Clinica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Biological variation of immunoglobulin heavy chain-light chain pairs in serum

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ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 2 May 2014
Accepted 4 May 2014
Available online xxxx

Keywords:
Biological variation
Free light chains
Heavy chain-light chain pairs
Freelite
Hevylite

ABSTRACT

Background: Assays for immunoglobulin heavy chain-light chain (HLC) pairs called Hevylite® have recently been developed. These assays can be useful in patients with hard to interpret serum protein electrophoresis peaks. Measurement of the biological variation of clinical laboratory tests can help clinicians better interpret laboratory results.
Methods: Serum samples were collected from 15 healthy donors and assayed with IgAκ, IgAλ, IgGκ and IgGλ Hevylite. The coefficients of the within-subject and between-subject biological variation, index of individuality (II), number of samples (*n*) required in the determination of the homeostatic setting points (HSP) and reference change values (RCV) were calculated.
Results: The coefficients of the within-subject biologic variation were all less than the between-subject biological variation. The II for all the assays and their κ/λ ratios were near or <0.6. The RCV ranged from 17 to 41%. The number of measurements to determine the HSP for an individual was 1 for the HLC ratios and between 2 and 9 for the individual isoforms.
Conclusions: II indicates it is better to use the patient's individual results rather than population based reference values, fewer measurements are required to determine the HSP for the HLC ratios than the individual isoforms and the RCV can now be used to aid in the interpretation.

1. Introduction

Polyclonal assays for immunoglobulin free light chains (FLC[1]) (Freelite®, The Binding Site, Ltd.) in serum have been used for many years in conjunction with protein and immunofixation electrophoresis to manage patients with multiple myeloma and other plasma cell disorders. Both the International Myeloma Working Group [1] and the National Comprehensive Cancer Network [2] have incorporated FLC into their guidelines for assessing patients with plasma cell disorders. Several studies on the biological variation (BV) of serum FLCs have recently been published [3–5].

A new group of assays for immunoglobulin heavy chain-light chain (HLC) pairs collectively called Hevylite® have recently been developed and cleared for use in the United States, Europe and Canada (The Binding Site, Ltd.). Just as the 2 FLC assays (1 each for the free kappa and free lambda light chains) can detect clonality in patients with multiple myeloma or other plasma cell disorders so can the Hevylite assays. The Hevylite assays are constructed by raising polyclonal antibodies to the interface between the immunoglobulin heavy chain and the light chain. As such, a specific pair of heavy and light chains is required to be present for a signal to be generated in the assay. These new assays now allow, for example, IgGκ to be measured separately from IgGλ and the IgGκ/λ ratio to be calculated. This is also true for patients with elevations in IgA or IgM isoforms. In a patient suspected of having a plasma cell disorder, a finding of any of the isoforms or their HLC κ/λ ratio (heavy chain–light chain kappa/lambda ratio) (HLCR) outside of the population based reference values would increase suspicion that the patient has a plasma cell disorder. For example, in a patient with an IgGκ myeloma the IgGκ concentration provides a measure of tumor burden, while the IgGλ concentration provides a measure of the degree of isotype specific immunosuppression of that particular patient. Isotype specific immunosuppression is only measurable with the Hevylite assays. Because of these features, the Hevylite assays have been found to be valuable for the prognosing and monitoring patients with multiple myeloma [6,7]. The IgA HLC tests are particularly helpful in patients with hard to interpret serum protein electrophoresis [8] peaks.

Analogous to the FLC assays the HLC assays provide 3 values for each pair of tests used. If the patient has an IgA myeloma then the IgA HLC

[1] Abbreviations: FLC; immunoglobulin free light chain, HLC, immunoglobulin heavy-light chain; κ, kappa immunoglobulin light chain; λ, lambda immunoglobulin light chain; II, index of individuality; HSP, homeostatic setting point; n; number of samples, *n* (italicized *n*) number of samples required to determine the HSP; RCV, reference change value; BV, biological variation; HLCR, heavy-light chain kappa to lambda ratio; CV, coefficient of variation; CV_A, coefficient of analytical variation; CV_I, coefficient of the within-subject biological variation; CV_G, coefficient of the between-subject biological variation; and MGUS, monoclonal gammopathy of undetermined significance.

http://dx.doi.org/10.1016/j.cca.2014.05.002
0009-8981/

assays can be used to quantify the serum concentration of IgAκ, IgAλ and the ratio of IgAκ/IgAλ or HLCR. Reference values have been established for all 6 isoforms and their ratios to guide clinical practice [9]. Similar to the work of Braga et al. [4] we carried out an evaluation of the biological variation of the HLC assays according to the methods established by Fraser [10].

2. Material and methods

2.1. Samples

The study inclusion criteria were that the subjects had no evidence of disease, nor current use of medication. Venous blood was obtained at the same approximate time from the donors. Blood samples were collected by the same phlebotomist with minimal stasis using vacuum collection tubes with no anticoagulants or gel for serum separation. The specimens were separated by centrifugation and the resulting sera were stored at −80 °C until analysis.

We collected 3 samples (from 5 donors) to 4 samples (from 10 donors) each from 15 total healthy donors on the same day every 2 weeks for 6 weeks. Healthy subjects were used for the study so that any fluctuation in the analytes would be due to normal biological variation and not any pathological process. In accordance with the University of California at San Francisco Committee on Human Research, informed consent was obtained from each donor in the study.

2.2. Testing with Hevylite assays

Six Hevylite assays have been constructed as previously described to independently measure 6 different immunoglobulin isoforms in serum: IgG-kappa (IgGκ), IgG-lambda (IgGλ), IgA-kappa (IgAκ), IgA-lambda (IgAλ), IgM-kappa (IgMκ) and IgM-lambda (IgMλ) [9]. There was insufficient volume of serum to perform assays on the HLC IgM isoforms. The sera were thawed, mixed and analyzed in duplicate with the HLC IgAκ/λ and HLC IgGκ/λ assay pairs. The HLC assays (The Binding Site, Ltd.) were performed on a BNII nephelometric analyzer (Siemens Healthcare Diagnostics, Inc.).

2.3. BV calculations

The Cochran's test was performed for outlier identification among observations and within-subject variances, whereas Reed's criterion was used for the identification of outliers among mean values of subjects using a Microsoft Excel spreadsheet designed by Frank Wians, PhD (Clinical Pathology Laboratories, Austin, TX) according to the method of Fraser et al. [11]. After exclusion of outliers, the Shapiro–Wilk test was applied separately to the set of results from each individual to check data distribution. The analytical, within-subject, and between-subjects components of variation were calculated by nested analysis of variance from replicate analyses [11]. Analytical variance was estimated from the duplicate results for each specimen, within-subject biological variance from the within-subject total variance (referred to as the variance of the mean of duplicate assays) minus one-half of the analytical variance, and between-subjects biological variance from the total variance of the data minus the analytical and intra-individual components. All the components of variance were then transformed to the relevant coefficient of variation (CV) using the overall means. The coefficient of analytical variation is indicated by CV_A, the coefficient of the within-subject biological variation is indicated by CV_I, and the coefficient of the between-subject biological variation is indicated by CV_G. The index of individuality (II) was calculated. The reference change value (RCV), i.e. the minimal significant difference ($P < 0.05$) between 2 consecutive HLC measurements on the same subject, was calculated using a parametric approach because the data distribution was normal [10]. The number of specimens that should be tested to estimate ($P < 0.05$) the HSP of an individual within ±10% was calculated [11].

3. Results

Table 1 shows the BV results after removing the outliers. For all parameters Shapiro–Wilk test accepted the normality hypothesis in a substantial proportion (≥94%) of subjects. All isoforms and ratios had an index of individuality close to or <0.6. The number of measurements to determine HSP of the κ/λ ratios was consistently 1 while the number for the individual isoforms ranged from 2 to 9.

Optimal, desirable and minimum analytical goals for the imprecision, bias and total error for HLC and HLC ratios were obtained from BV components, calculated as described in [10] (Appendix, 2 page 139) and are listed in Table 2 [12].

4. Discussion

From Table 1 it can be seen that for all cases the CV_I was less than the CV_G. The II which is calculated using both CV_I and CV_G, yields information about the utility of conventional population-based reference values. Since all the II for the Hevylite isoforms and their ratios were near or <0.6, the reference values for all isoforms and ratios are probably not as useful as letting the patient act as their own control when using these tests for monitoring. This information may be useful to clinicians when they are monitoring patients for a change in assay concentration over time. This data indicated that magnitude of the change in a patient's own results has more importance in interpretation than where the patient's test results are in comparison to the reference values that have been set for the assays.

Unfortunately we only had enough serum to test the HLC A and G assays. We did not have enough to perform the HLC M assays. This is a limitation of this study.

For all Hevylite assays and ratios tested, a normal distribution was present so the RCV up is equivalent to the RCV down. Consensus recommendations have been published for the use of tests in multiple myeloma and these include recommendations for assigning response or progressive disease depending upon laboratory tests [13]. Serum free light chains are incorporated into these guidelines and HLC tests are likely to be incorporated as soon as they come into more regular use. A decrease in the HLC isoform concentration or ratio > RCV could be an indication that the patient is having a significant response to current therapy. An increase in the HLC isoform concentration or ratio > RCV in a patient with a stringent complete response could be an indication of relapse. Knowing the boundary of normal patient fluctuation should be helpful to clinicians revising recommendations such as these.

The number of times the tests need to be run to establish the HSP of an individual was calculated. Clearly the use of the ratio will allow determination of the HSP with fewer measurements: see Table 1 κ/λ ratio for both IgA and IgG. This may help clinicians in practice when

Table 1
Biological variation for free light chain (FLC) and heavy chain-light chain (HLC) pairs.

Analyte	n	Mean	CV_A[a]	CV_I[a]	CV_G[a]	II	RCV[a]	n[b]
FLC-κ [4]	18	8.0[c]	1.2	8.1	14.1	0.33	22.6	2
FLC-λ	19	8.5[c]	0.9	7.0	27.5	0.07	19.6	2
FLC-κ/λ	18.5	0.98	1.7	4.5	15.3	0.09	13.4	1
FLC-κ [5]	18	NA	7.3	4.3	21	0.35	24	1
FLC-λ	18	NA	4.5	7.0	30	0.30	23	1
HLC A-κ[d]	15	1.1[e]	5.3	7.2	33.3	0.27	30	3
HLC A-λ	15	1.0[e]	3.8	8.0	35.8	0.25	32	3
HLC A-κ/λ	15	1.2	6.1	0.56	18.0	0.34	17	1
HLC G-κ	15	6.0[e]	9.4	12.3	24.9	0.62	41	9
HLC G-λ	15	3.6[e]	3.4	5.9	16.3	0.42	28	2
HLC G-κ/λ	15	1.7	4.7	1.5	24.6	0.20	23	1

NA = not available.
[a] %.
[b] Number of samples required to be within ±10% of the true HSP with 95% probability.
[c] mg/L.
[d] This study. A = IgA, G = IgG.
[e] g/L.

Please cite this article as: Finlay JA, Wu AHB, Biological variation of immunoglobulin heavy chain-light chain pairs in serum, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.05.002