

REVIEWS

Adv Clin Exp Med 2014, 23, 1, 127–133
ISSN 1899–5276

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Immunoglobulin Heavy Chain/Light Chain Pairs (Hlc, Hevylite™) Assays for Diagnosing and Monitoring Monoclonal Gammopathies

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Abstract

Immunofixation (IFE) is a standard method for detecting monoclonal immunoglobulins and characterizing its isotype. Recently clonality can also be determined by using immunoglobulin (Ig) heavy chain/light chain immunoassays – HLC, Hevylite™. HLC separately measures in pairs light chain types of each intact Ig class generating ratios of monoclonal Ig/uninvolved polyclonal Ig concentrations. Studies have shown that HLC and IFE are complementary methods. HLC assays quantify monoclonal proteins and identify monoclonality. It is possible to predict prognosis in multiple myeloma and to monitor response to treatment using HLC ratio. HLC ratio may serve as a parameter for myeloma induced immunoparesis and serve as a new marker for validating remission depth and relapse probabilities (*Adv Clin Exp Med* 2014, 23, 1, 127–133).

Key words: hevylite, free light chains, monoclonal gammopathy, multiple myeloma, remission.

HLC Assay Among Other Methods for Detecting, Immunochemical Characterizing and Quantifying Monoclonal Immunoglobulins

Because of the secreted monoclonal immunoglobulin (M protein), plasma cell proliferative disorders are generally classified among monoclonal gammopathies. These diseases include malignant disorders such as multiple myeloma (MM), plasmacytoma, plasma cell leukemia, and Waldenström macroglobulinemia; premalignant disease such as monoclonal gammopathy of undetermined significance (MGUS); and protein or low tumor burden diseases such as primary light chain amyloidosis, light chain deposition disease, and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) [1].

Serum protein electrophoresis (SPE) and immunofixation (IFE), are routinely used methods of identifying, characterizing and quantifying monoclonal proteins. SPE techniques have undergone

considerable improvements in the past few decades and now provided crisp resolution (especially in the β -globulin region) to detect many subtle electrophoretic differences in M proteins not readily distinguished by older 5-band gel techniques [2, 3]. However, problems in detecting and especially in quantifying M proteins still exist. By SPE, M proteins can migrate anywhere from the α to the γ region. They can be obscured by large proteins, such as haptoglobin in the α_2 region or more commonly by transferrin and complement component 3 (C3) in the β region [4]. This is particularly apparent for monoclonal IgA. Occasionally, IgM M proteins self-aggregate, making problems with detection and measurement.

IFE is the current gold standard for detecting and immunochemically characterizing M proteins. Unfortunately, IFE is not a quantitative technique.

At present, the M protein spike is commonly measured by densitometric scanning and concentrations of monoclonal immunoglobulin by quantification of protein bands in SPE is used for monitoring and evaluation of response to treatment. As noted above, when a relatively small M protein overlays a typical serum component, it can be difficult to obtain a precise measurement with this

technique. Nephelometry is also used for immunoglobulin measurements and is analytically accurate at their low concentrations. However, patients' samples also contain non-tumor origin polyclonal immunoglobulins of both κ and λ types that are included in the analysis so that results are clinically inaccurate at normal serum concentrations [5].

In 2009 Bradwell et al. [6] developed immunoglobulin heavy/light chain immunoassays – “Hevylite” (HLC). Availability of antibodies which bind to conformational epitopes spanning the junctional regions between bound κ or λ light chains and their respective heavy chain partners has

allowed the specific measurement of serum IgG κ , IgG λ , IgA κ , IgA λ , IgM κ and IgM λ concentrations (Fig. 1). In turn, this has enabled the calculation of IgG κ /IgG λ , IgA κ /IgA λ and IgM κ /IgM λ ratios (heavy/light chain or HLC ratios) for individual patients. Separate measurements of the κ and λ light chain types of IgG, IgA and IgM allow evaluation of individual tumor clones and give quantitative information about the immunosuppression of each non-tumor immunoglobulin. Measurement of molecule pairs, such as IgG κ /IgG λ , IgA κ /IgA λ , IgM κ /IgM λ , would indicate clonality in the same manner as serum free light chain (FLC) κ / λ ratios [7].

Bradwell et al. [6] proved that use of anti – HLC reagents could help in the vexing issue of detecting and providing reproducible measurements of M proteins that comigrate with other major serum components, such as transferrin or C3. Normal concentration ranges of HLC immunoglobulins and HLC ratios in blood donors sera are shown in Table 1 [6].

Until now, apart from a key Bradwell's et al. [6] publication, there appeared congress reports on the results of the use of nephelometric measurement of individual immunoglobulin κ / λ ratios in assessment of monoclonal gammopathies [8–11].

In the study performed at the Institute of Hematology and Transfusion Medicine in Warsaw the diagnostic value of HLC ratios was compared with the serum protein IFE results. Sera from 51 patients with monoclonal and biconal gammopathy including 34 with MM and 11 with Waldenström's macroglobulinemia were assayed.

Table 1. HLC concentrations and HLC IgG κ /IgG λ , IgA κ /IgA λ , IgM κ /IgM λ ratios in blood donor sera [6]

HLC	n	Median	95% range
IgG κ (g/L)	109	7.76	4.23–12.18
IgG λ (g/L)		4.00	2.37–5.91
IgG κ /IgG λ ratio		1.96	1.26–3.20
IgA κ (g/L)	191	1.27	0.43–2.36
IgA λ (g/L)		0.87	0.40–1.73
IgA κ /IgA λ ratio		1.40	0.58–2.52
IgM κ (g/L)	118	0.77	0.33–1.54
IgM λ (g/L)		0.50	0.20–1.10
IgM κ /IgM λ ratio		1.60	0.81–2.52

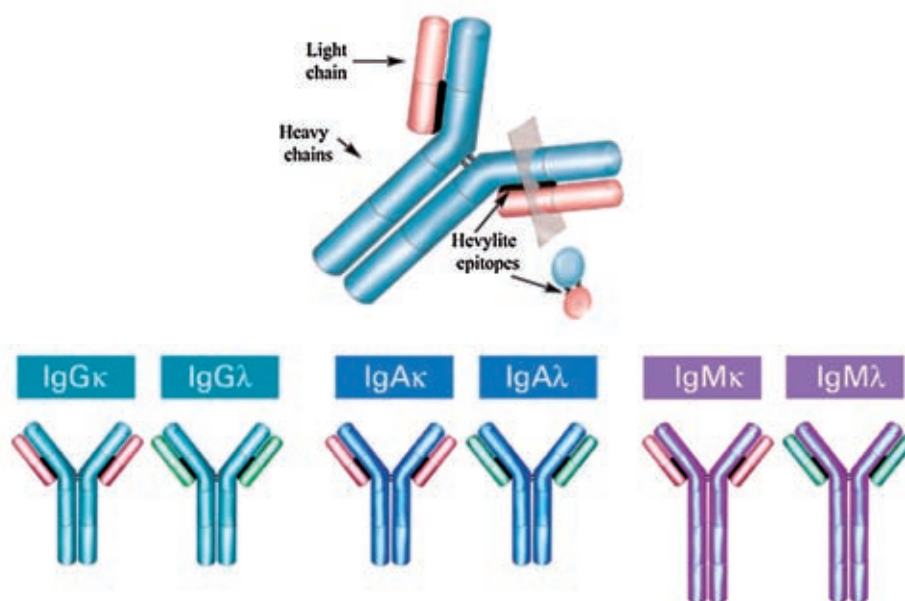


Fig. 1. Heavy chain/light chain pairs of IgG, IgA and IgM molecules showing the target epitopes for Hevylite immunoassays in black [5]

In the majority of MM patients the isotype specific monoclonal protein production level was greater than the upper limit of the normal range. In addition all of the patient tested had the appropriate abnormal HLC κ/λ ratios. IFE and HLC ratio results were concordant for 44 of the 51 IFE – monoclonal protein positive samples. IFE detected 2 “minor” IgG κ bands (1 oligoclonal in MM patient in complete response (CR) after autologous stem cell transplantation (ASCT), 1 in Hodgkin lymphoma patient in 10 year CR), and 1 IgA λ with polyclonal background (in a patient with MM and liver cirrhosis) that were not detected by HLC assay. In 6 cases with biclonal IgG κ + IgG λ gammopathy revealed in IFE, the HLC ratios were normal in 3, also in the three cases the HLC ratios revealed only one component IgG κ or IgG λ . In 4 cases with biclonal IgG + IgA gammopathy, including 3-IgG κ + IgA κ cases and one IgG κ + IgA λ , the HLC ratios were concordant with IFE. In one patient with Waldenström’s macroglobulinemia and 3 homogeneous bands IgG+IgA+ IgM in serum IFE analysis, the HLC assay correctly revealed only IgM κ /IgM λ abnormal ratio while IgG κ /IgG λ and IgA κ /IgA λ ratios were normal, as has been also proved in quantitative studies of total immunoglobulin concentration. Our results indicate that HLC and IFE are complementary methods. HLC assay provides numerical results [11].

Wechalekar et al. [12] tested serum samples in patients with light chain amyloidosis to assess HLC sensitivity compared with IFE. Among 46 light chain amyloidosis patients with no detectable serum or urinary bands and a normal serum FLC ratio the HLC ratio was abnormal in 9 cases (19%) identifying 2 IgA κ , 3 IgA λ and 4 IgG κ clones [5].

Recently, an assay for serum immunoglobulin FLC has become available for clinical use [7] and is currently applied to monitor patients with light chain amyloidosis, and MM [12–15]. The assay allows quantitation of kappa and lambda chains that are not bound to intact immunoglobulin molecules, and allows determination of clonality based on the kappa to lambda ratio. Katzmann et al. [15] defined the normal range using fresh and frozen sera from 127 healthy donors aged 21–62 years and frozen sera from 155 donors aged 51–90 years from the serum bank. The 95% reference interval for κ FLC was 3.3–19.4 mg/l, and that for λ FLC was 5.7–26.3 mg/l. Accepted the normal diagnostic range for FLC κ/λ ratio was 0.26–1.65. Patients with ratios greater than 1.65 have excess of κ FLC and are presumed to be producing clonal κ FLC. In turn, patients with ratios less than 0.26 contain excess of λ FLC and are presumed to be producing clonal λ FLC.

Comparison of HLC, FLC and IFE in Assessment of Remission in MM

In MM, concentrations of monoclonal immunoglobulin by quantification of protein bands in SPE is considered the best biomarker for monitoring and evaluation of response to treatment. However, as mentioned above SPE measurements can be inaccurate depending on the migration of the band. IFE is a more sensitive method but is not quantitative. Evaluation of HLC ratios is a quantitative addition to IFE [11]. Normalization of serum FLC ratio is considered a higher level of CR in MM and has been incorporated into the definition of stringent complete response (sCR) in the International Myeloma Working Group Uniform Response Criteria [16, 17].

In a study performed at the Institute of Hematology and Transfusion Medicine in Warsaw to compare IFE, HLC and FLC in assessment of remission we assayed sera from 44 MM patients who underwent ASCT. Of 44 patients in 26 (59%) after ASCT normalization of FLC ratio occurred. In 22 (84.6%) patients with normal FLC ratio also normalization of HLC ratio was noted but in 5 (19%) patients IFE was still positive. Concordance of three tests (IFE negative, HLC ratio normal, FLC normal) was found in 19 (73%) patients. In all IgA MM patients concordance of 3 tests (IFE negative, HLC ratio normal, FLC ratio normal) was found. Discrepancy between IFE and HLC and FLC results (IFE positive, HLC ratio normal, FLC ratio normal) was found in patients with IgG MM [18].

Avet-Loiseau et al. [19] compared the use of HLC assays to SPE and IFE for monitoring MM patients. Sequential archived sera from 156 patients enrolled onto the 2007-01 IFM trial were respectively analysed. Comparisons were made at CR. At presentation HLC ratio was abnormal in 43/43 IgA and 112/112 IgG MM patients. Post-ASCT 92% of IgA patients were negative by SPE with 63% of patients achieving a CR; 55% of patients had a normal IgA HLC ratio. For IgG patients post-ASCT, 45% were negative by SPE with 26% of patients achieving a CR. 39% of patients had a normal IgG HLC ratio. They concluded: “HLC ratios have a greater sensitivity than IFE for detection of minimal residual disease in IgA MM, but are slightly less sensitive in IgG MM” [19]. Decaux et al. [20] found that in 5 MM patients who achieved very good partial response (VGPR) serial serum samples analysis showed persistent normalization of HLC ratio after 6–9 months of treatment, whereas IFE was still positive.

Olivero et al. [21] analysed sensitivity of the HLC in minimal residual disease (MRD) assessment in comparison with IFE, serum FLC κ/λ ratio and the six-color flow cytometry (FC) from bone marrow aspirations. Both serum and bone marrow samples (1–3 per patient) from 27 patients enrolled in the IFM 2008 trial (15 IgG, 8 IgA and 4 light chain MM), were analysed at 3 times: respectively, pre-stem cell transplantation (n = 11, MRD 1 stage), post-ASCT (n = 25, MRD 2 stages) and post-consolidation (n = 23, MRD3). For 50 intact immunoglobulin MM samples, FC and IFE showed almost the same sensitivity: 60 and 58% respectively. HLC and FLC were abnormal in 46% and 30% respectively. In details FC MRD1 analysis showed better sensitivity than IFE and HLC: 100% vs. 80% and 70% respectively. They have not observed significant different sensitivities at MRD2 and MRD3 points between FC, IFE and HLC. FLC data showed the same low sensitivity at all points (30%). The HLC ratios were normal in 9 light chain MM samples while FC remained positive in 2.

Tovar et al. [22] studied the possible value of the serum HLC ratios in 42 MM patients in CR after stem – cell transplantation. They found that relative higher HLC ratio of the uninvolved immunoglobulin is predictor for a significantly longer progression free survival (PFS) and even overall survival (OS) in patients with MM in CR. They suggest that since HLC ratios provide a measure of tumour immunoglobulin production plus immunoparesis rather than a marker of MRD, this parameter is more likely a surrogate marker of robust immune recovery.

Preliminary results of our study and congress publications indicate that it is possible to monitor response to treatment using HLC ratio. HLC ratio may serve as a parameter for myeloma induced immunoparesis and serve as a new marker for validating remission depth and relapse probabilities [24–26] and as illustrates case reported by Willenbacher et al. [26] may serve as new diagnostic tool for rational treatment allocation, especially with respect to maintenance and consolidation strategies. However further studies are necessary to define more precisely the interest of this new assays among traditional methods.

Prognostic Value of HLC and FLC Assays in Monoclonal Gammopathies

Some congress publications [27–32, 34, 36] and our own study [33] provide data on prognostic value of HLC analysis in MM and MGUS.

Hari et al. [27] in order to assess the prognostic impact of HLC and FLC assays and to correlate them with SPE and IFE assessment, analysed 497 stored serum samples from patients enrolled in the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0102 clinical trial, sponsored by the NHLBI and NCI, of tandem autologous vs. tandem autologous –allogeneic hematopoietic cell transplantation. Samples were collected prior to the first ASCT. Of the 211 patients with baseline SPE response better than or equal to >VGPR, 188 also had an HLC remission (sensitivity = 89%). Similarly, all 56 patients in CR by SPE/IFE were in HLC remission. Compared with conventional CR assessment, sensitivity of HLC remission was 100%. FLC remission correlated with > VGPR disease state had sensitivity of 47%. It was found that there was a lower risk of treatment failure and superior PFS for patients who achieved an HLC remission. Normalization of FLC ratio among patients with > VGPR disease state did not impact PFS. These results indicate that abnormal HLC after induction therapy has a high negative predictive value for identifying patients not achieving CR by uniform response criteria and is also associated with shorter PFS after transplant.

Koulieris et al. [28] reported that the depth of response correlated to PFS and MM patients in sCR, CR and nCR had a longer PFS than the others. HLC ratios normalization only was a strong parameter of increased PFS (p = 0.016) after treatment at any line.

In 21 of 44 our MM patients who underwent ASCT, normalization of serum HLC and FLC ratio confirmed sCR. PFS in this group of patients was 24 months vs. 12 months (p < 0.07) in patients with CR, VGPR and PR [18].

Avet –Loiseau et al. [29,30] reported that HLC ratios at presentation were prognostic for PFS in the IFM 2005-01 myeloma trial. In that study, Kaplan Meier analysis indicated that more abnormal HLC ratios were associated with reduced PFS (> median for IgG κ and IgA κ patients, < median for IgG λ and IgA λ patients; P = 0.007). When using more extreme ratios (> 200 or < 0.01), the significance level was higher increased (P = 0.002). Cox regression analysis confirmed the association of the latter HLC ratios with reduced PFS (P < 0.001) and indicated that the association was independent of and more significant than that of β_2 M or albumin. The combined use of the extreme HLC ratios and β_2 M > 3.5 mg/L, in a risk stratification model, showed significant differences in PFS for patients with 0, 1, or 2 adverse risk factors (P = 0.000013). A more complex risk stratification model combining HLC ratios with the International Staging System also showed significant differences in PFS according to the number of risk factors (P = 0.0001).

The use of HLC ratios provides a measure of both tumor immunoglobulin production and immunoparesis. Probably, the combination of these two factors has a prognostic value. HLC measurements may be a useful addition to the current ISS assessments.

Also Ludwig et al. [31, 32] found that the ratio of monoclonal to polyclonal immunoglobulins assessed with the Hevlyte test predicts prognosis, is superior for monitoring the course of the disease and allows detection of monoclonal immunoglobulin in MM patients with normal or subnormal involved immunoglobulin isotype. In a study of 103 MM patients median OS of the entire group was 37.9 months. In multivariate analysis, β_2M , and HLC ratio were found as the only parameters correlating with survival. A three tiered risk stratification model utilizing $\beta_2M > 3.5$ mg/L, and HLC $>$ median value had a greater prognostic value than ISS ($= 0.001$ vs $p = 0.09$). Patients with 0 risk factors ($\beta_2M < 3.5$ mg/L, HLC ratio $<$ median) had a 50% survival time of 118 months, patients with 1 risk factor (either $\beta_2M > 3.5$ mg/L or HLC ratio $>$ median) had a 50% survival of 53 months and those with both risk factors ($\beta_2M > 3.5$ mg/L and HLC ratio $>$ median) had a 50% survival of 29 months ($p = 0.001$) [31]. In recent analysis when patients were stratified according to their presentation HLC ratios being moderately abnormal (0.022 – 45; $n = 51$) or highly abnormal (< 0.022 or > 45 ; $n=52$), survival was significantly shorter in those with highly abnormal ratios (median 32,1 months versus median not reached, $p = 0.016$). The survival rates at 5 years were 33.4% for the former and 58.9% for the latter group ($p = 0.01$). For patients with a highly abnormal FLC ratio (< 0.1 or > 30) a statistically non-significant tendency for shorter survival was noted (40.8 months versus median not reached, $p = 0.08$) compared to those with less abnormal FLC ratios (0.1–30). A risk stratification prognostic model with highly abnormal HLC and FLC ratios as risk factors at presentation was developed. OS was significantly different between patients with both, highly abnormal HLC and FLC ratios or only one, or none of these risk factors ($p = 0.01$). The median was not reached in patients with 0 or 1 risk factor and was 29.2 months in those with 2 risk factors. The respective five years' survival rates were 67.4%, 50.0% and 23.3% [32].

The aim of our recent study performed at the Institute of Hematology and Transfusion Medicine in Warsaw was to assess the prognostic impact of HLC and FLC assays in MM and IgM malignant lymphoma (ML) patients with real long term survival. Measurements of serum HLC and FLC concentrations were performed in 23 MM and 12 ML patients with survival exceeding 10 years and 43 (19 MM, 24 ML)

patients with survival not exceeding 5 years. HLC and FLC ratios at diagnosis were less abnormal in patients with survival exceeding 10 years than in patients with survival up to 5 years ($p = 0.03$). The differences in median values were manifold. However, in patients with survival over 10 years highly abnormal HLC ratio (< 0.022 or > 45) was found in 3 MM patients and 7 ML patients and highly abnormal FLC ratio (< 0.1 or > 30) was found in 5 MM patients and in 1 ML patient. In conclusion, serum HLC and FLC measurements at MM diagnosis provide prognostic information, despite that even in MM patients with survival exceeding 10 years in 15% of them at diagnosis serum HLC and FLC ratios may be highly abnormal [33].

A carried out in our previous study [11] evaluation of actual survival time of 21 IgM gammopathy patients diagnosed with MM or Waldenström's macroglobulinemia and mean follow-up time of six years showed no statistically significant difference in median survival depending on the results of the HLC ratios tested at diagnosis amounting 7 years in patients with HLC ratio values $<$ the median and 5.5 years for patients with values of HLC ratio $>$ the median ($p = 0.2738$). In the present study highly abnormal HLC ratio (< 0.022 or > 45) was found in 7 out of 12 (58%) IgM ML patients with survival exceeding 10 years [33].

However, Koulieris et al. [34] evaluated the prognostic value of IgM κ /IgM λ HLC ratios at diagnosis and the role of HLC ratio in disease monitoring in 31 patients with Waldenström's macroglobulinemia. Median IgM HLC ratio was significantly higher in patients requiring treatment at presentation than in those not requiring treatment. IgM HLC ratio correlated with bone marrow infiltration and time to first treatment. A simple risk stratification model utilizing IgM HLC ratio $>$ median, $\beta_2M > 5$ mg/L and abnormal LDH identified 3 prognostic groups with respect to survival ($p < 0.001$) in this series with a median follow – up of 59 months. Authors suggest that HLC IgM and IgM HLC ratio seem to separate patients with a more aggressive disease. Leleu et al. suggest that Hevlyte test might replace the current technique to measure IgM M-spike in the years to come [35].

IgG but not IgA HLC ratios have been shown to predict malignant transformation in MGUS patients [36].

HLC Assays in Diffuse Large B-cell Lymphoma

The aim of Jardin et al. [37] study was to determine the clinical and prognostic relevance of a quantitative assessment of HLC in the serum of

diffuse large B-cell lymphoma (DLBCL) patients. 407 adult patients included in the LNH03-B clinical trial program with confirmed DLBCL were analyzed. 244 patients were treated by R-CHOP/R-mini-CHOP and 163 by ACVBP/R-ACVBP regimens. FLC/HLC measurements were performed in sera collected at the time of inclusion before any chemotherapy. They found that both

elevated serum FLC and abnormal IgM kappa/lambda ratio are associated with unfavorable outcome in DLBCL treated by immunochemotherapy. In a multivariate analysis elevated FLC and age-adjusted international prognostic index (aa IPI), an abnormal IgM κ/λ ratio remained predictive of a shorter OS. They suggest that FLC and HLC can be used as simple circulating biomarkers.

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Conflict of interest: None declared

Received: 22.08.2012
Revised: 24.11.2012
Accepted: 20.02.2014