nositic value was also poor, with ~25% false negatives. By contrast, plasma cystatin C better reflected GFR with the strongest correlation with 51Cr-labeled EDTA clearance and no false negatives.

In a group of adult transplant recipients at steady-state renal function (average time since transplantation, 6 years), cystatin C was shown to have a better diagnostic value than serum creatinine for a GFR cutoff set at 60 mL/min (3). Despite a similar diagnostic value, 24-h creatinine clearance overestimated GFR by >20% in two-thirds of patients (3). The underestimation of GFR by plasma cystatin C we report here might be attributable to an inappropriate cutoff for cystatin C (too low), renal function not at steady state, or other unknown factors. Underestimation of GFR by serum cystatin C recently has been reported in pediatric transplantation (4). Serum cystatin C concentrations were 25% higher (range, 19–31%) in transplanted children vs non-transplanted children having the same GFR determined by inulin clearance (4). Interference with the cystatin C assay, tubular back-leakage of intact cystatin C into the circulation, or impaired filtration of antibody-cystatin C complexes have been suggested without definitive proof (4).

Despite the underestimation of GFR, plasma cystatin C appears superior to creatinine and 24-h creatinine clearance for evaluation of GFR in the postoperative follow-up of adult kidney transplant recipients. Moreover, GFR can be rapidly estimated from the reciprocal of the plasma cystatin C concentration, using a simple formula independent of age, body surface, and sex of the recipient.

References


Falsely Increased Values for Rabbit Immunoglobulin-based Nephelometric Immunoassays Attributable to Human Anti-Rabbit Antibodies

To the Editor:

A large number of serum proteins are routinely measured by automated immunonephelometric assays utilizing antisera derived from rabbits, goats, or sheep. Because this type of immunoassay uses polyclonal reagents derived from animals other than mice, it is free from interference by the human anti-mouse antibodies that commonly plague monoclonal antibody-based sandwich assays and competitive immunoassays (1). Nonspecific antibodies against murine immunoglobulins can be detected in the serum of a significant proportion of patients, with a prevalence estimate as high as 80% (2). The incidence of anti-rabbit antibodies in serum samples is considerably lower, with estimates between 0.1% and 5% (1, 3–6). Interference from human anti-rabbit antibodies has been documented for two-site immunoassays and radioimmunoassays for several hormone assays as well as for creatinine kinase MB (3–8). It recently was reported that anti-rabbit antibodies produced falsely increased C-reactive protein values when measured by nephelometric methods utilizing rabbit reagents (9).

Our clinical laboratory recently identified a sample with an abnormally high serum transthyretin value of 1406 mg/L (reference interval, 200–400 mg/L) that was being requested for assessment of nutritional status. The serum sample was analyzed by nephelometry using the automated BN II analyzer (Dade Behring), which uses rabbit anti-transthyretin antibodies. Other samples that were subsequently submitted for testing had transthyretin values of 1038–1107 mg/L (Table 1, specimens A–D). The increased transthyretin values could not be explained by the patient’s clinical history or medications. The patient was a 56-year-old female, status post chemotherapy for malignant B-cell lymphoma with an IgM monoclonal protein of 15 g/L. She was taking erythropoietin and furosemide. To investigate the presence of an interfering substance, transthyretin was remeasured using the Array automated nephelometer (Beckman Coulter). Results were considerably lower on reanalysis and were slightly increased or within the reference interval (Table 1). Because the Array uses antisera derived from goats whereas the BN II uses antisera from rabbits, the interference may have been attributable to anti-rabbit antibodies.
To determine whether the interference was limited to the transthyretin assay, haptoglobin was measured using the BN II and found to be within the reference interval of 750-3500 mg/L (Table 1). This was unexpected because the patient had received blood transfusions for a steadily declining hemoglobin and should have had extremely low or undetectable haptoglobin concentrations. When haptoglobin was remeasured using the Array, undetectable values were obtained (Table 1) that were more consistent with the clinical picture. Thus, the interference was not restricted to transthyretin and occurred with another BN II assay utilizing rabbit antisera.

To confirm that the false increase in transthyretin was the result of human antibodies against rabbit immunoglobulins, various classes of immunoglobulin were removed from the serum samples by affinity chromatography. After IgG was removed by passage through a protein G-Sepharose column, the transthyretin values were only slightly higher than results obtained by the Array (Table 1). A similar reduction in transthyretin was observed when a rabbit immunoglobulin column was used. When both IgG and IgM were removed by a protein L-Sepharose column, transthyretin values were comparable to those obtained by the Array, suggesting that IgM antibodies also contributed to the interference. Similar results were obtained when haptoglobin was examined (Table 1). As expected, IgG and IgM concentrations (measured by the Array) were substantially reduced after column chromatography using protein G and protein L, confirming that the expected immunoglobulin fractions had been depleted.

Because animal sera and immunoglobulins are routinely used as blocking agents to prevent anti-animal antibody interferences in other types of immunoassays (1), this approach was examined to determine whether it would eliminate the interference. The addition of rabbit serum or purified rabbit immunoglobulin to serum samples failed to reduce the falsely increased results for transthyretin and haptoglobin (data not shown).

In summary, this report demonstrates that human anti-rabbit antibodies can interfere with BN II nephelometric assays for transthyretin and haptoglobin (data not shown). The interference was usually classified as heterophile antibodies (10). However, the interfering antibodies were not multispecific, a feature of heterophile antibodies, because the interference was not observed in nephelometric assays using goat antisera. Nevertheless, I believe that this is the first report describing a human anti-rabbit interference in an automated immunonephelometric assay in the absence of a well-documented animal exposure. It is recommended that all unexplained increases in transthyretin and other nephelometric assays should be verified by repeat analysis using an assay that utilizes antisera from a different animal species.

Table 1. False-positive results with BN II nephelometric assays that use antisera derived from rabbits.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Transthyretin, mg/L</th>
<th>Haptoglobin, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BN II 1406</td>
<td>Array 496</td>
</tr>
<tr>
<td>B</td>
<td>1107</td>
<td>1200</td>
</tr>
<tr>
<td>C</td>
<td>1100</td>
<td>1200</td>
</tr>
<tr>
<td>D</td>
<td>1038</td>
<td>288</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IgG, g/L</th>
<th>IgM, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Protein G</td>
<td></td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>None</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>Rabbit immunoglobulin</td>
<td>5.8°</td>
<td>12.1°</td>
</tr>
<tr>
<td>Protein L</td>
<td>1.0°</td>
<td>0.9°</td>
</tr>
</tbody>
</table>

a Serum samples were obtained at 3- to 4-day intervals.

b Separate serum pools (prepared at different times) were used for each treatment group.

c Normalized to creatinine value for pretreated sample (to correct for dilution).

References


Anthony W. Butch

UCLA Medical Center
Department of Pathology and Laboratory Medicine
10833 Le Conte Ave.
Mailroom A2-179 CHS
Los Angeles, CA 90095-1713
Fax 310-794-4864
E-mail abutch@mednet.ucla.edu