17 - Intact immunoglobulin multiple myeloma - monoclonal immunoglobulins at presentation

In intact immunoglobulin multiple myeloma:

- IgG is the most common disease isotype, followed by IgA, IgD, IgM and IgE.
- An abnormal κ/λ sFLC ratio is detected in around 95% of patients.
- Monitoring concentration changes in the intact immunoglobulin is essential for assessing patient response.
- An abnormal IgG or IgA Hevylite® ratio (corresponding to the monoclonal immunoglobulin type) is found in 97 - 100% of both IgG and IgA multiple myeloma patients at diagnosis.
- Hevylite assays offer an alternative method to densitometric quantification in cases where small monoclonal proteins are obscured by other serum proteins.

17.1. Introduction

Approximately 80% of all multiple myeloma (MM) patients produce monoclonal intact immunoglobulins, with 95% of these also producing monoclonal serum free light chains (sFLCs). IgG intact immunoglobulin multiple myeloma (IIMM) accounts for more than half of all MM patients, and IgA IIMM accounts for a further 20% of cases (Table 17.1) [1]. Only around 1 - 2% of patients have IgD IIMM [2][3] and monoclonal IgM is present in less than 1% of MM patients [2] (more commonly found in Waldenström’s macroglobulinaemia, Chapter 32). IgE MM is extremely rare, with fewer than 50 cases reported in the literature [3]. Around 1-2% of MM patients are diagnosed with biclonal gammopathy (Sections 7.7 and 17.2) [1][4]. In such cases, two monoclonal proteins are identified that can differ in their heavy chains, light chains or both. It has been proposed that the larger (dominant) monoclonal protein is a product of the active MM clone(s), and the smaller (minor) monoclonal protein represents a separate benign MGUS-like clone (Chapter 13) [5].

Measurements of the monoclonal intact immunoglobulin and sFLC are essential for diagnosis and follow-up of IIMM, and form the basis of response criteria [6][7][8][9][10][11]. This chapter reviews intact immunoglobulin and sFLC measurements at diagnosis. The limitations of serum electrophoretic techniques used to quantify monoclonal intact immunoglobulins are discussed, along with heavy chain/light chain immunoglobulin (Hevylite, HLC) assays.
Table 17.1. Types of serum monoclonal proteins in 1027 patients with multiple myeloma. *Total does not equal 100% due to rounding.

### Table 17.1. Types of serum monoclonal proteins in 1027 patients with multiple myeloma

<table>
<thead>
<tr>
<th>Type</th>
<th>% of MM patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgGκ</td>
<td>34</td>
</tr>
<tr>
<td>IgGλ</td>
<td>18</td>
</tr>
<tr>
<td>IgAκ</td>
<td>13</td>
</tr>
<tr>
<td>IgAλ</td>
<td>8</td>
</tr>
<tr>
<td>IgMκ</td>
<td>0.3</td>
</tr>
<tr>
<td>IgMλ</td>
<td>0.2</td>
</tr>
<tr>
<td>IgDκ</td>
<td>1</td>
</tr>
<tr>
<td>IgDλ</td>
<td>1</td>
</tr>
<tr>
<td>K FLC only</td>
<td>9</td>
</tr>
<tr>
<td>L FLC only</td>
<td>7</td>
</tr>
<tr>
<td>Biclonal</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
</tr>
</tbody>
</table>

Although IIMM is characterised by the secretion of monoclonal intact immunoglobulins, around 95% of patients have abnormal sFLC concentrations (Table 17.2). Mead et al. assessed the frequency of sFLC abnormalities at presentation of IIMM according to immunoglobulin isotype. The study comprised 314 patients with IgG MM, 142 with IgA MM, 36 with IgD MM and 5 with IgE MM. Generally, sFLC concentrations were higher in IgA than in IgG patients, but highest in IgD patients (Figures 17.1A and B). Overall, 89% had abnormally high sFLCs, with the following breakdown: IgG 84%, IgA 92%, IgD 94%, and all five of the IgE MM patients. Some patients had normal or reduced concentrations of FLCs, but abnormal $\kappa/\lambda$ ratios, indicating monoclonality in association with bone marrow suppression. A study by Campbell and colleagues characterised monoclonal protein production in MM patients with biclonal gammopathy. In the majority (93%) of cases, the dominant tumour clone expressed a monoclonal intact immunoglobulin that was associated with monoclonal FLC production in 90% of cases, while a minority (6.9%) expressed FLCs only. In contrast, all minor clones expressed monoclonal intact immunoglobulins, which was associated with monoclonal FLC production in only 16% of cases (Section 7.8).
<table>
<thead>
<tr>
<th>Publication</th>
<th>Number of patients</th>
<th>% of patients with an abnormal κ/λ sFLC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mead et al. (2004)</td>
<td>497</td>
<td>89</td>
</tr>
<tr>
<td>Orlowski et al. (2007)</td>
<td>487</td>
<td>94</td>
</tr>
<tr>
<td>Owen et al. (2007)</td>
<td>207</td>
<td>95</td>
</tr>
<tr>
<td>Snozek et al. (2008)</td>
<td>576</td>
<td>95</td>
</tr>
<tr>
<td>Dispenzieri et al. (2008)</td>
<td>399</td>
<td>96</td>
</tr>
<tr>
<td>Katzmann et al. (2009)</td>
<td>467</td>
<td>97</td>
</tr>
<tr>
<td>Jeong et al. (2013)</td>
<td>159</td>
<td>87</td>
</tr>
</tbody>
</table>

It is noteworthy that the concentration of monoclonal immunoglobulins and monoclonal FLCs are not correlated in IIMM patients (Figure 17.2). However, in the majority of cases, changes in the intact immunoglobulin and the FLC are concordant (Chapter 18). Importantly, discordant responses may indicate the presence of multiple clones, which have been identified by Ayliffe et al. in a proportion of MM patients, using dual staining immunocytochemistry. In 82% of MM patients, single populations of cells were present that expressed either intact monoclonal immunoglobulin (with or without monoclonal FLC co-expression [74%]) or monoclonal FLC alone (8%). However, 18% of the samples contained a mixture of both cell populations. Therefore, measurement of both monoclonal intact immunoglobulins and sFLCs in IIMM patients provides information on the underlying tumour cell clones, which may evolve differentially over the course of the disease (Chapter 19).

### 17.3. International guidelines for the quantification of monoclonal immunoglobulins in IIMM

Once a monoclonal intact immunoglobulin is identified in a patient's serum, guidelines recommend that it is quantified using scanning densitometry of serum protein electrophoresis (SPE) gels (or capillary zone electrophoresis [CZE]), alongside nephelometric/turbidimetric quantitation of total immunoglobulins (Chapter 25). The serum concentration of the monoclonal intact immunoglobulin is an important determinant in choosing the correct method to monitor disease course. Whilst it is preferred that monoclonal proteins are monitored by densitometric quantification, in cases where small monoclonal proteins are obscured by other serum proteins (e.g. transferrin), nephelometric measurements may be more accurate.

Additionally, international guidelines recommend performing sFLC analysis at presentation as it provides important prognostic information (Chapter 20). This also gives a baseline measurement for monitoring oligosecretory patients (serum monoclonal protein <10 g/L and urine monoclonal protein <200 mg/24 hours) and all patients to allow early detection of relapse by FLC escape (Chapter 18).

### 17.4. Limitations of electrophoresis
Whilst serum electrophoresis and total immunoglobulin assays are both well-established techniques for the diagnosis and monitoring of IIMM, the measurement of a monoclonal protein spike remains a subjective method and users should be aware of their limitations (Section 4.2)[29]. Users should also be aware that therapeutic monoclonal antibodies administered to patients may increase the complexity of SPE interpretation (Section 18.3.5).

Firstly, monoclonal immunoglobulins can co-migrate with other major serum protein bands in gel electrophoresis, making identification and quantification inaccurate. This is often the case for monoclonal IgA, since its anodal electrophoretic migration positions it over other bands such as transferrin and complement component 3 (C3) in the $\beta$-region of gels in approximately 40% of cases. Co-migration with other serum proteins may also affect monoclonal IgM and to a lesser extent monoclonal IgG.

Secondly, quantification of monoclonal proteins at low concentrations (1 - 10 g/L) can be inaccurate. In the case of monoclonal proteins that migrate in the $\gamma$-region, it is usually impossible to avoid including a proportion of polyclonal IgG in the densitometric measurement. Whilst the coefficient of variation (CV) of SPE quantitation for intact immunoglobulin >10 g/L is acceptable (at less than 10%), for monoclonal proteins of <10 g/L that are detectable by SPE, the CV rises sharply and can be as high as 35% (Figure 17.3) [27][25]. This may in part reflect the additional biological variation of polyclonal immunoglobulins included in the SPE measurement [29]. At presentation, approximately 10% of MM patients are classified as having oligosecretory disease [35]. Below these thresholds, monoclonal proteins are deemed unmeasurable because quantification by electrophoretic techniques is unreliable. In addition, small monoclonal bands may be completely undetectable by SPE/CZE; this is a particular problem for monoclonal immunoglobulin concentrations of <3 g/L [36][37].

Thirdly, changes in plasma cell populations in IgG IIMM patients may not be accurately reflected by changes in monoclonal IgG concentrations quantified by SPE. The correlation between monoclonal IgG measurements by SPE and nephelometry is non-linear above 20 g/L (Figure 17.4) [28]. This is attributed to dye saturation of dense, narrow monoclonal IgG bands in the SPE gel [38] and leads to an underestimation of tumour burden by SPE at high monoclonal protein concentrations (Figure 17.5).

Furthermore, assessments of monoclonal IgG are affected by concentration-dependent catabolism via FcRn recycling receptors (Sections 3.5.3 and 18.4.5).

Fourth, exogenous substances, including therapeutic monoclonal antibodies (Section 18.3.5), antibiotics and contrast dyes, may produce false-positive results [39].

Finally, IgM monoclonal proteins may self-aggregate and precipitate at the point of application, making quantification by SPE inaccurate (Section 32.2). In some instances, IgM and IgA monoclonal proteins migrate as broadly restricted bands in the $\gamma$-
region after electrophoresis, making them difficult to distinguish from polyclonal background immunoglobulins \[40\]. In particular, IgA monoclonal proteins may be relatively broad because they are heavily glycosylated and can exist as monomers, dimers or multimers \[5\]. In such cases, HLC assays offer an alternative means of monitoring response (Chapter 18, Clinical case history 2).

17.5. Limitations of total immunoglobulin measurements

Total immunoglobulin measurements are unable to distinguish monoclonal from polyclonal immunoglobulin concentrations of the same isotype. Therefore, when IIMM patient samples contain significant concentrations of non-tumour polyclonal immunoglobulins, nephelometric results are inaccurate.

Nephelometry is also known to grossly overestimate monoclonal IgM concentrations. In one study, IgM values obtained by SPE and nephelometry were linearly correlated with a slope of 1.8 (CI 1.68 – 1.92), showing a systematic bias for higher values by nephelometry \[28\].

17.6. Immunoglobulin HLC immunoassays (Hevylite) at diagnosis

HLC immunoassays provide an additional tool for the management of patients with IIMM. The assays separately quantify the different light chain types of each immunoglobulin isotype (i.e. IgG\(_\kappa\), IgG\(_\lambda\), IgA\(_\kappa\), IgA\(_\lambda\), IgM\(_\kappa\) and IgM\(_\lambda\), Chapter 9). The molecules are assessed in pairs to produce HLC ratios (e.g. IgGk/IgG\(_\lambda\)) in the same manner as \(\kappa/\lambda\) sFLC ratios. The HLC ratio provides information on both the involved (monoclonal) immunoglobulin (e.g. IgGk in an IgG\(_\kappa\) patient) and the uninvolved (polyclonal) HLC-pair (e.g. IgG\(_\lambda\) in an IgG\(_\kappa\) patient). When the concentration of the HLC-pair is below the normal reference interval, alongside an abnormal HLC ratio, this is termed "HLC-pair suppression".

HLC assays are less labour-intensive and less subjective than serum electrophoretic techniques \[30][43\], and may overcome many of the known limitations of serum electrophoresis discussed above (including co-migration, dye saturation and broadly migrating monoclonal proteins, Section 17.4). For example, in Figure 17.6 two cases are presented in which monoclonal proteins co-migrate with other serum proteins bands, whereas HLC assays provide a quantitative result \[41\].

HLC assays were assessed in a large cohort of IIMM patients recruited to the IFM 2005-01 MM trial \[30\]. A total of 339 patients, comprising 245 with IgG (166 IgG\(_\kappa\), 79 IgG\(_\lambda\)) and 94 with IgA (60 IgA\(_\kappa\), 34 IgA\(_\lambda\)) isotypes were evaluated at presentation. The HLC data are summarised in Figure 17.7A and B, using IgGk/IgG\(_\lambda\) dot plots. The involved HLC concentration was greater than the upper limit of the normal range in the majority of patients (Tables 17.3 and 17.4), and all IIMM patients had the corresponding abnormal HLC ratio. Thirty-three percent (31/94) of the IgA patients could not be accurately quantified by SPE, due to co-migration with other serum proteins. An initial study of IgM Hevylite in IgM MM concluded that HLC ratios were concordant with IFE results, and that HLC-pair suppression identified patients with inferior outcome (Section 20.5) \[44\].
Table 17.3. Concentrations of IgG monoclonal proteins and IgG HLC in 245 patients with IgG MM.

<table>
<thead>
<tr>
<th></th>
<th>IgGκ MM</th>
<th>IgGλ MM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal Ig by SPE (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>40.5 8.02 - 90 (4.9 - 109)</td>
<td>46.0 10.86 - 85.02 (5.7 - 86.0)</td>
</tr>
<tr>
<td><strong>IgGκ HLC (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>33.0 5.60 - 91.8 (4.07 - 107)</td>
<td>0.41 0.12 - 5.84 (0.09 - 6.76)</td>
</tr>
<tr>
<td><strong>IgGλ HLC (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>0.33 0.072 - 2.03 (0.04 - 3.19)</td>
<td>35.5 4.77 - 91.12 (4.58 - 97.3)</td>
</tr>
<tr>
<td><strong>IgGκ/IgGλ HLC ratio</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>93.52 5.13 - 864 (3.94 - 1334)</td>
<td>0.018 0.001 - 0.878 (0.001 - 1.05)</td>
</tr>
</tbody>
</table>

Table 17.4. Concentrations of IgA monoclonal proteins and IgA HLC in 94 patients with IgA MM.

<table>
<thead>
<tr>
<th></th>
<th>IgAκ MM</th>
<th>IgAλ MM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal Ig by SPE (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>34.0 11.35 - 88.55 (10.0 - 98.0)</td>
<td>28.0 3.46 - 62.1 (2.2 - 67.0)</td>
</tr>
<tr>
<td><strong>IgAκ HLC (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>36.7 5.36 - 110.2 (3.49 - 125)</td>
<td>0.3 0.018 - 2.17 (0.018 - 2.65)</td>
</tr>
<tr>
<td><strong>IgAλ HLC (g/L)</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>0.072 0.017 - 1.13 (0.017 - 1.38)</td>
<td>30.1 1.95 - 49.9 (0.78 - 64.1)</td>
</tr>
<tr>
<td><strong>IgAκ/IgAλ HLC ratio</strong></td>
<td>Median 95% range Total range</td>
<td>Median 95% range Total range</td>
</tr>
<tr>
<td></td>
<td>462 11.2 - 6020 (8.8 - 7352)</td>
<td>0.01 0.018 - 0.255 (0.001 - 0.32)</td>
</tr>
</tbody>
</table>

Ludwig et al. evaluated the HLC assays in 100 IgG and 56 IgA MM patients, all of whom had corresponding abnormal HLC ratios at diagnosis. Of note, accurate quantitation of the monoclonal immunoglobulin by SPE densitometry was not possible in 46\% (26/56) of IgA patients and 4\% (4/100) of IgG patients due to co-migration with other serum proteins. Similarly, in a study by Mirbahai et al. comparing HLC data with SPE results at diagnosis of IgA MM all 210 patients had abnormal HLC ratios as determined by HLC assays (Figure 17.8A). However, accurate quantitation of the monoclonal protein was not possible in 40\% (83/210) of patients by SPE; three of these are shown in Figure 17.8B.

Katzmann et al. assessed the diagnostic sensitivity of HLC ratios in 365 IgG and 153 IgA MM patients at presentation. An abnormal HLC ratio was present in 97\% of both IgG (354/365) and IgA (148/153) diagnostic samples. In IgA MM, the HLC ratio
was more sensitive for detecting a monoclonal intact immunoglobulin than SPE (Section 4.2.5), and the authors recognised the utility of HLC assays in patients whose monoclonal protein migrates in the β-region.

In the IFM 2005-01 MM trial [30], the majority of IIMM patients had HLC-pair suppression (i.e. reduced concentrations of IgGκ in a patient with IgGλ IIMM). For IgGκ, IgGλ, IgAκ and IgAλ MM patients HLC-pair suppression was present in 99%, 92%, 93% and 69% of cases, respectively. For the IFM-2009 trial, 93% of IIMM patients had HLC-pair suppression at diagnosis [46]. Similar findings were reported by Ludwig et al. [47]. For one IFM IgAλ patient whose IgGλ HLC concentration was within the normal range (labelled 2 in Figure 17.7B), IgAκ HLC-pair suppression was present and the IgAκ/IgAλ ratio was abnormal. Interestingly, this patient and one additional patient (labelled 1 in Figure 17.7B) had highly elevated λ sFLC concentrations (8,500 mg/L and 103,000 mg/L, respectively). This was consistent with monoclonal λ sFLCs being the dominant monoclonal protein produced by the tumour. In such cases, sFLC analysis may be more informative for monitoring disease status (Chapter 18).

A number of studies have demonstrated that the degree of HLC-pair suppression varies greatly between patients (Tables 17.3 and 17.4) and during follow-up [30][47][46]. For example, Michallet et al. [46] reported a gradual increase of uHLC concentrations following stem cell transplantation and throughout consolidation, reaching a plateau during maintenance therapy.

The IFM 2005-01 MM trial was the first to demonstrate that patients with higher concentrations of involved HLCs tended to have lower concentrations of uninvolved HLCs. This negative correlation between suppression and production was more significant in IgG patients (IgGκ: r = -0.456; p<0.0001; IgGλ: r = -0.310; p=0.005) than in IgA patients [48]. Similar findings were reported by Ludwig et al. [47].

Ludwig et al. [47] defined three categories of HLC-pair suppression: no suppression; moderate suppression (below the lower normal limit and up to 50% suppression), and severe suppression (a >50% reduction below the lower normal limit). Severe HLC-pair suppression was present in 54.5% (85/156) patients at diagnosis and tended to be more prevalent in IgG patients than IgA patients, but this did not reach statistical significance (57% vs 48.2%, p=0.0764). Several studies have reported an association of severe HLC-pair suppression with a significantly shorter survival [46][47][49][50] as well as an increased incidence of bloodstream infections [50], and is described in more detail in Section 20.4.

Next
18 - Intact immunoglobulin multiple myeloma - monitoring monoclonal immunoglobulins

Previous
16 - Nonsecretory multiple myeloma

Figures

Figure 17.1. sFLC concentrations in IIMM.

(A) 314 patients with IgG MM compared with 282 normal sera. (B) 142 IgA, 36 IgD and 5 IgE MM patients compared with 282 normal sera.
17.2. Free light chains at diagnosis

Figure 17.2 Serum IgG and λ sFLC concentrations in 116 IgGλ MM patients.

(Pearson rank correlation $r = -0.0037$).

View source:
* 17.2. Free light chains at diagnosis

Figure 17.3. Median serum monoclonal protein concentration by SPE plotted against the coefficient of variation (CV) for individual patient’s serial samples.

Dashed lines indicate current recommendations for minimal values for monitoring. (Republished with permission of Clinical Chemistry[27]; permission conveyed through Copyright Clearance Center, Inc.).

View source:
* 17.4. Limitations of electrophoresis

Figure 17.4. Bland-Altman plot comparing SPE monoclonal protein values and quantitative immunoglobulins assessed by nephelometry for IgG monoclonal proteins.
Figure 17.5. IgG monoclonal protein concentration by SPE compared with total IgG by nephelometry.
Figure 17.6. Quantitative Hevylite results in patients with (A) IgAλ and (B) IgAκ monoclonal proteins that co-migrate with other serum proteins by SPE.
17.6. Immunoglobulin HLC immunoassays (Hevylite) at diagnosis

Figure 17.7. Serum HLC concentrations in MM patients at presentation.

(A) 166 IgGκ patients (blue circles) and 79 IgGλ patients (red circles). (B) 60 IgAκ patients (blue circles) and 34 IgAλ patients (red circles). Concentrations of λ sFLCs in samples 1 and 2 were 103,000 mg/L and 8,500 mg/L, respectively. Healthy blood donors (black diamonds) and 95% confidence limits (diagonal lines) are shown. (Reprinted by permission from Macmillan Publishers Ltd: Leukemia [39], copyright 2013).
17.6. Immunoglobulin HLC immunoassays (Hevylite) at diagnosis

Figure 17.8. HLC results for IgA MM at disease presentation.

(A) Serum IgA HLC concentrations in 210 IgA MM patients (145 IgAκ [blue squares] and 65 IgAλ [red squares]). Healthy blood donors (black squares) and 95% confidence limits (diagonal lines) are shown. All patients had abnormal HLC ratios, including three samples with unquantifiable monoclonal protein by SPE (numbered). (B) SPE gel of normal human sera (NHS) and the 3 IgA MM samples highlighted in (A) where the monoclonal protein was not quantifiable by SPE densitometry.

References

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32. Ludwig H, Milosavljevic D, Zojer N, Faint JM, Bradwell AR, Hubl W, Harding SJ. Immunoglobulin heavy/light chain ratios...
improve paraprotein detection and monitoring, identify residual disease and correlate with survival in multiple myeloma patients. Leukemia 2013;27:213-9


42. Mirbahai L, Fourrier NJ, Harper J, Harris J, Bradwell AR, Harding SJ. Monoclonal IgA proteins migrating into the b region of serum protein electrophoresis gels can be easily identified and quantified using IgAk and IgAl measurements. Clin Chem 2011;57:C-64a


