Evidence of Temperature-dependent Interference in an Immunonephelometric Assay by Monoclonal IgM

To the Editor:
Interference by monoclonal IgM has been described in the immunonephelometric assays of C-reactive protein, antistreptolysin O, ferritin, and complement C4 (1–6). This report describes the interference by monoclonal IgM (IgMκ) in the immunonephelometric assays of apolipoproteins A-I and B, haptoglobin, and immunoglobulins A and G.

The patient, a man 54 years of age, was admitted to a cardiac care unit with angina. His total plasma protein was 110 g/L, his serum cholesterol was 3.3 mmol/L, his apolipoprotein A-I was 1.5 g/L, and his apolipoprotein B was 2 g/L. Myeloma was suggested by the increase in total plasma proteins and verified by immunonephelometric assay of immunoglobulins and by immunofixation electrophoresis (Beckman reagents), which identified a κ monoclonal IgM. This monoclonal IgM was not a cryoglobulin because after 8 days at 4 °C, no flocculation was observed in the serum sample.

The apolipoproteins, immunoglobulins, and haptoglobin were assayed initially by immunonephelometry with a BNA and a BN II (Behring Nephelometer; Dade-Behring) and on a second sample with a BN 100 and again with a BN II. The BN II is operated at 37 °C, the others at room temperature (~23 °C). The results are shown in Table 1. The BNA (room temperature) appeared to overestimate all proteins except IgM (2.94 g/L), which was underestimated in the crude serum; after the crude serum was diluted, the IgM concentration was 60 g/L. The serum cholesterol value was consistent for apolipoproteins A-I and B with the BN II (37 °C) procedures but not with the room temperature methods.

To determine the frequency of this interference by monoclonal immunoglobulins, a double-blind study was conducted that involved determining the proteins with both a BN 100 and a BN II. Among 70 sera with monoclonal immunoglobulins (38 with monoclonal IgM, 12 with monoclonal IgA, 20 with monoclonal IgG), comparable interference occurred only for the index patient (Table 1B).

We therefore conclude that the monoclonal IgM could influence the BNA and BN 100 procedures. We (5) and others (1–4, 6) have reported interference between monoclonal IgM and latex particles in the immunonephelometric assay of C-reactive protein, antistreptolysin O, and ferritin. However, latex particles and supplement reagents for precipitation were not used in the immunoglobulin and haptoglobin assays by the BNA, BN 100, or BN II procedures. This rules out any dependent unselective precipitation of the reagents.

Thus, after excluding a cryoglobulin and the reagents as causal factors of the discrepancy in the presented data, we must conclude that the monoclonal IgMκ was the primary reason for the present unselective precipitation during the immunonephelometric assays of proteins, which did not occur when the temperature was increased to 37 °C. We point out the difficulty in taking into account this rare interference because it occurred only once among 38 samples containing monoclonal IgM checked in our laboratory.

Table 1. Specific proteins assayed by the BNA, BN 100, and BN II procedures.

<table>
<thead>
<tr>
<th>Protein</th>
<th>BNA, a g/L</th>
<th>BN II, b g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein A</td>
<td>1.58</td>
<td>1.10</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>2.03</td>
<td>0.75</td>
</tr>
<tr>
<td>IgA</td>
<td>4.69</td>
<td>1.72</td>
</tr>
<tr>
<td>IgG</td>
<td>6.13</td>
<td>4.78</td>
</tr>
<tr>
<td>IgM</td>
<td>60</td>
<td>59.1</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>1.94</td>
<td>1.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>BN 100, a g/L</th>
<th>BN II, b g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein A</td>
<td>1.44</td>
<td>1.18</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>1.85</td>
<td>0.97</td>
</tr>
<tr>
<td>IgA</td>
<td>2.63</td>
<td>1.41</td>
</tr>
<tr>
<td>IgG</td>
<td>5.07</td>
<td>4.53</td>
</tr>
<tr>
<td>IgM</td>
<td>34.8</td>
<td>40.2</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>2.46</td>
<td>1.95</td>
</tr>
</tbody>
</table>

Notes:
- a Room temperature.
- b 37 °C.

References

Robert Granouillet1
Françoise Rasclé2
Christine Bonneau2
Annette Chamson2
Jacques Frey1
Christian Perier1

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Complexed Prostate-specific Antigen and the “Prostate-specific Antigen Gap”

To the Editor:
Jung et al. (1) reported an apparent gap between total serum prostate-specific antigen (t-PSA) and the sum of free PSA (f-PSA) plus α1-antichymotrypsin-PSA (ACT-PSA) in patients with prostate carcinoma (PCa) but not in those with benign prostatic hypertrophy (BPH). The gap may be attributable to a variety of technical artifacts such as different recognition of multiple forms of f-PSA or t-PSA in the two groups of patients or the lack of equimolarity of the tests used (1). Of more interest, however, is the suggestion by the authors that the appearance of this “PSA gap” reflects an increase in minor PSA complexes in PCa patients that their assay for ACT-PSA does not detect. Indeed, they suggest that such an observation contradicts the high expectations (2) for the determination of ACT-PSA or the ratio of ACT-PSA to t-PSA to improve the differentiation between PCa and BPH.

The authors refer to a novel approach to the measurement of complexed forms of PSA (3) as a possible solution to the potential problem of minor PSA forms. This complexed PSA (c-PSA) assay is now available commercially for the Bayer Immuno-1 automated immunochemistry analyzer (Bayer Diagnostics). We recently used this assay along with measurements of t-PSA (Bayer Immuno-1), f-PSA (Abbott IMx; Abbott Diagnostics), and the ratio of f-PSA to t-PSA (Abbott IMx, both assays) on a prospective basis in men referred to a hospital urology clinic with t-PSA measurements between 3.0 and 22.0 μg/L. Of the 79 consecutive patients assessed to date, 21 (27%) were diagnosed with PCa by a combination of clinical presentation, digital rectal examination, prostatic volume, and transrectal ultrasound-guided prostatic biopsy (all patients). We found no gap between t-PSA (Bayer Immuno-1 assay) and the sum of f-PSA (Abbott IMx assay) and c-PSA (Bayer Immuno-1 assay) in patients with PCa or BPH. The sum of f-PSA and c-PSA was 100.6% (SD, 7.6%) of t-PSA in the BPH patients and 100.5% (SD, 6.2%) in the PCa patients.

We believe, therefore, that our data showing the lack of a PSA gap in both PCa and BPH patients, along with the observations of Jung et al. (1), may help solve the conundrum of why ACT-PSA does not perform as well as predicted.

References
1. Jung K, Brux B, Knabich A, Lein M, Sinha P, Schnorr D, Loening SA. A gap between total prostate-specific antigen and the sum of free prostate-specific antigen plus α1-antichymotrypsin-PSA complex in serum does not influence the recognition of the multiple forms of PSA and the sum of free PSA or total PSA in both groups, or lack of equimolarity), although we paid particular attention to this problem. It should be mentioned that Espana et al. (3), using homemade immunoassays for the determination of total PSA and α1-antichymotrypsin-PSA, recently found better discrimination with the ratio of α1-antichymotrypsin-PSA to total PSA than with the ratio of free to total PSA. Therefore, at present, our results and the mentioned contradictory data should only draw the attention of clinical chemists and physicians to the fact that there are possible biological rather than analytical reasons that contradict the high expectations placed on the determination of α1-antichymotrypsin-PSA (4). It is obvious that both basic experiments and clinical studies are necessary to solve the whole problem. We hope that a recently performed multicenter study using a newly developed α1-antichymotrypsin-PSA assay with changed architecture may help clarify this issue.

The authors of the Technical Brief cited above reply:

To the Editor:
Our observation of a prostate-specific antigen (PSA) gap in the serum of patients with prostate carcinoma (PCa) and the assumption of the increased occurrence of minor PSA forms in serum may explain the better discrimination between PCa and benign prostatic hyperplasia (BPH) patients by the free/total PSA ratio compared with the determination of the α1-antichymotrypsin-PSA complex or the ratio of α1-antichymotrypsin-PSA to total PSA that we observed in 112 untreated patients with PCa and in 34 patients with BPH (1). However, as pointed out in that report and in Ref. (2), we cannot exclude all possible technical reasons for the PSA gap discrepancies between BPH and PCa patients (e.g., calibration of the assays, different recognition of the multiple forms of free PSA or total PSA in both groups, or lack of equimolarity), although we paid particular attention to this problem. It should be mentioned that Espana et al. (3), using homemade immunoassays for the determination of total PSA and α1-antichymotrypsin-PSA, recently found better discrimination with the ratio of α1-antichymotrypsin-PSA to total PSA than with the ratio of free to total PSA. Therefore, at present, our results and the mentioned contradictory data should only draw the attention of clinical chemists and physicians to the fact that there are possible biological rather than analytical reasons that contradict the high expectations placed on the determination of α1-antichymotrypsin-PSA (4). It is obvious that both basic experiments and clinical studies are necessary to solve the whole problem. We hope that a recently performed multicenter study using a newly developed α1-antichymotrypsin-PSA assay with changed architecture may help clarify this issue.

References