The Utility of The Serum Heavy/Light Chain Assay as a Complementary Tool in the Monitoring of Patients with Plasma Cell Myeloma: a Report of Three Cases

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Abstract. We report three cases of plasma cell myeloma with obscure and discordant data for a monoclonal component. In this study, the results of serum heavy/light chain (sHLC) were retrospectively compared with those of conventional methods during disease monitoring. All three patients achieved a complete response and experienced a relapse during follow-up, and the sHLC ratio allowed early prediction of disease relapse and correlated well with other electrophoretic methods compared with the free light chain ratio. Therefore, we suggest that the sHLC assay may be useful as a complementary tool; it has a good correlation with conventional methods and sensitivity in assessing disease status and treatment response in patients with plasma cell myeloma.

Key words: Heavy/light chain, plasma cell myeloma, monitoring

Introduction

Plasma cell myeloma (PCM) is characterized by the production of monoclonal protein (M protein) in bone marrow and presents in serum and urine [1]. Conventional methods to detect monoclonal immunoglobulin (Ig), such as serum protein electrophoresis (sPEP), serum immunofixation electrophoresis (sIFE) and serum free light chain (sFLC) assay, are mainly used for the diagnosis and serial monitoring of PCM according to international guidelines [2]. Because of the heterogeneous disease course of PCM and the introduction of novel agents and stem cell transplantation, both a small level of M protein overlaying normal serum levels of proteins on protein electrophoresis and a weak band or an abnormal protein band (APB) on sIFE are often obscured. Discordant results when using conventional methods make it difficult to assess the patient’s disease status and treatment response. Although the sFLC has excellent sensitivity, it cannot detect whole Igs or those with only a heavy chain protein or little free light chain [3-5]. A novel method for quantifying serum intact Ig heavy/light chain (sHLC) pairs using antibodies specific for the junctional epitopes of each Ig was recently developed, and several studies have evaluated its performance in detecting M protein levels and determining disease progression and response to therapy. Those studies have correlated well with clinical responses to changes in sHLC ratios [5-10]. Here, we report three patients with obscure and discordant data during monitoring of PCM.

Materials and Methods

Patients and serial samples. In this study, we retrospectively analyzed serial data from three patients with PCM who had discordant results among different M protein measurements and developed APBs such as oligoclonal bands, isotype-switching bands, or single protein bands during monitoring. In all three cases, M protein was serologically identified from diagnosis. Fifty-four frozen sera samples from the three patients were assayed for the sHLC ratio. The results among those methods for detecting malignant plasma cell clones were compared, but only in the same periods. We evaluated only serological data for defining disease status in these patients. Each patient underwent a different treatment regimen with only chemotherapy or a combination of chemotherapy and autologous stem cell transplantation (ASCT) and/or
radiotherapy. All three patients achieved complete response (CR) after treatment and experienced a relapse at least once during monitoring. The diagnosis of PCM was established according to the 2008 World Health Organization classification [1], and the disease status of the patients was defined according to the European Blood and Bone Marrow criteria [2]. The study was approved by the Institutional Review Board of the Korea Cancer Center Hospital, and all patients gave written informed consent.

Laboratory methods. Sequential analysis of M protein markers was performed using the following methods. Total IgG and IgA (Roche Diagnostics, Mannheim, Baden-Württemberg, Germany) were analyzed using the Cobas Integra® 800 (Roche Diagnostics). Detection of the monoclonal components in sPEP and sIFE (Hydragel; Sebia, Evry, Essonne, France) was performed using the HYDRASYS 2 analyzer (Sebia). sFLC using polyclonal sheep antisera (Freelite®, Binding Site, Birmingham, England) was performed using the Roche/Hitachi Modular P800 (Roche Diagnostics, Naka, Japan). The heavy/light chain (HLC) pair assay was a nephelometric assay (SPAPLUS; Binding Site, Birmingham, England) using antibodies specific for the junctional epitopes of each Ig (i.e., IgGκ, IgGλ, IgAκ, and IgAλ) and allowed quantitative assessment of each Ig HLC combination (Hevylite®; Binding Site, Birmingham, England).

Association between sHLC assay and sFLC assay were tested using Pearson’s correlation analysis. The statistical analyses were performed using SPSS (SPSS version 19, IBM, Armonk, NY, USA).

Case Reports

Case 1. A 40-year-old woman was admitted to the hospital in August 2008 because of pain in the left leg that was unresponsive to treatment at local clinics. She presented with anemia (hemoglobin level of 10.8 g/dL) and an M protein level of 4.5 g/dL on sPEP. sIFE demonstrated monoclonal IgAκ type protein. The patient’s IgA concentration was markedly increased to 40.60 g/L (normal range: 0.845-4.99 g/L), and osteolytic lesions of the proximal tibia and humerus were revealed on simple radiography and magnetic resonance imaging. Bone biopsy of the proximal tibia on the left side confirmed plasmacytoma. The patient achieved CR in February 2009 after undergoing chemotherapy (5 cycles of vincristine, doxorubicin, and dexamethasone; VAD, vincristine, doxorubicin, dexamethasone; PAD, bortezomib, doxorubicin, dexamethasone; VD, bortezomib, dexamethasone). Earlier increases in the HLC ratio compared with the FLC ratio are marked with red arrows. Changes in the patient’s drug regimen are marked in boxes with arrows at the top of the figure. Abbreviations: ASCT, autologous stem cell transplantation; CR, complete response; FLC, free light chain; HLC, heavy/light chain; Ig, immunoglobulin; M, melphalan; NR, normal range; PAD, bortezomib, doxorubicin, dexamethasone; VAD, vincristine, doxorubicin, dexamethasone. *Oligoclonal bands (IgG, IgM, λ) were shown on serum immunofixation electrophoresis (sIFE). **Oligoclonal bands (IgG, IgM, λ) and original monoclonal bands (IgAκ) were shown on sIFE.
ratio became normal and remained so during follow-up, even though sPEP and sIFE showed an obvious monoclonal gammopathy pattern. After a recurrence of myeloma was confirmed, the patient restarted chemotherapy and achieved CR again. Oligoclonal bands were found after the second ASCT during monitoring. A second relapse was confirmed at the last follow-up in April 2011 based on abnormal serum measurements of M protein, although the sFLC ratio was normal.

**Case 2.** In December 2004, a 37-year-old man with a history of acute myocardial infarction was admitted to the hospital because of severe back and flank pain. IgAλ type PCM including anemia was diagnosed, with an M protein level of 5.5 g/dL on sPEP. Bone marrow aspiration and biopsy confirmed 35.3% plasma cell involvement. The patient achieved CR after chemotherapy with thalidomide-dexamethasone. After ASCT, oligoclonal bands including IgGκ, two κ, and λ bands appeared without M protein on sPEP, and the sFLC ratio was within the normal range. After two months, the patient had no abnormal findings on sIFE and sPEP until January 2010, when suspicious monoclonal bands started to present and persisted on sIFE with increasing M protein on sPEP during monitoring. During that time, sFLC ratio was normal until the M protein level reached 1.6 g/dL.

**Case 3.** A 69-year-old woman received a diagnosis of IgGλ type PCM in March 2007. The initial M protein level was 1.1 g/dL according to sPEP. Bone marrow aspiration and biopsy showed 13% plasma cells. The patient achieved CR after several cycles of chemotherapy (2 cycles of melphalan-pamidronate and 4 cycles of bortezomib-dexamethasone) in June 2007. During monitoring, weak bands indicating a suspicion of M protein on sPEP (0.3 g/dL) and an IgGλ band on sIFE were observed, but a normal sFLC ratio was observed. After six months, prominent monoclonal bands on sPEP and sIFE developed, and abnormal ratios were found on sFLC. The patient restarted chemotherapy with melphalan-pamidronate after confirmation of relapse. The M protein level on sPEP decreased during treatment, but abnormal results continued for seven months. However, an abnormal sFLC ratio was demonstrated for only four months. Two lambda bands appeared, but normal results were found for total IgG level, sPEP, and sFLC in January 2012. Abnormal results (M protein level of 0.4 g/dL) for sPEP and sIFE were detected after two months, but the sFLC ratio was normal.

**Results and Discussion**

PCM is considered an incurable malignancy, but the disease usually progresses slowly, and survival is improving with the development of novel treatments.Serial measurement of serum M protein levels is useful for identifying the response to therapy for PCM and can assist in making therapeutic decisions. Early detection of M protein activity also allows early treatment and defends against further
disease progression. Therefore, monitoring patients with serological testing is essential for the detection of relapse. Recently, a new method for quantifying intact immunoglobulin (Ig) heavy/light chain (HLC) was developed and several studies showed that sHLC ratios had good correlations with clinical responses and prognosis in PCM patients [5-10].

In this report, we retrospectively analyzed serial samples from three patients to determine the utility of HLC assay in the monitoring of PCM. The specific Ig HLC pairs showed equivocal and/or discordant results for sPEP, sIFE, and sFLC ratio during monitoring. The patient in Case 1 presented with an abnormal IgAκ/IgAλ ratio that correlated relatively well with total IgA level, M protein level of sPEP, and IgAκ band of sIFE, except the κ/λ ratio. During both the first and the second relapse, abnormal results were observed for sPEP, sIFE, total IgA level, and sHLC ratio, but not sFLC ratio. The sFLC ratio was abnormal during the early period of the first relapse but returned to normal. However, abnormal results were found for the other monoclonal parameters before the second CR (Figure 1). In Case 2, an abnormal IgAκ/IgAλ ratio persisted for longer during the early treatment course before ASCT; however, the sFLC ratio became normal. During the relapse, an abnormal result was detected earlier in the sHLC ratio than in the sFLC ratio (Figure 2). Lastly, in Case 3, an abnormal IgGκ/IgGλ ratio was observed in the sample that had previously showed weak bands. In addition, an abnormal transition of the sHLC ratio was observed earlier than that of the sFLC ratio during the first and second relapses (Figure 3). During monitoring, there was no relationship between the sHLC ratios and the sFLC ratios (r=0.237, p=0.415, Table 1) in Case 1, although good correlation between them was shown in Cases 2 and 3 (r=0.903, p=0.000 and r=0.702, p=0.000, respectively).

Because persistently normal FLC ratios were seen in these three patients who were suspected of experiencing a relapse on the basis of other serum parameters, this did not appear to be a proper tool to monitor disease progression in our series. Although the sFLC assay has been approved for diagnosis and monitoring with high sensitivity, and the relationship between the sHLC ratios and the sFLC ratios was highly comparable in Case 2 and 3, it could not
detect the M protein found earlier in sHLC assay during follow-up of the three patients, especially in determination of relapse. No correlation between the sHLC ratios and the sFLC ratios in Case 1 might indicate discordant amount of the production of heavy chains and light chains in malignant plasma cells during early relapse [11]. In addition, the sFLC assay was essentially unable to detect whole Igs, and low levels of monoclonal FLC in serum can be masked by sufficient levels of polyclonal heavy chains [3-5]. It was much more difficult to judge the disease state with normal results using conventional methods and/or sFLC assay around the time of relapse, even though other clinical findings or symptoms were abnormal. The use of serial sHLC assays in patients treated with chemotherapy and/or ASCT correlated well with other parameters for the monitoring of disease progression or regression.

In addition, all three cases involved APBs on sIFE during therapy. When APBs appeared, the sHLC ratio was measured and demonstrated normal values, except when oligoclonal bands (IgG, IgM, λ) were shown and in both original monoclonal bands (IgAκ) in Case 1 (Figure 1). The appearance of APBs causes difficulties in decisions regarding patient management, although immune reconstruction after chemotherapy and transplantation has been proposed in many studies [12-17]. The use of the sHLC assay may help to assess APBs.

Unfortunately, this study was based on a small number of patients, and the samples in each method were not strictly sequential from the time of diagnosis of PCM. Each patient had different intervals of follow-up, and the method of measurement of M protein was not always identical to the previous methods. Also, serial HLC measurements were not allowed from the first diagnosis of PCM. Well-designed further studies are needed to determine the beneficial effect of HLCs for the early detection of treatment response and relapse.

In our study, serial serum levels of M protein in sPEP, sIFE, sFLC, and sHLC ratio were all useful for monitoring disease activity during therapy, and the HLC assay was not always superior to the other conventional methods. To date, the HLC assay may not be a substitute for sIFE but can serve as an adjunct in the diagnosis of early recurrence. In summary, we present serial data demonstrating that the

### Table 1. The comparison between sHLC ratio and sFLC ratio in Case 1 (IgAκ type) during monitoring.

<table>
<thead>
<tr>
<th>Time (years) after diagnosis</th>
<th>sHLC ratio (IgAκ/IgAλ, normal range: 0.78-1.94)</th>
<th>sFLC ratio (κ/λ, normal range: 0.26-1.65)</th>
<th>Pearson’s correlation</th>
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<td>0.1</td>
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<tr>
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<td>0.41</td>
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<tr>
<td>1.6</td>
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<tr>
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<tr>
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<td>2.0</td>
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<td>0.84</td>
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<tr>
<td>2.6</td>
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<tr>
<td>→ 2.7</td>
<td>48.25</td>
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Abbreviations: sHLC, serum heavy/light chain; sFLC, serum free light chain. Arrows: the time of diagnosis of relapse.
sHLC ratio may be a useful monitoring tool for the interpretation of obscure or discordant findings for sPEP, sIFE, and sFLC ratio that may subsequently indicate relapse. Therefore, monitoring of the sHLC ratio may allow more knowledgeable patient management.

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References