Quantitative Determination of Immunoglobulins as a Diagnostic Tool for Paraproteinemia

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Quantitation of serum immunoglobulin was made in 14 cases of multiple myeloma and in one case of Waldenström’s macroglobulinemia, using an antibody-agar plate method.

Most of the patients with multiple myeloma showed a monoclonal increase of gamma globulin. One case of multiple myeloma and the case of Waldenström’s macroglobulinemia demonstrated a specific increase of gamma A and of gamma M, respectively.

The immuno-electrophoretic patterns of these serums were compared with the results of quantitative analysis by the antibody-agar plate. In most of the cases both methods recognized paraprotein, but in some cases only one of these two immunological methods could detect paraprotein. Therefore, both methods seem useful in diagnosing a paraproteinemia.

It should be stressed that Waldenström’s macroglobulinemia in this study was diagnosed by the antibody-agar plate method prior to the ultracentrifugal analysis.

Bone marrow studies and serum electrophoretic findings have been important tools for the diagnosis of multiple myeloma. To diagnose Waldenström’s macroglobulinemia, ultracentrifugation has been required. Although these remain basic means for the diagnosis of paraproteinemia, immuno-electrophoresis has also been found useful to diagnose this condition. Simple immuno-electrophoresis, however, is still insufficient for quantitation of immunoglobulins. Furthermore, it fails occasionally to produce a pathologic precipitin line in the presence of paraprotein. In such a case an antibody-agar plate method designed for immunoglobulin quantitation seems useful in identifying paraproteinemia.

The purpose of this report is to describe an application of the immunoglobulin quantitation to identifying and classifying monoclonal gammopathies, and to refer to the possibility that this simple method alone can diagnose Waldenström’s macroglobulinemia.

Received for publication, September 27, 1966.
MATERIALS AND METHODS

Materials

Serum samples were taken from 14 cases of multiple myeloma and one case of Waldenström's macroglobulinemia. These cases consisted of either patients admitted to Tohoku University Hospital from August, 1965 until July, 1966 or patients in other institutions. The serums of the latter cases were referred to us for examination during the same period.

All cases of multiple myeloma were proved to contain myeloma cells in bone marrow smears.

The case of Waldenström's macroglobulinemia showed increase of lymphocytes, but not of plasma cells, in the bone marrow and was proved to contain increased macroglobulin in serum by analytical ultracentrifugation (Fig. 1).

Table 1 shows total serum protein levels in all the cases examined and the results of their electrophoretic analysis.

![Fig. 1. Ultracentrifugal pattern of the serum from a case of Waldenström's macroglobulinemia. The 19 S macroglobulin is seen as a 'self-sharpening peak' indicated by 'c', while 'a' and 'b' represent 48 and 78 peaks, respectively.](image)

Methods

Immunoelectrophoresis was performed by a micromethod as described by Peetoom. Antibody-agar plates were obtained from Hyland Laboratories, Los Angeles, California (Immunoplates, list no. 85–124, 58–126, 85–130). Reference standards
TABLE 1. *Total serum protein and its electrophoretic analysis*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Total protein g/100ml</th>
<th>Electrophoresis (cellulose-acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alb %</td>
<td>α1 %</td>
</tr>
<tr>
<td>1</td>
<td>6.0</td>
<td>42.1</td>
</tr>
<tr>
<td>2</td>
<td>10.6</td>
<td>29.7</td>
</tr>
<tr>
<td>3</td>
<td>8.6</td>
<td>48.2</td>
</tr>
<tr>
<td>4</td>
<td>13.8</td>
<td>19.6</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>50.6</td>
</tr>
<tr>
<td>6</td>
<td>8.4</td>
<td>43.3</td>
</tr>
<tr>
<td>7</td>
<td>11.6</td>
<td>24.1</td>
</tr>
<tr>
<td>8</td>
<td>9.2</td>
<td>44.2</td>
</tr>
<tr>
<td>9</td>
<td>12.2</td>
<td>39.3</td>
</tr>
<tr>
<td>10</td>
<td>10.4</td>
<td>41.2</td>
</tr>
<tr>
<td>11</td>
<td>9.7</td>
<td>35.2</td>
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<tr>
<td>12</td>
<td>10.6</td>
<td>48.0</td>
</tr>
<tr>
<td>13</td>
<td>5.8</td>
<td>63.3</td>
</tr>
<tr>
<td>14</td>
<td>9.0</td>
<td>42.7</td>
</tr>
<tr>
<td>15</td>
<td>12.0</td>
<td>33.8</td>
</tr>
</tbody>
</table>

Normal | 6.5–8.3 | 62.8±1.4 | 3.2±1.0 | 8.6±1.0 | 10.1±0.4 | 15.3±1.1 |

No. 1-14 Multiple myeloma | No. 15 Primary macroglobulinemia

for each immunoglobulin were attached to these plates. Details of this method for immunoglobulin quantitation were described previously by one of us.\(^\text{11}\)

Regarding the preparation of the employed antisera the following information was kindly provided by Hyland Laboratories:

‘In regards to the immunizing antigens, the gamma G-globulin was isolated from pooled normal human serum by DEAE cellulose ion exchange chromatography. The gamma A-globulin was also isolated from normal serum by a modification of the Heremans’ technique. The gamma M-globulin was prepared by subjecting normal serum to low ionic strength dialysis, recovering the euglobulin and isolating the macroglobulin by Sephadex G-200 gel filtration. Each of the antigens was incorporated in complete Freund’s adjuvant and goats were immunized by subcutaneous injections of the antigen mixture. The anti-gamma A and anti-gamma M serums were rendered monospecific by absorption with cord serum. Antiserum to gamma G-globulin did not require absorption.’

**RESULTS**

1) *Immunoelectrophoresis*

Out of 14 cases of multiple myeloma 13 demonstrated a definite pathological line in immunoelectrophoresis with anti-whole human serum (Table 2). A couple of these examples are shown in Figs. 2 and 3. Among these 13 cases with definite pathological lines is included one case which did not show abnormality in the total protein or in electrophoretic pattern (Case 13).

One case of multiple myeloma (Case 14) and the case of Waldenström’s macroglobulinemia (Case 15) did not show definite abnormal lines, and both cases exhibited only questionable patterns. In the former a questionable thickening
TABLE 2. Results of serum immunoglobulin quantitation and also of immunoelectrophoretic analysis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>( \gamma G ) mg/ml</th>
<th>( \gamma A ) mg/ml</th>
<th>( \gamma M ) mg/ml</th>
<th>Immunoelectrophoresis (Anti-human serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>0.5</td>
<td>0.4</td>
<td>Definite pathological ( \gamma G )</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0.3</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.7</td>
<td>0.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>0.5</td>
<td>0.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>0.8</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>0.5</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>110</td>
<td>0.3</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
<td>62</td>
<td>0.6</td>
<td>0.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>4.5</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>10</td>
<td>64</td>
<td>0.5</td>
<td>0.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
<td>79</td>
<td>0.5</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>0.4</td>
<td>0.1</td>
<td>&quot;</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>0.5</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>52.0</td>
<td>0.2</td>
<td>Equivocal pathological ( \gamma A )</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>0.9</td>
<td>40.0</td>
<td>Abnormal precipitate around the well</td>
</tr>
</tbody>
</table>

Normal \( 18.5 \pm 3.9 \) \( 3.6 \pm 0.7 \) \( 1.3 \pm 0.4 \)

No. 1–14 Multiple myeloma  No. 15 Primary macroglobulinemia

Fig. 2 and Fig. 3. Immunoelectrophoresis using anti-whole human serum, 'a' denotes serum from multiple myeloma case, and 'n' indicates normal serum. Abnormal gamma \( G \) precipitin line is seen.
Fig. 4. Immunoelectrophoresis by anti-whole human serum, and ‘a’ is the serum from Case 14. Although not distinct in this picture, questionable thickening of gamma A line is seen.

Fig. 5. The serum from Case 15 is indicated by ‘a’ and abnormal precipitation of protein is seen around this well.

of gamma A line was observed and in the latter considerable amount of protein precipitated around the well (Figs. 4 and 5).

2) Quantitation of immunoglobulins

In Table 2 are shown the results of quantitative determination of gamma G, A, and M. Most of the myeloma cases showed an increase of gamma G with a decrease of gamma A and M. One case of plasmacytoma (Case 14) had a marked increase of gamma A and the case of Waldenström’s macroglobulinemia revealed a high value of gamma M. In these two cases, in spite of equivocal immunoelectrophoretic findings the diagnosis of A-multiple myeloma and of Waldenström’s macroglobulinemia became definite through the immunoglobulin quantitation.

Some cases of multiple myeloma demonstrated double rings suggesting that one ring is for normal immunoglobulin and the other for paraprotein. Although in G-multiple myeloma gamma G was not always markedly elevated, a decrease of gamma A and gamma M was characteristically observed. It should be noted that some of these cases were under treatment with various agents.
Fig. 6. Graphic representation of immunoglobulin distribution of 15 cases of paraproteinemia in this study.

DISCUSSION

Immunoelectrophoresis of the serum and urinary proteins of patients with multiple myeloma and Waldenström's macroglobulinemia has contributed much to the elucidation of these protein constituents. In Case 13 of Table 1 there was no abnormality in serum protein in routine laboratory analysis, but immunoelectrophoresis using anti-whole human serum showed a pathological line as is shown in Table 2. Since a pathological line can be present without a quantitative increase, immunoelectrophoresis gives an important clue to the diagnosis in such a situation.

The agar immunoelectrophoresis, however, has some shortcomings. It
Quantitation of Immunoglobulins in Paraproteinemia

occasionally fails to demonstrate clearly a paraprotein, when the abnormal protein does not migrate by electrophoresis because of its precipitation around the well. Even if this is not the case, it seems difficult to recognize a paraprotein of gamma M type. Furthermore, it is hard to evaluate the quantity of each immunoglobulin by simple immunoelectrophoresis, even though some modification can be employed.

These inconveniences can be covered by employing concomitantly the technique of antibody-agar plate. The principle of antibody-agar plate method for quantitative determination of immunoglobulins was described by various investigators, but Fahey and McKelvey described this method in details. The significance of variation in immunoglobulin levels was described also in various diseases.

Waldenström's macroglobulinemia in this study was definitely diagnosed by the quantitative determination of immunoglobulins, using the antibody-agar plate method (Fig. 7), although the immunoelectrophoretical findings exhibited an equivocal pattern. Later the analytical ultracentrifugation was performed to verify the finding obtained by the antibody-agar plate method.

An analytical ultracentrifugation requires an expensive apparatus and not every institution can afford one. In laboratories where it is not available, the antibody-agar plate method for immunoglobulin quantitation would be of valuable help. At the same time a thin-layer gel-filtration technique and Sephadex G-200 gel filtration method also may be helpful in diagnosing macroglobulinemia, when ultracentrifugation is not possible.

It may be noticed that quantitative determination of immunoglobulins is not always superior to immunoelectrophoresis for the diagnosis of paraproteinemia, as is exemplified by cases in which no increase of immunoglobulins is found and yet an abnormal protein is found in immunoelectrophoresis. Both methods are useful, the one as qualitative, the other as quantitative.

The immunological methods proved useful in studying protein abnormalities, but it should be kept in mind that these means are always indirect, and that there
is always a possibility of not identifying a paraprotein because of the absence of a corresponding antibody in the antiserum employed. In the present study there was one case which did not show any abnormal line in immunoelectrophoresis using one kind of anti-human serum but was proved to have a paraprotein by another kind of antihuman serum (Case 13). Furthermore, a paraprotein may contain an antigenic structure different from that of normal human globulin.28

On the other hand, a question occurs why paraproteins fall always into what Heremans designated as immunoglobulins,29 and not into other globulins than immunoglobulins. Could we interpret this fact as suggesting that a paraprotein is formed on immune basis? If so, the word 'paraprotein' or 'pathological or abnormal protein' may be a misnomer.30

Acknowledgment

The authors wish to thank Prof. T. Torikai for his valuable guidance and Dr. H. Tazawa, Dept. of Bacteriology, Tohoku University School of Medicine for performing the ultracentrifugal analysis.

References

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