Combined use of free light chain and heavy/light chain ratios allow diagnosis and monitoring of patients with monoclonal gammopathies: Experience of a single institute, with three exemplar case reports

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Abstract. Monoclonal gammopathies are characterized by serum monoclonal component (MC) plus an intact immunoglobulin and a free light chain (FLC), or a combination of both. The measurement of FLC with Freelite® is the standard practice recommended by International Myeloma Working Group guidelines. Recently, Hevylite® heavy/light chains (HLC) assays were introduced to specifically target junctional epitopes between the heavy and light chains of intact immunoglobulins, allowing the independent quantification of the involved (MC) and uninvolved (polyclonal immunoglobulin background) HLC isotype. Between January 2012 and March 2014, 90 patients were examined: 49 multiple myeloma (MM), 6 smoldering MM (SMM) and 35 monoclonal gammopathy of undetermined significance (MGUS). Of these 90 patients, 300 samples were collected at different times. The diagnostic and monitoring contribution of Hevylite A and G assays was assessed in all 90 patients examined. Additionally, 3 representative cases were selected. The Hevylite absolute values and ratio demonstrated high sensitivity and specificity with respect to serum protein electrophoresis and serum immunofixation. The combined use of Hevylite A and G with Freelite was particularly useful in dubious cases with more than one MC or with co-migrating components, as well as in the course of monitoring to assess the independent change of FLC and HLC, possibly reflecting the presence of clonal heterogeneity in the cohort. From this study, it can be concluded that FLC and HLC are independent, useful markers to monitor the MC and to assess with greater specificity and sensitivity the effect of therapy, thereby providing clinical support. Further studies are required to assess the prognostic potential of Hevylite in MGUS and SMM.

Introduction

Monoclonal gammopathies are characterized by the presence of a serum monoclonal component (MC) produced by clonal plasma cells. The MC can be an intact immunoglobulin alone or in combination with a free light chain (FLC), either κ or λ (1,2).

Precise and accurate MC quantification is one of the key parameters required to discriminate between monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM) and smoldering MM (SMM) (1,2). The monitoring of MC levels provides indications of the response to therapy and, with the advent of immunomodulatory drugs, MC quantification has also been identified as a quick method to assess the depth of response, providing prominent prognostic information (3,4).

Traditionally, quantification and characterization of the MC relies on serum protein electrophoresis (sPEP) and serum immunofixation (sIFE), respectively. A lack of standardization, limited sensitivity and subjectivity, to a certain extent, are the main limitations of these techniques (5,6).

The introduction of Freelite® (The Binding Site Group Ltd., Birmingham, UK) for the automatic nephelometric/turbidimetric measurement of serum (s)FLC in 2001 (7) radically changed the diagnostic procedure in clinical practice, and FLC is now an established tool for hematological diagnostics (8-10).

More recently, a new assay was introduced: Hevylite® (The Binding Site Group Ltd.) for heavy/light chain (HLC) quantification. Hevylite targets the junctional epitopes, allowing the measurement of couples of immunoglobulin (Ig)'κ/Ig'λ (IgGκ,

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IgGλ, IgAκ, IgAλ, IgMκ and IgMλ), thus, providing novel insight into the quantification and characterization of MC (11).

FLC and HLC assays have routinely been performed at San Gennaro Hospital (Naples 1 Local Health Center, Naples, Italy) for the follow-up of MM patients. The current study demonstrates the clinical utility of these assays, providing 3 examples of their combined use and displaying the advantages with respect to traditional tools.

Patients and methods

Patient selection and frequency of monitoring with FLC and HLC. Between January 2012 and March 2014, 300 samples were collected from 90 patients (49 MM, 6 SMM and 35 MGUS) treated in the Complex Operative Unit (U.O.C.) of Hematology of San Gennaro Hospital (Naples 1 Local Health Center). The mean age was 69.14 years, and the 49 MM patients were split into 21 IgGκ MM, 16 IgGλ MM, 6 IgAκ MM, 3 IgAλ MM and 3 light-chain MM (LCMM).

FLC and HLC were repeated every 3 months in MM patients under treatment, to assess their response to therapy. SMM patients were monitored every 6 months, whereas MGUS patients were monitored every 6-12 months, depending on their risk assessment. MGUS and SMM were re-assessed by the clinicians every 4 months.

Every month, a clinical evaluation was performed on the MM patients under chemotherapy; during follow-up they were monitored every 3 months.

This study was approved by the Ethical Committee of San Gennaro Hospital and written informed consent was obtained from all patients.

Treatment options. The MM patients received different treatment protocols. In total, 7 patients were eligible to receive autologous stem cell transplantation (ASCT); 3 received a bortezomib, thalidomide and dexamethasone regimen and the remaining 4 received a bortezomib, doxorubicin and dexamethasone (PAD) regimen. Among the remaining 42 patients, 16 received a melphalan, prednisone and bortezomib (MPV) regimen and 3 an MP regimen, while the patients who experienced relapse received lenalidomide and dexamethasone (Rd; 19 patients) or bortezomib and dexamethasone (VD; 4 patients) therapy. In detail, the therapeutic regimens were as follows: i) 1.3 mg/m² bortezomib (Velcade®) on days 1, 4, 8 and 11, 50 mg/day thalidomide on days 1-14, 100 mg/day thalidomide on days 15-28, and 40 mg dexamethasone on days 1-4 and 8-11 (4 cycles every 28 days); ii) PAD regimen: 1.3 mg/m² bortezomib on days 1, 4, 8 and 11, 30 mg/m² liposomal doxorubicin (Caelix®) on day 1, and 20 mg dexamethasone on days 1-2, 4-5, 8-9 and 11-12 (6 cycles every 28 days); iii) MPV regimen: 1.3 mg/m² bortezomib on days 1, 8, 15 and 22, 9 mg/m² melphalan on days 1-4, and 60 mg/m² prednisone on days 1-4 (9 cycles every 28 days); iv) MP regimen: 9 mg/m² melphalan on days 1-4 and 60 mg/m² prednisone on days 1-4 (6 cycles every 28 days); v) Rd regimen: 25 mg lenalidomide on days 1-21, and 20 mg dexamethasone on days 1-4, 9-12 and 17-20 (every 28 days until to progression); and vi) VD regimen: 1.3 mg/m² bortezomib on days 1, 4, 8 and 11, and 40 mg dexamethasone on days 1, 4, 8 and 11 (6 cycles every 28 days).
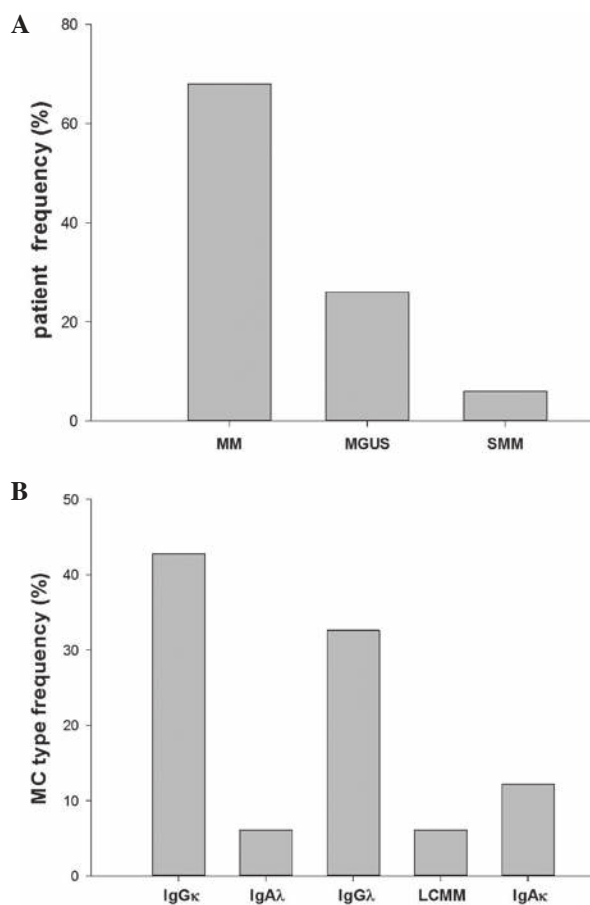
Figure 1. MGUS, MM and SMM patient frequency and MC type distribution in the study population. (A) Percentage of patients with MGUS, MM and SMM, accounting for 6, 26 and 68, of the population, respectively. (B) MC type frequency in the population, consisting of 42.8% IgGκ, 6.1% IgAλ, 32.6% IgGλ, 6.1% LCMM and 12.2% IgAκ MM patients. MM, multiple myeloma; SMM, smoldering MM; MGUS, monoclonal gammopathy of undetermined significance; Ig, immunoglobulin; MC, monoclonal component.

Laboratory tests. Serum samples were analyzed with the standard diagnostic work up for MC (12-14), and stored at -20°C. sPEP with 0.8% agarose gel was used for separating β1/β2 bands, and black staining was analyzed by scanning densitometry (Hydrasys 2; Sebia, Lisses, France) to quantify the CM in % and g/dl. sIFE and urine IFE on agarose gel with acid violet staining (Sebia) were employed for confirmation of clonality and subsequent typing.

Total IgA, IgG and IgM (Roche Diagnostics, Basel, Switzerland) were measured using serum immunifixation, as well as azotemia and creatinemia for the assessment of renal function.

Freelite and Hevylite (The Binding Site Group Ltd.) assays were used for FLC determination and independent HLC isotype quantification, respectively. The Hevylite assay relies on the targeting of junctional epitopes between the heavy and light chains of intact Igs. The associated measurements were performed on turbidimetric platform SPAplus® (The Binding Site Group Ltd.).

Measurements of these parameters were used to derive IgGκ/IgGλ, which were compared with reference ranges. A HLC ratio (HLCr) outside of the reference range was considered to be indicative of a clonal process.
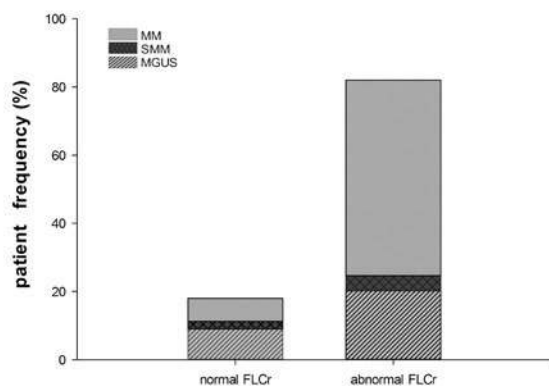
Figure 2. Abnormality of FLCr in the overall population, and MGUS, SMM and MM frequency. FLC abnormality was present in 82% of all patients; among these samples, 69.9% were MM patients, 24.6% were MGUS patients and 5.5% were SMM patients. With regard to the remaining 18% of patients without FLCr abnormality, 37.5% were MM patients, 50% were MGUS patients and 12.5% were SMM patients. MM, multiple myeloma; SMM, smoldering MM; MGUS, monoclonal gammopathy of undetermined significance; FLC, free light chain; FLCr, FLC ratio.
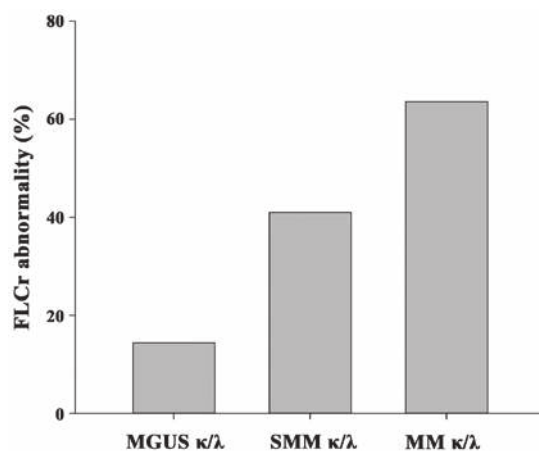

Figure 3. κ/λ FLCr abnormality in the three types of patients: MGUS, SMM and MM. κ/λ ratio abnormality in the three categories of MGUS, SMM and MM patients, accounted for 14.4, 41.0 and 63.6% of cases, respectively. MM, multiple myeloma; SMM, smoldering MM; MGUS, monoclonal gammopathy of undetermined significance; FLCr, free light chain ratio.

Patients were categorized using FLC ratio (FLCr) prognostic values, which were broadly identified using receiver operator characteristic analysis with final cut-offs being identified by trial and error.

Results

MGUS, MM and SMM frequency. The study population consisted of 49 MM patients (37 IgG, 9 IgA and 3 LCMM), 35 MGUS and 6 SMM. Patients were selected from the population treated at the U.O.C. of Hematology of San Gennaro Hospital. This accounts for the lower percentage of MGUS with respect to MM, as low-risk MGUS patients are usually referred to a general practitioner.

In the study population, the majority of MC cases were IgGκ, followed by IgGλ at 30% and IgAκ at 11%. Only a small percentage (5%) of LCMM was found (Fig. 1).

FLC abnormality was present in 82% of all patients (MM, SMM and MGUS) when considering the first sample received, which was regarded as the diagnostic sample (Fig. 2).

In total, 69.9% of the samples were MM patients, 24.6% were MGUS patients and 5.5% were SMM patients; however, care must be taken when assessing these results due to the limited number of SMM patients (6).

Notably, ~70% of the intact immunoglobulin MM (IIMM) samples had an abnormal FLC (15), thus supporting the use of FLCr as an additional marker to diagnose and monitor IIMM.

FLCr abnormality: Distribution across MGUS, SMM and MM patients. The FLCr is an important indicator of FLC pair suppression, with prominent prognostic meaning in MM patients at diagnosis and in the course of treatment (9); a high degree of abnormality correlates with reduced overall survival and progression-free survival times (16). In the present study population, ratio abnormality was compared in the three categories of MGUS, SMM and MM (Fig. 3), to assess the level of pair suppression. The majority of the patients had an IgGκ MC; therefore, this type of patient was focused upon.

The mean value of the IgGκ/IgGλ ratios was obtained, and a rising trend of the FLCr level was found in the three categories, suggesting that the severity of the FLC imbalance could be associated with overt disease.

Selected clinical cases
Case 1: Evolution of an IgGλ MC patient to double MC and then LCMM (Fig. 4). A 60-year-old man was diagnosed with IgGλ MM in March 2006 and received VAC (2 mg/m² vincristine on day 1, 30 mg/m² liposomal doxorubicin on day 2, 100 mg cyclofosfamide on days 1-4; 6 cycles every 28 days) at the Hematology Unit of S. Giovanni Bosco Hospital (Naples, Italy). In February 2009, the patient commenced therapy with the Rd regimen at the U.O.C. of Clinical Pathology of San Gennaro Hospital, and was routinely monitored with sPEP, sIFE and FLC.

From March 2012, HLC was introduced at the U.O.C. of Clinical Pathology of San Gennaro Hospital and was used together with FLC.

In October 2012 the patient had a small IgGλ MC (4.7 g/l), quantified by sPEP; FLCλ (620 mg/l) was significantly above the normal range (NR) of 5.7-26.3 mg/l and the FLCr was highly abnormal at 0.01 (NR, 0.26-1.65). IgGλ HLC (4.26 g/l) was within the NR (1.91-6.74 g/l), but the IgGκ (1.82 g/l) was below NR (3.84-12.07 g/l), thus the IgG HLCr abnormality was consistent with sIFE. This suggested the presence of persistent monoclonality even with relatively low MC levels, aiding in the assessment of a small MC together with the response to therapy.

In February 2013, a secondary small IgGλ MC was identified through sIFE and sPEP, and FLCλ increased had to 1,049 mg/l. By contrast, IgGλ HLC levels were decreased with respect to the previous time point (3.23 g/l), while IgGκ HLC (1.88 g/l) remained below the NR. HLC pair suppression assisted in identifying monoclonality even in the presence of small electrophoretic peaks that were hardly quantifiable with sPEP.

In April 2013, therapy was suspended due to a vertebral collapse requiring kyphoplasty, as well as severe anemia requiring blood transfusions.
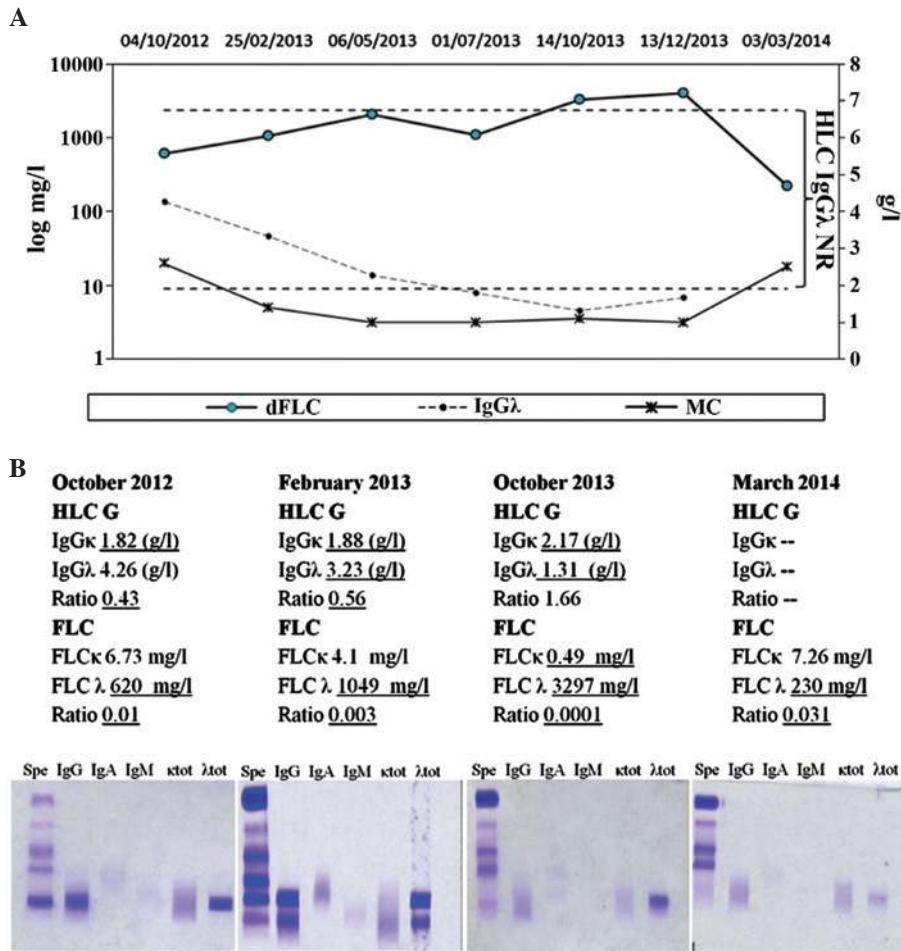
Figure 4. Case 1: Hevylite® G allowed quantification of the primary and secondary MC. (A) Hevylite G and Freelite® absolute values and ratio, and (B) immuno-fixation in an IgGλ MM patient. As the intact immunoglobulin clone appeared to respond to therapy, with normalization of HLC G values, FLCs did not ever normalize, suggesting the presence of a secondary, independent clone of light chain-producing plasma cells, which prompted a change of treatment and autologous stem cell transplantation. Underlined values are outside the NR. HLC, heavy/light chains; FLC, free light chain; Ig, immunoglobulin; MC, monoclonal component; dFLC, difference between FLCκ and FLCλ; NR, normal range; Spc, serum protein components; tot, total.

When the patient returned in October 2013, although HLCr was restored to within the NR, in agreement with the sIFE, the IgGκ and IgGλ levels were below the NR. As MC disappeared in response to therapy, with normalization of Hevylite values and ratio, Freelite never normalized and emerged a secondary clone producing free light chains [light chain escape (LCE)]. FLCλ reached 3,297 mg/l and the patient was diagnosed with LCMM. Treatment with the PAD regimen was then commenced and the patient was eligible for ASCT. Double ASCT was performed in another center prior to returning to the San Gennaro Hospital in March 2014. A marked reduction in FLCλ to 230 mg/l was observed, which was abnormal but significantly lower with respect to the previous values.

For this patient, the combined monitoring of FLC and HLC supplied more detailed information compared with sPEP and sIFE. At the most recent follow-up in June 2016, the patient had commenced therapy with pomalidonmide (4 mg daily for 21 days, every 28 days) and exhibited stable disease.

Case 2: FLC and HLC persistent abnormality correctly identifies monoclonality of MCs in the presence of secondary clones and predicts clinical relapse (Fig. 5). A 78-year-old woman was diagnosed with IgAλ and FLCλ MM in October 2012. The IgAλ MC, estimated by sPEP, was 22.4 g/l,

while FLCλ was 258.6 mg/l. The patient underwent 5 cycles of therapy with MP. In November 2012, a partial response was obtained and the sPEP did not show any monoclonal peak. Hevylite IgAκ and IgAλ were within the NR; importantly, the HLCr was also normal at this time point, suggesting that patient had responded to therapy. However, FLCλ (48 mg/l) and FLCr (0.01) remained above the NR, thus indicating the presence of residual disease. The shorter FLC half-time compared with IgA meant that an earlier FLC response was expected with respect to intact Igs. Therefore, this could be suggestive of clonal heterogeneity with coexisting FLC and HLC clones, and possibly a diverse sensitivity to the chemotherapy agent.

The patient was constantly monitored and, in June 2013, the sPEP profile suggested the reappearance of an MC visible in the γ zone, confirmed by sIFE as IgAλ. Densitometry measurement of the MC indicated a 5.8 g/l peak, whereas IgAλ HLC was 11.5 g/l with HLC pair suppression of IgAκ and an altered HLC A ratio. The Freelite ratio did not normalize and in September 2013, the patient experienced a clinical relapse, revealed by the MC increasing to 11.8 g/l and an IgAλ HLC level amounting to 15.3 g/l. Relapse was confirmed during follow-up. The patient received 6 cycles of the VD regimen, but monitoring with sPEP was not reliable, as the polyclonal
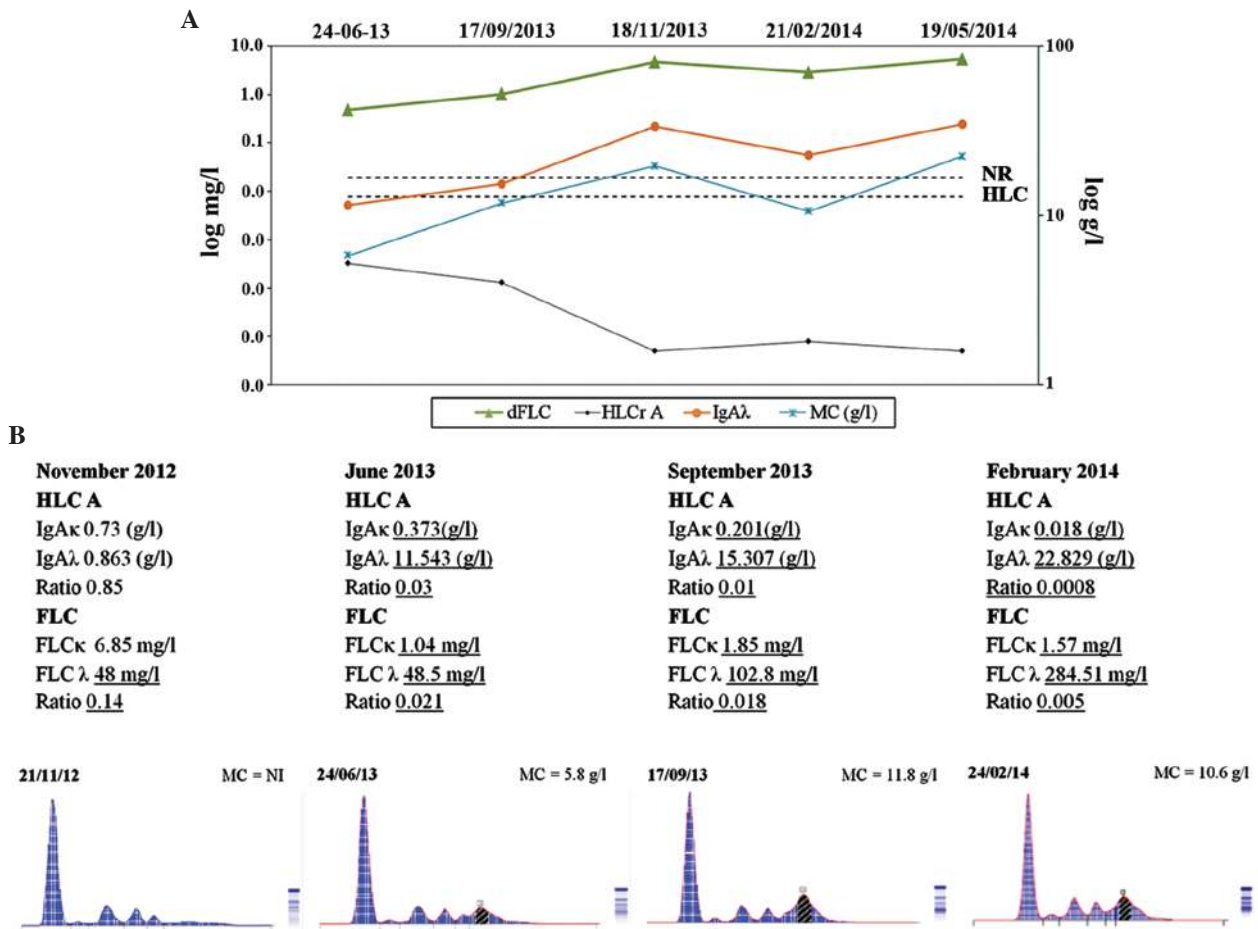
Figure 5. Case 2: HLC and FLC persistent abnormality predicts relapse prior to the clinical symptoms. (A) Hevylite® G and Freelite® absolute values and ratio, and (B) sPEP profile in an IgAλ and FLCλ MM patient. The high FLCλ (48 mg/l) and FLCr (0.14) levels in November 2012 indicated the presence of residual disease for this patient who previously underwent 5 cycles of therapy with melphalan and prednisone. From June 2013, the sPEP profile showed an MC in the γ zone, which appeared increased in the subsequent monitoring. This was accompanied by the observation of FLC and HLC absolute and ratio abnormal values. Underlined values are outside the NR. HLC, heavy/light chains; FLC, free light chain; Ig, immunoglobulin; MC, monoclonal component; dFLC, difference between FLCκ and FLCλ; NR, normal range; HLCr, HLC ratio; sPEP, serum protein electrophoresis; NI, not identified.

Igs in the γ zone interfered with an accurate IgA quantification, resulting in an underestimation of the MC (19.60 g/l by sPEP vs. 33.5 g/l IgAλ HLC). Being unable to quantify the MC by sPEP, HLC A was chosen in combination with Freelite to monitor the patient. In February 2014, a reduction in FLCλ and IgAλ HLC was observed. However, FLCr and HLCr were highly abnormal, suggesting a significant degree of pair suppression at the expense of the uninvolved FLCκ and IgAκ HLC. Despite an apparent reduction in the MC upon densitometric analysis (10.6 g/l), the severe degree of pair suppression, revealed by the ratios, suggested that there was no specific tumor killing by chemotherapy. This was confirmed in May 2014 through the observation of an increase of all biochemical disease markers: IgAκ. 0.0010 g/l (NR 0.57-2.08 g/l); IgAλ, 23.79 g/l (NR 0.44-2.04 g/l); Ratio, 0.0004; FLCκ, 1.22 mg/l (NR 3.3-19.4 mg/l); FLCλ, 298.57 mg/l (NR 5.71-26.3 mg/l); Ratio, 0.004. No further treatment was planned and the patient succumbed in July 2014.

Case 3: Clonal heterogeneity in a patient with IgAκ MM and a secondary MC (Fig. 6). In August 2012, a 77-year-old woman was diagnosed with IgAκ MM accompanied by diffuse osteolytic lesions at the U.O.C. of Hematology of San Gennaro Hospital. Treatment commenced with 2 cycles of PAD, followed by 4 cycles of VD, with 8 monthly injections of zoledronate (4 mg) to prevent skeletal fractures.

The monoclonal IgAκ was visible in the β2 zone of the sPEP migration pattern, a frequent occurrence with IgA MCs, thus hindering identification and accurate measurement of the monoclonal peak. The patient also exhibited elevated FLCκ (27 mg/l).

In June 2013, the patient suffered from severe anemia and infiltration of the mandibular bone by clonal plasma cells, prompting a change of therapy and the beginning of treatment with the Rd regimen as salvage treatment.

In September 2013, the IgAκ (9.08 g/l; NR 0.57-2.0 g/l) and FLCκ (378 mg/l; NR 3.3-19.4 mg/l) levels remained stable and, thus, above the NR. This suggested that no response to therapy occured. At the same time, a secondary MC was identified in the γ zone of the migration pattern, and sIFE results were suggestive of IgGκ and FLCλ.

However, when sIFE was compared with FLC and HLC, no abnormality was observed in the λ chains and Hevylite ratio G (0.76) clearly indicated the presence of an IgGλ.

This case is a clear example of how sIFE interpretation can be challenging even for a skilled operator when more than one MC is present, with FLC and intact Igs.
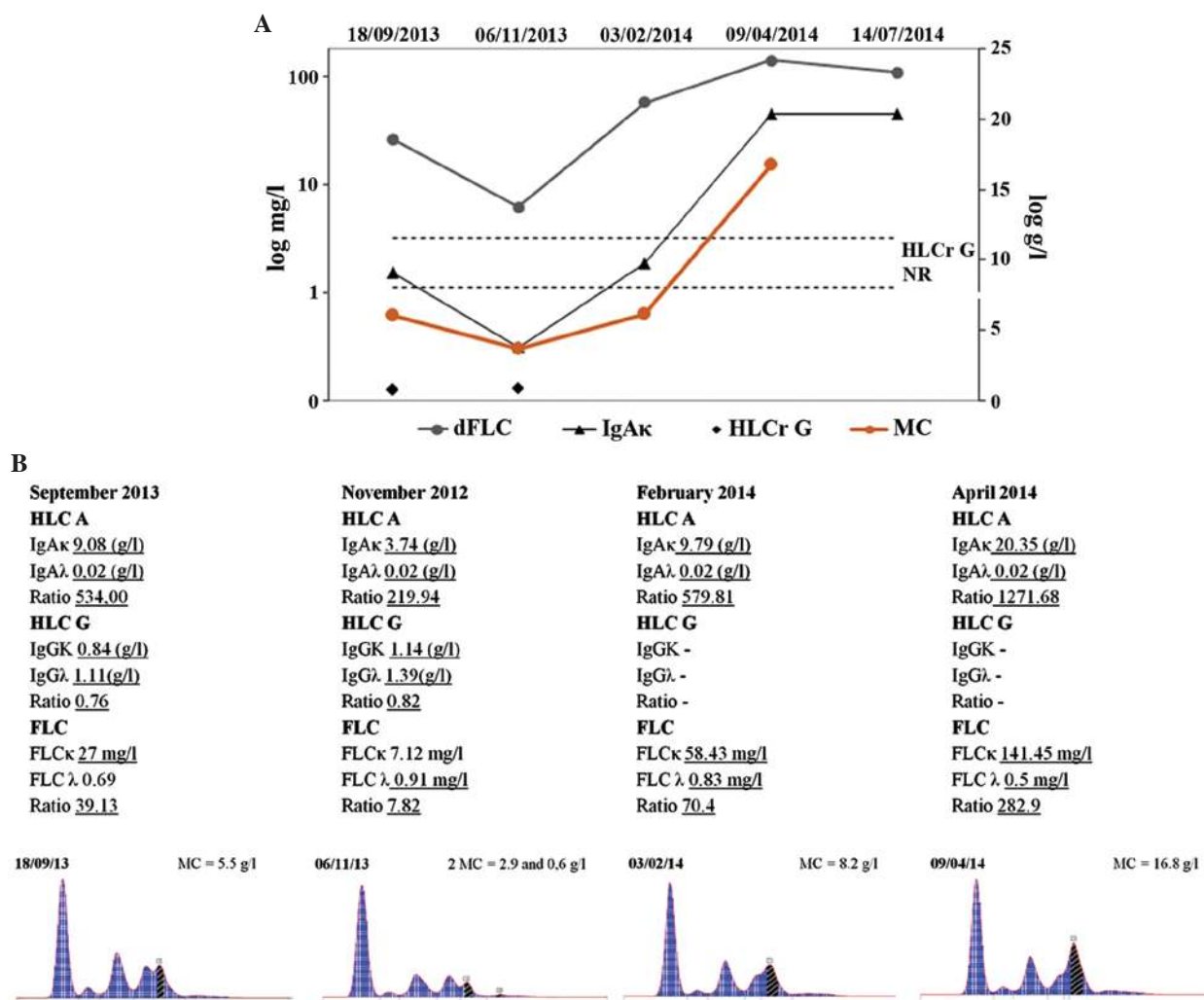
Figure 6. Case 3: HLC and FLC persistent abnormality predicts relapse prior to the clinical symptoms. (A) Hevylite® G and Freelite® absolute values and ratio, and (B) sPEP profile in an IgAκ MM patient. This patient exhibited a monoclonal IgAκ in the β2 zone of the sPEP migration pattern. In September 2013, it was identified a secondary MC in the γ zone of the migration pattern, and Hevylite and Freelite assisted in clarifying the isotypes, allowing for subsequent monitoring. The IgGκ and IgGλ levels were below the NR; IgAκ was increased above the NR (9.08 g/l), whereas the IgAλ was suppressed (0.017 g/l), with a highly abnormal HLC A ratio. In November 2013, there was an improvement in the biochemical parameters of IgGκ and IgGλ, the HLC ratio became closer to the NR, and there was a reduction in dFLC and HLC A. In February 2014, the secondary IgG MC was no longer visible, whereas the IgA HLC and FLC were increased and highly above the NR. HLC A and FLC steadily increased over the next months, until the patient succumbed in October 2014. Underlined values are outside the NR. HLC, heavy/light chains; FLC, free light chain; Ig, immunoglobulin; MC, monoclonal component; dFLC, difference between FLCκ and FLCλ; NR, normal range; HLCr, HLC ratio; sPEP, serum protein electrophoresis.

Hevylite and Freelite assisted in clarifying the isotypes and allowed for subsequent monitoring.

Notably, the IgGκ and IgGλ levels were below the NR, possibly suggesting a condition of systemic immunoparesis. IgAκ was increased (9.08 g/l) above the NR, whereas the IgAλ uninvolved chain was suppressed (0.017 g/l), with a highly abnormal HLC A ratio of 534.

The patient continued to receive lenalidomide and dexamethasone. At the following time point in November 2013, there was an improvement in the biochemical parameters of IgGκ and IgGλ, the HLCr became closer to the NR, and there was a reduction in the difference between FLCκ and FLCλ (dFLC) and HLC A.

In February 2014, the patient returned to hospital and the secondary MC IgG was no longer visible upon sIFE, whereas the IgAκ HLC (9.79 g/l) and FLCκ (58.43 mg/l) levels were increased and highly above the NR. IgAκ HLC (20.35 g/l) and FLCκ (141.45 mg/l) steadily increased over the next

months despite treatment with the Rd regimen, until the patient succumbed in October 2014.

Discussion

Accurate quantification of the MC is fundamental for forming a differential diagnosis and to assess the response to therapy (17), as well as to define immune system reconstitution in patients undergoing ASCT (18). Traditional routine tests include sPEP and sIFE, which occasionally lack sensitivity and specificity. On the other side, the current advances in molecular biology and cytogenetics have led to a major breakthrough in the development of newer and highly resolutive technologies, although they are not easily accessible for all patients, thus confirming the requirement for quick, reliable and standardized serum markers (19).

Within this scenario, serum immunoglobulin FLC measurement with polyclonal antisera represents an important diagnostic tool for monitoring patients (8).

Unlike total light chain assays, Freelite allows separate measurement of sFLC κ and λ, thus allowing the determination of the κ/λ ratio (FLCr), which serves as a reliable marker of monoclonality that is particularly relevant for a diagnosis (10). On the other hand, concerning the monitoring, it is also important to consider the dFLC and the individual concentrations of involved and uninvolved FLC. The degree of the Freelite ratio abnormality has been associated with disease progression. When considering IgGκ patients, which were the most abundant in the present study population, in agreement with previous reports (20), a trend of greater FLC abnormality in MGUS, SMM and MM IgG patients was observed.

FLC is a reliable marker that can be used together with traditional tests to diagnose and monitor all monoclonal gammopathies (21). In the present study, it was observed that the FLCr was abnormal in 82% of all patients, including SMM and MGUS patients, supporting the use of FLC in LCMM and non-secretory MM monitoring (22,23).

However, the clinical utility is not restricted to these patients. FLCs are also fundamental for IIMM: In 80% of IIMM, FLC determination is required. As shown in the first case, FLCs were independent markers of disease and allowed identification of LCE (24).

Hevylite has been used since 2012 in San Gennaro Hospital and the current study presents 3 exemplar cases where the introduction of Hevylite, together with Freelite, provided additional information with respect to standard techniques. Similarly to FLC, HLCrs are powerful and sensitive markers of monoclonality, providing novel insight into the balance between the involved and uninvolved Igs (25,26).

Clinical case 1 is a perfect example of how FLC and HLC can be reliable markers of disease, particularly for small MCs. Double MCs can be difficult to evaluate since the migration patterns could prevent accurate quantifications (27). At the same time, total Igs cannot distinguish between the MC and the polyclonal background (28). Hevylite, in this case, allowed the quantification of the IgGλ MC and confirmed the disappearance of the IgG component.

The combined use of FLC and HLC is emerging as a novel strategy to detect MC and to enable better monitoring of patients, allowing the depth of response to treatment as well as the minimal residual disease (MRD) to be assessed; it can also be used to detect relapse in MM patients (29). Clinical case 2 demonstrated Freelite sensitivity for residual disease, in spite of clinical remission and low MC levels assessed by sPEP. In this case, the MC was not accurately quantifiable with sPEP, but only Hevylite allowed precise MC measurement and also identified its reappearance, anticipating the clinical relapse. FLCλ and FLCr did not normalize, thus suggesting persistence of the disease, and Hevylite G also became abnormal several months prior to clinical relapse.

A noteworthy point of discussion within the clinical community is the requirement for earlier indicators of relapse and reliable markers of MRD that could support the intention to treat (30). In clinical case 2, the persistent FLCr abnormality and the early identification of relapse by HLCr, with respect to clinical symptoms, can be taken as an example of how a careful and evidence-based selection of novel markers could lead to a change in the paradigm of clinical practice (31).

The scientific evidence in support of HLC shows that it has greater sensitivity, providing quantitative results also in the absence of an MC detectable by sPEP; in addition, it can clarify dubious sIFE patterns (32,33).

The third case presented in the current study is a clear example of the advantages of Hevylite and Freelite over sIFE; this case also highlights the usefulness of these assays for laboratory specialists and clinicians. With regard to their use in the laboratory, these techniques could change the usual way of monitoring patients, possibly replacing traditional tests and providing more rapid, quantitative and qualitative results all in one. The potential clinical use of Hevylite and Freelite could be even wider and have a strong impact on prognosis (34,35).

As the attention focused on these novel biomarkers increases, the most pressing issue will be their application in the improvement of patient management.

Potentially, HLC could become part of a panel of parameters required to support the intention to treat prior to the observation of a severe clonal expansion accompanied by the aggravation of symptoms. All these parameters move in the same direction as the recently updated International Myeloma Working Group criteria for MM diagnosis, introducing the concept of 'myeloma defining events', which are expected to become as important as CRAB (calcium elevated, renal failure, anemia, bone lesions) symptoms (10,36). At the same time, the possibility to closely monitor the response to therapy, or the lack thereof, could provide indications to support a change of treatment or an adjustment in the frequency of monitoring.

Certainly, more evidence will be required prior to the implementation of such a paradigmatic change. However, in our experience, the benefits of Freelite and Hevylite monitoring in patients with monoclonal gammopathies are already visible. In this regard, the present clinical cases, clearly demonstrating the positive advantages of HLC and FLC assessment with respect to traditional tests, could provide valid support in illustrating the beneficial impact of these assays for the clinical and laboratory management of MM patients.

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