Original Article

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Comparison of Hevylite™ IgA and IgG assay with conventional techniques for the diagnosis and follow-up of plasma cell dyscrasia

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Abstract

Background: Heavy/light chain assay allows the characterization and quantification of immunoglobulin light chains bound to heavy chains for each Ig'k and Ig'λ immunoglobulin class, discriminating between the involved/uninvolved isotypes in plasma cell dyscrasia. The Ig'k/Ig'λ ratio (heavy/light chain ratio) enables to monitor the trend of monoclonal component during therapy and disease evolution.

Objective: In this study, we evaluate the impact of the heavy/light chain assay in monitoring multiple myeloma patients in comparison with conventional techniques.

Methods: Serum samples of 28 patients with IgG or IgA monoclonal component were collected for a mean of 109 days and analyzed. The heavy/light chain assay was compared with classical immunoglobulin quantification (Ig'Tot), serum immunofixation electrophoresis, serum protein electrophoresis, and serum-free light chains quantification. Serum samples from 30 healthy patients were used as control (polyclonal).

Results: Heavy/light chain ratio and serum immunofixation electrophoresis were comparable in 86% of the cases, and free light chain ratio and heavy/light chain ratio in 71.8%. Heavy/light chain assay and Ig'Tot measurements showed a concentration-dependent agreement in monoclonal patients. The heavy/light chain assay was able to quantify the monoclonal component migrating in SPE β region: this occurred in 10% of our IgG and 50% of our IgA patients.

Conclusions: The concordance scores indicate that heavy/light chain and Ig'Tot assays show differences at high monoclonal component values. The heavy/light chain ratio, serum immunofixation electrophoresis, and free light chain ratio showed partial concordance. Our study confirmed that, in the context of heavy/light chain assay, heavy/light chain Ig'k and Ig'λ absolute values and heavy/light chain ratio are both important tools to monitor the presence of monoclonal component that are difficult to be identified in SPE.

Keywords

Heavy/light chain assay, monoclonal component quantification, plasma cell dyscrasia, immunoglobulin

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Introduction

The monoclonal component (MC) quantification is typically performed in plasma cell dyscrasia with serum protein electrophoresis (SPE) followed by scanning densitometry and nephelometry (Ig'Tot).[1] Serum immunofixation electrophoresis (S-IFE) and urine

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immunofixation electrophoresis (U-IFE) are used to confirm the diagnosis and characterize the MC. These approaches are simple and cheap,[2] but present many disadvantages: peaks densitometric measurement in SPE is semi-quantitative and monoclonal spike (M-spike) quantification can be inaccurate, depending on the migration of the band.[2,3] Nephelometric MC quantification does not discriminate between MC and normal polyclonal immunoglobulins, which become particularly relevant at low MC concentrations.[4] IFE is a non-quantitative assay and requires expert interpretation.[5] Despite their limitations many physicians use Ig'Tot quantification to monitor monoclonal proteins, and some use both the M-spike and Ig'Tot quantification to monitor treatment.[6] In 2009 the Binding Site developed a new immunoassay (the Hevylite^{TM} Assay, The Binding Site Ltd., Birmingham, United Kingdom) to accurately quantify the involved and uninvolved immunoglobulin isotype.[3] This test uses sheep polyclonal antibodies directed against the junctional epitopes (constant region) between the heavy chain and the light chain (heavy/light chain (HLC)): thus HLC antibodies recognize the different immunoglobulin class, i.e. IgGκ, IgGλ, IgAκ, IgAλ, IgMk, and IgMλ. These molecules are then measured in pairs, e.g. IgGκ/IgGλ, to calculate the ratios of involved and uninvolved immunoglobulins like the serum free light chain (FLC) κ/λ ratios (3,7). Preliminary studies employing the HLC assay have shown that HLC ratios (HLCr) may be of use in screening, monitoring, and risk stratifying of patients with multiple myeloma (MM), Amyloid Light-chain (AL) amyloidosis, Waldenström's macroglobulinemia, and MGUS (monoclonal gammopathy of undetermined significance). In addition, they may provide the only sensitive marker of disease progression in some patients.[4]

Here, we compared both HLCr and HLC absolute values of monoclonal immunoglobulins with data obtained performing S-IFE, free light chain ratio (FLCr), and SPE densitometric quantification. In addition, we evaluated the correlation and agreement of Ig'Tot in comparison with Ig'k + Ig'λ HLC quantification in both healthy donors (polyclonal) and patients with plasma cell dyscrasia (monoclonal).

Material and methods

Clinical study and patients' samples

During a one-year period, 28 patients (monoclonal) presented to the Haematology ward and Haematology day hospital of Azienda Ospedaliera Spedali Civili of Brescia (AOSCB) for laboratory screening or follow-up of monoclonal gammopathy were included in the study. Samples included were either from patients with suspect onset of MM (newly diagnosed), plasma cell dyscrasia difficult to monitor, and MM patients after autologous bone marrow transplantation. Clinical diagnoses of patients were determined by local physicians. For each patient, serum was collected sequentially after each control visit in the Laboratory of Biochemical Chemistry after processing for routine assays, according to International Myeloma Working group[7]: SPE, S-IFE, nephelometric measurement of immunoglobulin heavy chains (Ig'Tot), and FLC quantification. On the day of the withdrawal, an aliquot of the serum sample was frozen at –80°C for further analysis. Thirty blood donors were used as healthy controls (polyclonal). The institutional review board of AOSCB approved the study in adherence with the Declaration of Helsinki. All traceable identifiers were removed before analysis to protect patient confidentiality, and all samples were analyzed anonymously.

Laboratory analysis

HLC assay

Immunoglobulin HLC pairs (IgGk/IgGλ, IgAk/IgAλ) were measured using latex-enhanced immunoassay (Hevylite^{TM}, The Binding Site, Birmingham, UK) on the turbidimetric platform Binding Site SPA_{PLUS} analyzer. Quantifications of these parameters were used to determine the Ig'k/ig'λ ratio (HLCr), which was compared with reference ranges.[8,9] The diagnostic ranges had been previously established by the manufacturer: IgGk 3.84–12.07 g/L; IgGλ 1.91–6.74 g/L; Gk/Gλ 1.12–3.21; IgAk 0.57–2.08 g/L; IgAλ 0.44–2.04 g/L; Ak/Aλ 0.78–1.94. HLCr outside the reference range was considered to be indicative of clonal proliferation.[3]

SPE

Agarose gel electrophoresis and staining were performed with the Microgel system on a G26 automated instrument (Interlab, Rome, Italy). Gels were visually interpreted for the presence of monoclonal gammopathies and peaks densitometric scanning was performed using the "Labware" software (Interlab).

S-IFE

S-IFE was performed with the Microgel system on a G26 automated instrument (Interlab, Rome, Italy). Heavy chains were stained using anti-IgG, -IgA, -IgM antibodies. Light chain k or λ antibodies were used. Identification of the paraprotein isotype and light chain was performed by visual interpretation.

Quantification of immunoglobulins by nephelometry (Ig'Tot)

IgA, IgG, IgM immunoglobulins (Ig'Tot) were measured by a nephelometric immunoassay (Dimension Vista System Flex® Reagent Cartridge) on a Dimension Vista 500 (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). The diagnostic ranges were established by the manufacturer: IgG 6.9–15 g/L, IgA 0.85–4.1 g/L, IgM 0.4–2.4 g/L.

FLC assay

Serum FLC concentrations were measured using latex-enhanced immunoassay (Freelite™, The Binding Site, Birmingham, UK) on SPA_{PLUS} analyzer. K FLC (reference range: 3.3–19.4 mg/L), λ FLC (reference range: 5.7–26.3 mg/L), and k/λ FLCr (reference range: 0.26–1.65) ranges were previously established by the manufacturer. An abnormal FLC concentration was defined as an increased k FLC and/or λ FLC concentration and/or abnormal ratio of k FLC to λ FLC.[1]

Statistics

Patients (monoclonal) were divided into two groups (IgA and IgG) according to MC isotype diagnosed with S-IFE. Polyclonal IgA and IgG (polyclonal) were quantified on healthy donor samples and analyzed for each isotype separately. For correlation analysis, Pearson's correlation coefficient and linear regression equation were calculated. The evaluation was performed between Ig'Tot concentration of the involved isotype and the sum of the HLC quantifications of the involved and uninvolved isotype (Ig'k + Ig'λ) in both monoclonal and polyclonal groups. For agreement analysis between the two methods, Bland–Altman (BA) test and linear regression of difference and average were determined. All statistics were performed using GraphPad software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Evaluation of concordance between S-IFE, FLC, and HLC test

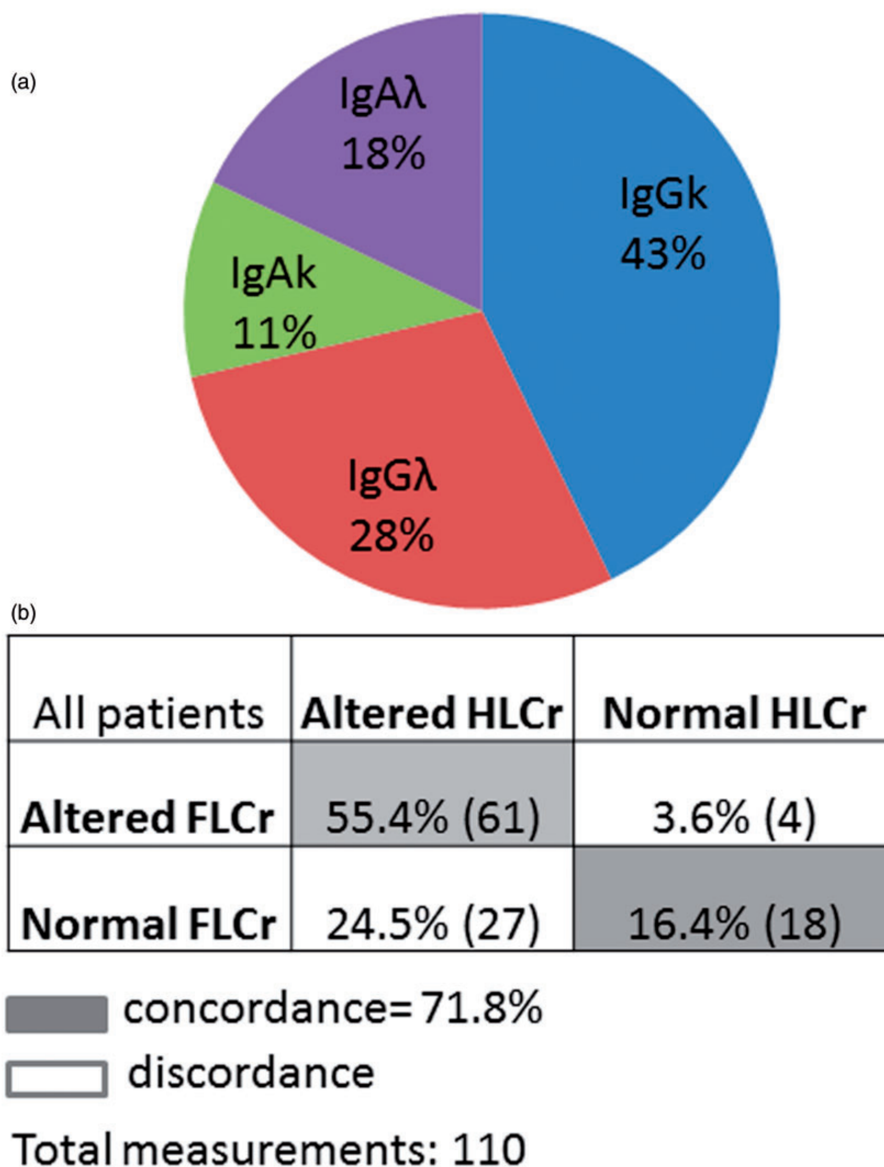
Sequential analysis on serum samples from 28 patients admitted to the Haematology ward and sent to the laboratory of Biochemical chemistry of AOSCB for screening and follow-up of monoclonal gammopathy was performed. Patients were followed for a mean of 109 days (range 26–204 days) between February 2013 and February 2014 and serum samples were collected during each control visit. All patients were selected for diagnosis (10/28 suspect onset of MM, 13/28 difficult to monitor plasma cell dyscrasia, 5/28 MM patients after autologous stem cell transplantation) and paraprotein production. According to S-IFE, the majority of patients included in the study presented a IgGk MC (Figure 1(a)). SPE, Ig'Tot, FLC, and the HLC assay were performed for all samples.

Qualitative S-IFE results were compared with HLCr. Concordance, among the two tests, was set as described: positive S-IFE and altered HLCr for the same isotype or negative S-IFE and normal HLCr. HLCr showed a concordance with S-IFE of 86% with slight differences among each MC: 85% IgGk, 82% IgGλ, 92% IgAk, 87% IgAλ.

FLCr was also compared with HLCr and 71.8% of samples showed concordance between the two tests (Figure 1(b)).

Ig'Tot and HLC assay correlation and agreement

We compared the Ig'Tot concentration with the summed concentration of the Ig'k + Ig'λ HLC assay from 30 healthy donors serum samples (polyclonal) both for IgG and IgA isotype. We assumed that the Ig'Tot concentration of the involved isotype and the sum of the HLC quantifications of the involved and uninvolved isotype (Ig'k + Ig'λ concentration) could be the same. As shown in Figure 2(a) and (b) in polyclonal patients, IgATot and IgAk + IgAλ HLC showed a good correlation (Pearson correlation coefficient r: 0.93, 95% confidence interval (CI) 0.86–0.96) and agreement (BA bias: 7.8 [absolute value], 95% limits of agreement [LA]: from –49.93 to 65.86, difference versus average linear regression $X = -0.071$ [slope of the line], not significantly different from 0),[10] in IgG polyclonal (Figure 2(c) and (d)) the two tests show a quite good correlation (r: 0.86, 95% CI 0.73–0.93) and agreement (BA bias –190.8, BA 95% LA: from −495.7 to 114.2 difference versus average linear regression $X = -0.054$, not significantly different from 0) between the two methods. We applied the same statistical analyses at IgA or IgG MC and we noticed that in IgA monoclonal patients the two methods still correlate (r: 0.96, 95% CI 0.92–0.99) while this correlation is weaker in IgG (r: 0.79, 95% CI 0.67–0.87) (Figure 3(a) and (b)). BA analysis for both IgA and IgG shows that there is a concentration-dependent discordance, especially in IgG MC patients (Figure 3(c) and (d)): at high MC concentration agreement among the two test is completely lost. The LA of BA tests are wider in monoclonal patients than in polyclonal even after log transformation of the original data (data not shown).[11] At high MC concentrations there is also an opposite trend in IgA and IgG patients. IgATot values are lower than IgAk + IgAλ



All patients	Altered HLCr	Normal HLCr
Altered FLCr	55.4% (61)	3.6% (4)
Normal FLCr	24.5% (27)	16.4% (18)

Figure 1. FLC ratio versus HLC ratio concordance. (a) Monoclonal component distribution, according to S-IFE, of patients included in the study. (b) Concordance analysis for the FLC Ratio (FLCr) and HLC Ratio (HLCr). Concordance was set as described: both altered or normal FLCr and HLCr (gray areas). Discordant results were set if one ratio was altered and the other one was normal (white area). Percentage and number of measurements (in brackets) are also shown.

HLC quantification (difference versus average linear regression $x = -0.36$, significantly different from 0: p value <0.001; BA bias –122) while, in IgG patients, IgGTot values are higher than IgGk + IgGλ HLC quantifications (difference versus average linear regression $x = 0.2$, significantly different from 0: p value = 0.018, BA bias 459.2).

Comparison between SPE and HLC

In this study we evaluated the MC quantification of the γ peak with the HLC measurement of the involved isotype and HLCr for every patient (supplementary data, Figures 1 to 4). In 76% of patients, HLC quantification of the involved isotype shows a similar trend with MC quantification during the monitoring time as shown in Figure 4(a), Patient 3.

In this study we confirmed that HLC quantification is an important tool and HLCr is a very helpful parameter to monitor MC, which cannot be quantified by densitogram scan. Indeed this problem occurred in 11/28 patients: for six patients (patient 2, 4, 5, 13, 34, 39, supplementary data, Figures 1 to 3) MC quantification was not possible at one or more time points while in five patients (patient 1, 11, 15, 28, 31, supplementary data, Figure 4) the MC migrates in the β region of SPE

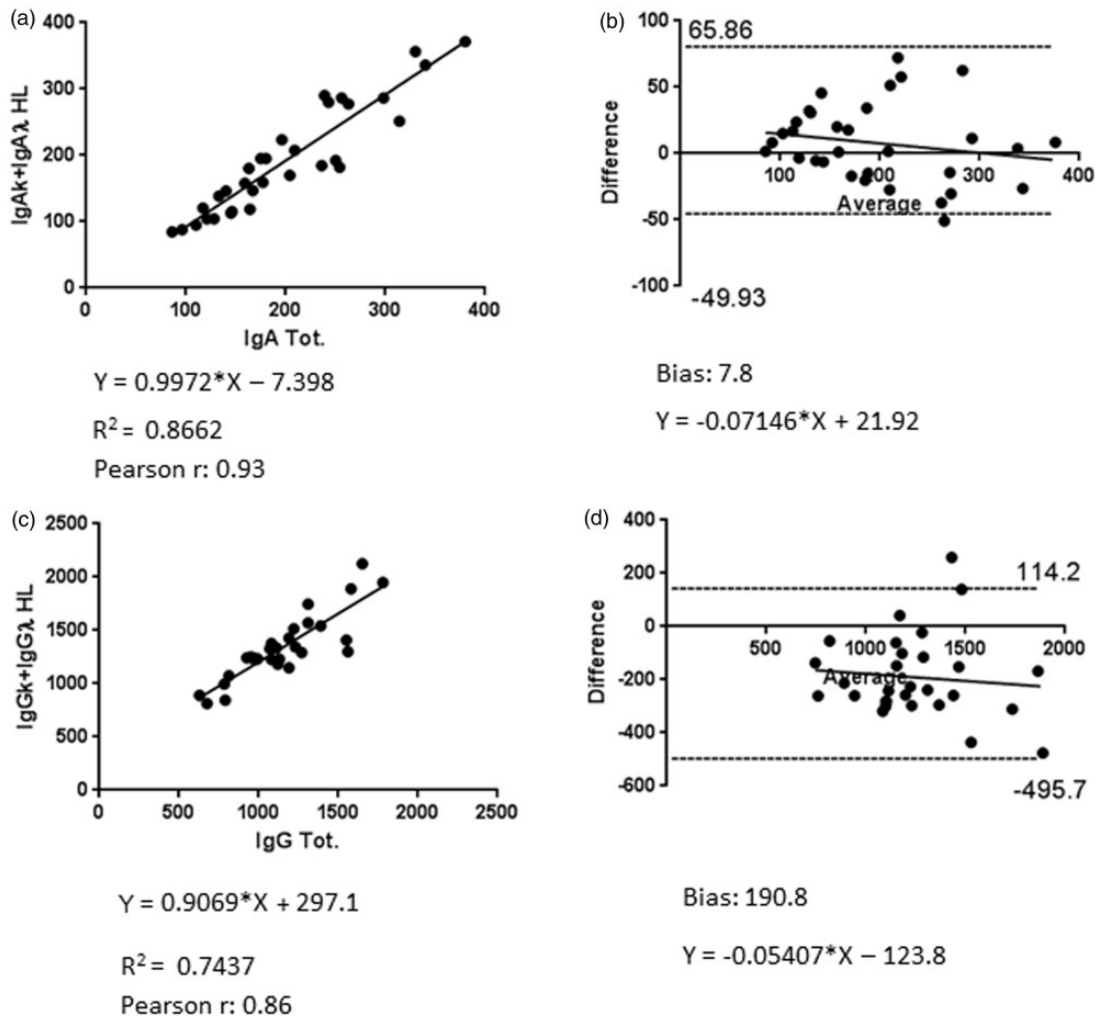


Figure 2. Correlation and agreement of polyclonal IgG and IgA samples. Plots represent linear regression analysis of Ig'Tot concentration with summed concentration of the Ig'k + Ig'λ HLC assay from polyclonal serum samples both for IgA (a) and IgG (c) isotype. Linear regression equation, R square, and Pearson correlation factor r were also calculated and represented. Bland–Altman test plots of Ig'Tot concentration with summed concentration of the Ig'k + Ig'λ HLC for polyclonal IgA (b) and polyclonal IgG (d). Linear regression equation and the bias of the average of the differences were calculated for every graph.

densitogram. Among the last group, 80% were IgA patients and 20% IgG patients, confirming that monoclonal IgA immunoglobulins frequently migrate in β region.[4,12,13] In contrast, HLC quantification was obtained for all patients and HLCr calculated. Figure 4(b) shows the follow-up of patient 11 after autologous bone marrow transplantation. In 2011 the patient was admitted to the hospital and diagnosed as a IIIA IgAK MM. After four cycles of chemotherapy and autologous bone marrow transplantation, MM completely remitted and patient underwent the follow-up. After two years the patient presented lower back pain (T0). S-IFE showed the presence of an IgAk MC not quantifiable due to its migration in β region at SPE densitogram. IgATot was out of the reference ranges (27.1 g/L), but k FLC value was normal (k 14.5 mg/L). The λ FLC concentration was under the test's detection limits (λ < 0.01 mg/L) so the k/λ FLC ratio was not computable (NQ). The IgAk/IgAλ ratio was strongly altered (IgAk/IgAλ 184.88, IgAk 35.13 g/L, IgAλ 0.19 g/L) while the IgGk/IgGλ ratio was normal (IgGk/IgGλ 2.32, IgGk 2.94, IgGλ 1.26) confirming the presence of an IgAk MC. The patient started a new cycle of treatment and after 14 days (T14) the clinical picture was unchanged with elevated IgATot (27.52 g/L), k/λ FLC ratio NQ, altered IgAk/IgAλ ratio, and MC migrating in β in SPE. As T34 IgATot decreased (5.11 g/L), k/λ FLC ratio normalized and only IgAk/IgAλ ratio remained altered (IgAk/IgAλ 48.89, IgAk 9.04 g/L, IgAλ 0.185 g/L). After the third (T55) and fourth cycle (T96) of therapy, according to IgATot and k/λ FLC ratio, the clone seemed to

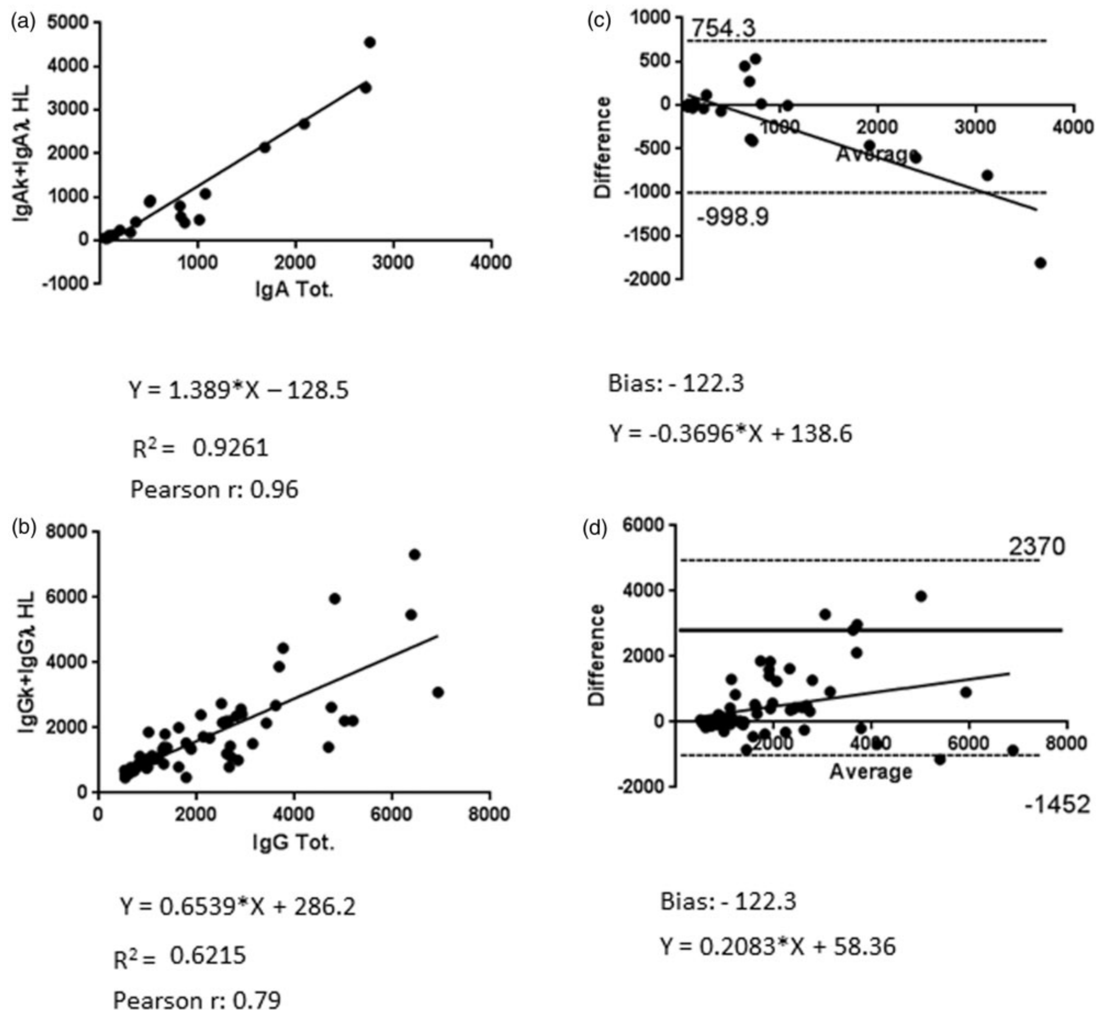
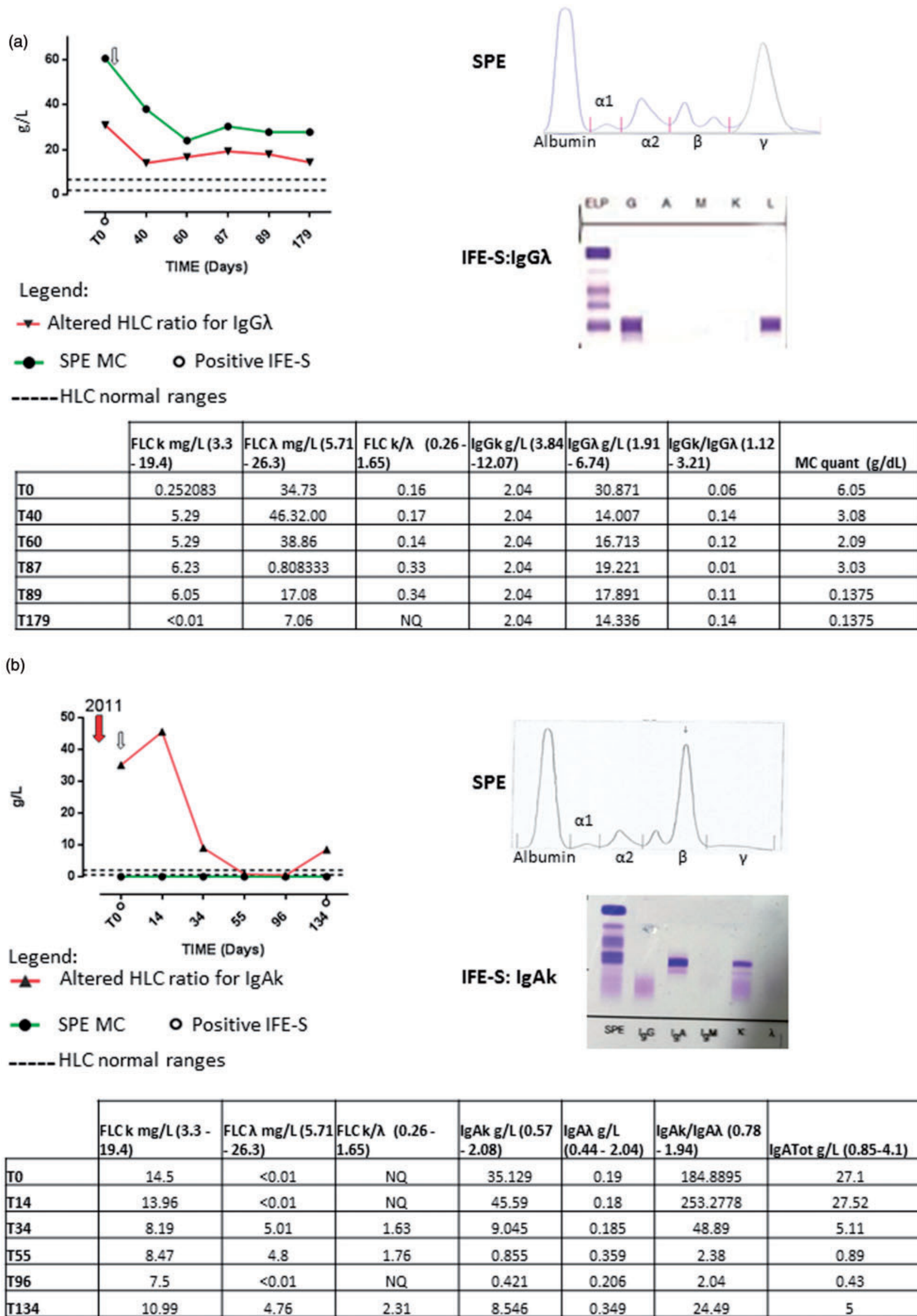


Figure 3. Correlation and agreement of monoclonal IgG and IgA samples. Plots represent linear regression analysis of Ig'Tot concentration with summed concentration of the Ig'k + Ig'λ HLC assay from monoclonal serum samples both for IgA (a) and IgG (c) isotype. Linear regression equation, R square, and Pearson correlation factor r were also calculated and represented. Bland–Altman test plots of Ig'Tot concentration with summed concentration of the Ig'k + Ig'λ HLC for monoclonal IgA (b) and polyclonal IgG (d). Linear regression equation and the bias of the average of the differences were calculated for every graph.

respond to treatments and only the IgAk/IgAλ ratio highlighted the suspect of therapy resistance (IgAk/IgAλ ratio 2.38 (T55), 2.04 (T96)). At T134 this suspect was confirmed because all monitored parameters increased (IgATot 5 g/L, k/λ FLC ratio 2.31, IgAk/IgAλ 24.49) and S-IFE became positive again, indicating an evolving relapse.

Discussion

HLC assay quantifies immunoglobulins using polyclonal antibodies targeted at unique junctional epitopes between heavy-chain and light-chain constant region of intact Ig. This allows to distinguish between the Ig'k and Ig'λ and to calculate the ratio of the two isotypes, identifying the involved and uninvolved isotype in a monoclonal gammopathy. Since 2009 this test has been proposed to support routine lab assays (SPE, S-IFE, FLC assay, nephelometric measurement of immunoglobulin heavy chains of serum), and in some cases to overcome their limits in the management of patients with monoclonal gammopathy.[14] In our study we compared the qualitative assay S-IFE with the quantitative data obtained from HLCr: in most cases results are comparable, especially for the IgAk MC. Among discordant patients, in 73% of cases HLCr were within normal limits, but S-IFEs were positive even if the MC trend decreased (with HLC measurements and SPE quantification). Our results confirm the utility of the HLC assay to identify and quantify the MC, even when SPE and S-IFE are difficult to be evaluated.



	FLC k mg/L (3.3 - 19.4)	FLC λ mg/L (5.71 - 26.3)	FLC k/λ (0.26 - 1.65)	IgGk g/L (3.84 -12.07)	IgGλ g/L (1.91 - 6.74)	IgGk/IgGλ (1.12 - 3.21)	MC quant (g/dL)
T0	0.252083	34.73	0.16	2.04	30.871	0.06	6.05
T40	5.29	46.32.00	0.17	2.04	14.007	0.14	3.08
T60	5.29	38.86	0.14	2.04	16.713	0.12	2.09
T87	6.23	0.808333	0.33	2.04	19.221	0.01	3.03
T89	6.05	17.08	0.34	2.04	17.891	0.11	0.1375
T179	<0.01	7.06	NQ	2.04	14.336	0.14	0.1375

	FLC k mg/L (3.3 - 19.4)	FLC λ mg/L (5.71 - 26.3)	FLC k/λ (0.26 - 1.65)	IgAk g/L (0.57 - 2.08)	IgAλ g/L (0.44 - 2.04)	IgAk/IgAλ (0.78 - 1.94)	IgATot g/L (0.85-4.1)
T0	14.5	<0.01	NQ	35.129	0.19	184.8895	27.1
T14	13.96	<0.01	NQ	45.59	0.18	253.2778	27.52
T34	8.19	5.01	1.63	9.045	0.185	48.89	5.11
T55	8.47	4.8	1.76	0.855	0.359	2.38	0.89
T96	7.5	<0.01	NQ	0.421	0.206	2.04	0.43
T134	10.99	4.76	2.31	8.546	0.349	24.49	5

Figure 4. Monoclonal samples characterization. (a) The graphs show trend of HLC quantification of the involved isotype, HLC ratio (red line, triangles), and band densitometric quantification of SPE (green line) in sequential serum measurements for patient 3. Table shows patient characteristics for all the data points analyzed. Patient 3 SPE densitogram and serum immunofixation electrophoresis (S-IFE) highlighting an IgGλ MC migrating in γ region of SPE. (b) The graphs show trend of HLC quantification of the involved isotype, HLC ratio (red line, triangles), and band densitometric quantification of SPE (green line) in sequential serum measurements for patient 11. Table shows patients characteristics for all the data points analyzed. Patient 11 SPE densitogram and S-IFE highlighting an IgAK MC migrating in β region of SPE.

According to the International Myeloma Working Group (IMWG) guidelines,[7] in addition to S-IFE and SPE, serum FLC should be measured at diagnosis for all patients with MGUS, smoldering or active MM, solitary plasmacytoma, and AL amyloidosis. FLC assay is routinely used to monitor disease activity and relapse after therapy or autologous transplant even if 85% of MM secrete a whole paraprotein, 13% secrete only FLC, and 2% are oligo/non-secretory MM.[15] All our patients were selected for whole immunoglobulin production and FLCr failed to detect MC alteration in 25% of the cases. In this scenario, HLC assay can be a valuable support to FLC assay to monitor MM evolution, especially in those patients with an intact immunoglobulin MC.[16]

Nephelometric measurement of immunoglobulin heavy chains of serum (Ig'Tot) is in the repertoire of tests to document and measure the immunoglobulins.[1] In normal practice nephelometric measurement of immunoglobulin heavy chains of serum (IgG, IgA, IgM) is used to monitor the MC behavior even if there are some limits: when the paraprotein production increases the involved isotype should increase, while usually there is a consequential immunoglobulin suppression of the uninvolved isotype.[16] Despite the quantitative results, this test cannot distinguish between the involved/uninvolved isotype as HLC assay and this can lead to aspecific antibody reactivity that alter the MC quantification. When the immunoglobulins are in the reference ranges, as seen in polyclonal patients, the two tests (Ig'Tot and Ig'k + Ig'λ HLC) show a certain correlation (IgA polyclonal linear regression equation $y = 0.9972X - 7.398$; IgG polyclonal $y = 0.9069 + 297.1$), but they are not perfectly in agreement. Our results appeared less satisfactory than the data obtained by Bradwell et al.,[14] however this could be due to the limited number of our dataset. Instead, in the presence of a high MC concentration (monoclonal patients) the agreement is completely lost and quantifications obtained with the two assays are more discordant. Our results highlight that, similar to FLC measurements, for patients producing paraproteins the antiserum could display different antigen–antibody interactions generating macromolecular immunocomplexes giving rise to different light absorption/diffraction properties affecting the analytical measurements.[17] These data confirm the differences between the two methods and the lack of accuracy monitoring MC only with the Ig'Tot measurement.[14]

According to the IMWG, one of the gold standard tests for MM screening is SPE.[7] This technique allows a semi-quantitative measurement of the MC with a densitometric scan of γ peak in the electrophoretic pattern.

In our study we confirmed that HLC assay is a useful tool to monitor MC migrating in β region of the SPE, where it is obscured by proteins such as transferrin, β lipoprotein, and C3, which preclude quantification.[4] We monitored 28 patients for MM screening and follow-up for a median of 95 days and HLC assay was always able to quantify the MC component, even if not quantifiable in SPE. This problem is quite common since SPE has a limit of quantification of 0.5–2 g/L depending on the migration characteristics of MC-Ig bands[14] and can be due to patient immunosuppression. Furthermore, we confirmed that HLC quantification could be a sensitive assay to detect early relapse especially in patients presenting not quantifiable MC (patients 11, Figure 4(b) and patient 15, supplementary Figure 5).[4] In the small group of patients with MC migrating in β region, we compared HLCr with FLCr. In 2/5 patients (patient 11 and 15, Figure 4 and supplementary Figure 5) the ratios were different in one or more data points. In 3/5 patients HLCr and FLCr were comparable (patient 1, 28, 31, supplementary Figure 4). These differences highlight the importance of considering every single patient as a unique case. In conclusion, the findings presented indicate that HLC assay can support traditional methods to characterize MC at disease onset and monitor monoclonal gammopathies along therapy and follow-up processes. Similar to FLC assay, HLC can be a robust alternative to invasive procedure, such as osteo-medullary biopsy, to monitor MM progression.

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Declaration of conflicting interests

None declared.

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Ethical approval

The institutional review board of Azienda Ospedaliera Spedali Civili of Brescia approved this study (REC number: SFLC01).

Guarantor

DR.

Contributorship

DR and LP conceived and designed the experiments. LP and FM performed the experiments. LP, GM, GDN, FM, were involved in sample collection. DR, LP, GDN, FM, GM, LC, and AR analyzed the data.
LP and GDN wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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