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# Heavy/light chain assay in the monitoring of multiple myeloma

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## Summary

Serum protein (SPE) and immunofixation electrophoresis (IFE) have been extensively validated for the routine use of identifying, characterising and quantifying monoclonal proteins. However, accurate quantitation of IgA monoclonal proteins can be difficult when they migrate in to the β fraction, due to co-migration with transferrin and complement components. The heavy/light chain (HLC) immunoassay is an additional tool for measuring intact immunoglobulin monoclonal proteins. Therefore, we aimed to examine the clinical utility of the HLC assay for the disease monitoring of IgG and IgA multiple myeloma (MM) patients.

A total of 177 samples from 30 MM patients (21 IgG and 9 IgA) were analysed retrospectively with median number of six follow up samples per patient (range 3–13). Serum free light chains (sFLC) and HLC were quantified using Freelite and Hevylite immunoassays. Details of M-protein concentration, β-globulin levels, total immunoglobulin levels and disease treatment response were obtained from the laboratory and patient information system. Passing–Bablok regression analysis was performed to compare (i) M-protein quantification with involved HLC (iHLC) and (ii) total immunoglobulin with summated HLC pairs for each immunoglobulin type (e.g., IgGκ+IgGλ).

For 127 IgG MM samples, IgG iHLC levels showed a good correlation with SPE quantification (iHLC $y=0.96x+4.9$; $r=0.917$) and summated HLC showed a good correlation with total IgG concentration (summated HLC $y=0.94x+5.74$; $r=0.91$). In total, 95/127 (75%) IgG MM follow-up samples had an abnormal HLC ratio and 122/127 (96%) had a positive SPE, probably due to the lower sensitivity of HLC assay in detecting clonality in patients with IgG MM. Consistent with this, one patient assigned a very good partial response by International Myeloma Working Group criteria would be assigned a complete response based on HLC measurements. For 50 IgA MM samples, 42/50 (84%) had an abnormal HLC ratio. Conversely, 50/50 (100%) of M-proteins showed β fraction migration and were difficult to accurately quantify by SPE. Therefore, M-protein concentration and iHLC did not correlate as well in IgA MM ($y=1.9x-8.4$; $r=0.8$) compared to IgG MM. However, there was good correlation between total IgA and summated IgA HLC (IgAκ+IgAλ $y=1.35x-0.33$; $r=0.95$). Of the 8/50 (16%) IgA samples with a normal HLC ratio, 6/8 (75%) were consistent with the disease status being in complete remission. Interestingly, in one IgA MM patient, SPE and IFE were negative, but the serum FLC ratio and involved FLC were highly abnormal, consistent with the presence of light chain escape.

Our data suggest HLC measurements could add value to the current disease monitoring of MM patients. In IgG MM patients, the M-protein level correlated well with HLC values. The HLC assay complements the serum FLC assay and is especially useful for monitoring of IgA MM patients who display M-proteins migrating in the β region on SPE.

*Key words:* Multiple myeloma; monoclonal protein; heavy/light chain; free light chain.

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## INTRODUCTION

Multiple myeloma (MM) is a malignant plasma cell disorder that accounts for approximately 10% of all haematological cancers.[1,2] Diagnosis of MM is based on the detection of 10% or more clonal bone marrow plasma cells and at least one myeloma defining event.[3] Precise quantitation of monoclonal protein (M-protein) plays an important role in diagnosis, monitoring, prognosis and in detecting disease relapse.[4,5] Detection and quantitation and characterisation of the M-protein are commonly performed by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE), respectively.

Despite considerable improvement in SPE techniques, problems in identifying and quantifying an M-protein band still exist. M-proteins can co-migrate with other serum proteins and can be present anywhere from $\alpha_2$-to $\gamma$-region on SPE. This is commonly observed in patients with IgA MM due to co-migration M-proteins with transferrin and complement components. If M-protein is present in low concentrations or in the presence of concurrent infection or inflammation giving a dense polyclonal background, M-protein may fail to resolve as a discrete band. Arbitrary gating can cause significant variation in the quantitation by densitometry[6,7] and often leads to confusion for both clinicians and patients. Serum IFE is a more sensitive method and currently

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the gold standard for detection and characterisation of M-protein. However, it is not a quantitative technique. The International Myeloma Working Group (IMWG)[8] recommended quantification of total immunoglobulins (Ig) by nephelometry, but this assay cannot discriminate monoclonal Ig from normal polyclonal Ig. Overestimation of M-protein concentration by SPE, particularly in IgG and IgM paraproteinaemias, has been reported.[6,9]

Measurement of free κ and free λ light chains plays a key role in diagnosis, monitoring, and prognosis for many patients with a monoclonal gammopathy. These assays are advocated by the IMWG as an essential component of the primary screening algorithm for suspected monoclonal plasma cell disorders.[10] Markedly increased baseline clonal free light chain (FLC) concentrations are associated with poor prognosis in amyloid light chain (AL) amyloidosis, MM, and virtually every plasma cell disorder.[10]

In 2009, a new immunoassay to measure heavy/light chain (HLC) assay was introduced.[11] This novel assay utilises antibodies which bind specifically to target epitopes at junctional regions between bound κ or λ light chains and their respective heavy chain molecules. With that, the HLC assay can separately quantify IgGκ, IgGλ, IgAκ, IgAλ, IgMκ or IgMλ concentration and therefore it provides more specific information on the evaluation of individual tumour clones than total Ig quantification by conventional techniques. The calculation of molecule pairs (such as IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ) or more commonly known as heavy/light chain ratio (rHLC) serves as an indicator of clonal expansion in the same way as serum FLC κ/λ ratio.[12] Studies employing HLC assay have shown that HLC ratios may be of use in screening, monitoring and risk stratifying patients with plasma cell disorders.[13–15]

The aim of this study was to examine the clinical utility of the HLC assay in relation to that of standard conventional methods for quantification of intact Ig M-proteins and for monitoring IgG and IgA MM patients.

## MATERIAL AND METHODS

This study was conducted in a tertiary care centre. To evaluate the usefulness of the HLC assay, we used frozen archived sera of MM patients who had been monitored in our hospital, from June 2015 to December 2017. The selection criteria were: (a) diagnosis of MM; (b) IgG MM migrating in γ-fraction and IgA in β-fraction; (c) availability of a minimum of three post-treatment frozen sera. We excluded cases of other monoclonal gammopathies such as monoclonal gammopathy of undetermined significance (MGUS), primary amyloidosis, Waldenström macroglobulinaemia and light chain disease. Samples from 21 patients with IgG MM and nine patients with IgA MM were chosen, so a total of 177 (127 IgG MM and 50 IgA MM) samples with a median number of six follow-up samples per patient (range 3–13) were included in the study. All samples were coded and anonymised prior to analysis. The clinical information of the patients was retrieved from the patient record system of the hospital. The response to the treatment for MM was assigned according to IMWG criteria.

A complete laboratory assessment was performed at the time of the patient's follow-up which included quantification of total Ig, SPE and IFE. These data were retrieved from the laboratory information system. Total IgG, IgA and IgM were quantified using the BN ProSpec System (Siemens Healthcare Diagnostics, Germany) by using Siemens reagent. SPE was performed based on the principle of zone electrophoresis using agarose gel [Hydragel 15 Protein (e), Hydrasys System; Sebia, France]. M-proteins were identified and quantified using peak fractionation with perpendicular gating followed by densitometric scanning using the Hydrasys 2 scan. In all IgA MM cases where the quantification of M-protein was difficult due to its comigration to β fraction, the M-protein concentrations were determined by total quantification of β fraction. IFE was performed using Hydragel 4IF kits (Hydrasys System; Sebia). All procedures were carried out strictly according to the manufacturers' protocols. The study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the institution.

Serum FLC and HLC levels were quantified using Freelite and Hevylite immunoassays (The Binding Site Group Ltd, UK), respectively, and performed on the SPAPLUS turbidimeter (The Binding Site Group Ltd) according to the manufacturer's instructions. Reference range for HLC isotypes (IgGκ, IgGλ, IgAκ, IgAλ) and their ratios quoted by the manufacturer were used for the study.

### Statistical analysis

Passing–Bablok regression analysis was performed to compare HLC assay with standard conventional laboratory tests. We compared M-protein quantification with involved HLC (iHLC), total Ig with summated HLC pairs for each Ig type (e.g., IgGκ+IgGλ) and serum HLC ratio with FLC ratio. The correlation coefficient ($r^2$), slope, y-intercept were calculated for the linear regressions. Significance was defined as $p<0.05$. Statistical analysis was performed using MedCalc Statistical Software version 18 (MedCalc Software, Belgium).

## RESULTS

The characteristics of the patient population in this study are shown in Table 1. There were in total 177 samples from 21 IgG and nine IgA MM patients. Median age of patients was 66 years (range 54–81) and male-to-female ratio was approximately 1:1. The patients were followed up for a median of 20.5 months (range 6–30). To evaluate HLC assays for disease monitoring in MM patients, we quantified IgG or IgA HLC-pairs for all the samples and compared the results with total Ig quantification, SPE and IFE findings as well as serum FLC.

### IgG multiple myeloma

In 127 IgG MM samples, 95 (75%) samples had both detectable M-protein and an abnormal rHLC. Five samples had both SPE and rHLC negative and the remaining 27 samples had positive SPE but rHLC was within the reference interval (Table 2). Among these, 16 samples were from patients who achieved 'very good partial response', two samples from 'partial response' and four from 'progressive disease'. The other five samples were from one IgGκ MM patient who developed monoclonal IgGλ during the course of treatment. We noted that one patient, who achieved very good partial response due to treatment, had M-protein concentration ranging from 1.7 to 5.0 g/L on five subsequent follow-up samples over a period of 16 months. These samples showed abnormal HLC values on only three occasions, although the IgG rHLC remained within the reference interval.

We compared the M-protein concentration to the iHLC IgG quantification in samples with both M-proteins positive and an abnormal iHLC (Fig. 1). The slope, intercept and correlation coefficient obtained were 4.972, 0.963 and 0.92, respectively.

The summated HLC (IgGκ+IgGλ) and total Ig concentration were compared (Fig. 2). Passing–Bablok regression analysis for 127 IgG MM samples gave a correlation coefficient of 0.91 [summated IgG HLC vs total IgG: $y=0.94x+5.74$; confidence interval (CI) 0.87–0.93; $r^2=0.91$]. Both analyses showed good correlation between two parameters.

### IgA multiple myeloma

All IgA samples had IgA M-protein that migrated to β-globulins. Therefore, the reported measurement of M-protein

**Table 1** Characteristics of the study population

| | All | IgG patients | IgA patients |
|---|---|---|---|
| Samples | 177 | 127 | 50 |
| No. patients | 30 | 21 | 9 |
| Age, years (median) | 66 (54–81) | 66 (57–77) | 66 (54–81) |
| Sex, male/female | 14/16 | 8/13 | 6/3 |
| Median follow-up, months | 20.5 (6–30) | 18.0 (6–30) | 20.5 (12–29) |
| Involved Ig level, g/L | – | 18.6 (5.1–84.1) | 11.2 (0.4–63.2) |
| FLC ratio | 4.1 (0–7702.9) | 2.8 (0–7702.9) | 11.5 (0–719.7) |
| HLC ratio | 6.6 (0–4787.7) | 9.3 (0–662.9) | 4.0 (0–4787.7) |

Values are shown as median and range.
FLC, free light chain; HLC, heavy/light chain.

**Table 2** IgG and IgA MM samples: SPE, HLC-pair ratios and FLC ratio in follow-up samples

| Follow-up sample (post-treatment) | *n* | Abnormal SPE[a] | Abnormal rHLC | Abnormal FLC |
|---|---|---|---|---|
| IgG MM | 127 | 122 (96) | 95 (75) | 92 (73) |
| IgA MM | 50 | 27 (54) | 42 (84) | 37 (74) |

Data are *n* (%).
FLC, free light chains; MM, multiple myeloma; rHLC, heavy/light chain ratio; SPE, serum protein electrophoresis.
[a] Presence of M-proteins.

included quantitation of β-globulins also. This explains why M-protein concentration and iHLC did not correlate well in IgA MM ($y=1.9x-8.4$; $r^2=0.8$) samples as compared to IgG MM samples. Forty-two of the 50 samples (84%) had an abnormal rHLC (Table 1). Of the eight IgA samples with a normal HLC ratio, the disease status in six of the samples was complete remission as per IMWG criteria. One had partial response to treatment and the other one had relapse of the disease with the appearance of excess κ light chain after complete remission. Similar to IgG MM, a good correlation was observed between summated IgA HLC (IgAκ+IgAλ) and total IgA ($y=1.35x-0.33$; $r^2=0.95$) (Fig. 3).

### Monitoring of the multiple myeloma patients

Figure 4 illustrates the iHLC Ig and nephelometric total Ig in two IgG MM patients (6 follow-up samples each) and two IgA MM patients (4 follow-up samples each). We observed that the total Ig fluctuated over different sampling points in each patient and so did the iHLC Ig in which both parameters showed parallel changes to that of M-proteins. The observed iHLC Ig at all sampling points displayed a lower concentration than total Ig quantification in both IgG and IgA MM.

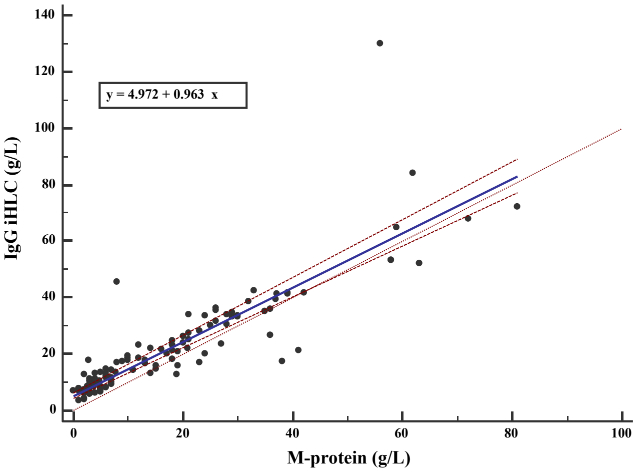

**Fig. 1** Comparison of IgG involved HLC and M-protein concentration. The slope, intercept and correlation coefficient were 4.972, 0.963 and 0.92, respectively.

### HLC and FLC ratios

Ratios of FLC and HLC for all patient samples were compared. Both rFLC and rHLC were tallied and consistent with the disease status in about 80% of total samples (141/177). An abnormal rHLC in the presence of normal rFLC was noted in 23/177 samples, including one sample from an IgA MM patient who was in complete remission. On the contrary, about 8% of total samples had a normal HLC ratio but an abnormal FLC ratio.

## DISCUSSION

Turbidimetric/nephelometric Ig HLC assays have been made available for general use since 2009 with the trade name of Hevylite. An abnormal rHLC indicates monoclonality, and the iHLC concentration provides a quantitation for M-protein. It is particularly advantageous in situations where electrophoretic quantification of the M-protein is not possible.[16,17] Hence, we assessed the clinical utility of the HLC assay in relation to that of standard conventional methods for quantification of M-protein and for the monitoring of IgG and IgA MM patients.

In our study, 75% of samples showed an abnormal rHLC, whereas positive SPE was noted in 96% samples (Table 2). In a study by Katzmann *et al.*, both serum SPE and rHLC showed similar results in monitoring of IgG MM.[17] Contrary to their findings, in our study, SPE was positive in more samples compared to rHLC. The patients who achieved very good partial response had normal rHLC and showed presence of M-protein levels less than 2 g/L. Similar to our findings,

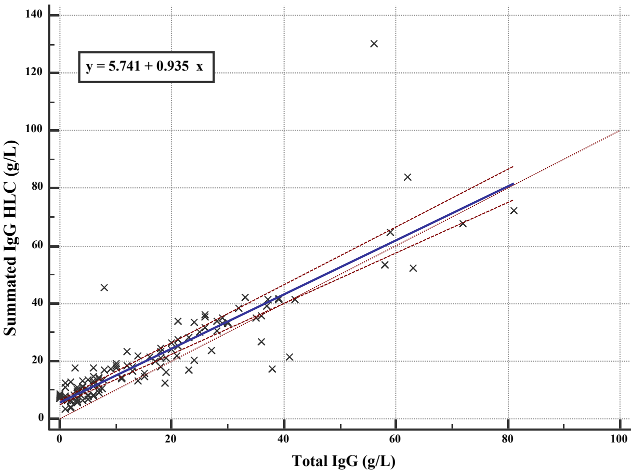

**Fig. 2** Comparison of summated IgG HLC and total IgG concentration. Passing–Bablok regression analysis for 127 IgG MM samples showed y=0.94x+5.74; CI 0.87–0.93; $r^2$=0.91.

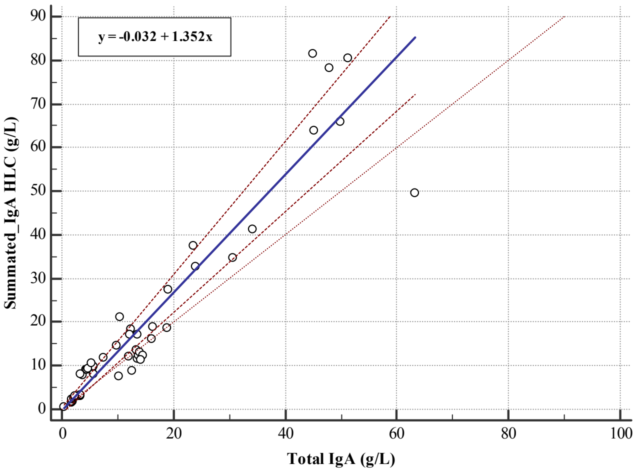

**Fig. 3** Comparison of summated IgA HLC and total IgA concentration. Linear regression of summated IgA HLC (IgAκ+IgAλ) and total IgA showed good correlation (y=1.35x–0.33; $r^2$=0.95).

Suehara *et al.* noted normal rHLC in patients with IgG MM who had very good partial response, and attributed this to lower sensitivity of IgG HLC assay for detecting monoclonal Ig compared to IgM and IgA HLC assay.[18] Apart from that, in one patient with IgGκ MM on follow up, rHLC was normal; this is due to appearance of intact IgGλ during treatment. Similar to our findings, Paolini reported normal rHLC in a patient with biclonal gammopathy with different isotypes.[19]

Nephelometry quantitation of Ig is unable to distinguish between the involved and uninvolved Ig of the respective isotype. Figure 4 shows that the observed iHLC Ig display a lower concentration than total Ig quantification at all sampling points, in both IgG and IgA MM. This explains the fact that HLC assay measures iHLC and its concentration would more accurately represent the tumour burden in patients. In our study, an abnormal iHLC result was noted in more than half of the patients with total Ig levels within the normal reference interval, signifying presence of M-protein. Regression analysis showed a good correlation between summated Ig HLC and total Ig quantification. Other studies have also reported a good agreement between summated HLC and total Ig results for patients with monoclonal gammopathy.[16,17,20]

In IgA MM, M-protein concentration and iHLC did not correlate well ($r^2$=0.80). This is mainly because all our IgA MM follow-up samples showed β fraction migration and it was difficult fractionating the β-migrating IgA M-proteins from other β-components. Similar findings have been reported by Katzmann *et al.*[17] Total IgA and summated concentration of IgA HLC were noted to correlate well, which confirm the findings of Eckold *et al.*[21] The IMWG recommends measurement of total IgA for monitoring IgA MM response to therapy. Since total IgA correlates well with summated IgA HLC, this test may have added value in monitoring the patient. HLC assay has been shown to better assess the clonality of involved Ig isotype than simple measurement of the total involved Ig. Therefore, it is expected to provide a more precise measure of the amount of monoclonal Ig after treatment, which is important for response assessment in patients with MM.[18] We identified FLC escape in one patient whose SPE and IFE were normal and did not exhibit an increased intact monoclonal Ig and HLC ratio, but had an increased serum FLC ratio. Subsequent follow up of this patient showed relapse of the disease. This result also illustrates the importance of disease monitoring using FLC analysis as well as HLC ratio when there is a suspicion of clinical relapse as suggested by Brioli *et al.*[22]

FLC ratios concurred with HLC ratios in the majority of cases; however, some results were obviously discordant. This discordance shows variability in clonal evolution during the course of MM. The original clone may persist as a single

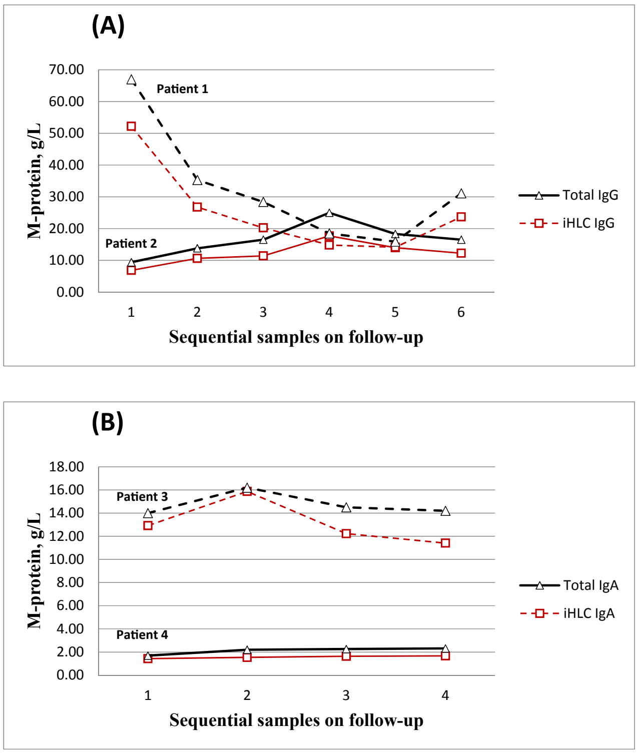

**Fig. 4** Total Ig quantification and involved HLC (iHLC) Ig of sequential serum samples. The dotted line and the solid line each represent two different patients. The M-protein concentration for each sequential serum sample is measured by nephelometry (triangle) and iHLC (square). (A) Two IgG MM patients with six sequential follow-up samples. (B) Two IgA MM patients with four sequential follow-up samples. The iHLC Ig levels showed consistently lower concentration than total Ig quantification at all sampling points.

clone, with possible emergence of subclones gaining capability to produce intact Ig.[23,24] Our data showed that normal rHLC values did not necessarily indicate normal rFLC values, or vice versa. Similar observations have been reported previously.[14,25]

This study was limited by the small sample size and the lack of standardised or predefined schedules for response assessments. Each patient had different intervals of follow-up, and they were in different clinical stages of disease, receiving different anti-myeloma therapies. Also, we did not include MGUS, amyloidosis and IgM MM patients.

In conclusion, our data suggest HLC measurements could add value to the current disease monitoring of MM patients. In IgG MM patients, the M-protein level correlated well with HLC values. The HLC assay complements the serum FLC assay and is especially useful for monitoring of IgA MM patients who display M-proteins migrating in the β-region on SPE.

**Conflicts of interest and sources of funding:** The Binding Site sponsored the reagents and the instruments for HLC assay.

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