Original Study

Abnormal Heavy/Light Chain Ratio and Matched Pair Suppression Increase Residual Disease Detection Sensitivity in Patients With Multiple Myeloma With Deep Responses

Kanji Miyazaki, Kenshi Suzuki

Abstract

Heavy/light chain (HLC) assay can quantify involved as well as uninvolved immunoglobulin pairs. We compared the sensitivity between HLC assay and other examinations in patients with multiple myeloma. The lower the monoclonal protein levels, the more the possibility that the patients had normal HLC ratios and no matched pair suppression. Abnormal HLC ratios and HLC-matched pair suppression can increase the sensitivity.

Background: Heavy/light chain (HLC) assay can quantify involved as well as uninvolved immunoglobulin pairs and is used to detect monoclonal proteins. **Patients and Methods:** We compared the sensitivity between HLC assay and serum protein electrophoresis, serum immunofixation electrophoresis (IFE), and free light chain (FLC) assay in patients with symptomatic multiple myeloma (n = 111) whose responses were stable disease or better. **Results:** Among patients with negative IFE and normal FLC ratios, 84.4% (38 of 45) and 80% (36 of 45) exhibited normal HLC ratios and no pair suppression, respectively (13.3% [6 of 45], moderate pair suppression and 6.7% [3 of 45], severe pair suppression). The lower the monoclonal protein levels, the more the possibility that the patients had normal HLC ratios and no matched pair suppression (both $P < .000001$). HLC ratios or pair suppression combined with IFE results and FLC ratios were more sensitive for detecting monoclonal proteins than were IFE results and FLC ratios alone ($P = .016$ and .0039, respectively). A combination of all 4 methods (IFE, FLC, HLC, and pair suppression) was far more sensitive than were IFE findings plus FLC ratios alone ($P = .00024$). **Conclusion:** Abnormal HLC ratios and HLC-matched pair suppression can increase the sensitivity for detecting residual disease in patients with multiple myeloma with deep responses.

Clinical Lymphoma, Myeloma & Leukemia, Vol. ■, No. ■, ■-■
Keywords: Free light chain, Hevylite, Immunofixation, Minimal residual disease, Pair suppression

Introduction

Recently, minimal residual disease (MRD) detection in patients with multiple myeloma (MM) has become imperative because many patients with MM achieve deep responses, and the relation between the depth of response and survival has been elucidated.[1] Patients with deeper responses have better progression-free survival and overall survival.[1] MRD negativity in the bone marrow detected using multiparameter flow cytometry immunophenotyping is also associated with superior progression-free survival.[2] In 2016, the International Myeloma Working Group advocated criteria for response and MRD assessment.[3] Currently, achieving deep response is an important goal for the treatment of MM.

Heavy/light chain (HLC) assay (Hevylite; Binding Site, Birmingham, United Kingdom) can provide accurate quantification of involved as well as uninvolved immunoglobulin (Ig) pairs.[4] One study reported that an abnormal HLC ratio was detected in approximately 25% of patients with complete response (CR).[5] In addition, HLC-matched pair suppression, which indicates the suppression of the uninvolved Ig pair, at the best response was correlated with subsequent survival.[6] HLC ratios as well as pair suppression at the best response are useful. HLC assay can be performed using peripheral blood and does not require bone marrow examination. Furthermore, it is easier to perform than multiparameter flow cytometry or next-generation sequencing, which requires bone marrow samples.

Department of Hematology, Japanese Red Cross Medical Center, Tokyo, Japan

Submitted: Aug 2, 2017; Revised: Jan 16, 2018; Accepted: Jan 30, 2018

Address for correspondence: Kanji Miyazaki, MD, Department of Hematology, Japanese Red Cross Medical Center, 4-1-22 Hiroo, Shibuya-ku, Tokyo 150-8935, Japan Fax: 81-3-3400-1604; e-mail contact: miyazaki_kanji@med.jrc.or.jp

2152-2650/$ - see frontmatter
https://doi.org/10.1016/j.clml.2018.01.008

To the best of our knowledge, there is no report on the utility of HLC ratios as well as pair suppression for residual disease detection. In this prospective study, we compared the sensitivity of HLC assays with that of serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (IFE), and free light chain (FLC) assay in patients with MM. The aim of this study was to explore the sensitivity of HLC assays, particularly in patients with MM with deep responses.

Patients and Methods

Patients

This prospective study included patients with symptomatic MM, whose responses were stable disease or better, as defined by the International Myeloma Working Group.[3,7] Patients with Bence Jones proteins only, IgD or IgE disease type, or coexisting amyloidosis were excluded from this study. Blood samples were analyzed using SPEP with or without immunosubtraction (CAPILLARYS 2; Capillary System; Sebia, Evry-Paris, France), serum IFE (LSI Medience, Tokyo, Japan), FLC assay (LSI Medience), and HLC assay (Hevylite; Binding Site) between March 2016 and June 2017 at the Japanese Red Cross Medical Center, Tokyo, Japan. Simultaneous bone marrow or urine examinations were not performed in this study. All patients provided written informed consent before the HLC assay tests were performed. The institutional review board of the Japanese Red Cross Medical Center approved this study.

Heavy/Light Chain Assay

Heavy/light chain assay was performed according to the manufacturer's instructions, which are described elsewhere.[4-6] The reference ranges for IgG κ and IgG λ were 3.84 to 12.07 g/L and 1.91 to 6.74 mg/L, respectively, and the IgG κ/λ ratio was 1.12 to 3.21. The reference ranges for IgA κ and IgA λ were 0.57 to 2.08 g/L and 0.44 to 2.04 mg/L, respectively, and the IgA κ/λ ratio was 0.78 to 1.94, according to the protocols of Binding Site. The limit of detection was 0.05 g/L for IgG κ, 0.03 for IgG λ, 0.007 g/L for IgA κ, and 0.005 g/L for IgA λ. HLC-matched pair suppression was defined as reduction below the lower limit of normal of the respective isotypes: $>$ 50% reduction was defined as severe HLC-matched pair suppression and $\leq$ 50% as moderate HLC-matched pair suppression.[6]

Other Commercially Available Tests

Serum protein electrophoresis, IFE, and FLC assays were commercially available. The evaluators were uninformed of the results of the HLC assay, and the results were used in this study without our re-evaluation. IFE was performed using a commercially available kit (Helena Laboratories, Saitama, Japan). The limit of detection for serum IFE used in this study was 50 mg/dL. The FLC assay was performed using Freelite (Binding Site). The reference ranges for κ and λ were 2.42 to 18.92 mg/L and 4.44 to 26.18 mg/L, respectively, and the κ/λ ratio was 0.248 to 1.804, according to LSI Medience. The limits of detection for the FLC assay were $<$ 0.5 mg/L and $>$ 3800 mg/L. SPEP with or without immunosubtraction was performed using CAPILLARYS 2, according to the manufacturer's instructions, which are described elsewhere.[8,9] SPEP with immunosubtraction is more sensitive for monoclonal protein detection than is SPEP alone.[9]

Statistical Analysis

Heavy/light chain ratios were classified as positive or negative and HLC pair suppression results as severe, moderate, or within normal limits. We explored the sensitivity of HLC and other assays in patients with MM who were undergoing treatment. The combined results of the SPEP, IFE, and FLC assays were used as the reference standard. More than 2 independent nonparametric samples were compared using the Kruskal–Wallis test. Binomial proportion confidence intervals were calculated, and the differences in sensitivity were evaluated using the McNemar test. All statistical analyses were performed using SPSS version 18.0 (IBM Corp, Armonk, NY).

Results

A total of 111 patients were evaluated (Table 1). The median age of the patients (61 male and 50 female) was 68 years (range, 39-89 years). The number of patients with IgG and IgA disease types was 86 and 25, respectively. One patient exhibited false positivity for FLC: a patient with IgG λ type myeloma had an abnormally high κ ratio (κ, 26.5 mg/L; λ, 14.5 mg/L; ratio, 1.828); this sample was considered to represent the normal values for FLC in this study. No false positive results for HLC ratio and pair suppression were observed.

The results of SPEP, IFE, FLC, and HLC examinations are shown in Table 2. The HLC ratios of patients with $>$ 1000 mg/dL of monoclonal proteins detected using SPEP were all abnormal (26 of 26). The HLC ratios of patients with 500 to 1000 mg/dL of monoclonal proteins were 87.5% (14 of 16) and 12.5% (2 of 16) for abnormal and normal ratios, respectively. Among the patients with positive IFE and $<$ 500 mg/dL of monoclonal proteins, 53.3% (8 of 15) having abnormal and 46.7% (7 of 15) having normal ratios of HLC were noted. All of the patients (9 if 9) with negative IFE and abnormal FLC ratios had normal HLC ratios. Among the patients with negative IFE as well as normal FLC, 15.6% (7 of 45) had abnormal and 84.4% (38 of 45) had normal HLC ratios. The lower the detected monoclonal protein levels, the greater the number of patients with normal HLC ratios; this finding was significant ($P < .000001$).

Table 1 Patient Characteristics

Characteristic	Patients With Multiple Myeloma (n = 111)
Age, Years	
Median	68
Range	39-89
Sex, n (%)	
Male	61 (55)
Female	50 (45)
Heavy Chain Type, n (%)	
IgG	86 (77)
IgA	25 (23)
Involved Light Chain Type, n (%)	
κ	71 (64)
λ	40 (36)