Use of Immunoglobulin Heavy- and Light-Chain Measurements Compared with Existing Techniques as a Means of Typing Monoclonal Immunoglobulins

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Using automated measurements of kappa and lambda light chains and IgG, IgA, and IgM, we assessed the utility of the kappa/lambda ratio and the heavy chain/light chain ratio in characterizing monoclonal immunoglobulins previously identified by sensitive electrophoresis on agarose gel and typed by immunofixation or immunoelectrophoresis. We examined 348 selected samples, of which 165 contained monoclonal components, finding that 93.4% were detected and 89% correctly typed by this approach. Eight samples were shown to contain monoclonal light chains that were not visible as bands on electrophoresis but demonstrated abnormalities of the kappa/lambda or heavy chain/light chain ratio.


Monoclonal immunoglobulins (monoclonal gammapathies) arise from malignancies of B-cell origin or from restricted immune responses due to hyperstimulation of one or a few clones. They may be of any immunoglobulin class, and monoclonal immunoglobulin light chains may be present, either alone or in conjunction with intact immunoglobulin molecules. They are undetectable by simple immunoglobulin quantification, because they may be present despite normal values for total immunoglobulin concentrations.

Monoclonal gammapathies are identified as single or multiple bands, known as "paraproteins," on serum protein electrophoresis (SPE), but the sensitivity of different types of electrophoretic procedure for detecting them varies considerably (1). Their presence is confirmed and their immunoglobulin class is established by either immunoelectrophoresis (IEP) (2) or immunofixation (IFE) (3).

The recent development of quantitative assays for kappa (κ) and lambda (λ) light chains, either attached to intact immunoglobulin molecules or free, has led to the proposal that disturbance of the normal κ to λ ratio and of the normal light to heavy chain ratio may be used to detect or confirm most immunoglobulin abnormalities, which now requires IEP or IFE (4). We have examined 348 sera by the Kallestad Immunochemical Evaluation (ICE) system of automated heavy-chain and light-chain assays on two different instruments, one turbidimetric and one nephelometric, and compared the findings with those by agarose electrophoresis followed by IEP or IFE. With this approach we detected 93.4% of the paraproteins detected by a sensitive agarose electrophoresis system and correctly typed 89% of the latter.

In addition, eight cases with serum monoclonal light chains were found that had not previously been identified by bands on electrophoresis.

Materials and Methods

Samples

In center 1, a district general hospital, all samples found to contain paraproteins or demonstrating abnormal immunoglobulin concentrations during four months were included in the study. We also took as controls 100 normal samples at random from clinical samples demonstrating no abnormality on electrophoresis or immunoglobulin measurements.

In center 2, a reference center, the same procedure was used but, because more "difficult" samples are referred to this laboratory, the sample mix is biased towards paraproteins of low concentration.

Both centers defined paraproteins as bands seen on agarose gel electrophoresis (Paragon; Beckman Instruments Inc., Brea, CA 92621-6209). They were typed by IFE in center 1, and by IEP in center 2, after separation on agarose. Antisera used for IFE and IEP were supplied by Atlantic Antibodies, Scarborough, ME 04074. Concentrations of monoclonal immunoglobulins were measured by densitometry of agarose electrophoretic separations (5). Table 1 gives the characteristics of the paraprotein-containing samples.

Quantification of Immunoglobulins

Center 1 used an endpoint turbidimetric assay procedure on a centrifugal analyzer (Cobas Bio, Roche). The antisera and calibrators were supplied by Kallestad Diagnostics, Austin, TX 78701, in the form of the Quantimeter-2 test kit. Center 2 used kinetic nephelometry in the Beckman Immunochemistry System (ICS; Beckman Instruments). For heavy-chain assays we used ICS reagents, for light-chain assays the Quantimeter-2 reagents.

Assays for heavy chains were calibrated by using the calibrator SPS-01 (PRU Central Antiserum Purchasing

<p>| Table 1. Characteristics of Samples Containing Paraprotein Bands on Electrophoresis |
|--------------------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Paraprotein concn, g/L</th>
<th>Associated immunoglobulin changes</th>
<th>No. of samples</th>
<th>Abnormal</th>
<th>Correctly typed</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10 Polyclonal increase</td>
<td></td>
<td>Total</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>17</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Immunosuppressed</td>
<td></td>
<td>58</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>5-10 Polyclonal increase</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Immunosuppressed</td>
<td></td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>&lt;5 Polyclonal increase</td>
<td></td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>27</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Immunosuppressed</td>
<td></td>
<td>14</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

* Not included are three samples containing IgD paraproteins, which cannot be typed by the ICE system or IFE/IEP by using only IgG, IgA, and IgM antisera, and one sample with multiple monoclonal bands.

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1. Nonstandard abbreviations: SPE, serum protein electrophoresis; IEP, immunoelectrophoresis; IFE, immunofixation; ICE, Immunochemical Evaluation; ICS, Immunochemistry System.

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Unit, Sheffield, U.K.). Light-chain measurements in both laboratories were calibrated with Kallestad reference material, which has values for κ and λ light-chain concentrations assigned on a calculated "immunoglobulin equivalent molar weight" basis and verified by analytical-recovery studies with affinity-purified monoclonal and polyclonal light chains and immunoglobulins. Between-batch reproducibility (CV) was similar for all the analytes; for the Cobas Bio it was 3.7% and for the Beckman ICS 4.0%. Sixty samples were analyzed by each of the two laboratories, with no significant bias noted between the two instruments for any of the assays. Results of linear-regression analysis were as follows (x = center 1, y = center 2): IgG, slope 1.15, intercept −0.68, r = 0.98; IgA, slope 1.14, intercept −0.20, r = 0.96; IgM, slope 1.02, intercept −0.19, r = 0.99; κ, slope 1.06, intercept −0.32, r = 0.99; λ, slope 1.17, intercept −0.1, r = 0.99.

Immunochemical Evaluation (ICE)

The ICE approach to evaluation of patients' samples depends on the κ/λ ratio and (or) the heavy-chain/light-chain ratio falling outside the reference interval for sera containing monoclonal gammopathies. The reference intervals we used for the immunoglobulins were those routinely used in our laboratories, obtained by excluding the highest and lowest 2.5% of results for 1000 normal samples. Those for the κ/λ ratio and for the percentage difference between the total amount of heavy and light chains were similarly obtained from results for the 183 samples in this study that did not contain paraproteins. They are: IgG, 5.3–16.5 g/L; IgA, 0.8–4.0 g/L; IgM, 0.5–2.0 g/L; κ/λ ratio, 1.29–2.61; percentage difference between heavy and light chains, −7% to +12%.

The antisera used for light-chain measurement are specific for light-chain exposed epitopes and thus will measure free light chains as well as light chains attached to intact immunoglobulin molecules. An abnormal κ/λ ratio may thus indicate the presence and light-chain class of an intact monoclonal immunoglobulin or of monoclonal light chains. Because the light-chain measurements are calibrated on an immunoglobulin equivalent molar weight, the difference between the sum of IgG + IgA + IgM and κ + λ should not exceed the cumulative imprecision of the assays, unless free light or heavy chains are present or the paraprotein is IgD or IgE in type.

The correct typing of a paraprotein by use of the ICE system depends on allocating the light-chain type (from the κ/λ ratio) and the heavy-chain class. The heavy-chain class can be allocated by calculating the excess (monoclonal) light-chain concentrations and comparing them with the concentration of each heavy chain. If only one heavy chain is present in sufficient concentration to account for the concentration of monoclonal light chain, then this is the class allocated to the paraprotein.

The monoclonal light-chain concentration is established as follows: A κ/λ ratio >2.61 indicates excess κ light chains. If one assumes a normal κ/λ ratio among the residual light chains, they will have a maximum concentration equal to the λ chain concentration × 2.61. Thus κ λ = (λ × 2.61) represents the minimum concentration of monoclonal κ chains. Similarly, if the κ/λ ratio is <1.29, the concentration of monoclonal λ chains is λ = (κ/1.29).

The heavy-chain class of the paraprotein can be simply allocated if a single heavy chain has a concentration equal to or greater than that of the monoclonal light chain. To allow for assay imprecision, we allocated a heavy chain only if the heavy-chain concentration exceeded 110% of that of the monoclonal light chain. Samples containing biclonal or oligoclonal bands, or polyclonal hypergammaglobulinemia together with a monoclonal immunoglobulin, may have more than one heavy chain fulfilling this criterion, in which case they cannot be typed by this method. Samples in which no heavy chain is present in sufficient concentration similarly cannot be typed. In all such cases, further investigation by IFE/IEP is indicated.

Results and Discussion

We examined 348 serum samples by SPE and quantitative measurements of IgA, IgG, IgM, κ, and λ. If a paraprotein was visible or any abnormality of the κ/λ ratio or light-chain excess was demonstrated, it was characterized by IFE or IEP and classified accordingly (Tables 1 and 2). We studied 215 samples in center 1 and 133 samples in center 2. Overall, 165 samples contained monoclonal components, of which 157 contained paraprotein bands seen on electrophoresis, 103 were normal, 24 showed immunosuppression, and 56 showed a polyclonal increase in immunoglobulins.

The κ/λ Ratio

The mean κ/λ ratio for the 183 samples not containing monoclonal components was 1.79 (95% confidence limits, 1.29–2.61). Pranis et al. (6) found a mean ratio of 1.9, with 95% confidence limits of 1.35–2.70; Lammers and Gressner (7) found a reference range of 1.2–2.8, although they do not state how this was derived.

Of samples containing a monoclonal component, 93.4% demonstrated a κ/λ ratio outside our reference range and could thus be considered as identified by ICE. Caron et al. (4), examining a group of 500 samples containing monoclonal gammapathies, found abnormal κ/λ ratios in 89%; Schultz and Fink (8), studying 41 sera containing paraproteins, found abnormal light-chain concentrations in 95%; Moya and Miller (9) found abnormal ratios in 86.5% of a group of 52 sera with paraproteins; Guinan et al. (10) found abnormal ratios in 82% of 61 sera with paraproteins; Hoyer et al. (11) found abnormal light-chain concentrations in 71% of 38 sera containing paraproteins.

Ten paraproteins did not have abnormal κ/λ ratios and thus could be considered as "not identified" by ICE. The mean paraprotein concentration was 4.1 g/L (range, 0.5 to

### Table 2. Comparison of Findings by ICE and by SPE Followed by IFE or IEP

<table>
<thead>
<tr>
<th>SPE and IFE category</th>
<th>No. of samples</th>
<th>No. with abnormal κ/λ</th>
<th>No. correctly typed</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG&lt;sub&gt;κ&lt;/sub&gt;</td>
<td>52</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>IgG&lt;sub&gt;λ&lt;/sub&gt;</td>
<td>44</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>IgA&lt;sub&gt;κ&lt;/sub&gt;</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>IgA&lt;sub&gt;λ&lt;/sub&gt;</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>IgM&lt;sub&gt;κ&lt;/sub&gt;</td>
<td>21</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>IgM&lt;sub&gt;λ&lt;/sub&gt;</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>IgD</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Light chains alone</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Multiple monoclonal bands</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>24</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Polyclonal increase</td>
<td>56</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>103</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only five detected by SPE.
11.0 g/L; concentrations <1 g/L were calculated as being 1 g/L). In general, as would be expected, these paraproteins were present in low concentrations (Table 1). None of these sera demonstrated immunosuppression, and three had concomitant polyclonal increases in other immunoglobulins, which could be expected to obscure the increased light chain produced by the monoclon. Two had paraprotein concentrations of 10 g/L or greater, and we are surprised that these did not yield an abnormal κ/λ ratio. Possibly these light chains were unreactive in the assay, owing to steric effects inhibiting antibody binding (13). This notion is supported by the fact that abnormal κ/λ ratios were seen in other paraproteins of all immunoglobulin classes at concentrations as low as 1 g/L.

One sample, categorized independently, contained multiple monoclonal bands (two IgGκ, one IgGλ, and one IgAκ) and demonstrated a normal κ/λ ratio, which probably reflected the relative concentrations of the different monoclonal immunoglobulins.

Of 191 samples, 21 did not contain a paraprotein band on SPE but demonstrated an abnormal κ/λ ratio. Monoclonal light chains were detected by IFE in eight of these, six of which showed immunosuppression.

Typing of Paraprotein

Samples containing a paraprotein categorized by IFE and IEP were allocated a heavy-chain class by comparing the concentration of the monoclonal light chain with that of each heavy chain (see Materials and Methods). Those in which this agreed with the previous findings were classified as having been correctly typed by ICE. If it was not possible to type the heavy chain in this way, these were classified as unypeable by ICE. This, together with the κ/λ ratio, never resulted in incorrect typing, but it did fail to type 11% of paraproteins, mainly paraproteins in low concentration (Table 1). Although IgD, IgE, light chain, and biclonal paraproteins cannot be typed by this technique, the results compare well with other studies in which 66% (10), 71% (11), 89% (4), and 90% (9) of paraproteins were correctly categorized.

The Heavy Chain/Light Chain Ratio

The percentage difference obtained by subtracting the total light-chain concentration from the total heavy-chain concentration was found by Caron et al. (personal communication) in 472 normal blood donors to show a mean of +3.6% (SD 4.3%). They suggest on an empirical basis that a difference exceeding +10% may indicate the presence of free light or heavy chains. The 2.5 and 97.5 percentiles for the difference between heavy chains and light chains in our study were −7% to +12%. Examining the samples from center 1 that fell outside these limits showed heavy-chain excess in 16 samples, seven of which contained paraproteins. We did not search for free heavy chains in these sera; although this may be one explanation for the finding, we think that decreased reactivity with antisera to light chains in paraproteins (12) is a more likely explanation.

Light chains were in excess in 30 samples. IFE showed monoclonal light chains, either alone or in association with intact immunoglobulin paraproteins, in 23 of these. Seven of eight samples that contained monoclonal light chains alone demonstrated both an abnormal κ/λ ratio and >10% excess of light chains. One sample, however, showed a κ/λ ratio of 2.3 with a 32% excess of light chains. The evidence suggests that the percentage difference is a useful parameter for indicating the presence of monoclonal light chains particularly if accompanied by an abnormal κ/λ ratio.

In summary, we conclude:

- In this study the ICE system detected 93.4% of the paraproteins previously seen on SPE. These were of all immunoglobulin classes, including IgD. Low concentration in serum was the main reason for failure. Electrophoresis remains essential in the detection of paraproteinaemia.
- The ICE system correctly typed 89% of paraproteins previously detected by SPE. The remainder were unypeable; none was incorrectly typed. Automated κ and λ measurement (7, 13) in addition to electrophoresis and immunoglobulin assays could replace the majority of IFE and IEP testing.
- In our study, eight light-chain monoclonal gammopathies were found that were not detected by SPE. Measurement of κ and λ may thus be a useful method of detecting this clinically important form of monoclonal gammopathy.

We gratefully acknowledge the gift of Quantimetric-2 test kits from Kallestad Diagnostics.

References