Biological Variation of Procalcitonin in Healthy Individuals

To the Editor

Procalcitonin (PCT) is a stable 116-amino acid precursor molecule to the hormone calcitonin. High serum concentrations have been reported in patients with severe systemic bacterial or fungal, but not viral, infections (1).

Information on the biological variation of PCT is not available; this is limiting because the clinical utility of laboratory data can be affected by physiologic variation (2). Here we report the results of a study to determine the biological variation of PCT.

To investigate the biological variation of this analyte, we followed the same protocol that we used for the study of the biological variation of N-terminal pro-B-type natriuretic peptide (3). We took five blood specimens from each of 16 apparently healthy laboratory workers (5 men and 11 women; age range, 43–62 years) twice a week (Tuesdays and Fridays) over a 17-day period. None of the workers smoked, took any medication, or consumed substantial quantities of alcohol. In accordance with Helsinki Declaration II, the design and execution of the experiment were explained thoroughly to the participants, and informed consent was obtained. Blood was collected under standardized conditions to minimize sources of preanalytic variation. After an overnight fast, a blood specimen was taken by conventional venipuncture between 0800 and 0900 in a single run in duplicate. PCT concentrations were determined by the time-resolved amplified cryptate emission (TRACE) methodology on Kryptor (Brahms Diagnostic) by the same analyst, who followed the assay manufacturer’s recommendations. The mean PCT concentrations for men and women were not different (P = 0.93). The analytical (CV A) and intra- (CV I) and interindividual (CV G) components of variation were calculated by nested ANOVA. The reference change value (RCV) for significant changes in serial results (P <0.05), the index of individuality, and the desirable quality specifications for imprecision (I), bias (B), and total error (TE) were calculated using the following formulae:

\[
\text{RCV} = 2^{1/2} \times 1.96 \times (\text{CV}_A^2 + \text{CV}_I^2)^{1/2}
\]

\[
I < 0.5(\text{CV}_I)
\]

\[
B < 0.25(\text{CV}_A^2 + \text{CV}_G^2)^{1/2}
\]

\[
\text{TE} < 1.65 I + B(\alpha < 0.05).
\]

The number of specimens that should be collected to estimate (P <0.05) the homeostatic set point of an individual within ±10% [1.96\times (\text{CV}_A^2 + \text{CV}_G^2)/100] was also evaluated. The results are reported in Table 1.

The TRACE precision assay does not meet the goal [neither the desirable nor the minimum analytical precision, CV A ≤0.50(\text{CV}_I) and CV A ≤0.75(\text{CV}_G)], respectively] based on biological variation, at least at the concentrations measured in our population. The error added to clinical signal through analytical noise is 37% (2). Improvement is therefore required if this assay is to be offered on a routine basis. Finally, the low index of individuality shows that the use of population-based reference limits is inadequate for interpretation.

Table 1. Mean values; estimated mean analytical (\text{CV}_A), intraindividual (\text{CV}_I), and interindividual (\text{CV}_G) variation; and derived indices for serum PCT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean, μg/L</th>
<th>\text{CV}_A %</th>
<th>\text{CV}_I %</th>
<th>\text{CV}_G %</th>
<th>I</th>
<th>RCV, %</th>
<th>n</th>
<th>Imprecision, %</th>
<th>Bias, %</th>
<th>TE, %</th>
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<tbody>
<tr>
<td>All</td>
<td>0.040</td>
<td>15</td>
<td>16</td>
<td>22</td>
<td>0.7</td>
<td>60.6</td>
<td>18</td>
<td>8.1</td>
<td>6.9</td>
<td>20.3</td>
</tr>
<tr>
<td>Men</td>
<td>0.043</td>
<td>14</td>
<td>19</td>
<td>21</td>
<td>0.9</td>
<td>65.5</td>
<td>21</td>
<td>9.4</td>
<td>7.1</td>
<td>22.6</td>
</tr>
<tr>
<td>Women</td>
<td>0.038</td>
<td>15</td>
<td>14</td>
<td>23</td>
<td>0.6</td>
<td>57.0</td>
<td>16</td>
<td>7.0</td>
<td>6.7</td>
<td>18.3</td>
</tr>
</tbody>
</table>

* II, index of individuality.

References


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False-Negative Result in the Detection of an IgM Monoclonal Protein by Capillary Zone Electrophoresis

To the Editor:

Capillary zone electrophoresis (CZE) of serum proteins has developed into a rapid and sensitive analytical technique that is gaining impact in clinical laboratories because of its suitability for automation (1,2). We report a case of plasmacytic lymphoma with an IgM monoclonal gammopathy that was difficult to detect by CZE.
The patient was a previously healthy 49-year-old man who was referred because of palpable large (1.5-cm) lymph nodes bilateral in the submandibular region, in the neck, and in the axillae. Computed tomography scans revealed several large (1–1.5 cm) mediastinal and retroperitoneal lymph nodes. His blood hemoglobin concentration was 120 g/L, his leukocyte count was 6.6 $\times$ 10$^9$/L, and his platelet count was 361 $\times$ 10$^9$/L with a normal differential count. Serum total lactate dehydrogenase activity was 4.8 (cutoff for reference interval 8) kat/L, and serum $\beta_2$-microglobulin concentration was 2.6 ($\leq$ 2.7) mg/L. Biopsy from one of the cervical lymph nodes showed a plasmacytic non-Hodgkin lymphoma with the following immune phenotype: CD20+, IgM+, $\lambda$+, $\kappa$-, CD5-, CD10-, CD23-, bcl-2+, cyclin D1-. Bone marrow trephine biopsy showed 50–60% cellularity, dominated by normal erythropoiesis but with a paratrabeular lymphoid infiltration with $\lambda$-clonal, CD20+ cells showing plasmacytoid differentiation. There were no lymphoplasmacytic cells in the bone marrow smear.

CZE of serum proteins performed with the multichannel, automated Paragon CZE 2000 instrument with seven capillaries in parallel and software version 1.6.02 (Beckman Coulter) did not reveal any obvious monoclonal paraprotein, and there were no detectable decreases in the concentrations of polyclonal immunoglobulins (Fig. 1A). However, immunochemical quantification of IgG, IgA, and IgM with use of the Image system (Beckman Coulter) revealed a high IgM concentration of 32 g/L.

To further investigate the possibility of an IgM-type paraprotein, we treated 500 $\mu$L of serum from the patient with 5 mg of d/l-penicillamine (cat. no. P-5125; Sigma) at 37 °C for 1 h, a treatment that cleaves disulfide bonds in the IgM pentamer through the thiol activity of penicillamine. CZE analysis of the treated sample revealed a monoclonal gammopathy (Fig. 1B) that was also visible in an untreated sample after agarose gel electrophoresis (Fig. 1C) and was classified by agarose immunoelectrophoresis as a monoclonal IgM-$\lambda$ protein.

Problems with the detection of monoclonal components by CZE have been described previously (3–5), and a report similar to this one has appeared (6). This case underscores the necessity of combining CZE analysis of serum proteins with immunochemical quantification of the immunoglobulins, using a system with thorough testing against antigen excess to avoid false-negative results. It also describes an old experimental procedure that may be useful when a monoclonal IgM-type paraprotein is suspected and the CZE analysis is inconclusive: penicillamine treatment of the sample.

References


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Ethanol Precipitation Is Not Reliable for Selectively Removing Nonmonoclonal Peaks Seen in the Fibrinogen Region on Capillary Zone Electrophoresis of Serum Proteins

To the Editor:
The enhanced resolution of capillary zone electrophoresis (CZE) provides superior sensitivity for the detection of monoclonal (M) proteins in serum samples compared with agarose gel electrophoresis (AGE). This comes at the cost, however, of a lower specificity, calculated as 78% in our hands for the Paragon CZE 2000® with software version 1.602 (Beckman) (1). Abnormalities in the electropherogram that cause confusion are most frequently located in the anodal part of the γ-globulin fraction. Notably, fibrinogen, which remains in incompletely clotted serum samples, migrates at the junction between the β- and γ-fractions. Even when following the strict procedure of keeping serum samples for a minimum of 4 h at room temperature before electrophoresis, we found a suspected fibrinogen or possible M-protein peak in 39 (8.6%) of 454 consecutive routine samples in our laboratory.

Recently, a simple method consisting of cold-ethanol precipitation was described for the removal of fibrinogen from serum specimens before agarose protein electrophoresis (2). We evaluated the use of this method in the routine analysis of serum samples by CZE in which a peak at the fibrinogen location could cause confusion with the detection of small M-proteins.

Absolute ethanol was added to the specimens at a final concentration of 100 mL/L. Precipitation was induced by cooling for 15 min in an ice bath, after which samples were centrifuged at 1100 g and the supernatant was removed for subsequent CZE analysis. Preliminary experiments demonstrated that this method completely removed the fibrinogen peak from plasma samples on CZE as well as on AGE (Fig. 1A). Clear monoclonal peaks (9–31 g/L) in the γ-globulin area were not affected (data not shown). These data are consistent with the findings reported by Qiu et al. (2). However, fibrinogen was still detectable by immunofixation on postprecipitation plasma samples (Fig. 1A). It should be noted that the β2 peaks are clearly diminished after the procedure (Fig. 1). This could be a consequence of C3-complement turnover, but changed electrophoretic mobilities of some proteins as a result of denaturation by ethanol has also been suggested (3).

When we used ethanol precipitation to treat seven serum samples with suspected fibrinogen peaks, we found that these peaks disappeared on CZE in only three of the samples. To determine whether the peaks in the fibrinogen region that did not disappear represented M-proteins, we analyzed serum samples that were submitted for both electrophoresis and immunofixation and that showed a peak in the fibrinogen area after CZE. Electropherograms on CZE before and after ethanol precipitation were judged by three independent observers. Of the 12 samples thus analyzed, 7 displayed a peak in the fibrinogen area after ethanol precipitation: 4 samples with a M-protein on immunofixation (located in the β-γ area), but also 3 samples with no M-protein detectable (for an example, see Fig. 1C). Of the five samples in which the peak in the fibrinogen area disappeared, three had no M-protein on immunofixation but two were positive, again in the β-γ area (for an example, see Fig. 1B). It should be noted that these two samples were judged still to contain a small peak in the fibrinogen area by one observer; the other results were completely consistent over the three observers. Removal by cold-ethanol precipitation of a M-protein that behaves as a cryoglobulin could also explain some of these observations. Thus, some CZE samples with a peak in the fibrinogen area were still suspect for harboring a M-protein after ethanol precipitation, although they contained no M-protein by immunofixation. Conversely, two of three observers judged two samples to be devoid of fibrinogen after precipitation (because the abnormal peak in the fibrinogen area had disappeared), even in the presence of a M-protein in this area on immunofixation.

We performed immunofixation for the detection of fibrinogen on some samples with suspect fibrinogen peaks: fibrinogen was present in one sample before and after ethanol precipitation on CZE (Fig. 1B). The other four samples were devoid of fibrinogen by immunofixation, and none of them had unambiguous M-proteins, despite the presence of an altered morphology at the β-γ region (Fig. 1C). Ibrahim et al. (3) recently concluded that the ethanol precipitation method is neither specific nor selective for fibrinogen.

In conclusion, the cold-ethanol-precipitation method described by Qiu et al. (2) was effective in removing fibrinogen from plasma samples as judged by CZE and AGE, without interfering with the detection of major M-proteins. When used on truly problematic serum samples, however, in which the presence of a peak in the fibrinogen area can be caused either by fibrinogen or by a small M-protein, this method was unreliable in our hands to make this distinction. We therefore recommend that all samples that are questionable