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IgMκ-IgMλ pair quantitation in the clinical laboratory practice

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ABSTRACT

Background: New Hevylite® assay quantifies the immunoglobulin classes, including IgM bound to light chains, allowing distinguishing immunoglobulins involved and uninvolved in plasma cell disorders.

Objective: To compare data obtained by IgM Hevylite® (IgM-HLC) assay with conventional methods used in routine laboratory practice for monitoring IgM plasma cell disorders.

Methods: Serum samples (n = 122) from 50 patients with IgM monoclonal protein (MP) identified by Immunofixation (IFE) before the beginning of the study were collected during monitoring from December 2012 to September 2014 (2 Waldestrom's macroglobulinemia, 4 NH-lymphoma, 44 MGUS) and were assessed using IgM Hevylite® (HLC) assay, Capillary Electrophoresis (CE), Immunofixation (IFE), serum Free Light Chain (FLC) assay and total IgM measurements.

Results: IgM MP was detected by IFE in 85/122 samples (71 IgMk, 10 IgMl, 4 IgMk/IgMl), while in 37/122 was undetectable although CE measured small MP, probably as a consequence of disease stimulating inflammatory immuno-response. Among the 85 positive samples, the HLC ratio but not the FLC ratio was altered in 36 samples while in 4 sera only FLC was altered. Out of 37 IFE negative samples 24 had normal HLC and FLC ratios.

Conclusions: Since the partial overlap of abnormalities identified by HLC and FLC assays, IgM Hevylite assay can provide valuable information on the evolution of IgM monoclonal disease and may support the recognition of a transitory monoclonality leading to an improvement in routine laboratory practice.

1. Introduction

Monoclonal gammopathies encompass a broad spectrum of diseases characterized by a clonal expansion of B lymphocytes secreting monoclonal complete immunoglobulins or only free light chains (FLC). Serum Protein Electrophoresis (SPE), serum ImmunoFixation Electrophoresis (IFE), and serum Free Light Chain (FLC) immunoassay are incorporated into the algorithms of guidelines for assessing patients with plasma cell disorders. These guidelines recommend how to identify and quantify monoclonal intact immunoglobulins for diagnosis, staging risk factors and monitoring therapy response [1,2]. SPE shows limits in detecting and quantifying monoclonal protein (MP), failing to resolve a discrete spike size because of the presence of polyclonal immunoglobulins or other proteins co-migrating with MP in the same electrophoretic band. Particularly, IgM monoclonal components are difficult to distinguish and, occasionally, can form self-aggregate making their detection and measurement difficult [3,4]. IFE identifies MP, but it is unable to quantify it. The FLC immunoassay is a quantitative technique that allows identifying imbalance between κ and λ chain concentrations due to clonal excess by kappa/lambda ratio (κ/λ) [2].

Recently, a new assay for immunoglobulin heavy chain-light chain (HLC) pairs, called Hevylite®, has been developed to identify and quantify the different immunoglobulin classes and the associated light chain types using novel polyclonal antibodies [5]. The heavy chain-light chain pairs are measured by immunoreaction with polyclonal antibodies anti-Ig isotype and, similarly to FLC, an HLC ratio is computed, allowing to discriminate between involved and uninvolved immunoglobulins [6]. A number of studies using the new Hevylite assay have been performed to screen, monitor, and stratify risk in patients with multiple myeloma and MGUS (Monoclonal Gammopathy of Undetermined Significance), secreting monoclonal proteins of IgG and IgA classes [6–10], while only few articles evaluating the ability of this assay to quantify IgM HCL pairs have been published [6,9,11,12].

In the present study we assessed the concentration of IgMκ and IgMλ using Hevylite® assay in serum samples from individuals with MGUS-IgM, Waldestrom's macroglobulinemia, and non-Hodking lymphoma. The results were then compared to data obtained by Capillary Electrophoresis (CE), IFE and FLC to evaluate whether the new Hevylite® assay can provide additional information in routine laboratory practice with benefits for patients with IgM MP.

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2. Materials and methods

2.1. Samples and assays

One hundred and twenty-two serum samples from 50 patients with IgM monoclonal protein (MP) identified by Immunofixation (IFE) before the beginning of the study were collected during monitoring (December 2012–September 2014). Each patient may have had more than one sample.

Patients' age ranged from 50 to 100 years (average age equal to 74 years) except for a 23 year-old man with chronic kidney disease. Four patients had a diagnosis of non-Hodking lymphoma, 2 of Waldestrom's macroglobulinemia (1 treated in 2010), and 44 of MGUS associated to other diseases including: cancer, chronic kidney disease, diabetes, chronic HCV infection, heart failure, and respiratory failure. After collection, samples were stored at −80 °C until further use. Written informed consent was obtained from all individuals and the study was conducted in accordance with the Declaration of Helsinki.

On all frozen samples routine examinations for the identification and monitoring of monoclonal gammopathies were performed. These included CE as a screening assay performed by Capyllaris according to manufacturer's recommendations (Sebia, Evry, France) and IFE performed by Hydrasis (Sebia, Evry, France) for MP identification, IgM measurement and FLC performed by SPA Plus (Binding Site, Birmingham, England). In addition IgMκ – IgMλ Hevylite® (IgM HLC) assays were performed by turbidimetric method on SPA Plus as indicated by the manufacturer.

Concentration of total serum IgM was determined with a specific antibody by turbidimetry giving a measuring range of 0.2–7.5 g/L, and reference range of 0.35–2.42 g/L; samples found to contain high levels of IgM HLC were pre-diluted 1:10 by the analyser and rerun. Those with low levels were rerun undiluted.

The FLC concentrations were measured in mg/L, IgMκ – IgMλ Hevylite® in g/L; κ/λ FLC ratio and IgMκ/IgMλ ratio were calculated accordingly.

The HLC reference intervals for IgMκ and IgMλ concentration were 0.19–1.63 g/L and 0.12–1.01 g/L respectively, while the IgMκ/IgMλ ratio range was 1.18–2.74; the limit of quantitation was 0.017 g/L and measuring range 0.09–4.97 g/L [6]. Samples were routinely diluted 1:20; when the concentration was higher than 7.5 g/L they were automatically diluted 1:40, those higher than 15 g/L were manually diluted depending on need, while those with concentration lower than 0.5 g/L were rerun *in toto*.

Samples with κ and λ FLC levels above the analytical ranges of 180 mg/L and 165 mg/L, respectively, were rerun after 1:100 or 1:1000 dilutions. The FLC reference intervals were those suggested by the manufacturer, i.e. 3.3–19.4 mg/L and 5.7–26.3 mg/L, for κ and λ respectively, and the κ/λ ratio ranged between 0.26 and 1.65.

A control sample was obtained by pooling serum samples from 11 healthy blood donors. This control sample was measured in triplicate for 5 days to determine the analytical imprecision of IgMκ – IgMλ Hevylite® assay according to CLSI EP15A2 (2009).

2.2. Statistical analyses

All statistical analyses and procedures were performed using MedCalc Software for Windows, version 15.4.5.

Passing-Bablok regression analysis was used to compare the concentration of total IgM with the sum of IgMκ and IgMλ levels obtained using Hevylite, and to determine the relationship between MP quantitation and the corresponding IgM isotype concentration [13].

Receiver Operating Characteristic (ROC) analysis was performed to compare HLC and FLC diagnostic performance in the study population considering IFE as the gold standard in order to define the "disease" and "healthy" patient status [14]. Statistical significance was considered with a p-value < 0.05.

3. Results

3.1. HLC analytical variability and correlation with total IgM concentration

The analytical variability of HLC was evaluated using the control sample (pool of 11 sera). Mean IgMκ and IgMλ concentrations were 0.55 g/L and 0.31 g/L, respectively. The total and within-run imprecisions were 3.3% and 0.7% for IgMκ; 4.9% and 3.8% for IgMλ.

The comparison of total IgM concentration to the sum of IgMκ and IgMλ measured by HLC provided a Passing-Bablok regression equation of "$y = 0.02 + 1.04x$". Both the intercept (0.02, 95%CI −0.04–0.08) and the slope (1.04, 95%CI 0.99–1.09) were not significantly different from 0 and 1 respectively, revealing an agreement within the IgM concentration range of 0.3–81.0 g/L (Fig. 1A).

3.2. IFE results

IgM isotype was detected by IFE in 85/122 samples (69.7%) from 35 patients while 37/122 sera (30.3%, 15 patients) showed undetectable IFE. Among the 85 IFE positive samples, 71 (from 29 patients) were IgMκ, 10 (from 4 patients) were IgMλ, and 4 (from 2 patients) were characterized by biclonality IgMκ-IgMλ (Table 1).

3.3. HLC, FLC, CE in IFE positive samples

Out of the 71 IgMκ samples, 61 (85.9%) resulted to have an HLC ratio higher than the reference range (from 3 to 100) while the FLC ratio was outside the reference range (from 1.65 to 12) only in 41 samples (57.7%) (Table 1).

The Passing-Bablok regression analysis showed significant differences between IgMκ HLC concentrations and IgM MP measured by CE (Fig. 1B) having a regression equation ($y = -0.05 + 2.51x$) with a slope significantly different from 1 (2.51, 95%CI 1.93–3.24). Moreover, 1 sample collected from a patient with WM, and 2 from non-Hodking Lymphoma patients showed consistent absolute differences between HLC and CE measurements: 48.5 g/L, 25.2 g/L, and 30.2 g/L, respectively (first sample HLC = 80.7 g/L, CE = 31,5 g/L; second sample HLC = 31,42 g/L CE = 6,4 g/L; third sample HLC = 37,32 g/L CE = 7,2 g/L). Additionally 11 samples out of 61 showed a suppression of uninvolved IgMλ isotype.

All 10 (100%) IgMλ IFE positive samples exhibited an HLC ratio <1.18, while the FLC ratio was altered (<0.16 or >1.65) in 4 samples (40%, 2 different patients) (Table 1).

Passing-Bablok regression between IgMλ concentrations (HLC) and MP (CE) ($y = 2.14 + 0.13x$), showed a significant difference for both intercept (2.14 95%CI 0.06–2.62) and slope (0.13 95%CI 0.01–2.34) from 0 and 1, respectively (Fig. 1C). Two samples had IgMλ ranging from 10.3 to 11.2 g/L, while IgM MP was within 4.0–4.3 g/L. Four other samples showed IgMλ concentrations ranging from 3.1 to 5.8 g/L and MP beyond 20 g/L.

Biclonal IgMκ and IgMλ IFE was confirmed in 4 sera from 2 patients (with non-Hodking Lymphoma or with MGUS) showing high concentrations of both IgMκ and IgMλ HLC, and total IgM. Because of biclonality, the HLC ratio was close or within the reference range (Table 1).

3.4. HLC, FLC, CE in IFE negative samples

Among the 37 samples with negative IFE, 27 (73.0%) had normal HLC ratio and the remaining 10 (27.0%) were characterized by slightly altered values. FLC ratios were just above reference range only in 4/37 sera (10.8%) (Table 1).

When the HLC and the CE MP concentrations measured in these 37 samples were compared by Passing-Bablok regression ($y = 0.30 + 0.11x$), a disagreement in the results was observed since both the intercept (0.30 95%CI 0.07–0.39) and the slope (0.11 95%CI 0.01–0.37) were significantly different from 0 and 1, respectively (Fig. 1D).

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