β-Trace Protein Is Not Better than Cystatin C as an Indicator of Reduced Glomerular Filtration Rate

To the Editor:
Recent studies suggest that serum cystatin C [reviewed in Refs. (1, 2)] and β-trace protein (3, 4) identify reductions in glomerular filtration rate (GFR) better than creatinine does. Woitas et al. (5) compared for the first time the diagnostic performance of cystatin C, β-trace protein, β2-microglobulin, and creatinine in relation to inulin clearance. They noted that we reported a higher diagnostic accuracy of β-trace protein in a study with 115 diabetic patients (4). As explanation, Woitas et al. (5) offered different patient characteristics.

In this letter we would like to compare our data with regard to the diagnostic accuracy of cystatin C in comparison with β-trace protein in our diabetic cohort. We have expanded our former investigations of β-trace protein with the additional determination of cystatin C measured by two methods: N Latex Cystatin C, from Dade Behring, on the BNA analyzer; and Cystatin C PET-Kit, from Dako, on the Hitachi 717. Our data supplement the findings of Woitas et al. (5).

The ROC plots and calculated areas for creatinine, β-trace protein, and cystatin C, based on 80 mL·min⁻¹·1.73 m² as the cutoff for GFR, are shown in Fig. 1. The areas for cystatin C (Dade Behring) and β-trace protein do not differ significantly (0.89 and 0.85, respectively; P = 0.334). The ROC areas for cystatin C measured by the Dako method and for creatinine are smaller than the ROC areas for cystatin C measured by the Dade Behring method (P < 0.01). Using samples stored at −80 °C, we repeated the measurements of cystatin C by the Dade Behring method after 12 months and confirmed these results (area under the ROC curve ± SE, 0.88 ± 0.033). At 95% sensitivity, the specificity of cystatin C was 0.62 (95% confidence interval, 0.51–0.72), whereas the specificity of β-trace protein was only 0.27 (95% confidence interval, 0.18–0.37). Thus, the Dade Behring cystatin C discriminates even better than β-trace protein and our data confirm, in a quite different patient population, the conclusion of Woitas et al. (5) that cystatin C may be the best low-molecular weight protein marker at present to indicate reduced GFR.

References

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Optimization of Erythrocyte Folate Extraction

To the Editor:
Erythrocyte folates are commonly extracted for assay by 10-fold dilution of EDTA-anticoagulated blood (EDTA-WB) in 10 g/L ascorbic acid. It is assumed that this procedure achieves two goals: the complete lysis of erythrocytes and the exposure of intracellular folate polyglutamates to plasma conjugase enzyme (γ-glutamyl hydrolase) at a pH near 4.5, which favors their conversion to assayable folate. These EDTA-WB hemolysates require a further 20-fold (or more) dilution before analysis by microbiological assay with Lactobacillus rhamnosus.

A recent study of red cell folate extraction (1), using a microbiological assay, challenged these assumptions. The authors noted that adjusting the pH of a 10 g/L ascorbic acid diluent from pH 2.7 to pH 4 or 4.25 with 1 mol/L NaOH before EDTA-WB dilution (1 in 10) and incubation produced significantly increased whole-blood folate (WBF) yields (>10%) from fresh, unfrozen hemolysates. They also recommended the inclusion of a cell-lysing agent in extraction diluents, noting that saponin (100 mg/L) could substantially increase WBF yields, depending on hemolysate pH (1). We evaluated these findings using our “in house” microbiological assay (2) and fresh clinical EDTA-WB samples. We compared the WBF concentrations of 25 individual EDTA-WB samples after extraction in 10 g/L ascorbate solutions at pH 2.7, 4.0, and 4.25, respectively (to give hemolysate pH values of 4.0, 4.7, and 5.2)

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as described (1), and added saponin (100 mg/L) to a portion of each as a separate control. We noted no significant difference by paired t-test in the folate concentrations of EDTA-WB extracted at pH 4 (158 ± 83 μg/L; mean ± SD), at pH 4.7 (154 ± 90 μg/L), or at pH 5.2 (153 ± 83 μg/L). The addition of saponin (100 mg/L) to these diluents had no significant effect on results.

Wright et al. (1) used 0.1 mol/L sodium phosphate buffer, pH 6.2, to dilute both red cell hemolysates and folic acid calibrators before microbiological assay, whereas we used 5 g/L sodium ascorbate (25 mmol/L). We note that a 40-fold dilution of a pH 4 hemolysate in 5 g/L sodium ascorbate (pH ~7.4) achieves a pH of 5.8. We prepared fresh blood hemolysates in ascorbate at pH values of 4.0, 4.7, 5.2, 5.5, and 7.0 and analyzed them after an additional 40-fold dilution in either 5 g/L sodium ascorbate or in 0.1 mol/L sodium phosphate buffer (pH 6.2). In several studies, hemolysates at each pH yielded consistently and significantly higher folate concentrations (>20%) when diluted in sodium ascorbate (25 mmol/L). Consequently, we evaluated the recovery of spectrophotometrically determined (3) concentrations of supplemental 5-methyltetrahydrofolate (kindly provided by Eprova, Lernemack, Switzerland) and pteroylglutamic acid (Merck) from sodium ascorbate and from phosphate buffer both before and after dilution of pH 4 hemolysates (n = 16). The yield was <70% of added folate monoglutamate from phosphate buffer and >95% from sodium ascorbate-diluted hemolysates (Table 1); full equimolar yields of these folate calibrators were obtained from plain phosphate or ascorbate in the absence of added hemolysate. We also noted that dilution of hemolysate in sodium ascorbate produced clear solutions, whereas those diluted in phosphate buffer tended to be cloudy. This may represent isoelectric precipitation of protein (4) and include hemoglobin, red cells, and red cell debris.

Our preliminary studies of erythrocyte hemolysis in 10 g/L ascorbic acid, as monitored by cytometric red cell counting using the Sysmex XE 2100, yielded surprising results. In a typical experiment, the percentages of erythrocytes remaining in fresh hemolysates at pH 4.0, 4.7, 5.2, 5.5, 6.0, 6.3, and 7.0 after 60 min at 20 °C were 13.5%, <5%, <5%, <5%, 65%, 100%, 100%, and 100%, respectively, whereas they were 34.6%, 15.4%, 5.8%, <5%, <5%, <5%, and <5%, respectively, in the ascorbate-treated solutions that included saponin (100 mg/L). Hemolysis was more rapid at pH 4.7 and 5.2 than at pH 4 and 5.5, and at pH 6, 6.3, and 7, more than 90% of cells remained apparently intact for hours. Saponin, the traditional hemolyzing agent, consistently inhibited total cell lysis in ascorbate at the important pH values of 4.0, 4.7, and 5.2, which favor plasma conjugase activity, and a large cohort of cells remained intact. We note that Triton X-100 (1 and 2 mL/L) effects complete cell lysis (>95%) after 5 and 15 min, respectively, with identical pH 4.0 hemolysates. We have used Triton X-100 previously for this purpose (5).

We fully agree with Wright et al. (1) that the inclusion of a suitable hemolyzing agent is a logical support to erythrocyte folate extraction. We also agree that cells appearing intact after extraction in ascorbate may have revealed the loss of some hemoglobin and folate. However, the authors’ observations of significantly increased erythrocyte folate yields from EDTA-WB hemolysates near pH 5.0 compared with pH 4.0 are suspect and need further investigation. They may be overly influenced by assay sensitivity and the incompleteness of cell lysis even in the presence of saponin. In addition, their overall findings were based on experiments using blood samples from “a female regular donor”. We urge caution in the modification of existing assay protocols without comprehensive evaluation using blood samples from many donor sources.

### Table 1. Comparison of the recoveries of supplemental 5-methyltetrahydrofolate and pteroylglutamic acid from ascorbate-treated whole blood hemolysates (n = 16) diluted in either sodium ascorbate or sodium phosphate buffer (0.1 mol/L, pH 6.2) prior to microbiological assay.

<table>
<thead>
<tr>
<th>Hemolysate*</th>
<th>WBF, μg/L</th>
<th>Recovery, %</th>
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<tbody>
<tr>
<td>A</td>
<td>108.5 (±47.8)</td>
<td>96.1 (±12.7)</td>
</tr>
<tr>
<td>B</td>
<td>90.9 (±48.7)</td>
<td>68.6 (±11.4)</td>
</tr>
</tbody>
</table>

* A, hemolysates diluted in 5 g/L sodium ascorbate; B, hemolysates diluted in sodium phosphate buffer (0.1 mol/L, pH 6.2).

** Mean (SD).

© 5-CH₃THF, 5-methyltetrahydrofolate; PteGlu, pteroylglutamic acid.

References


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