11.5. Comparison of Hevylite results with other immunoglobulin tests

11.5.1. Comparison of Hevylite and total immunoglobulin measurements

In general, the agreement between summated Hevylite (e.g. IgG\(\kappa\) + IgG\(\lambda\)) and total immunoglobulin assays (e.g. IgG) is good; indeed, comparison of summation data for blood donor sera was used to validate the assays during development\(^{[6]}\) and summation checks are routinely used as part of the manufacturing quality control (Sections 9.4.4 and 9.7). Other studies have also reported a good agreement between summated Hevylite and total immunoglobulin results for normal sera\(^{[2][3][5]}\).

Eckold et al.\(^{[1]}\) compared summated IgG, IgA and IgM HLC concentrations with total immunoglobulins for patients with monoclonal gammopathy, and reported an excellent linear correlation (Figure 11.3). Similar findings were reported by Jacobs et al.\(^{[6]}\) when 27 samples from the Dutch External Quality Assessment M-protein scheme and the international standard ERM-DA470k were analysed.

Kenyon et al.\(^{[7]}\) compared summated Hevylite with total immunoglobulins for 518 IgG and IgA MM patients at diagnosis. A total of 254/518 (49%) of summated Hevylite values fell outside the recovery range (defined as 80 – 120% total immunoglobulin values). Despite repeating 254 measurements, only one sample (an IgAk) demonstrated Hevylite antigen...
Boyle et al. [3] compared total IgM and summed IgM HLC values (IgMκ + IgMλ) for sera from 110 normal donors and 78 Waldenström’s macroglobulinaemia (WM) patients. There was a good correlation between the results (Linear regression $R^2 = 0.90$, Passing-Bablok regression $y=-0.32 + 1.17x$, Figure 11.5A), although Bland-Altman analysis demonstrated that IgM values measured using IgM Hevylite assays were slightly higher than those measured using total IgM assays (bias = 1.8 g/L, Figure 11.5B). Similar findings were reported by Manier et al. [8] and Sarto et al. [9].

As part of an evaluation of HLC assays for monitoring multiple myeloma (MM) patients, Katzmann et al. [2] compared total IgA quantification and the IgA iHLC for 149 IgA MM samples. Passing–Bablok linear regression gave a slope of 1.124 (95% CI 1.015–1.194), $r = 0.97$ (Figure 11.4A), indicating a good agreement. Jacobs et al. [5] also compared iHLC and total immunoglobulins for monitoring 4 patients with MM, and concluded that both assays showed a parallel response. The iHLC concentration was lower than the total immunoglobulin value, but the authors comment that iHLC may be a more accurate representation of M-protein concentration because it measures less polyclonal background than total immunoglobulin assays. Theoretically, the more a tumour suppresses the production of polyclonal immunoglobulin (of the same immunoglobulin class), the closer will be the correspondence between iHLC concentrations with both the total immunoglobulin concentrations and the monoclonal immunoglobulin concentrations, as measured by densitometry of SPE gels.

### 11.5.2. Comparison of Hevylite and immunoglobulin measurements by SPE

In an in-house study, the IgG iHLC concentration was compared with the monoclonal concentration, determined by SPE scanning densitometry, for 160 IgG MM patients. In the majority of patients, there was good agreement in the concentration determined by the two methods but some samples did show variance (Figure 11.6). Lopez-Anglada et al. [10] reported similar results. Possible explanations for the variance include inaccurate SPE measurements, for example if the monoclonal protein co-migrated with other serum proteins (e.g. transferrin), or dye saturation underestimated the concentration of IgG monoclonal proteins. This is discussed in more detail in Section 17.4. In addition, when a high concentration of polyclonal immunoglobulins is present, Hevylite measurements may be less sensitive than serum electrophoretic techniques for detecting a monoclonal protein (e.g. MGUS; Chapter 13).

Katzmann et al. [2] compared the iHLC concentration with the monoclonal protein concentration determined by SPE for 114 IgG and 41 IgA MM serum samples. Each sample had a band present on SPE (M-spike) and a corresponding abnormal HLC ratio. There was a linear correlation between the iHLC concentrations and the M-spike for both IgG and IgA patients (Figure 11.7 and 11.4B). Other studies have also reported a good correlation of IgA and IgG iHLC and/or dHLC concentration with the SPE M-spike [3][6][11][12][13]. For example, Chae et al. [14] compared IgG or IgA M-Spike concentrations with the corresponding dHLC or iHLC value for samples from 115 IgG and 61 IgA MM patients. There was a strong linear correlation between iHLC or dHLC and M-protein concentration for both IgG and IgA M-proteins (both $p <0.0001$). In the latter patient group, iHLC measurements had
a stronger correlation with M-protein quantification than dHLC (p=0.002), and the authors concluded that iHLC values have a potential role for monitoring IgA MM, an isotype for which M-protein quantification is notoriously inaccurate (Section 17.4).

Katzmann et al. [2] noted that the correlation of iHLC with total IgA (Figure 11.4A; r=0.97) was better than the correlation of iHLC with the SPE M-spike (Figure 11.4B; r=0.87), and suggested that this was most likely to be due to the difficulty of quantitating β-migrating monoclonal proteins that co-migrate with other serum proteins (Section 17.4). Katzmann et al. [2] study also compared the relative changes in the iHLC and serum M-spike concentration for 13 IgG patients for whom diagnostic and four follow-up samples were available. The relative changes of the mean iHLC and mean M-spike were not statistically different (p>0.75; Figure 11.8).

Boyle et al. [3] compared monoclonal IgM concentrations determined using the involved IgM HLC concentration or SPE densitometry for 66 WM patients with a quantifiable M-spike. The agreement between the two assays was poor, and a higher value was reported by the IgM HLC assays in most cases (Linear regression R^2 = 0.49; Passing-Bablok regression y = -4.4 + 1.98x; Bland-Altman systematic bias = 10.1 g/L, Figure 11.9). Similar findings were reported by Manier et al. [8] and Sarto et al. [9]. The lack of agreement between electrophoretic techniques and automated IgM immunoassays has been reported previously [15][16], and was also demonstrated by Boyle et al. [3]. One explanation for these findings is the polymeric nature of IgM molecules, which causes an overread by nephelometric assays [16]. It should be noted that whilst the numerical agreement between IgM HLC and SPE densitometry is poor, there is an excellent correlation between the responses assigned using the two methods (Section 32.4.2).

11.5.3. Comparison of Hevylite and immunofixation electrophoresis

In general, the concordance between the M-protein type determined by Hevylite and immunofixation electrophoresis (IFE) is very good [4][5][6][7][8]. A number of studies have shown excellent agreement at diagnosis of MM (Section 17.6) and Waldenström’s macroglobulinaemia (Section 32.4.1). Other groups have compared Hevylite and IFE in a range of samples submitted for routine serum electrophoresis [5][6]. For example, Jacobs at al. [6] compared the M-protein type determined by Hevylite and IFE in 166 routine clinical samples, as well as 27 samples from patients with known monoclonal gammopathy. They demonstrated high concordance (Cohen κ coefficient of 0.84) between both methods, and 93% of tested samples had identical results. Similar results were reported by Paoloni et al. [5].

Occasionally, discrepancies occur between Hevylite and immunofixation results during follow-up of patients with monoclonal gammopathies. These can be broadly grouped into two scenarios, which are discussed below.

1) IFE positive, normal HLC ratio: in IgG patients, recycling of IgG by the FcRn receptor may result in the persistence of small IgG monoclonal proteins in the serum, causing IFE to remain positive long after the tumour has been eradicated and HLC pair suppression has ended (Section 18.4.5). In addition, in a minority of cases, the diagnostic sensitivity of IFE may be superior to that of the HLC ratio. For example, if a small IgG monoclonal protein is present with a large amount of polyclonal IgG [19]. Finally, on rare occasions, Hevylite antigen excess may cause a sample value to be falsely low. If antigen excess is suspected,
an additional sample dilution should be performed (Section 11.4).

2) IFE negative, abnormal HLC ratio: in some patients, the HLC ratio may be more sensitive for detecting a monoclonal immunoglobulin than IFE. This is more common for IgA and IgM where there is less polyclonal production so smaller amounts of monoclonal immunoglobulin can produce an abnormal HLC ratio \[4\]. An abnormal HLC ratio may indicate residual disease in IIMM patients whose electrophoresis results have normalised following therapy (Section 18.4.3).

Figures

**Figure 11.3. Correlation of summated HLC with total immunoglobulin concentrations**

Patient sera contained (A) IgA (n=106); (B) IgG (n=106); and (C) IgM (n=28) monoclonal proteins. (Reproduced from with permission from Clinical Laboratory Publications).

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- 11.5.1. Comparison of Hevylite and total immunoglobulin measurements

**Figure 11.4.** (A) A plot of total IgA versus IgA iHLC concentrations for 149 patients with IgA MM. Passing–Bablok linear regression has a slope of 1.124, \( r = 0.97 \). (B) A plot of M-spike versus iHLC concentrations for 41 patients with IgA MM. Passing–Bablok
Figure 11.5. Comparison of total IgM and summated IgM Hevylite results.
(A) Passing Bablok linear regression and (B) Bland-Altman correlation and agreement. Data generated using sera from 110 normal donors and 78 WM patients. (Reproduced from[3] with permission from AACR journals).

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Figure 11.6. A plot of M-spike versus iHLC concentration for 160 IgG MM patients.

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- 11.5.2. Comparison of Hevylite and immunoglobulin measurements by SPE

Figure 11.7. A plot of M-spike versus iHLC concentrations for 114 patients with IgG MM,
that had both an M-spike and an abnormal IgG HLC ratio.

Passing–Bablok linear regression has a slope of 1.02 (95% CI 0.89–1.13), $r = 0.87$. (Republished with permission of Clinical Chemistry permission conveyed through Copyright Clearance Center, Inc.)

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Figure 11.8. Mean M-spike % change and mean iHLC % change for sequential samples from 13 IgG MM patients (with standard error of the mean).

Note: Sample 1 = diagnostic sample defined as 100%. Samples 2–5 = post-treatment, follow-up samples. P value derived from unpaired Student t-test. (Republished with permission of Clinical Chemistry permission conveyed through Copyright Clearance Center, Inc)

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- 11.5.2. Comparison of Hevylite and immunoglobulin measurements by SPE

Figure 11.9. Comparison of SPE densitometry and involved IgM Hevylite results for IgM monoclonal proteins.

References


