Monitoring IgA Multiple Myeloma: Immunoglobulin Heavy/Light Chain Assays

Jerry A. Katzmann,1,2* Maria A.V. Willrich,1 Mindy C. Kohlhagen,1 Robert A. Kyle,2 David L. Murray,1 Melissa R. Snyder,1 S. Vincent Rajkumar,2 and Angela Dispenzieri2

BACKGROUND: The use of electrophoresis to monitor monoclonal immunoglobulins migrating in the β fraction may be difficult because of their comigration with transferrin and complement proteins.

METHODS: Immunoassays specific for IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMA heavy/light chain (HLC) were validated for use in the clinical laboratory. We assessed sample stability, inter- and intraassay variability, linearity, accuracy, and reference intervals for all 6 assays. We tested accuracy by verifying that the sum of the concentrations for the HLC-pairs accounted for the total immunoglobulins in each of 129 healthy sera, and that the HLC-pair ratios (rHLCs) were outside the reference interval in 97% of 518 diagnostic multiple myeloma (MM) samples.

RESULTS: We assessed diagnostic samples and posttreatment sera in 32 IgG and 30 IgA patients for HLC concentrations, rHLC, and total immunoglobulins and compared these nephelometry results with serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). In sample sets from patients with IgG MM, the sensitivity of SPEP was almost the same as for rHLC, and no additional advantage was conferred by running the sensitivity of SPEP was almost the same as for rHLC, resis (IFE). In sample sets from patients with IgG MM, the SPEP, rHLC, and IFE identified monoclonal immunoglobulin in most cases can be identified and quantified as an M-spike on serum protein electrophoresis (1–3). As a complementary test, Ig quantification by nephelometry is useful in patients with high concentrations of monoclonal IgG (4) and in patients with monoclonal IgA whose electrophoretic migration is in the β fraction. Quantitative serum free light chain (FLC) κ/λ ratios and FLC concentrations also can be used in light chain myeloma for detection and quantification of monoclonal light chains (5, 6).

CONCLUSIONS: The use of IgA HLC assays for monitoring β-migrating monoclonal proteins can substitute for the combination of SPEP, IFE, and total IgA quantification.

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1 Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology, and 2 Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN.

* Address correspondence to this author at: Mayo Clinic, 200 1st St. SW, Rochester, MN 55905. Fax 507-266-4088; e-mail katzmann@mayo.edu.

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Monitoring plasma cell proliferative diseases such as multiple myeloma (MM) is usually straightforward, since the monoclonal immunoglobulin in most cases can be identified and quantified as an M-spike on serum protein electrophoresis (1–3). As a complementary test, Ig quantification by nephelometry is useful in patients with high concentrations of monoclonal IgG (4) and in patients with monoclonal IgA whose electrophoretic migration is in the β fraction. Quantitative serum free light chain (FLC) κ/λ ratios and FLC concentrations also can be used in light chain myeloma for detection and quantification of monoclonal light chains (5, 6).

Monoclonal IgA proteins that migrate in the β fraction may be obscured by healthy β-migrating proteins and may not electrophorese as a discrete band. When the IgA band is indistinguishable from the healthy β components, the protocol in our laboratory is to perform immunofixation electrophoresis (IFE) if the β fraction is increased to ≥1.6 g/dL and to fractionate M-spikes on serum protein electrophoresis by gating if the β fraction is ≥2 g/dL. As the amount of monoclonal protein changes over time and falls or rises around 2 g/dL, the arbitrary gating of the M-spike is confusing for clinicians and patients. Although the monoclonal band is obscured, the IgA can still be quantified by nephelometry. We recommend that clinicians order both immunofixation and electrophoresis to document the continued presence of the monoclonal IgA and, additionally, nephelometric quantification of IgA. The complementary use of nephelometry for quantification is supported in the 2014 International Myeloma Working Group recommendations (7). When the IgA concentration falls into the reference interval, it is not clear if monoclonal IgA is still present and, therefore, if IFE is still needed.

In 2009, Bradwell et al. reported immunoassays specific for epitopes defined by combinations of the individual Ig heavy chains with either κ or λ light chains (8).
These heavy/light chain (HLC) reagents can separately quantify IgGκ, IgGλ, IgAκ, IgAA, IgMκ, or IgMA. The HLC reagents can therefore provide more specific information than Ig quantification, and the HLC-pair ratio (rHLC) (e.g., IgAκ/IgAA) is an indicator of clonal expansion. The rHLC assessment of clonality has been reported to be useful for diagnosing and monitoring MM patients (9) and also for the diagnosis and monitoring of broadly migrating monoclonal IgGs that are difficult to identify by electrophoretic methods (10). The IgA HLC assays should also prove useful for monitoring β-migrating monoclonal IgA proteins (8, 11). The aims of this study were to validate the performance of the HLC assays for routine use in the clinical laboratory and to examine the utility of these assays in relation to that of electrophoretic assays and Ig quantification for monitoring monoclonal proteins.

Methods

We quantified total IgG, IgA, and IgM with Siemens immunoassay reagent sets on the BNII immunonephelometer (Siemens Diagnostics) and IgGκ, IgGλ, IgAκ, IgAA, IgMκ, and IgMA with Hevylite™ reagents provided by the manufacturer (The Binding Site, Birmingham, U.K.). Serum protein electrophoresis (SPEP) was performed by the Mayo Clinic Institutional Review Board.

We determined intraassay variability with 20 replicate assays on 3 sera for each of the 6 HLC assays. The 3 sera for each assay represented low, healthy, and high concentrations. We determined interassay variability for each HLC assay with frozen serum aliquots containing healthy and increased concentrations of each HLC. The assays were performed over the course of 3 months and on 3 different BNII instruments.

We determined linearity for each HLC assay by 2-fold dilution series of healthy sera as well as a serum containing a monoclonal protein at increased concentration. The expected concentrations on the basis of the dilution scheme were compared to the measured values, and the lower limits of linearity were determined by at least 80% recovery of expected concentration.

We assessed accuracy with the healthy donor serum sets for each of the 3 HLC-pairs, comparing the sum of the HLC-pair to the total Ig quantification by nephelometry of the appropriate isotype. In addition, 518 sera from patients with IgG or IgA MM were tested, and rHLCs were compared to the immunotype of the monoclonal protein.

For evaluation of disease monitoring, frozen sera were identified according to the following criteria: (a) diagnosis of MM, (b) IgG migration in γ fraction or IgA in β fraction, and (c) availability of the diagnostic sample plus 2–5 posttreatment samples. Thirty-two IgG MM samples sets were identified: 28 with 4 posttreatment samples, 3 with 3 posttreatment samples, and 1 with 2 posttreatment samples. Thirty IgA MM sample sets were identified: 26 with 4 posttreatment samples, 1 with 5 posttreatment samples, and 3 with 3 posttreatment samples.

All queries to the Laboratory Information System or Dysproteinemia database followed a protocol on the “Natural History of Monoclonal Gammopathies,” approved by the Mayo Clinic Institutional Review Board.

We performed comparisons of continuous variables in 2 different groups with the Student t test or Wilcoxon rank sum test. Method comparison data were analyzed by Passing–Bablok regression analysis. The correlation coefficient (r), slope, and y-intercept were calculated for the linear regressions. Significance was defined as P < 0.05. JMP 10.0.0 (SAS Institute) and Analyse-it for Microsoft Excel 3.76 (Analyse-it Software) statistical software packages were used for data analyses.

Results

The HLC assays were validated for variability, linearity, stability, and recovery of total Ig. The mean intraassay and interassay variability for the 6 assays in healthy sera was 2.3% and 4.9%, respectively. The HLC assays were linear to low concentration limits of 14 mg/dL (IgGκ and IgGλ), 4 mg/dL (IgAκ), 2 mg/dL (IgAA), and 1 mg/dL (IgMκ and IgMA). After 7 days at room temperature, 7 days at 4 °C, or 3 freeze/thaw cycles, the mean decreases of the HLC assays
were 2.5%, 2.2% and 3.4%, respectively, and the maximum decreases were 4.7%, 4.9%, and 6.7%. The recovery for the sum of the concentrations for each HLC-pair of healthy donors compared to the total IgG, IgA, or IgM concentrations were mean (SD) 100% (8%), 107% (10%), and 104 (14)%, respectively.

The central 95% RIs for the concentrations of the 6 Ig HLCs were calculated (Table 1). These are similar to previously published RIs (12) except that the 2.5th percentiles for the IgGk, IgMk, and IgMA ranges in the original description of the assays (8) fall slightly above those determined with this data set. We expanded the data set for the rHLCs by including frozen serum samples that were negative for monoclonal Ig in the Olmsted MGUS prevalence study, and we defined both central 95% and 99% RIs. The central 99% intervals were used in this study to define clonality.

The frequency of abnormal rHLCs was determined in frozen diagnostic samples of 365 IgG and 153 IgA MM patients. Both IgG and IgA had rHLC concentrations outside the central 99% RI in 97% of cases (354/365 IgG MM, 148/153 IgA MM). Previous studies reported that in IgG, IgA, and IgM MGUS, the HLC-pair ratios were abnormal in 56%, 97%, and 90% of cases (12). The high frequency of abnormal IgA HLC-pair ratios in MM and MGUS suggests its potential utility for monitoring clonality and concentration in posttreatment IgA MM.

To evaluate HLC assays for monitoring MM, we tested diagnostic and posttreatment sera from 32 IgG patients with migration in the y fraction and 30 IgA patients with β-fraction migration. We quantified IgG or IgA HLC-pairs as well as total IgG or IgA and retrieved SPEP and IFE results if they were available in the medical record. Among the 32 IgG patients, there was a patient with a monoclonal IgGk that was detected by IFE who had no M-spike and IgG rHLC results in the RI (Table 2). A second MM patient with an IgG rHLC concentration in the RI had a 1.9 g/dL M-spike on diagnosis. Over the course of 7 years, this patient’s M-spike decreased to 1.4 g/dL, and eventually the SPEP and IFE became negative, whereas the IgG rHLC remained in the RI. The frequency of abnormal IgG rHLC was similar to the frequency of SPEP abnormalities (Table 2). Presumably because of the sensitivity of SPEP in this group (87%), only 103 of the 155 (66%) sera were tested by IFE, and the observed rate of positive IFEs was 88%.

Among the 155 IgG MM samples, 114 samples had both an M-spike and an abnormal rHLC, and these were used to compare the M-spike quantification to the involved HLC (iHLC) quantification. The graph of iHLC and M-spike concentration (Fig. 1) has a Passing–Bablok linear regression slope of 1.016 (95% CI 0.8947–1.129), r = 0.87. The same analysis for total IgG vs M-spike has a slope of 1.317 (95% CI 1.225–1.413), r = 0.92 (data not shown).

There were 13 patient sample sets of 30 IgG MM with a diagnostic sample and 4 posttreatment samples that each contained an M-spike and an abnormal rHLC. For each of the 13 patients, the diagnostic sample’s M-spike and iHLC value was defined as 100%, and subsequent values were expressed as a percentage of this initial value. The percentage changes for each time point were averaged, and the sequential results are shown in Fig. 2. The relative changes of the mean M-spike and iHLC were very similar (P > 0.75).

All 30 IgA patients had IgA monoclonal proteins that migrated in the β fraction, and the initial serum samples for all 30 were positive by IFE. In 7 patients, however, the initial samples had a symmetric β fraction <2 g/dL and therefore did not have a quantifiable M-spike, and 1 patient had an IgA rHLC within the RI (Table 3). Among the 23 patients whose initial sample contained an M-spike, 18 had a symmetric β fraction ≥2 g/dL and were fractionated as M-spike, whereas 5 had β-migrating IgA M-spike distinct from the β band. Among the 30 IgA patients, 2 sample sets had 3 posttreatment samples and 1 sample set had 5 posttreatment samples. Unlike the IgG patients, 92% of the posttreatment

| Table 1. HLC concentration and HLC-pair ratios: reference intervals. |
|-----------------------------|------------------|------------------|
| **Analyte** | **n** | **Central 95% RI** | **Central 99% RI** |
| IgAx (mg/dL) | 129 | 53–262 |  |
| IgAα (mg/dL) | 129 | 38–181 |  |
| IgGk (mg/dL) | 129 | 434–1080 |  |
| IgGλ (mg/dL) | 129 | 177–531 |  |
| IgMk (mg/dL) | 119 | 22–161 |  |
| IgMA (mg/dL) | 119 | 10–94 |  |
| IgAx/igAα | 278 | 0.70–2.21 | 0.53–2.52 |
| IgGk/igGA | 276 | 1.17–3.61 | 1.06–4.46 |
| IgMk/igMA | 267 | 0.93–2.79 | 0.79–4.61 |

| Table 2. IgG MM: SPEP and HLC-pair ratios in diagnostic and posttreatment sera.a |
|-----------------------------|------------------|------------------|
| **Sample** | **n** | **Abnormal SPEP** | **Abnormal HLC-pair ratio** |
| Diagnostic serum samples | 32 | 31 (97) | 30 (94) |
| Posttreatment serum samples | 123 | 104 (85) | 106 (86) |
| All samples | 155 | 135 (87) | 136 (88) |

a Data are n (%).

b M-spike or small, fuzzy band.
samples had IFE assays in the medical record. The rate of IgA rHLC outside the RI (56%) was higher than the rate of SPEP abnormalities (28%) and was similar to that of the IFE results (61%) in all serum samples. The total IgA quantification was increased above the upper RI end point (>356 mg/dL) in 87% of diagnostic samples; however, the increase persisted in only 40% of posttreatment serum samples.

Total IgA quantification and the IgA iHLC for the 149 IgA MM samples were compared, and Passing–Bablok linear regression gave a slope of 1.124 (95% CI 1.015–1.194) (Fig. 3A), \( r = 0.969 \). Because of the overlap of the M-spike and the healthy \( \beta \) peak, it is difficult to accurately fractionate the M-spike, and only 41 samples had both an M-spike and an abnormal rHLC. These 41 samples were used to compare the M-spike to the iHLC concentrations (Fig. 3B). The Passing–Bablok linear regression gave a slope of 1.054 (95% CI 0.9105–1.289), \( r = 0.87 \). When the \( \beta \) fraction and the iHLC concentrations of all IgA samples were compared, the linear regression slope was 1.11, \( r = 0.82 \). However, the regression line was shifted so that the intercept on the vertical axis indicated the \( \beta \) fractions were almost 1 g/dL (977 mg/dL) larger than the iHLC concentrations. When the comparison of \( \beta \) fractions to iHLC was restricted to \( \beta \) fractions <2 g/dL, the correlation coefficient dropped to 0.33.

**Discussion**

Monitoring monoclonal gammopathies involves the 2 separate steps of identification and quantification of a monoclonal protein. Monoclonal Ig that migrates in the \( \gamma \) fraction has a background of diffuse polyclonal Ig, and the restricted migration of a monoclonal band signals the continued presence of clonal disease. The suppression of polyclonal Ig synthesis that often occurs in MM may make detection of the monoclonal band even easier than in the more common, asymptomatic MGUS. SPEP monitoring of IgG MM provides an efficient assay for both recognition and quantification of a monoclonal protein. If the monoclonal Ig migrates in the \( \beta \) fraction, however, the \( \beta \) peak may obscure M-spike recognition and quantification. In this study, we retrieved the IFE results for IgG and IgA MM patients from the medical record, and the
difficulty with recognition of β-migrating abnormalities was evident from the frequency of IFE being ordered during disease monitoring. The IgG posttreatment sera had IFE performed in 58% of the samples, whereas the IgA posttreatment sera had IFE performed in 95% of the samples. The need to document the continued presence of monoclonal IgA required IFE for these β-migrating proteins. In the absence of an M-spike, these samples also required quantitative total IgA for monitoring therapy, and the International Myeloma Working Group now recognizes the need for IgA nephelometry for monitoring IgA MM response to therapy (7). Considering that approximately 21% of MM patients have an IgA monoclonal protein (12), and that in our cohort, 50% of all samples had an IgA quantification result in the RI, IgA HLC assays appear to have a potential role for monitoring myeloma in this population.

The development of HLC assays provides another method for recognizing and quantifying serum clonal Ig. Although changes in HLC concentration have not yet been incorporated into recommendations for response

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>SPEP M-spike quantified</th>
<th>Abnormal HLC-pair ratio</th>
<th>Positive IFE</th>
<th>Total IgA above RIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic serum samples</td>
<td>30</td>
<td>23 (78)</td>
<td>29 (97)</td>
<td>30 (100)</td>
<td>26/30 (87)</td>
</tr>
<tr>
<td>Posttreatment serum samples</td>
<td>119</td>
<td>18 (15)</td>
<td>54 (45)</td>
<td>56 (50)</td>
<td>48 (40)</td>
</tr>
<tr>
<td>All serum samples</td>
<td>149</td>
<td>41 (28)</td>
<td>83 (56)</td>
<td>86 (61)</td>
<td>74 (50)</td>
</tr>
</tbody>
</table>

* Data are n (%).
+ >356 mg/dL.
* n = 111.
* n = 141.
Fig. 3. Comparison of total IgA and IgA M-spike concentrations to IgA iHLC.
(A), Distribution of total IgA and IgA iHLC for the 149 IgA MM samples. Passing–Bablok linear regression has a slope of 1.124, \( r = 0.97 \). (B), Distribution of M-spike and iHLC for 41 samples with both an M-spike and an abnormal HLC-pair ratio. Passing–Bablok linear regression has a slope of 1.054, \( r = 0.87 \).
criteria (1), total IgA and IgA HLC nephelometry will likely be incorporated as the HLC assays become more widely used and as the difficulties in quantifying β-migrating IgA proteins become more widely recognized. The discontinuity inherent in gating or not gating β-migrating IgA for quantification by SPEP as disease responds or progresses is confusing to clinicians and patients. An abnormal rHLC indicates whether the monoclonal protein is still present, and the iHLC concentration provides the concentration of the monoclonal protein.

We validated the HLC assays for their performance in the clinical laboratory, and the stability, intra- and interassay variability, linearity, and accuracy were all found to be acceptable. We also verified the Rs and used the 99th percentile reference range for the rHLC to define clonal excess. In a previous study of patients with MGUS, the rHLC detected 56%, 97%, and 90% of IgG, IgA, and IgM MGUS (13). In the current study, we tested 365 IgG and 153 IgA MM diagnostic samples, and 97% of the cases were clonal by the rHLC. The suppression of polyclonal Ig synthesis is presumably why the rHLC is more sensitive in MM. Although the assay is not sensitive enough to use as a routine screening method for MM, the 97% sensitivity in MM and MGUS indicates that almost all IgA MM patients can be monitored by HLC for both clonality and quantification.

We found that in IgA MM, the rHLC is more sensitive for detecting the monoclonal immunoglobulin than SPEP in both diagnostic and posttreatment samples. Monitoring IgA patients requires either the combination of an SPEP, IFE, and quantification of total IgA or an IgA HLC-pair assay. If the HLC is abnormal, no IFE is needed and the iHLC provides quantification. iHLC had a linear correlation to the M-spike in both IgG and IgA MM (Figs. 1 and 3B). The correlation of IgA iHLC was better with total IgA quantification (r = 0.97) than with SPEP M-spike (r = 0.87). That is almost certainly a result of the difficulty of fractionating β-migrating monoclonal proteins away from the healthy β components and emphasizes the difficulty of monitoring β-migrating IgA proteins with M-spike. The reported biologic variability and reference change values of the iHLC (14) are similar to the variability of M-spike and Ig quantification (13).

In IgG MM patients, SPEP appears to be almost as sensitive as the rHLC for detecting the continued presence of the monoclonal Ig. SPEP therefore provides a method for documenting the monoclonal Ig and quantifying it as an M-spike or simply a small, fuzzy abnormality. Once the SPEP abnormality is no longer visible, an IFE is required to define a complete serologic response. The use of HLC assays should therefore not be routinely applied to monitoring all MM but will simplify the laboratory process in MM patients whose monoclonal protein migrates in the β fraction. In this study, we have evaluated the use of IgA HLC reagents for β-migrating IgA proteins, but it should be recognized that HLC reagent pairs may also be useful for IgM and IgG monoclonal proteins that migrate in the β fraction. The laboratory will need to play an active role in guiding test orders, and the laboratory’s role will continue to expand as more techniques such as mass spectrometry become available for monitoring monoclonal Igs (15, 16).

In addition to monitoring IgA MM, there are some monoclonal proteins that have very broad migration, and it may be difficult to distinguish between a broad monoclonal band and a restricted polyclonal Ig population. The use of HLC assays may help document clonality in the context of ambiguous IFE results (10).

Conclusion

HLC assays can be used to monitor IgA MM and provide information similar to the combination of SPEP, IFE, and IgA quantification. Triaging of β-migrating IgA MM samples to IgA HLC assays should simplify monitoring of these patients.

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