nonpathologic and pathologic samples. The nearly identical values for inter- and intraday imprecision demonstrate the stability of this method.

Quantitative results from a group of 1368 control newborn specimens (<7 days of age), 14 true-positive MMA and PA cases, 50 false positives, and 1 false negative are provided in Table 1. In addition to using visual interpretation, we determined presumptive positive results using several different concentrations of C3 in combination with C3 to C2 and C3 to palmitoylcarnitine (C16) ratios. The median concentrations of C3 for true- and false-positive groups were 8.7 and 5.4 μmol/L, respectively. These concentrations were markedly higher than that of the control values (1.5 μmol/L). The single false-negative result for C3 was 2.7 μmol/L, clearly within the control range. Overlap of false-positive results with either control or true-positive results was observed, as seen in the ranges provided in Table 1.

The median concentration of C2 in the false-positive group was more than twice that of the control and true-positive groups. It would be expected, therefore, that calculation of the ratio of C3 to C2 would improve the discrimination of false from true positives. The median C3/C2 ratio for the true-positive group was almost eightfold higher than the ratio for control group and more than fourfold higher than the ratio for the false-positive group.
More importantly, the ranges of C3/C2 values demonstrated that the true-positive results overlapped with neither the false-positive nor the control group. Other indices (18), such as the acyl/carnitine, C3/FC (data not shown), and C3/C16 (Table 1) ratios, were each two- to sevenfold higher than control values. A threefold decrease in the median concentration of FC for the true-positive group (5 μmol/L) compared with controls (17 μmol/L) was observed.

For each presumptive positive newborn blood specimen, a second blood sample was obtained. Quantitative results from 255 control infants ≥7 days of age (and for which no metabolic disorder was found) are provided in Table 1. Results for 11 repeat specimens from confirmed PA and MMA cases and 50 repeat specimens from false positives are also provided in Table 1. Results for a second specimen analysis from confirmed PA and MMA cases were still high, with a median C3 concentration of 8.8 μmol/L. Furthermore, an ∼10-fold increase in the C3/C2 and C3/C16 ratios for the true positives was observed relative to both the control and false-positive groups. The median C3 concentration in the second specimens from the group with initial false-positive results was nearly equivalent to that of the controls.

MS/MS cannot clearly differentiate between various subtypes of MMA, including the mutaseα and mutaseγ Cbl defects. Newborns and infants with presumptive positive results were referred to metabolic centers where further confirmatory studies, such as gas chromatographic–mass spectrometric analysis of organic acids, were performed. For PA (n = 7), the median (range) C3 was 22 (4–87) μmol/L and the C3/C2 ratio was 1.77 (0.66–4.2), whereas for MMA (n = 6), the median (range) C3 was 8.3 (6.3–10) μmol/L and the C3/C2 ratio was 0.81 (0.56–1.22). In one case of vitamin B12 deficiency, C3 was 10 μmol/L whereas the C3/C2 ratio was 0.67. Within the group of confirmed MMA cases, three mutaseγ cases showed no significant difference from two Cbl C cases with the single exception of the false-negative Cbl C case whose results are provided in Table 1.

Of the 908 543 newborn blood specimens analyzed in this laboratory since 1992, 14 positive cases for either MMA or PA were found. Specifically noted were 7 cases with PA, 7 cases with MMA, 3 cases with mutaseα, 2 cases with Cbl C, 1 case with maternal vitamin B12 deficiency, and 1 undetermined, representing 1 true-positive case in 64 896 infants screened using the method described here. One false-negative case was found among 908 543 infants screened. Of the 14 cases with positively identified and confirmed MMA or PA, 9 have survived whereas 1 has died. No information is accessible in the four remaining cases.

MS/MS analysis of C3 in filter-paper blood specimens is sufficiently selective, sensitive, and reproducible to be used as a newborn-screening assay for MMA and PA. A low calculated extraction efficiency (80%) may be attributable in part to an underestimation of blood volume in the filter-paper blood specimen as a result of cell lysis or spot size as described previously (19). Development of quality assurance/quality control for C3 and other acylcarnitines is in progress with some preliminary results published recently (20).

The use of molar ratios such as C3/C2 improves diagnostic efficacy, reduces false-positive results attributable to generalized increases in short-chain acylcarnitines, and potentially minimizes false-negative results. Although the data show that some differentiation between MMA and PA can be observed in the profiles, it is insufficient for diagnosis. Comparison of data between newborns and older, at-risk infants revealed some overlap in results from control and true-positive specimens. In many cases, the original newborn specimen was more reliable in the preliminary diagnosis of MMA and PA. Specimens that were substantially abnormal after the first analysis or remained abnormal on repeat analysis required organic acid analysis of urine specimens and other confirmatory tests to reach an accurate diagnosis of MMA or PA and to properly differentiate various subtypes so that the appropriate treatment could be initiated.

The database of nearly 1 million infants allows more reliable estimates of disease frequencies. Our finding of 1 child in 64 896 with either MMA or PA demonstrates that disorders previously thought to be rare are not and suggests that routine newborn screening should be considered. Although some infants with acute onset of these disorders may suffer harm and perhaps death before a newborn-screening test is performed, many infants with later-onset forms of MMA and PA will derive benefit from newborn screening because their responses to early intervention will generally translate into improved clinical outcomes. With more experience, greater numbers of MMA and PA cases may be identified early, and with increased knowledge in the treatment of these disorders, the clinical outcome for affected infants can be expected to improve.

References

Evaluation of Turbidimetric High-Sensitivity C-Reactive Protein Assays for Cardiovascular Risk Estimation, Ahmad Hamwi, Thomas Vukovich,* Oswald Wagner, Helmut Rumpold, Roswitha Spies, Martina Stich, and Carina Langecker (Institute of Medical and Chemical Laboratory Diagnostics, University Hospital of Vienna, AKH Leitstelle 5H, Waehringerguertel 18, A-1090 Vienna, Austria; * author for correspondence: fax 43-1-40400-5389, e-mail thomas.vukovich@kimcl.akh.magwien.gv.at)

Low-grade inflammation has been recognized as an important feature of atherosclerosis (1); therefore, markers of inflammation have been investigated for risk estimation of cardiovascular events (2, 3). However, in low-grade inflammations, C-reactive protein (CRP) concentrations are often lower than the measuring range of traditional CRP assays. For this purpose new assays, so-called high-sensitivity CRP (hs-CRP) assays, have been developed that cover a measuring range two orders of magnitude lower than those of the traditional assays (4). On the basis of hs-CRP results obtained in several cohort studies (5–8), an algorithm using hs-CRP and lipid values was recently proposed for cardiovascular risk assessment (9).

The aim of the present study was to evaluate four turbidimetric assays, suited for clinical chemistry analyzers, for analytical performance within the concentration range 0.7–3.9 mg/L, which has been proposed for cardio-

![Table 1. Summary of precision and linearity data.](https://academic.oup.com/clinchem/article-abstract/47/11/2040/5639240)