Interference of Monoclonal Antibody Therapies with Serum Protein Electrophoresis Tests

To the Editor:

During routine serum protein electrophoresis (SPE), we recently detected an apparent IgG heavy-chain gammopathy in a 51-year-old woman with a 5-year history of IgD multiple myeloma. Other relevant laboratory values at the time included the following: serum creatinine, 159 μmol/L (reference interval, 62–97 μmol/L); IgG, 2.55 g/L (reference interval, 6.00–17.00 g/L); IgM, <0.25 g/L (reference interval, 0.35–2.90 g/L); and IgA, 0.40 g/L (reference interval, 0.40–4.00 g/L). The rarity of an IgA, 0.40 g/L (reference interval, 0.35–2.90 g/L); and IgM, 2.55 g/L (reference interval, 6.00–17.00 g/L); IgM, <0.25 g/L (reference interval, 0.35–2.90 g/L); and IgA, 0.40 g/L (reference interval, 0.40–4.00 g/L). The rarity of an IgG heavy-chain gammopathy in combination with an IgD multiple myeloma was readily detected by immunofixation electrophoresis as an IgGκ monoclonal protein (data not shown). The majority of the monoclonal antibodies electrophoresed in the middle of the γ-globulin region, whereas rituximab and trastuzumab electrophoresed toward the cathode. The therapeutic monoclonal antibodies tested represent both chimeric human–mouse immunoglobulins (rituximab, siltuximab, infliximab, cetuximab) and humanized antibodies (trastuzumab, bevacizumab, adalimumab), suggesting that this class of drugs may broadly interfere with SPE methods.

Siltuximab (Centocor), a high-affinity antibody to human interleukin-6, is currently being used in clinical trials for the treatment of a number of malignancies, including multiple myeloma. Siltuximab is a chimeric immunoglobulin comprising the variable antigen-binding region of a mouse antibody and the constant region of human IgG1κ (1). It binds to human interleukin-6, a survival factor for myeloma cells (2).

We obtained siltuximab from the manufacturer and used 2 different SPE platforms, CAPILLARYS II (Sebia) and HYDRASYS (Sebia), to test it as an interferent. Siltuximab was analyzed alone or spiked into nonpathologic reference sera at concentrations of 50–600 mg/L, the reported interval of circulating drug concentrations (3, 4). Siltuximab was evident as an IgGκ monoclonal protein at a threshold of approximately 100 mg/L and was detected by all methods tested, including capillary electrophoresis, immunosubtraction (not shown), agarose gel electrophoresis, and immunofixation electrophoresis (Fig. 1). The capillary and immunofixation methods were more sensitive to monoclonal therapy interference than agarose gel SPE.

Given the detection of siltuximab in these systems, we tested other widely prescribed monoclonal therapies at their reported mean peak serum drug concentrations: rituximab (Rituxan), trastuzumab (Herceptin), bevacizumab (Avastin), infliximab (Remicade), cetuximab (Erbitux), and adalimumab (Humira). Each monoclonal antibody was detected by all methods tested, except for siltuximab, which was not detected by immunofixation electrophoresis (Fig. 1). The capillary and immunosubtraction methods were more sensitive to detect therapeutic monoclonal antibodies by their electrophoretic mobility. In particular, the cathodal migration of rituximab and trastuzumab may raise a suspicion of interference. Any of these approaches may be useful in identifying and handling potential monoclonal therapy interference.

Several other therapeutic agents, notably antibiotics and radio-opaque agents, have been reported to interfere with SPE (5). To our knowledge, this report is the first of a therapeutic monoclonal antibody interfering with SPE. The interference of these therapies may be of clinical importance in patients being investigated or monitored for plasma cell dyscrasias. Individuals receiving monoclonal therapy may be subject to unnecessary follow-up testing, and patients with IgGκ-producing disease may be misconstrued as experiencing recurrence or resistance to treatment. We suggest that the possibility of false-positive SPE results needs to be considered, given the increasing use of these agents in clinical practice.
Fig. 1. Detection of monoclonal immunoglobulin therapy by SPE and immunofixation electrophoresis (IFE).

The top panel shows agarose gel electrophoresis and capillary electropherograms. The reference serum trace (black) is overlaid on the siltuximab-spiked serum electropherogram (red); red arrows denote the migration of siltuximab. The middle and bottom panels show IFE results for siltuximab spiked into saline (middle left) and the results of physician-ordered IFE testing for 3 patients receiving siltuximab therapeutically. Results for patient #1 are for the IgD/H9260 index case described in the text. The immunofixation antisera are indicated at the bottom of each gel. Rf, acid-fixed reference pattern; +, anode; −, cathode; ·, sample-application point.

References


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Specificity of Serum and Urine Protein Electrophoresis for the Diagnosis of Monoclonal Gammopathies

To the Editor:

Serum protein electrophoresis (SPE) is the hub of laboratory testing for monoclonal gammapathies and is a component of almost all diagnostic panels (1–3). The sensitivity of SPE as a screening test, however, is dependent on how SPE abnormalities are defined (4). Discrete, quantifiable electrophoretic bands (M-spikes) are almost always monoclonal proteins. Subtle abnormalities such as fuzzy γ bands, increased β or α fractions, or decreased γ fractions may reflect the presence of a monoclonal protein, or they may have other causes. Because these subtle abnormalities can reflect serious diseases, laboratories often reflex samples with SPE abnormalities to immunofixation electrophoresis (IFE) for confirmation, even if IFE was not ordered. To evaluate the specificity of these reflex triggers, we reviewed IFE results for reflexed protein electrophoresis assays.

We performed SPE, urine protein electrophoresis (UPE), and urine IFE with agarose gel reagents on the Helena REP system (Helena Laboratories) and performed serum IFE with Sebia Hydragel reagent sets (Sebia). All SPE assay results that were reflexed between October 1 and November 3, 2008, and all UPE results reflexed between November 1 and November 30, 2009, were categorized. The triggers for reflexing SPE results were abnormal discrete bands (M-spikes), fuzzy or questionable fuzzy bands, hypogammaglobulinemia (〈5.5 g/L), increased β fractions (between 16 g/L and 19 g/L; β fractions 〈20 g/L were fractionated as M-spikes), and an increased α₂ fraction (〈14 g/L). UPE abnormalities were M-spikes, fuzzy bands, or the patient having a previously identified serum monoclonal protein.

Of 5992 sera for which SPE (but not IFE) was ordered, 790 samples (13.2%) were reflexed to serum IFE because of an abnormality that had not previously been characterized. IFE identified 43% of the abnormalities as a monoclonal protein (100% of all M-spikes and 28% of the other SPE abnormalities; see Table 1).

Among the 206 fuzzy, restricted bands, 112 monoclonal proteins (54%) were detected. Four of the 112 IFE-positive results were monoclonal free light chains. Among the 263 questionable fuzzy bands, 47 (18%) were monoclonal proteins. Of 85 hypogammaglobulinemic bands, 10 (12%) were positive, and 5 were monoclonal free light chains.

Of 2826 urine samples for which UPE (but not urine IFE) was ordered, 135 (4.8%) were reflexed to urine IFE, which identified 56% as monoclonal: 89% of M-spikes, 51% of fuzzy bands, and 44% of urine samples that had a known serum monoclonal protein. IFE confirmed all 169 serum M-spikes in this study as monoclonal proteins (100% specificity), and 23 of the 26 urine M-spikes were positive by IFE (89% specificity). Although fibrinogen and hemoglobin bands are not uncommon in serum samples, most laboratories

Table 1. Reflexed SPE cases (n = 5992).

<table>
<thead>
<tr>
<th>SPE Abnormality</th>
<th>Reflexed SPE cases, n</th>
<th>IFE positive (specificity), n</th>
<th>Monoclonal free light chain by IFE, n</th>
</tr>
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<tbody>
<tr>
<td>All abnormalities</td>
<td>790 (13.2%)</td>
<td>341 (43%)</td>
<td>9</td>
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<tr>
<td>M-spike</td>
<td>169</td>
<td>169 (100%)</td>
<td>—</td>
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<tr>
<td>Fuzzy band</td>
<td>206</td>
<td>112 (54%)</td>
<td>4</td>
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<tr>
<td>Questionable fuzzy band</td>
<td>263</td>
<td>47 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Hypogammaglobulinemia (〈5.5 g/L)</td>
<td>85</td>
<td>10 (12%)</td>
<td>5</td>
</tr>
<tr>
<td>Elevated β fraction (16–19 g/L)</td>
<td>10</td>
<td>1 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Elevated α₂ fraction (14–14 g/L)</td>
<td>48</td>
<td>2 (4%)</td>
<td>0</td>
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<tr>
<td>Broad or extra α₂ fraction</td>
<td>9</td>
<td>0 (0%)</td>
<td>0</td>
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