Clinica Chimica Acta 479 (2018) 7–13

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Heavy/light chain assay as a biomarker for diagnosis and follow-up of multiple myeloma

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ARTICLE INFO

Keywords:
Heavy-light chain assay
Multiple myeloma
Biomarker

ABSTRACT

Background: The heavy-light chain (HLC) assay enables the accurate measurement of each isotype-specific heavy and light chain (i.e., IgGκ, IgGλ, IgAκ, and IgAλ) and the derivation of an HLC-pair ratio. However, to date, only limited data have validated the usefulness of serial HLC measurements in the routine follow-up of intact immunoglobulin multiple myeloma (MM) patients.

Methods: A total of 36 diagnostic and 671 post-treatment sera from 115 IgG and 61 IgA MM patients were assessed with capillary zone electrophoresis, immunosubtraction electrophoresis, total immunoglobulin measurement, free light chain, and HLC assay. The correlations between M-protein levels and the HLC and FLC assay-derived parameters were examined and the clinical significance of the biomarkers was evaluated according to patients' status.

Results: Involved HLC (iHLC) was the best biomarker correlating with M-protein concentration in both IgG and IgA MM, and could provide a surrogate marker substituting M-protein levels to follow the course of the disease, especially in β-migrating IgA M-proteins. The distribution of iHLC values as well as HLC-pair ratios (rHLC) yielded significantly different results among the various response categories in both IgG and IgA MM. In addition, we detected 2 cases in which an abnormal rHLC in a stringent complete remission (sCR) sample was a marker of early non-symptomatic relapse.

Conclusion: In this study of a cohort of 176 patients in a routine clinical setting, we have provided evidence of the clinical utility of real world HLC assays for the identification of M-proteins and to monitor M-proteins with an emphasis on IgA monoclonal gammopathies.

1. Introduction

Multiple myeloma (MM) is a malignant disorder characterized by the proliferation of a single clone of plasma cells that usually secrete a monoclonal immunoglobulin (M-protein) with a unique variable region sequence. This unique structure of each M-protein makes them highly specific for each plasma cell clone. However, the variability also poses a challenge in the diagnosis and monitoring of MM, and no single test can confidently diagnose or monitor all MM patients [1]. Therefore, panels of tests have been recommended to increase the sensitivity and efficiency, and include tests such as protein electrophoresis (PEP), immunofixation electrophoresis (IFE), immunoglobulin quantitation, and free light chain (FLC) quantitation. However, problems with sensitivity and inaccuracy as well as a lack of standardization can limit the usefulness of PEP and IFE, and FLC assay cannot be used for approximately 4% of MM with normal FLC concentrations at initial diagnosis [2].

The heavy-light chain (HLC) assay is a recently developed immunoassay that uses polyclonal antisera with specificity for unique conformational epitopes that form at the junction of the heavy- and light-chain constant regions of each immunoglobulin (Ig) molecule [3]. This novel assay enables the accurate measurement of each isotype-specific heavy and light chain (i.e., IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMλ) and the derivation of an HLC-pair ratio (rHLC), which can serve as an indicator of clonality. Hence, the HLC assay is an important addition to the methods to detect and measure M-proteins.

However, to date, there are limited data available regarding the

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https://doi.org/10.1016/j.cca.2018.01.010
Received 2 May 2017; Received in revised form 7 December 2017; Accepted 7 January 2018
Available online 08 January 2018
0009-8981/

Table 1
Clinical characteristics of patients and samples.

Variable	Total	IgG MM	IgA MM	*P*-value
Patient characteristics				
Number of patients	176	115	61	
Percentage of total	100%	65%	35%	
Age (years), mean	63	62	65	.0619
Sex (male/female)	88/88	52/63	36/25	.1132
Number of samples (median)	3	3	3	.556
IgGκ/IgGλ-IgAκ/IgAλ		61/54	27/34	
Patients with normal FLC concentrations	5	4	1	
Sample characteristics				
Number of samples	671	439	232	
Samples at diagnosis	36	20	16	
Samples at follow-up	635	419	216	
Complete response		52	30	
Very good partial response		103	61	
Partial response		159	58	
Stable disease		102	56	
Progressive disease		3	11	
M-protein (median)		0.8 g/dL	0.52 g/dL	.0082
measurable M-protein (≥1 g/dL)		65.50%	34.50%	.0267
M-protein migration in β fraction	53	5	48	< .0001
Median follow-up (days)	84	77	114	< .0001

usefulness of serial HLC measurements in the routine follow-up of MM patients. Here, we aimed to assess the clinical value of HLCs and HLC-derived parameters in routine clinical laboratory using a series of 671 samples from 176 MM patients at diagnosis and during follow-up.

2. Materials and methods

2.1. Patients

A retrospective study was performed in which patients with IgG and IgA MM and who also had PEP, IFE, FLC, and HLC performed at the time of diagnosis and/or monitoring were selected. The cohort consisted of 176 patients examined at Seoul St. Mary's Hospital for diagnosis or monitoring of IgG or IgA MM. This included 115 patients with IgG and 61 IgA MM patients. The cohort and consecutive samples derived from the cohort were highly heterogeneous and represented a wide range of disease statuses (Table 1) as well as medical interventions (Supplementary Table 1). The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC16RISI0737).

2.2. Laboratory methods

The detection and quantitation of M-proteins was performed using capillary zone electrophoretic assay (CZE), with the CAPILLARYS 2 automated system (Sebia, Norcross, GA, USA). Immunochemical characterization of M-proteins was performed using immunosubtraction electrophoresis by CZE. To quantify M-proteins, the percentage of the M-protein derived from CZE was multiplied by the total protein concentration determined by colorimetric assay using biuret reagent (Seikisui Medical Co., Ltd. Tokyo, Japan) on a Hitachi 7600 analyzer (Hitachi, Tokyo, Japan). IgG and IgA were quantified by a turbidimetric immunoassay using Pureauto reagents (Sekisui Medical, Tokyo, Japan) on the Hitachi 7600 analyzer. The FLC assay was performed by immunoturbidimetry using Freelite reagents (The Binding Site, Birmingham, UK) on the Hitachi 7600 analyzer, and the HLCs were quantified by immunoturbidimetry using Hevylite reagents (The Binding Site) on a SPAPLUS analyzer (The Binding Site).

2.3. Definitions

Involved HLC (iHLC) referred to the actual value of the intact Ig heavy chain/light chain pair of the M-protein isotype. Uninvolved HLC (uHLC) referred to the value of the polyclonal immunoglobulin heavy chain/light chain pair of the same class of heavy chain isotype, but with a different light chain isotype from the M-protein. dHLC referred to the difference between iHLC and uHLC of the same class of heavy chain isotype. The HLC-pair ratio (rHLC), by default, is defined as IgGκ/IgGλ or IgAκ/IgAλ. To simplify the interpretation of this study, we have also used the involved/uninvolved rHLC with the monoclonal HLC in the numerator and the uHLC of the same class of heavy chain isotype as the denominator. Involved FLC (iFLC), dFLC, and FLC-pair ratio (rFLC) was defined as previously detailed [4]. The 95th percentile reference intervals provided by the manufacturer for HLC concentrations and rHLCs, FLC concentrations, and rFLCs were used.

2.4. Response assessment

International Myeloma Working Group (IMWG) criteria for general response assessment [5] were used. We also applied a response assessment according to HLC, using identical criteria as for the FLC-based response criteria as recommended by IMWG [5]. For the response assessment, a normal rHLC is complete remission (CR), and if rHLC is abnormal, then a < 50% reduction in iHLC is stable disease (SD), a ≥50 to < 90% reduction is partial response (PR), and > 90% is very good partial response (VGPR). Concordance between tests was assessed by Kappa analysis.

2.5. Statistical analyses

Continuous data are presented as mean (standard deviation) for normally distributed data and median (range) for non-normally distributed data, and categorical data are presented as numbers (%). Normality was assessed using the D'Agostino-Pearson normality test. Since M-protein concentration and the concentrations of HLC and FLC assay-derived parameters are non-normally distributed, Spearman's rank correlation was used to examine the correlations between M-protein levels and the individual HLC or FLC assay-derived parameters. The correlation analysis involved multiple measurements (median number of 3 consecutive samples) per patient, for most of the patients (75%). In this study, we assumed independence of all observations regardless of which subject they are clustered within [6].

To examine whether one of the HLC or FLC derived parameters was more highly correlated with the M-protein level in the same group of individuals, we tested the difference between two dependent correlations. When several correlations have been retrieved from the same group, this dependence within the data can be used to increase the power of the significance of the test, and hence a dependent correlation analysis: a statistical method for comparison of correlations from dependent samples was performed as described previously [7]. To compare the correlations between the same variable and M-protein levels between IgG and IgA MM, we tested the difference between independent correlations using Fisher's z transformation. The null hypothesis in both dependent and independent correlation analyses was that there is no difference between the two specific correlation coefficients under consideration.

To assess whether distribution of iHLC and rHLC values was different according to response categories, one-way analysis of variance analysis and the Kruskal-Wallis test were performed, respectively. The null hypothesis was that there is no difference between iHLC or rHLC values according to response categories. Scheffé's test was used for post-hoc analysis.

MedCalc version 14.12.0 (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses. $P < .05$ was considered statistically significant.