Case Report

Biphenotypic plasma cell myeloma: two cases of plasma cell neoplasm with a coexpression of kappa and lambda light chains

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Abstract: Plasma cell neoplasm (PCM) is a medullary and extra medullary proliferation of clonal plasma cells that occurs due to accidental translocation of proto-oncogenes into immunoglobulin (Ig) gene loci. While the majority of plasma cell neoplasms are monoclonal, up to 2% of the PCMs [1] considered being biclonal based on electro-phoretic analysis, characterized by secretion of paraprotein with two distinct heavy chains or light chains are possible and present unique diagnostic challenges. Methods: Traditionally protein electrophoresis has been used to diagnose, characterize, and monitor progression of plasma cell neoplasm. To characterize neoplastic plasma cells, in our institution, other ancillary studies, including in situ hybridization, flow cytometric analyses of plasma cell surface markers and cytoplasmic immunoglobulins with DNA ploidy, are also utilized routinely. Results: We present two cases of plasma cell myeloma in which the neoplastic plasma cells shows production of cytoplasmic kappa and lambda light chain, with secretion of free lambda light chain only. Co-expression of kappa and lambda light chain by the same neoplastic plasma cells is a rare but reported phenomenon. Conclusions: Our study indicates that serum electrophoresis alone could mischaracterize biphenotypic myeloma as monotypic plasma cell myelomas in the absence of additional testing methods.

Keywords: Biphenotypic plasma cell myeloma, light chain coexpression

Introduction

Plasma cell myeloma (PCM) is a second most common hematological malignancy accounting for 13% of all hematological cancers [1, 2]. Plasma cell myeloma is characterized by proliferation of immunoglobulin producing/secreting plasma cells in the bone marrow, presence of paraprotein in the blood or urine and associated end organ damage. The diagnosis of plasma cell myeloma is based on a combination of clinicopathological and radiological features. Incidence of plasma cell myeloma increases with age and majority of the patient population is over 50 years old. These patients typically presents with sign and symptoms associated with anemia, hypercalcemia, bone abnormalities, renal dysfunction and systemic infections. The exact etiology of MM is not yet known. However, exposure to certain chemical (dioxins, cleaners and solvents), radiation and viral infections (HSV8, HIV, HepB and EBV) have been associated with the development of plasma cell myeloma [2].

Plasma cells are terminally differentiated, non-dividing effector B cells. It develops from naïve marginal zone B cells and follicular B cells after antigen encounter [3]. The most important function of plasma cells is to produce immunoglobulin which is central to the body’s adaptive immune response to foreign antigens. Immunoglobulin is a secretory or cell surface bound protein which is composed of two heavy (α, γ, δ, ε or μ) and two light chain (κ or λ) peptides that together form a tetrameric complex. Based on the presence of distinct heavy chain polypeptide sequence, immunoglobulin can be classified and sub classified into IgA (1-2), IgG (1-4), IgD, IgE and IgM [4]. Human genome harbors three known gene loci for immunoglobulin synthesis including heavy chain gene locus on chromosome 14q32, kappa light chain gene locus on chromosome 2p11.2 and lambda light
chain gene locus on chromosome 22q11.1. During the process of B cell maturation, these loci undergo a series of genetic alterations including chromosomal gene rearrangement, somatic hypermutation, class switch recombination and allelic exclusion. These molecular events not only play a critical role in survival of maturing B cell, but it also confine plasma cells in producing immunoglobulins that is antigen specific as well as class and isotype restricted [3, 4]. Although myriads of chromosomal alterations are known to occur in plasma cell myeloma, including those involving immunoglobulin gene loci, the neoplastic plasma cells in majority of these cases retain the ability to produce either complete immunoglobulin or at least fragment paraproteins.

Based on the presence or absence and the type of paraproteins secreted, plasma cell myeloma can be broadly categorized into those that secrete complete immunoglobulin, light chain only, non-secretory and very rarely non-producer myeloma. Recently, the analysis was done by International Myeloma Working Group to study expression pattern of different paraproteins in patients with multiple myeloma. Based on their analysis of 10,000 myeloma cases, the frequency at which these paraproteins were observed are as follows, IgG (60%), IgA (24%), IgD (2%), IgM (0.5%), very rarely IgE (only 39 reported cases), light chain only myeloma (11%) and non-secretory myeloma (less than 1%). Interestingly, up to 2% of myeloma cases were also found to secrete more than one paraprotein [5]. Majority of these cases secrete two different heavy chain isotypes or subclasses, which classified as biclonal plasma cell myeloma [6]. And there are only very few reported cases in the literature in which the myeloma cells were found to be expressing both kappa and lambda light chain [7-9]. Unfortunately, most of these cases have not been studied in enough details to confirm whether these paraproteins are the product of one or two independent myeloma clones within the same individual. Here we report two cases of plasma cell myeloma in which the same neoplastic clones simultaneously express both

Figure 1. Plasma cell myeloma expressing IgG heavy chain with co-expression of kappa and lambda light chain. (A) Hematoxylin and eosin stain showing diffuse infiltration of bone marrow by myeloma cells. (B) Immunohistochemical staining of bone marrow biopsy showing diffuse positive staining for CD138. (C) and (D) In-situ hybridization detecting kappa and lambda light chain mRNA in bone marrow biopsy.
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kappa and lambda light chain as confirmed by three independent assays.

Methods and results

Case report 1

A 52 year old male with a 13 year history of lambda light chain myeloma was referred to our institution for workup and management of myeloma complications including pathologic fracture of femur, renal failure and amyloidosis associated cardiomyopathy.

Serum protein electrophoresis by capillary electrophoresis (Sebia Inc., Norcross, GA) revealed monoclonal lambda light chain gammopathy, consistent with the reports from outside institution (OSH). At that time, quantitative serum immunoglobulin analysis showed decreased IgA (30 mg/dl; reference range 40-230 mg/dl) and IgM (47 mg/dl; reference range 70-400 mg/dl) levels with a normal IgG (741 mg/dl; reference range 700-1600 mg/dl) level. Serum immunoglobulin free light chain study reveals increased lambda free light chain (45 mg/dl; reference range 0.57-2.63 mg/dl) and normal kappa free light chain (1.09 mg/dl; reference range 0.33-1.94 mg/dl) levels. Hematoxylin and eosin stained slides (Dako. Inc.) of the bone marrow biopsy confirmed diffuse (~40%) involvement of bone marrow by sheets of polymorphous appearing plasma cells showing cellular and nuclear pleomorphism and prominent nucleoli. These cells were diffusely positive for CD138 on immunohistochemical analysis (Ventana medical system, Oro Valley, AZ). Interestingly, in situ hybridization studies (Ventana medical system, Oro Valley, AZ), targeting light chain mRNA showed coexpression of kappa and lambda light chain mRNA by neoplastic plasma cells (Figure 1). However, antibodies targeting heavy chain proteins of IgM, IgG, IgA and IgD failed to detect expression of heavy chain peptides by neoplastic plasma cells (data not shown). To determine whether these plasma cells coexpress light chains, we performed two different methods of flow cytometric analysis of bone marrow aspirate (BD Biosciences, CA). Immunophenotypic flow cytometric analysis identified aberrant plasma cell population expressing bright CD38, CD56 and CD138 with dual expression of cytoplasmic kappa and lambda light chains (Figure 2). A second flow cytometric assay based on quantification of the DNA and cytoplasmic immuno-

Figure 2. Multi-parameter immunophenotypic flow cytometric analysis showing cytoplasmic co-expression of kappa and lambda light chain protein by CD38, CD56 and CD138 positive neoplastic plasma cells.
globulin content [10], identified aberrant population of plasma cells with hyperdiploid DNA with coexpression of kappa and lambda light chain (Figure 3). Chromosomal analysis performed on trypsin-giemsa banded metaphase cells identified multiple clones with complex karyotype. The karyotype was 49~50, XY, add (6) (q13), der (8) t (1; 8) (? q12; p23), +9, del (13) (q12q14), der (14) t (11; 14) (q12~13.1; q32), add (17) (p11.1), +21, +22 (cp2) 49, idem, der (14), +der (14) add (14) (p13) t (11; 14) (q12~13.1; q32), -16 (cp4) 49~50, idem, +add (6) (q13), +t (16; 17) (q11.2; p13), -add (17) (p11.1), -22 (cp6)/46, XY [8].

The patient was initially treated with four cycles of combination chemotherapy including VAD, DCEP, CAD and DCEP, followed by autologous bone marrow transplant. He was in complete remission for over four years and received maintenance therapy that included interferon gamma and dexamethasone. Following his first relapse, he was given induction therapy with M-VTD-PACE, followed by a second autologous bone marrow transplant and consolidation therapy with two cycles of VTD-PACE. He was maintained on VRD regimen for over 8 years, after which he presented with pathologic fracture of femoral shaft. He was then referred to our institution for further evaluation and management.

Case report 2

A 58 year old female presented to her primary care physician at OSH complaining of increasing fatigue, temperature and cough with production of green sputum. Blood work up showed severe anemia, normal WBC and platelet counts and elevated total serum protein level. Chest X-ray revealed features suggestive of left lower lobe pneumonia and a soft tissue lesion involving the first rib. She was admitted to the hospital, transfused with 2 units of packed RBC and appropriate antibiotics were started to treat her pneumonia. A CT scan of the chest, performed to further evaluate a soft tissue lesion, showed multiple lytic lesions of the thoracic vertebrae and ribs. A CT guided biopsy of the first rib lesion was performed which on microscopic examination revealed plasma cell neoplasm. She was then referred to our institution for further evaluation and management.

The blood work up performed in our institution showed normal blood counts, hemoglobin and creatine levels. Serum protein analysis revealed elevated total serum protein levels, elevated IgG lambda (8100 mg/dl; reference range 700-1600 mg/dl) and free lambda light chain (103 mg/dl; reference range 0.57-2.63 mg/dl) levels with decreased IgA (30 mg/dl; reference range 40-230 mg/dl) and IgM (<20 mg/dL; reference range 70-400 mg/dl) levels. Urine immunofixation electrophoresis (Sebia Inc, Norcross, GA) confirmed the presence of free lambda light chains in urine. Imaging studies including whole body PET-CT and MRI scans revealed extensive involvement of axial skeleton with multiple lytic lesions involving the skull, ribs, thoracic and lumbar vertebrae, sacrum and pelvis with extra medullary soft tissue extension especially within the thoracic region.

Microscopic examination of the bone marrow revealed a hypercellular marrow (80% cellularity) comprising predominantly of atypical plasma cells with abundant basophilic cytoplasm, eccentric nuclei and perinuclear hof. Tumor cells were diffusely positive for cytoplasmic IgG
and CD138 by immunohistochemistry. Interestingly, in-situ hybridization studies performed to detect light chain mRNA revealed dual expression of kappa and lambda light chain by the neoplastic plasma cells (Figure 4). To define the coexpression of light chains on these plasma cells performed two different kinds of flow cytometric analysis of bone marrow aspirate. Consistently, immunophenotypic flow cytometric analysis of bone marrow aspirate identified aberrant plasma cell population expressing CD38, CD138 and CD45 (dim) with a dual expression of cytoplasmic kappa and lambda light chains (Figure 5). Furthermore, second flow cytometric assay to quantify the DNA and cytoplasmic immunoglobulin content [10], identified aberrant population of plasma cells with hyperdiploid DNA and coexpression of kappa and lambda light chain (Figure 6). Chromosomal analysis performed on trypsin-giemsa banded metaphase cells identified multiple chromosomal abnormalities, consistent with multiple myeloma. The karyotype was 54, X, add (X) (p22.3), +5, +7, +9, -10, +11, +14, +15, +15, +19, add (19) (p13.3), +21.

The patient was treated with induction therapy that includes combination of M-VTD-PACE followed by autologous bone marrow transplant. She then received consolidation therapy that includes two cycles of VTD-PACE. She is currently under complete remission.

Discussion

Plasma cell myeloma (PCM) results from malignant transformation of PCs or their precursors. The analysis of paraprotein production in multiple myeloma usually involves serum and/or urine protein electrophoresis (SPEP/UPEP) and immunofixation. These methods most often used diagnostic and as well as follow up modalities provide fast and cost effective assessment of patient’s status. But the sole reliance on these techniques to assess the characteristics of plasma cell myeloma can be misleading. Case report-1, is a perfect example of such scenario, where the patient, based on SPEP and UPEP results, was diagnosed with lambda light chain producing plasma cell myeloma for over a decade. The thorough work up of this patient, at our institution, to included additional ancil-

Figure 4. Plasma cell myeloma expressing IgG heavy chain with co-expression of kappa and lambda light chain. (A) Hematoxylin and eosin stain showing diffuse infiltration of bone marrow by myeloma cells. (B) Immunohistochemical staining of bone marrow biopsy showing diffuse positive staining for CD138. (C) and (D) In-situ hybridization detecting kappa and lambda light chain mRNA in bone marrow biopsy.
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Lary studies on bone marrow biopsy and aspirate, like immunohistochemistry (IHC), in-situ hybridization (ISH), and flow cytometry analysis to detect light chain expression pattern at the mRNA and protein levels identified a population of neoplastic plasma cells showing dual expression of kappa and lambda light chains (Figures 1, 2). Biphenotypic nature of these neoplastic cells was further confirmed using a different flow cytometry method determined by quantitative DNA analysis on neoplastic plasma cells in S-phase of the cell cycle (Figure 3) [10]. Similarly, using the above mentioned techniques, we were able to accurately classify a patient with a new diagnosis of biphenotypic light chain myeloma secreting IgG lambda (Figures 4-6). We thus recommend performing comprehensive workup as previously described for accurate classification of multiple myeloma, at least in patients with the first time diagnosis.

There is only handful of cases reported in the literature showing biphenotypic expression of light chain myeloma. Similarly, the current case adds to the rarity of this condition.

Figure 5. Multi-parameter flow cytometric analysis showing cytoplasmic co-expression of kappa and lambda light chain protein by CD38, CD45 (dim) and CD138 positive neoplastic plasma cells.

Figure 6. Flow cytometric analysis of bone marrow aspirate to assess DNA contents showing diploid (normal) and hyperdiploid (neoplastic) cell population (A). Note the presence of cytoplasmic kappa (B) and lambda (C) light chain by hyperdiploid cells.
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light chain in multiple myeloma [7-9]. The frequency of kappa or lambda light chain production observed in plasma cell myeloma (PCM) is similar to that in B-cells of healthy individual. Previous studies confirm that 0.5-2% of normal B cells population show dual expression of kappa and lambda light chain [9, 11, 12]. There are multiple reports in the literature describing dual immunoglobulin expressing B-cell lymphoproliferative disorders, and may be the neoplastic counter parts of these B cells leads to the formation of B cell lymphoma with dual surface immunoglobulin expression [13-15]. Whether biphenotypic light chain myeloma are the result of neoplastic transformation of these dual light chain expressing B-cells is not yet known. But if that is the scenario, then one should expect more frequent occurrence of biphenotypic light chain myeloma than reported, unless their normal counterpart are comparatively more resistant to oncogenic transformation. Interestingly, all reported cases of plasma cell neoplasms with dual expression of light chains, have been shown to secrete only lambda light chain [7-9]. Majority of the times, the immunoglobulin characterization of the PCM involves, techniques that detect secreted serum free immunoglobulins or its fragments but not its cytoplasmic form. The reported low incidence of biphenotypic light chain myeloma could be the result of erroneous misclassification of PCM due to low incidence of serologic and cytoplasmic light chain expression correlation studies by the institutions.

Assembly of a functional IgH-IgL protein complex on the cell surface, the so-called B-cell receptor (BCR), allows B cells to escape apoptosis and proceed with maturation [16, 17]. The possibility of biphenotypic myeloma to be the product of neoplastic transformation of these naive B cells cannot be excluded either. Ig heavy chain gene (IGH) rearrangement (VDJH rearrangement) precedes Ig light chain gene rearrangement, mediated by tightly regulated enzymatic machinery operating at the DNA level. The process of allelic exclusion ensures that once a functional gene rearrangement has been achieved, the other IGH allele is generally excluded from further recombination attempts [1, 18].

Neoplastic transformation in plasma cell myeloma has traditionally been categorized into either pre-switch or post-switch event based on the type of heavy chain produced by neoplastic plasma cells. Naive mature B cells normally produce IgM and IgD heavy chains, which are the first two segments in immunoglobulin heavy chain gene locus. Following exposure to antigen and activation by helper T cells and several cytokines, the B cells undergo a process of class switching. It is characterized by rearrangements within the heavy chain gene locus resulting in different groups of plasma cells producing and expressing IgG, IgA or IgE [3, 19]. Thus it seems logical that neoplastic transformation in plasma cell myeloma producing IgM or IgD heavy chain might have occurred before class switching, whereas in IgG, IgA or IgE producing myeloma’s it is a post switch event.

After successful IGH recombination, the Ig light chain loci proceed to rearrange. Majority of the B cells becomes committed to producing specific type of light chain very early during the maturation process. Initial gene rearrangement happen at the Ig kappa locus (IGK), and if a functional IGK rearrangement is not achieved the Ig lambda locus (IGL) undergoes recombination. Usually, rearrangements of the IGL locus are accompanied by deletion of the non-functional IGK rearrangements. The mechanism responsible for imparting such restrictions is known as allelic exclusion. It involves alteration in genomic DNA resulting in deletion of other light chain gene alleles [1, 3, 19, 20]. Neoplastic transformation of these B cells into plasma cells before the allelic exclusion can thus render these cells with the capabilities to express both kappa and lambda light chain, especially if the cytogenetic abnormalities involves these light chain genes and drives its expression. Interestingly, the molecular mechanisms involved in the process of allelic exclusion are not yet well characterized. One another possibility could simply be the mutations in light chain gene that hinders recognition by DNase during the process of allelic exclusion. So, the formation of biphenotypic myeloma could be due to failure of allelic exclusion machinery.

Despite the nature of cell of origin, the observation that biphenotypic light chain myeloma shows dual cytoplasmic light chain expression and secretion of only kappa or lambda light
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chain indicates that non secreting light chain is not functionally active. The reasons for such functional inactivation were not explored in these cases, but could potentially be at any level failure of synthetic machinery including transcription, translation or posttranslational. Strong cytoplasmic staining of light chain mRNA and protein suggests overexpression of misfolded and/non secretory light chain proteins that overwhels the capacity of cellular degradation machinery. Why this functionally inactive allele is not excluded from the genome as seen in majority of normal B cells needs further scientific venture. Another reason for non-secretion of light chains may be associated with dysregulation of XBP-1 generation, since the XBP-1s encodes a more stable active Ig protein [18].

Described true biclonal myeloma based on the presence of more than one IgH subtype must be carefully reviewed because both serum M components may share the same clonal origin with an identical variable region rather than being 2 independent clones. The cases of biclonal myelomas may be truly independent myeloma clones, with ongoing CSR detectable in subclones derived from the same myeloma progenitor or, alternatively, lack of allelic exclusion within the same myeloma cell [20]. Analysis to identify common karyotypic and cytogenetic abnormalities in these rare biphenotypic light chain myeloma and its comparison with other myeloma phenotypes may help identify unique genetic differences to shed light on DNA modulating machinery. Biphenotypic myeloma thus has potential to serve as a model to characterize the molecular details of allelic exclusion.

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Disclosure of conflict of interest

None.

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