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Characteristics of unexpected protein bands in multiple myeloma patients after autologous stem cell transplantation

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ABSTRACT

Objectives: The aim of this study is to investigate the characteristics of unexpected protein bands (UPBs) in patients with multiple myeloma (MM).

Design and methods: Individuals diagnosed with MM ($n = 193$) were enrolled. Their medical records and IFE patterns were reviewed.

Results: Of the patients that underwent ASCT, 54% developed UPBs. The median time for UPB appearance and duration was 1.8 and 5.7 months, respectively. IFE revealed 74.1% of UPBs to be of the immunoglobulin G type and 72.2% to be of the κ-type. At UPB appearance, 42.6% of patients were defined as sCR or CR, and 50.0% of the patients satisfying the CR criteria had an abnormal FLC ratio. Of the patients who developed UPBs, five relapsed. Among these, four patients showed disappearance of the previous IFE oligoclonality and reappearance of the original paraprotein at relapse.

Conclusions: Close follow-up of UPBs is critical for evaluating MM therapeutic response and disease progression. The presence of monoclonal bands may indicate relapse of disease, but in the vast majority of cases with UPBs, it does not; instead, it most likely represents a transient phenomenon caused by the immune response.

Introduction

Multiple myeloma (MM) is characterized by the presence of paraproteins, immunologically identical monoclonal antibodies generated by clonal proliferation of plasma cells. Although MM-related paraproteins can switch isotypes during relapse, paraproteins usually remain unchanged throughout disease progression [1].

The recommended clinical laboratory tests for the diagnosis and follow-up of MM include complete blood cell count, determination of calcium and creatinine levels, serum free light chain (FLC) assay, urine or serum protein electrophoresis (EP), immunofixation electrophoresis (IFE), cytogenetic tests, and others [2–4]. Paraproteins can be identified by EP and IFE, although EP is not sensitive enough to detect small amounts of paraproteins and immunoglobulin (Ig) heavy and light chain class. Consequently, the initial EP should always be performed in combination with IFE to confirm monoclonality, and to determine the Ig heavy and light chain class [5].

The widespread use of autologous stem cell transplantation (ASCT) and the introduction of novel drugs have contributed enormously to the treatment outcome and prognosis of MM [6,7]. However, in patients who have undergone ASCT, the isotypes and locations of IFE bands frequently differ from that observed at diagnosis [1,8,9], which can complicate the interpretation of results. For these reasons, this study aimed to investigate the characteristics and clinical implications of unexpected protein bands (UPBs) by retrospective review of medical records and serum or urine IFE test results of MM patients who had undergone ASCT.

Methods

Study population

A retrospective study was conducted on MM patients treated at the Asan Medical Center in Seoul, Korea from January 2007 to January 2012. A total of 193 patients were included in the study. Of these, 100 patients (49 males and 51 females; median age 59 years) underwent ASCT and 93 MM patients (54 males and 39 females; median age 70) did not undergo ASCT. Induction chemotherapy was implemented as prescribed by a physician. The study was approved by the Institutional Review Board of the Asan Medical Center, in accordance with the World Medical Association's (WMA) Declaration of Helsinki.

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Immunofixation electrophoresis

The Beckman Paragon Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) was used for detection of paraprotein isotypes from January 2007 to April 2009, and the Hydragel 4IF reagent set (Sebia, Paris, France) was used from April 2009 to January 2012. In our laboratory, the lower limit of detection of an M-protein on an immunofixation gel was 12 mg/dL. UPBs were defined by the appearance of Ig bands in different locations or by the presence of different isotypes. Each UPB was categorized as either oligoclonal bands or an isotype switch. The presence of two or more discrete Ig bands of the same isotype was interpreted as oligoclonal bands. The presence of a protein band with a heavy or light chain type that was different from the original paraprotein was interpreted as an isotype switch.

Measurement of hemoglobin, calcium, creatinine and free light chain

Patients' hemoglobin, calcium, and creatinine levels at diagnosis were determined. Hemoglobin was measured by an XE-2100 (Sysmex, Kobe, Japan). Creatinine and calcium were measured by a TBA 200FR (Toshiba Medical Systems, Tokyo, Japan). A serum FLC measurement (Freelite™; Binding Site Ltd., Birmingham, UK) was performed by Siemens Dade Behring Nephelometer II analyzer System (BNII). All assays were performed following the manufacturer's instructions.

Statistical analyses

Statistical analysis was performed using the SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA). A chi-square test was used to analyze the difference in UPB incidence between the ASCT and non-ASCT group. An independent sample *t*-test was used to analyze UPB incidence based on age, initial hemoglobin, serum creatinine, and calcium levels. Fisher's exact test was used to analyze UPB incidence based on the use of novel or conventional drugs. A P value <0.05 was considered to indicate statistical significance.

Results

Patients

Of the 100 patients who had undergone ASCT, 65 patients received novel agents. Among these novel treatments, PAD (bortezomib, doxorubicin, and dexamethasone) was the most frequently used regimen, in 36/65 patients (55%). Thirty-five patients were treated with conventional drugs only. Of these patients, 27/35 (77%) were treated with a VAD (vincristine, doxorubicin, and dexamethasone) regimen.

Unexpected protein bands

UPBs were detected in 54% of patients that underwent ASCT (Fig. 1), while UPBs were detected in only 5/93 patients (5.4%) that did not undergo ASCT ($P< 0.001$). In patients who underwent ASCT, the median time for the appearance and duration of UPBs was 1.8 months (range 0.7–26.4 months) and 5.7 months (range 0.9–25.9 months), respectively. The most common heavy chain isotype was IgG, observed in 74.1%, and the most common light chain isotype was κ, observed in 72.2% of the patients (Table 1). Oligoclonal bands were observed in 38/54 (70.4%) patients with UPBs and an isotype switch was noted in 16 (29.6%) patients (Table 2). Based on the type of induction chemotherapy, UPBs appeared in 24/35 (68.6%) patients treated with conventional drugs and 30/65 (46.2%) patients treated with novel drugs. The incidence of UPBs in patients treated with novel drugs was significantly lower than that of patients treated with conventional drugs ($P = 0.037$).

To determine the association between UPB and clinical status, response criteria according to the International Myeloma Working Group (IMWG) at the time of UPB appearance and disappearance were evaluated. At the moment UPB appeared, the response criteria were categorized as a stringent complete response (sCR) in 10 (18.5%), complete response (CR) in 13 (24.1%), very good partial response (VGPR) in 6 (11.1%), partial response (PR) in 9 (16.7%), stable disease (SD) in 12 (22.2%), progressive disease (PD) in 3 (5.6%), and relapse in 1 (1.9%) patient. UPBs disappeared in 37/54 patients. The other 17 patients had persistent UPB until the last follow-up or loss to follow-up. In 37 patients, evaluation of the response criteria at the time of UPB disappearance resulted in categorization as sCR of 13 (35.1%), CR of 10 (27.0%), VGPR of 5 (13.5%), PR of 1 (2.7%), and SD of 8 (21.6%) patients.

Age, hemoglobin, calcium, and creatinine levels at diagnosis

For the 54 patients with UPBs, the median age at diagnosis was 60 (range 40–70), and median hemoglobin, calcium, and creatinine levels were 10.3 g/dL (range 6.2–17.1 g/dL), 9.2 mg/dL (range 7.4–13.0 mg/dL), and 0.9 mg/dL (range 0.4–10.6 mg/dL), respectively. For 46 patients without UPBs, the median age at diagnosis was 59 (range 46–68), and median hemoglobin, calcium, and creatinine levels were 9.5 g/dL (range 5.4–14.0 g/dL), 9.1 mg/dL (range 7.4–14.1 mg/dL), and 1.0 mg/dL (range 0.4–6.8 mg/dL), respectively. No differences were found in the age ($P = 0.558$), and hemoglobin ($P = 0.103$), calcium ($P = 0.295$), and creatinine ($P = 0.542$) levels, between the groups.

Free light chain ratio

At the time of UPB onset, 48 patients were analyzed by FLC assay, and 25 (52.1%) showed an abnormal FLC ratio (FLCR). Fourteen patients had an FLCR higher than the normal range, which indicates overproduction of κ light chains. However, of these patients, only four showed κ-type UPB, another four patients had λ-type UPB, and six

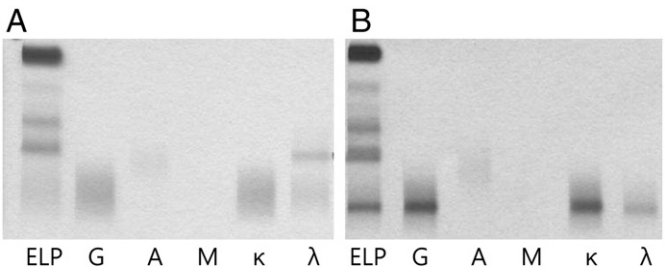

Fig. 1. Representative serum immunofixation electrophoresis (IFE) of a patient who underwent autologous stem cell transplantation (ASCT) for management of multiple myeloma. (A) Free λ monoclonal band at diagnosis. (B) IgG κ and λ type bands detected two months after ASCT.

Table 1
Isotypes and their frequency of unexpected protein bands (UPBs) in 54 multiple myeloma patients who developed UPBs after autologous stem cell transplantation.

Subtype	Number of patients (% frequency)
IgG κ	18 (33.3)
IgG κ and IgG λ	9 (16.7)
Free λ	7 (13.0)
IgG λ	6 (11.1)
IgG κ and free λ	6 (11.1)
Free κ and free λ	3 (5.6)
Free κ	2 (3.7)
IgG κ and IgA λ	1 (1.9)
IgM λ	1 (1.9)
IgA λ	1 (1.9)

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