Quantification of β-region IgA monoclonal proteins – should we include immunochemical Hevylite® measurements?

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Abstract: Accurate measurement of IgA monoclonal proteins presents a significant challenge to laboratory staff. IgA heavy/light chain (Hevylite, HLC) analysis is an alternative methodology for monoclonal protein assessment, giving an independent measure of IgAκ and IgAλ concentrations. Clonality is assessed by calculating the ratio of involved immunoglobulin to background uninvolved immunoglobulin concentrations (e.g. IgAκ/IgAλ in an IgAκ patient). Here we discuss the challenges faced by the laboratory in IgA monoclonal protein assessment, and compare the performance of Hevylite assays with electrophoresis and total IgA results. We present data which validates the use of Hevylite for response assessment: in most cases, Hevylite provides comparable response assignment to that provided by serum protein electrophoresis (SPE) and total IgA; in other cases Hevylite provides additional information, such as detection of residual disease or relapse.

Keywords: electrophoresis; Hevylite; IgA; immunoglobulin; monoclonal protein.

Introduction

In the quaternary structure of IgA molecules, the immunoglobulin domains of adjacent α-heavy and κ or λ light chains are in close proximity, for example, the heavy chain Cα1 constant domain closely pairs with the light chain CL constant domain [1]. This is the structural basis for immunoglobulin heavy/light chain (Hevylite, HLC) immunoassays, which are based on antisera specific for junctional epitopes that span the heavy and light chain constant domains [2]. These turbidimetric/nephelometric assays separately quantify the light chain types of each immunoglobulin class (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ). In humans it is estimated that there are at least $10^{13}$ unique antibody structural variants to allow recognition of a vast number of potential pathogens [3]. Therefore, polyclonal antisera were chosen as the basis of Hevylite assays, to allow full recognition of all potential structural variants of immunoglobulins.

IgA Hevylite assays are CE-marked and FDA-approved turbidimetric/nephelometric immunoassays suitable for routine use by clinical diagnostics laboratories. A comprehensive, independent study of IgA Hevylite assays in a routine clinical laboratory setting reported acceptable stability, intra- and inter-assay variability, linearity and accuracy [4].

Hevylite assays are measured in pairs to produce ratios (e.g. IgAκ/IgAλ) in the same manner as κ/λ serum free light chain (sFLC) ratios, to provide information on immunoglobulin clonality. Katzmann et al. [5] reported that an abnormal IgA HLC ratio was present in 97% (148/153) IgA multiple myeloma (MM) patients at diagnosis. Similar findings were reported by Boyle et al. [4]. In both studies, the involved HLC (iHLC) concentrations (e.g. IgAκ in an IgAκ patient) showed good agreement with the monoclonal protein concentration determined by scanning densitometry of SPE gels [4, 5], and summed HLC pairs (e.g. IgAκ+IgAλ) correlated well with total immunoglobulin measurements [4]. Moreover, the reported biological variation of iHLC values was comparable to the variability of monoclonal protein measurements by SPE scanning densitometry and total immunoglobulin measurements [4].

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Challenges with IgA quantification

In normal individuals, polyclonal IgA is too diffuse to be seen by SPE [6]. In contrast, IgA monoclonal proteins are usually visible as a band (or several bands) in the β- or γ-region (in 41% and 48% of cases, respectively) [4]. However, densitometric quantification of monoclonal bands can be challenging, even to experienced users, for several reasons. Firstly, monoclonal proteins may co-migrate with other serum proteins bands, for example, IgA monoclonal proteins may co-migrate with transferrin and complement component C3 that are found in the β1- and β2-regions, respectively. This is an important issue, as co-migration may affect >40% of IgA monoclonal proteins [4]. Secondly, IgA monoclonal proteins may appear as diffuse or multiple bands due to oligomerisation of the immunoglobulin [7]. Thirdly, dye saturation may lead to underestimation of monoclonal protein concentrations by SPE, this is a particular issue for IgG monoclonal proteins that migrate in a narrow, dense band [8]. Finally, the quantification of small monoclonal proteins may be inaccurate due to interference from the background polyclonal immunoglobulins [9].

Current International Myeloma Working Group guidelines state that whilst it is preferred that monoclonal intact immunoglobulins (>10 g/L) are monitored by densitometric quantification, in cases where they are obscured by other serum proteins bands, nephelometric total immunoglobulin measurements may be more accurate. However, total immunoglobulin assays are unable to distinguish between polyclonal and monoclonal immunoglobulins. This becomes important following treatment, when the monoclonal protein concentration falls, and total IgA values enter the normal range [10].

Hevylite assays provide a quantitative result for both the involved and uninvolved immunoglobulins of a particular isotype (e.g. IgAκ and IgAλ), and an abnormal HLC ratio provides evidence of clonal synthesis. Hevylite assays allow quantification of monoclonal proteins that co-migrate with other serum proteins bands, which is often the case for monoclonal IgA. This is illustrated in Figure 1 and Table 1, and confirmed in a number of reports [5, 11, 12]. Hevylite assays can also help quantify monoclonal proteins that migrate as broad bands, multimers, or that are difficult to distinguish. An example of an IgA monoclonal protein that migrates as a broad band is shown in Figure 1 (Sample 2), and another is described in a published case study by Donato et al. [13].

If a small IgA monoclonal protein is present on a background of polyclonal IgG (i.e. an alternate immunoglobulin isotype), Hevylite assays will provide a more accurate result of clonal synthesis than electrophoresis, as it is usually impossible to avoid including a proportion of polyclonal IgG in the densitometric measurement. In a recent study 29/157 IgA patient sera were not accurately quantifiable by SPE, whereas all were measurable by Hevylite [4]. In IgA monoclonal gammopathy of undetermined significance (MGUS), the monoclonal protein concentration is often low (<10 g/L in 49% of cases), but the concentration of polyclonal background immunoglobulins also tend to be low, for both the non-tumour isotypes (i.e. IgG and IgM) and the uninvolved HLC-pair (i.e. IgAκ in an IgAκ patient) [14]. Consequently, the vast majority of IgA MGUS (97%) patients have an abnormal HLC ratio at diagnosis [14].

Variations in protocols for reporting monoclonal IgA electrophoresis results can lead to discrepancies in results from different laboratories [15]. Some groups recommend that when a monoclonal protein is located in the β-region, the quantification should be reported to include total “β + paraprotein” concentration [15]. Whilst this approach simplifies reporting, it results in inaccurate monoclonal protein quantitation. Other laboratories recommend that when the total β-region is <20 g/L, monoclonal proteins within this region are “non-quantifiable”, and that immunofixation electrophoresis (IFE) should be performed instead [5]. However IFE is a labour-intensive and non-quantitative technique, and Katzmann et al. [5] reported that, using this strategy, IFE was required in 95% of IgA multiple myeloma post-treatment samples. In contrast, Hevylite assays provide numerical values, allowing simplified reporting which could improve the consistency of reported results by different laboratories.

Response assessment

A number of publications have reported the utility of IgA Hevylite for monitoring IgA monoclonal gammopathies. In many cases, the response measured using IgA HLC analysis is comparable to that of SPE/total IgA. For example, Adie et al. [16] compared the iHLC response with that defined using SPE or total IgA for 61 IgA patients at 254 time points. iHLC response criteria were based on those of SPE/total IgA (e.g. a partial response was defined as a 50% reduction in iHLC). Weighted kappa analysis showed a near perfect agreement between the responses assigned using iHLC and SPE/total IgA (Table 2).

In other cases, IgA Hevylite provides additional information. For example, in the same study, of the 27 IgA patients who were assigned as having a complete response (CR) by SPE/total IgA, 2/27 were classed as only having a
very good partial response (VGPR) by IgA HLC. This may be explained by the increased sensitivity of the HLC ratio to detect residual disease in some patients. Similar findings have been reported by others [10, 11]. In a monitoring example reported by Ludwig et al. [11] the patient’s monoclonal IgAκ became undetectable by IFE, consistent with a CR, but the IgAκ/IgAλ HLC ratio remained abnormal. After further follow-up, the HLC ratio normalised. The authors also describe three MM patients in whom a HLC abnormality pointed to imminent relapse while
IFE was still negative. Similar findings were reported by Batinic et al. [10].

HLC immunoassays may offer a sensitive method of quantifying monoclonal immunoglobulin concentrations in oligosecretory MM patients (defined as having serum monoclonal protein <10 g/L and urine monoclonal protein <200 mg/24 h). Ludwig et al. [11] reported that at presentation, all 18 oligosecretory MM patients (11 IgA, 7 IgG) had an abnormal HLC ratio at presentation. Similar findings were reported by Boyle et al. [4]. Young and colleagues [17] studied the use of HLC assays for monitoring eight oligosecretory MM patients (5 IgA, 3 IgG) and concluded that changes in Hevylite values were in concordance with clinical assessment during follow-up.

**Conclusions**

IgA Hevylite assays should be considered for monitoring all patients with IgA monoclonal proteins, and may reduce the requirement for total IgA and IFE assays. Hevylite assays accurately quantify monoclonal proteins that co-migrate with other serum proteins or that have a diffuse electrophoretic migration, and simplify the reporting of results. When tumour burden is low, the HLC ratio provides information on immunoglobulin clonality.

As Hevylite assays become more widely available and difficulties in quantifying IgA monoclonal proteins are more widely recognised, it is hoped that Hevylite assays will be incorporated into International guidelines for the routine monitoring of IgA patients, to allow accurate monoclonal protein quantitation and the assessment of residual disease.

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