Strategy to Diagnose Monoclonal Gammopathies in Serum: High-Resolution Electrophoresis, Immunofixation, and $\kappa/\lambda$ Quantification

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Identification of monoclonal gammopathies in serum has involved electrophoresis of serum proteins, immunoelectrophoresis (IEP), and quantification of IgG, IgA, and IgM. Recent innovations in technology—including high-resolution electrophoresis (HRE), immunofixation (IFX), and quantification of kappa- and lambda-containing immunoglobulins—allow for more rapid and precise assessment of serum for monoclonal proteins. We present a series of guidelines to determine when high-resolution electrophoresis and quantification of immunoglobulins (including kappa and lambda) are sufficient and when additional IFX is required to characterize the monoclonal gammopathy. Of the samples studied, 88% were correctly diagnosed by HRE with quantification of immunoglobulins and $\kappa/\lambda$; only 12% required that IFX be performed. The guidelines allow us to detect monoclonal gammopathies quicker and more efficiently by avoiding redundant IEP or IFX testing. For the vast majority of cases, these guidelines allow for a correct diagnosis within one day. After one year of follow-up since completion of the study, no undetected cases of monoclonal gammopathy have eventuated.

Additional Keyphrases: immunofixation · immunoelectrophoresis · laboratory efficiency · immunoglobulin quantification · index of monoclonality · screening

Traditional methods to detect serum monoclonal gammopathies included electrophoresis of serum proteins on cellulose acetate; quantification of IgG, IgA, and IgM; and immunoelectrophoresis (IEP). Unfortunately, because cellulose acetate electrophoresis fails to detect small monoclonal proteins and some proteins that migrate in the beta region (1, 2), IEP and quantification of IgG, IgA, and IgM were necessary to screen serum from patients for whom a monoclonal gammopathy was part of their differential diagnosis. Although this did detect the vast majority of monoclonal proteins, the use of IEP in this process was inefficient. Further, it was often difficult to detect monoclonal gammopathies by IEP when only small quantities of the monoclonal proteins were present or when IgM or IgA polymeric monoclonal proteins of larger molecular mass were present (3–5). These larger or self-aggregating proteins often required reduction and repeated examination or chromatography with repeated electrophoresis to detect the monoclonal protein.

The advent of high-resolution electrophoresis (HRE), immunofixation (IFX), and quantification of kappa-$\kappa$ and lambda-$\lambda$-containing immunoglobulins facilitated the detection of monoclonal proteins. HRE allows small monoclonal proteins to be distinguished more readily than is possible with the cellulose acetate method (1, 6). IFX is performed more quickly than IEP (in less than one day for some methods), and detects both small monoclonal proteins and large, polymerized monoclonal proteins more readily than IEP (3, 7–11). Quantification of $\kappa$- and $\lambda$-containing immunoglobulins provides objective information that can be used in conjunction with electrophoresis to screen for monoclonal proteins (12–16).

There is considerable controversy as to which combination of these available procedures will provide sensitive and specific diagnosis of monoclonal gammopathies and efficiencies for laboratory utilization. Here, we evaluate a series of guidelines for combined use of these newer techniques to streamline and improve the detection and characterization of monoclonal gammopathies in serum. With this strategy, we detected most monoclonal gammopathies in one day, missed no monoclonal gammopathies, and cut needless IFX or IEP procedures by almost 90%.

Materials and Methods

Specimens

From July 1, 1986, to June 30, 1987, we received 336 sera at the Clinical Immunopathology Laboratory at The University of Michigan Hospital to determine whether a monoclonal gammopathy was present. Control samples consisted of 94 sera obtained from healthy hospital workers, ages 19–58 y.

Strategy for Detecting Monoclonal Gammopathy in Serum

We analyzed each serum by a HRE in agarose, and quantified the IgG, IgA, IgM, $\kappa$, and $\lambda$ by nephelometry. We used the following criteria to determine whether further studies were needed:

1. If the HRE result was normal, or showed an obvious pattern such as acute phase reaction, nephrotic pattern, etc. (7), and the quantities of immunoglobulins and the $\kappa/\lambda$ ratio were normal, we concluded that further studies on the serum for monoclonal protein were not indicated.

2. If the HRE showed a polyclonal increase in the gamma (\(\gamma\)) or beta (\(\beta\)) and \(\gamma\) region with an accompanying increase in one or more of the heavy chain isotypes and a normal $\kappa/\lambda$ ratio, the sample was interpreted as having a polyclonal increase in the appropriate immunoglobulin type.

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2 Nonstandard abbreviations: IEP, immunoelectrophoresis; HRE, high-resolution electrophoresis; and IFX, immunofixation.

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3. If the HRE revealed an immune complex pattern ("tented restriction in the y region") with a normal $\kappa/\lambda$ and a polyclonal increase in $\gamma$ globulins, the sample was interpreted as showing a tented restriction in $\gamma$ region of the type that often occurs in patients with circulating immune complexes (17, 18).

4. If the HRE result was normal or showed a polyclonal increase in $\gamma$ globulins, but the $\kappa/\lambda$ ratio was abnormal, we performed IFX.

5. If the HRE demonstrated an obvious monoclonal restriction and one heavy-chain iso-type was increased, and the $\kappa/\lambda$ ratio was abnormal, we concluded that a monoclonal gammopathy was present (type specified) and we recommended urine studies to rule out a Bence Jones protein.

6. If the HRE demonstrated a monoclonal restriction, but the $\kappa/\lambda$ ratio was normal, we performed IFX.

7. If our files indicated that the patient had a previously documented serum monoclonal protein, we performed HRE with quantification of the $\gamma$-migrating restriction (19). If the restriction was in the beta region, we quantified the specific iso-type of the monoclonal protein by nephelometry to follow the patient's course. $\kappa/\lambda$ determinations and IFX were not performed on these samples unless there was a change in the migration pattern on HRE.

8. If the patient had hypogammaglobulinemia on HRE, or on quantification of IgG, we recommended a urine study to rule out Bence Jones protein.

**High-resolution electrophoresis.** For HRE we used the Panagel (Worthington Diagnostics, Freehold, NJ) agarose system, applying 5-μL samples of a fourfold-diluted serum to the application mask. After allowing this to absorb into the gel for 7 min, we placed the cooling block on the gel and applied current (200 V, 90–240 mA) across the gel for 40 min. Barbital–sodium barbital buffer, pH 8.6, was used. We fixed the gels with picric acid (1.5 g in 120 mL of dilute acetic acid, 170 mL/L) for 10 min, followed by two 3-min washes in dilute (50 mL/L) acetic acid solution. After pressing and drying the gels, we stained them for 10 min with Amido Black, 1 g/L in dilute acetic acid. Gels were destained in three successive rinses (1 min, 10 min, and 10 min) with a mixture of glacial acetic acid, methanol, and distilled water, 1/3/7 by vol. The gels were dried and scanned with a densitometer ("Appraise"; Beckman Instruments, Brea, CA) (19).

**Immunofixation.** This was done as described in detail previously (7). Briefly, we adjusted the concentration of the serum samples so that the concentration of the protein of interest was ~1 g/L (based on the nephelometric results), and submitted samples to agarose electrophoresis as above. For each immunoglobulin to be assayed—typically, IgG, IgA, IgM, $\kappa$, and $\lambda$—we applied a separate sample of appropriately diluted patient's serum to the gel. IgD or IgE was also occasionally quantified. After electrophoresis, we overlaid the samples with a strip of cellulose acetate containing 50 μL of specific antiserum (Kallestad Labs., Austin, TX). After 60 min, we washed the gels with buffer and stained them with Coomassie Blue, 50 g/L, in ethanol/glacial acetic acid/water (4.1/1/4.5 by vol). After destaining as described above, we dried the gels and examined them for specific restriction as described previously (7).

**Nephelometry.** IgG, IgA, IgM, $\kappa$, and $\lambda$ were quantified with the Auto ICS (Beckman) system as described previously (19). Antisera specific for IgG, IgA, and IgM (Beckman) and for $\kappa$- and $\lambda$-containing immunoglobulins (Kallestad) were reacted with appropriate dilution of the patients' sera. This nephelometer is programmed to make specific dilutions of the serum sample.

**Results**

**Concentrations of serum immunoglobulins in the control population.** The normal ranges for IgG, IgA, IgM, $\kappa$, and $\lambda$ concentrations in our control population were similar to those described by others (12, 20, 21). The single most useful variable for determining monoclonality was the average $\kappa/\lambda$ ratio, 1.91 (SD 0.36). The ratios we measured ranged from 1.15 to 3.43. Results for six of the 94 samples fell outside of mean ± 2 SD range, as would be expected statistically.

**Application of the strategy to detect monoclonal gammopathies.** Table 1 summarizes the results of applying our strategy to the 336 samples submitted for determination of monoclonal proteins. As shown, the vast majority of cases (88%) could be interpreted with the information provided by HRE and immunoglobulin quantification. Indeed, 180 (54%) of the cases were either normal samples or had insignificant HRE findings with regard to monoclonal gammopathy. Most abnormal electrophoretic patterns were those typical of acute-phase reactions, although other patterns were also seen.

Of the 81 monoclonal gammopathies detected in this study, 49 (60%) were evident from the combination of data from HRE and immunoglobulin quantification. These were obvious by the combination of a restriction seen on electrophoresis and an abnormal $\kappa/\lambda$ ratio. Also, almost all polyclonal expansions were obvious, obviating IFX; only three (6%) of the 51 cases with polyclonal expansion required IFX before we were certain that a monoclonal gammopathy was not present. We did not repeat IFX, or even quantification of $\kappa$ and $\lambda$, when the monoclonal gammopathy had been previously defined in our laboratory. Therefore, in 18 cases the HRE with quantification of IgG, IgA, and IgM sufficed to monitor the patient's status. Any change in the electrophoretic pattern (not just in the quantification) would indicate the need for an IFX assay. Such was not necessary in any of the cases during this study. In the past, we performed IFX on samples from patients who developed a second monoclonal band.

IFX was necessary in only 41 (12%) of the cases. Most of these involved detecting small monoclonal gammopathies where the monoclonal protein produced a small restriction in the electrophoresis gel, but the amount of monoclonal protein was too small to significantly alter the $\kappa/\lambda$ ratio.

**Table 1. Diagnostic Results of Screening 336 Serum Samples for Assessment of Monoclonal Gammopathy**

<table>
<thead>
<tr>
<th>Result</th>
<th>No.</th>
</tr>
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<tbody>
<tr>
<td>Normal or insignificant HRE with normal G, A, M, $\kappa$, $\lambda$</td>
<td>180</td>
</tr>
<tr>
<td>Monoclonal by HRE and G, A, M, $\kappa$, $\lambda$ alone</td>
<td>49</td>
</tr>
<tr>
<td>Polyclonal by HRE and G, A, M, $\kappa$, $\lambda$ alone</td>
<td>48</td>
</tr>
<tr>
<td>Known previous monoclonal with consistent HRE</td>
<td>18</td>
</tr>
<tr>
<td>Subtotal of patients with no need for IFX</td>
<td>295 (88%)</td>
</tr>
<tr>
<td>IFX needed to diagnose monoclonal</td>
<td>32</td>
</tr>
<tr>
<td>IFX needed to diagnose polyclonal</td>
<td>3</td>
</tr>
<tr>
<td>IFX needed to confirm normal</td>
<td>2</td>
</tr>
<tr>
<td>IFX needed to rule out monoclonal in a specimen with a minor $\kappa/\lambda$ abnormality</td>
<td>4</td>
</tr>
<tr>
<td>Subtotal of samples needing IFX for diagnosis</td>
<td>41 (12%)</td>
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</tbody>
</table>

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Concentration, g/L  
1.10  
IgGk  
0.25  
Small  
lgG  
IgDic  
1.74  
Weak  
Faint  
1.00  
<.25  
0.50  
0.72  
4.88  
Weak  
2.74  
0.80  

Whereas the utility of k/\lambda to facilitate the diagnosis in cases with large monoclonal gammopathies is obvious, the literature is less clear as to whether determining k/\lambda offers useful information in samples when only minor or no alterations are seen on electrophoresis of serum proteins. In the present series, we identified 10 patients with monoclonal gammopathies who had either no or minor alterations by serum protein electrophoresis but who had an abnormal k/\lambda ratio (Table 2). In these cases, the abnormal k/\lambda ratio was a major factor in deciding to perform the IFX, which confirmed the presence of a monoclonal gammopathy. Only three of these samples had monoclonal proteins that were not seen on initial examination of the gel. One, from a patient with neuropathy associated from an IgM monoclonal protein, showed only hypogammaglobulin and an abnormal k/\lambda on our screening. Both of the others were from patients with \beta-migrating IgA monoclonal gammopathies, which were obscured by transferrin and C3 (Figure 2). Two of the 10 samples were from patients with multiple myeloma: a newly diagnosed patient with rare IgDk gammopathy, whose serum displayed a broad \gamma band on electrophoresis, and a patient with treated IgGk myeloma, in whose serum hypogammaglobulinemia was evident, but with a very faint, broad, slow \beta band that could be mistaken for residual fibrinogen (Figure 3). The remaining five samples also had weak bands, which, by our strategy, would require IFX even if the k/\lambda ratio had been normal. However, the k/\lambda results were useful because some of these bands might have been missed by less-experienced observers.

Monoclonal gammopathies detected. There was little difference in the ability to detect a monoclonal protein of any particular isotype by these methods. Of considerable interest was the ability of the methods to detect monoclonal gammopathies in specific clinical situations. In Table 2, we review the clinical diagnoses of the patients with monoclonal gammopathies newly detected by our laboratory during this study (some were known monoclonal gammopathies detected outside laboratories, but were not on file in our laboratory). All four cases of Waldenström's macroglobulinemia and 14 (87.5%) cases of myeloma were correctly identified by electrophoresis with quantification of immunoglobulins and k/\lambda. Only two cases of myeloma required IFX. One of these was previously detected at another institution. As expected, most cases (58%) were monoclonal gammopathies of undetermined significance. Several cases with small monoclonal proteins were associated with neuropathy. Other cases included chronic lymphocytic leukemia and lymphoma, detection of which usually required IFX. Cases of plasmacytoma and amyloidosis were detected by HRE and k/\lambda ratio alone.

With 12 months of follow-up since completion of the study group, no then-undetected monoclonal gammopathies have been found. We recognize that it may take several years for small monoclonal proteins to evolve into full-blown clinical disease, but patients who had sufficient symptoms to initiate a request for monoclonal gammopathy evaluation should have shown evidence of progression in this time if the monoclonal process was responsible for their symptoms.

Discussion
In the present study we applied a strategy to screen for and characterize monoclonal gammopathies in all serum samples that were sent to the laboratory for evaluation of a possible monoclonal gammopathy. Previously, each sample would have been analyzed by electrophoresis on cellulose acetate; quantification of IgG, IgA, and IgM; and IEP. The latter evaluation took a minimum of three days and occasionally as long as a week when reduction by 2-mercaptop-
ethanol or column-chromatographic purification was required for us to characterize the monoclonal proteins. When using this older strategy, we observed that most of the samples turned out to be normal, or that many were monoclonal proteins that we had already characterized (22). Furthermore, most of the clinically significant monoclonal gammopathies (those that were useful in defining lymphoreticular proliferations or amyloid) were obvious on cellulose acetate electrophoresis. However, the combination of cellulose acetate electrophoresis and quantification of IgG, IgA, and IgM was ineffective for screening samples, because these procedures were not sufficiently sensitive to detect small monoclonal gammopathies.

By combining HRE with quantification of \( \kappa \) and \( \lambda \), we were able to effectively screen serum samples for the presence of monoclonal gammopathies. The essence of this strategy is to perform IFX on any sample that has abnormalities suggesting a monoclonal process, as detailed in the Methods section. Further, we stress to new users of this strategy that they should perform IFX whenever they are uncertain about a diagnosis. We found that, with increased experience, the number of redundant IFX declined considerably. With this strategy, we eliminated 88% of unnecessary IFX, and our reports on most patients who have monoclonal gammopathies are available the same day that the sample is drawn. This hastens the diagnostic process and also results in a net saving of the time for the skilled medical technologists.

The few samples that did require IFX were typically small monoclonal gammopathies, most of which fell into the category of monoclonal gammopathy of undetermined significance. Samples from two patients with multiple myeloma required IFX for diagnosis. One had been receiving treatment at another institution, the other had an IgG\( \kappa \) monoclonal protein. Neither had been evaluated previously in our laboratory. A few of the samples requiring IFX were from patients with chronic lymphocytic leukemia or lymphoma. Earlier studies indicated that the incidence of monoclonal proteins in patients with these conditions was small (23), but more recent work indicates that the vast

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**Table 3. Clinical Diagnoses of Monoclonal Gammopathies**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>( \kappa/\lambda )</th>
<th>IFX*</th>
</tr>
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<tbody>
<tr>
<td>MGUS</td>
<td>47</td>
<td>26(55%)</td>
<td>21(45%)</td>
</tr>
<tr>
<td>Myeloma</td>
<td>16</td>
<td>14(87.5%)</td>
<td>2(12.5%)</td>
</tr>
<tr>
<td>Waldenström's</td>
<td>4</td>
<td>4(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Neuropathy associated</td>
<td>8</td>
<td>3(37.5%)</td>
<td>5(62.5%)</td>
</tr>
<tr>
<td>with monoclonal protein</td>
<td>3</td>
<td>1(33%)</td>
<td>2(66%)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>1</td>
<td>1(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>50(82%)</td>
<td>31(58%)</td>
</tr>
</tbody>
</table>

*Cases detected by the high resolution screen with quantification of serum immunoglobulins.

*Cases requiring IFX to detect the monoclonal protein. All were detected by the screen, but required IFX for characterization.
The majority of these patients will have such proteins in their serum or urine (11, 24). Optimal detection requires IFX, isolectric focusing, or affinity chromatography (11, 24–26). The most common histopathological pattern is a diffuse lymphoma of small lymphocytes, with or without plasmacytoid differentiation (27). Interestingly, while one might have assumed that these patients would have IgM as the predominant immunoglobulin type, a recent study by Noel and Kyle (24) indicates that IgG was present in 51% while IgM was found in only 38% of the patients. Some monoclonals detected from about 2% of patients with lymphoma were shown to possess cold agglutinin activity (28). Monoclonal gammapathies were also reported in patients with small noncleaved follicular center cell lymphomas (8, 29). The one case of bicalonal gammapathy in the present series also required IFX for characterization. The need for IFX in such cases was noted previously (16, 30). Bicalonal gammapathies were reported recently in several cases of non-Hodgkin’s lymphoma and leukemia (30–33). It is likely that the increased numbers of these cases reported recently reflects the greater sensitivity of HRE and IFX.

A few recent studies explored the possibility of using \( \kappa \) and \( \lambda \) quantification to replace the standard methods of serum protein electrophoresis and IEP. Predictably, large monoclonal proteins were virtually always detected and smaller monoclonals were often missed (21). This strategy also yields significant numbers of false positives for monoclonal gammapathies (21). Clearly, \( \kappa \) and \( \lambda \) without electrophoresis and IFX or IEP as backups are inadequate as a screen. In the present study, cases with polyclonal increases as well as cases with hypogammaglobulinemia had occasional \( \kappa/\lambda \) outside of the normal range. With IFX, it was obvious that these cases did not represent monoclonal gammapathies. However, when used with HRE, and when viewed in the context of the strategy described in this paper, \( \kappa \) and \( \lambda \) quantification provides the information one needs to analyze correctly the vast majority of the serum samples submitted.

Normal values for \( \kappa \) and \( \lambda \) in some laboratories will differ from manufacturer’s recommendations. Normansell (12) found the mean \( \kappa/\lambda \) ratio to be 1.77, with a standard deviation of 0.337. This is similar to our \( \kappa/\lambda \) of 1.91 and standard deviation of 0.360. Theoretically, the total of \( \kappa + \lambda \) should equal IgG + IgA + IgM. In fact, these do not add up perfectly, because of the heterogeneity of both reagent antisera and of the antigens in this system (12, 13, 34). Another interesting feature reported by Normansell was that for 47% of pediatric sera the heavy/light chain ratio was outside of the normal range. Other workers (20), however, have found the mean \( \kappa/\lambda \) ratio for children to be the same as that for adults (1.81, SD 0.4). Our laboratory does not routinely quantify \( \kappa/\lambda \) or do IFX on pediatric sera. We found that most clinicians order IEP on children as part of an evaluation for immunodeficiency. While one can find tiny monoclonal proteins in the serum of children with Burkitt’s lymphoma, this is clearly not the way to screen for that diagnosis (8).

In the present study, the key to being able to screen for monoclonal proteins is the combination of the sensitivity of HRE with \( \kappa \) and \( \lambda \) and the judicious use of IFX to answer specific questions. The method for HRE used in this study allows for densitometric scanning, which can be useful as an adjunct in interpretation (2, 7, 19). IFX is an especially good technique to detect the small monoclonal proteins that cannot be readily distinguished with HRE and \( \kappa/\lambda \) alone. However, it is important to dilute each sample according to the amount of serum immunoglobulin present individually. When standard dilutions are used, antigen-excess effects can result in a false-negative result (7, 35, 36).

Methods for detecting monoclonal gammapathies will continue to improve. Several methods to improve sensitivity include isoelectric focusing, Western blotting, and two-dimensional electrophoresis. Whether such methods will be clinically useful and practical for diagnostic laboratories remains to be determined. The present study presents a strategy that allows for the rapid, accurate diagnosis of monoclonal gammapathies by screening the serum with HRE and quantification of IgG, IgA, and IgM, and \( \kappa \) and \( \lambda \). When necessary (see Methods), IFX is performed to be sure that small monoclonal proteins are not missed. With the strategy outlined in this study, IFX was necessary only 12% of the time. Considering that we are a large referral hospital, we anticipate that the percentage of samples requiring IFX would be smaller in the average community hospital or in a large commercial laboratory.

References

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