

Follow-up of IgD- κ multiple myeloma by monitoring free light chains and total heavy chain IgD: A case report

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Abstract. Immunoglobulin (Ig)D- κ multiple myeloma (MM) is a rare neoplastic disease characterized by an aggressive and rapidly progressing course, which constitutes only a very small proportion of all MM cases. In the present report, the clinical case of a 51-year-old Caucasian woman diagnosed with IgD- κ MM is described. The patient underwent different chemotherapeutic treatments subsequently to a single autologous stem cell transplantation. Despite the inherent difficulty of monitoring IgD levels and performing serum immunofixation electrophoresis, the clinical outcome of the patient was almost uniquely monitored by measuring the levels of κ and λ free light chains (FLCs) and total heavy chain IgD. The data suggest the non-invasive potential and usefulness of FLCs evaluation for early detection of stringent complete remission, follow-up and early detection of disease relapse. In addition, this diagnostic procedure has successfully been employed for the therapeutic monitoring of the present patient, and may represent a very helpful, non-invasive tool for the follow-up of IgD myeloma patients without the requirement of serial bone marrow aspirate.

Introduction

Multiple myeloma (MM) is a plasma cell (PC) disorder that induces anemia, skeletal destruction, renal failure and hypercalcemia (1). It is the second most common hematological malignancy, and it is characterized by the presence of a monoclonal immunoglobulin (Ig) expressed and secreted in the bone marrow, where there is a collection of abnormal PCs that interfere with the production of normal blood cells (2).

IgD MM constitutes $\leq 2\%$ of all MM cases, displays generally an aggressive phenotype (often with renal failure) and is usually characterized by poor prognosis (3). IgD- κ occurs only in 3-4% of all IgD MM cases, and is also associated with the difficulty of obtaining a reliable diagnosis and of conducting a constant and precise follow-up (4).

The diagnostic panel for IgD- κ MM should include PC flow cytometry characterization, fluorescence *in situ* hybridization (FISH), serum protein electrophoresis (SPE), serum immunofixation electrophoresis (sIFE), measurement of serum free light chains (sFLCs) and total heavy chain IgD, and urinary IFE for Bence Jones (BJ) protein (5).

Diagnostically, with novel agent therapy (including thalidomide, bortezomib and lenalidomide) specifically integrated with autologous stem cells transplantation (ASCT) when feasible, the survival for IgD MM is improved; however, the outcomes remain inferior to those achieved in patients with other myeloma isotypes, thus highlighting the requirement for better and more innovative approaches in treatment and monitoring (6).

The present report describes the follow-up of a case of an IgD-K MM patient, who often refused to undergo a bone marrow aspirate, even in certain critical phases of the disease. Thus, given the occasional inability to obtain bone marrow aspirate samples, at times when a relapse was suspected, minimal residual disease (MRD) was alternatively monitored uniquely by serological evaluation of FLC and total heavy chain IgD levels (7,8).

The current report presents the case of a long survival patient monitored almost exclusively by sFLC and IgD measurements as an essential, non-invasive marker. Written informed consent was obtained from the patient [medical records no. 4249 on June 10, 2013 at Hematology and Stem Cell Transplantation Unit, Italian National Cancer Institute 'Regina Elena' (Rome, Italy)].

Case report

In March 2007, a 51 year-old woman presented for the first time at the Hematology and Stem Cell Transplantation Unit of the Italian National Cancer Institute 'Regina Elena' with

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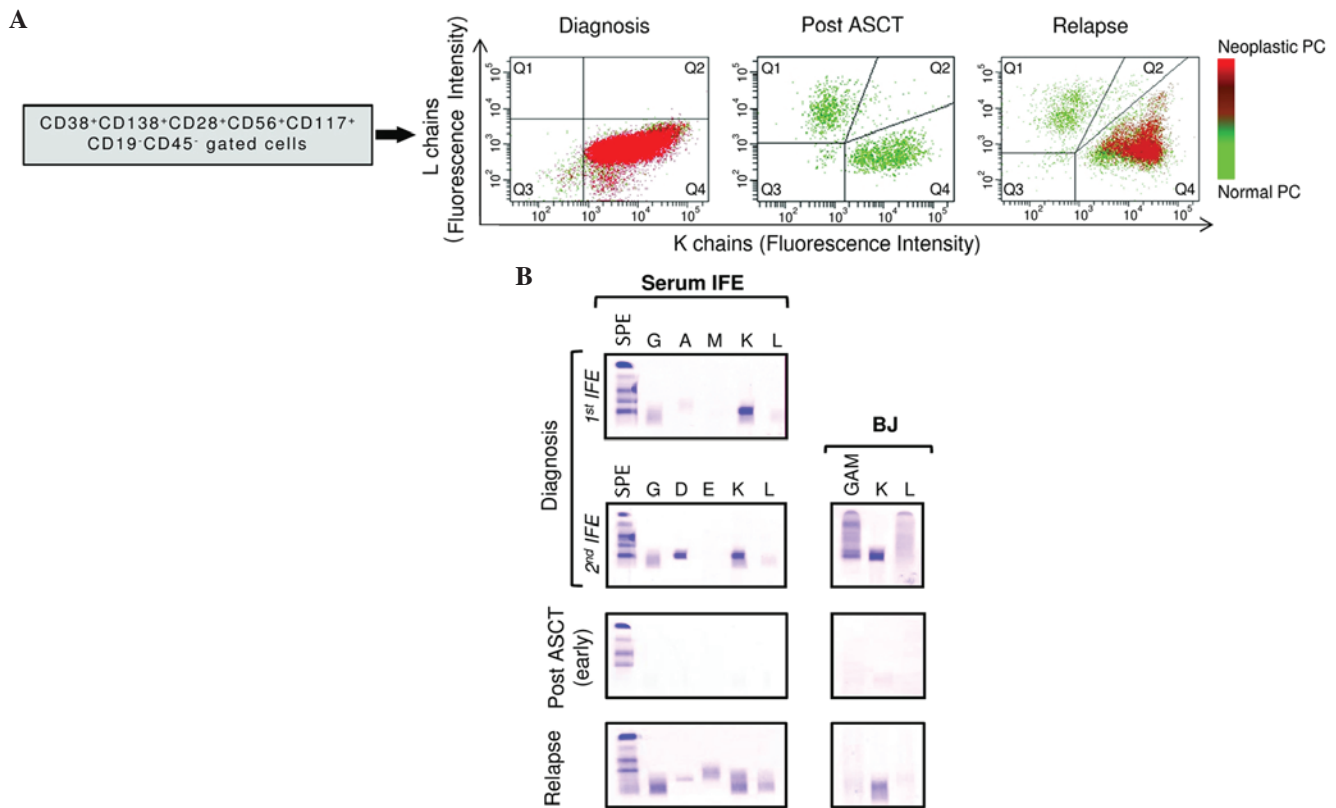


Figure 1. Flow cytometric analysis and IFE detection during patient monitoring. (A) Flow cytometric evaluation of the expression of κ and λ chains in normal vs. malignant PCs at diagnosis, upon ASCT and at relapse. Q1-Q4 represent the distinct regions analyzed by flow cytometry, where Q1 comprises λ -positive PCs and Q4 contains κ -positive ones. The green color in the plots represents normal PCs, whereas the red color depicts the presence of neoplastic PCs. These bone marrow aspirates indicate the presence of neoplastic cells at diagnosis, which disappear following ASCT, while they are still present at the time of relapse. Their progress was coherent with the values of serum free light chains tested (B) IFE and Bence Jones protein at diagnosis, pre/post ASCT and during relapse. The term 'early' inside parentheses refers to the first post-ASCT timepoint. IFE was performed with the immunoglobulin antisera indicated above each lane. IFE, immunofixation electrophoresis; CD, cluster of differentiation; PC, plasma cell; ASCT, autologous stem cells transplantation; BJ, Bence Jones; GAM, mixed antisera against immunoglobulins G, A and M; SPE, serum protein electrophoresis.

multiple osteolytic lesions. PC flow cytometry characterization (FACSCanto™; BD Biosciences, Franklin Lakes, NJ, USA) identified an infiltration (23% of bone marrow population) of cluster of differentiation (CD)38⁺CD138⁺CD28⁺CD56⁺CD117⁺CD19⁻CD45⁻ tumor PCs, with κ -sFLC restriction, as illustrated by flow cytometric analysis at diagnosis (Fig. 1A). Bone marrow examination by FISH revealed no abnormalities.

IFE and BJ protein IFE on urine evidenced the presence of an IgD- κ monoclonal component and κ light chains, respectively (Fig. 1B). In addition, sFLC quantification (The Binding Site Group, Ltd., Birmingham, UK) revealed a marked increase in κ -sFLC with an abnormal FLC κ/λ ratio (Table I). Total heavy chain IgD quantification (The Binding Site Group, Ltd.) confirmed the presence of elevated IgD levels (Fig. 1B and Table I).

The average and standard deviation values of β 2-microglobulin (β 2 M) levels were 7.47 and 3.77 mg/l, respectively (normal range, 0.80-2.20 mg/l). Other classical serological parameters [calcium (Ca)-renal-anemia-bone criteria] were altered. The diagnosis was an IgD- κ MM stage IIIA.

According to the current clinical practice at the Italian National Cancer Institute 'Regina Elena', the patient underwent chemotherapy with two cycles of vincristine, doxorubicin (Adriblastina®) and dexamethasone (VAD regimen) from April until May 2007 (9). The patient was then treated with

two cycles of cyclophosphamide (Endoxan Baxter®) from July until November 2007, and four subsequent cycles of bortezomib (Velcade®), Myocet® and dexamethasone (BMD regimen) from November 2007 until February 2008 (Fig. 2 and Table I). The FLC measurements and total IgD evaluations commenced upon ASCT (10) and were extended until the relapse phase (Fig. 2). Following the first VAD cycle, bone marrow stem cells were collected.

The patient was classified as 'non responder' to the different chemotherapeutic agents; however, the patient was selected for ASCT in April 10, 2008 (Fig. 2). Following ASCT, severe complications occurred, including pneumonia with *Morganella morganii* infection and then sepsis. Based on these observations, the hematological asset of the patient was re-evaluated upon ASCT, and bone marrow immunophenotyping revealed a 0.1% of PC population in the lymphocytes region. As displayed by post-ASCT flow cytometric analysis, the sFLC κ/λ ratio decreased, and no presence of neoplastic PCs was detected (Fig. 1A). In parallel, sIFE appeared without a monoclonal component, and the level of BJ protein was less pronounced overtime (Fig. 1B). Furthermore, these parameters were associated with a marked reduction in κ -sFLC (Fig. 3), suggesting that the patient was effectively responding to the treatment. However, osteolytic lesions were still present but did not progress overtime.

Table I. FLC/IgD values in the course of monitoring with BMD chemotherapy and ASCT.

Therapy	Therapy schedule	Date	FLCs ^a			Heavy chain total IgD (7.7-132.1)
			κ (3.3-19.4)	λ (5.7-26.3)	κ/λ ratio (0.26-1.65)	
Pre-BMD	-	Oct 10 2007	5,889.00	8.30	709.00	8,678.00
BMD	1st cycle, basal	Oct 31 2007	4,812.00	3.44	1,398.00	ND
	1st cycle, 5 days	Nov 16 2007	3,518.00	2.61	1,348.00	ND
	2nd cycle, basal	Dec 12 2007	3,352.00	2.82	1,188.00	5,028.00
	2nd cycle, 5 days	Dec 14 2007	2,539.00	2.09	1,215.00	ND
	2nd cycle, 15 days	Dec 24 2007	2,484.00	2.74	906.00	3,862.00
	2nd cycle, 28 days	Jan 7 2008	3,432.00	2.93	1,171.00	4,966.00
	3rd cycle, 5 days	Jan 11 2008	2,651.00	2.26	1,173.00	ND
	4th cycle, basal	Feb 4 2008	3,460.00	8.13	425.00	3,957.00
	4th cycle, 7 days	Feb 11 2008	2,627.00	8.00	328.00	4,050.00
	4th cycle, 15 days	Feb 19 2008	3,580.00	8.13	440.00	3,503.00
Pre-ASCT	-	Apr 10 2008	5,149.00	8.00	643.00	8,406.00
Post-ASCT	15 days	May 9 2008	42.09	14.10	2.91	ND
	2 months	Jul 23 2008	19.92	71.40	0.28	6.44
	4 months	Sep 3 2008	27.41	59.32	0.46	ND
	6 months	Nov 3 2008	30.08	28.91	1.04	6.44
	17 months	Sep 17 2009	25.83	17.77	1.45	ND
	21 months	Jan 20 2010	24.26	26.43	0.92	8.65
	24 months	Apr 19 2010	27.95	21.80	1.28	ND
	43 months	Feb 27 2012	26.97	17.66	1.53	12.50
	48 months ^b	Jul 2 2012	37.02	16.62	2.23	208.00
	56 months ^b	Mar 6 2013	262.00	14.78	14.78	190.00
	59 months ^b	Jun 10 2013	692.00	11.27	61.40	748.00

^aValues expressed in mg/l. Numbers in parentheses and inside the table represent the normal ranges. ^bRelapse. BMD, bortezomib, Myocet[®] and dexamethasone chemotherapy; ASCT, autologous stem cell transplant; ND, not determined; FLC, free light chain; Ig, immunoglobulin.

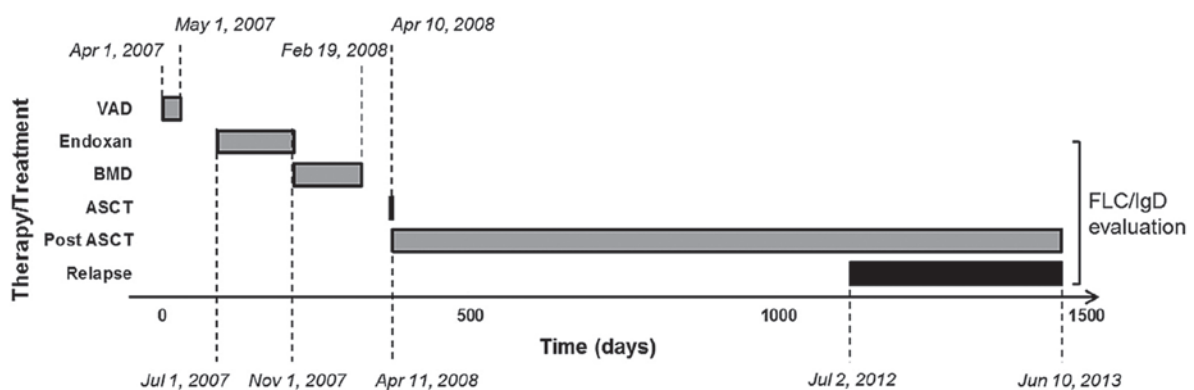


Figure 2. Overall view of the patient therapy and follow-up. The diagram represents the therapeutic strategies adopted for monitoring of the patient from July 2007 to June 2013. Free light chain/immunoglobulin D measurements were initiated in parallel with Endoxan Baxter[®] treatment. The gray boxes depict the duration of each treatment, inclusive of the beginning/end dates depicted in italics. The black box indicates the relapse interval of the patient. VAD, vincristine, Adriablastina[®] and dexamethasone chemotherapy (two cycles); BMD, bortezomib, Myocet[®] and dexamethasone chemotherapy (four cycles); ASCT, autologous stem cell transplant; FLC, free light chains. Ig, immunoglobulin.

In November 2008, the patient was diagnosed with Guillain-Barré syndrome. Due to the several complications occurred during the course of this syndrome, the patient

refused a second ASCT and other bone marrow aspirates. Therefore, the patient was regularly monitored with sFLC and total IgD measurements in order to assess the response to the

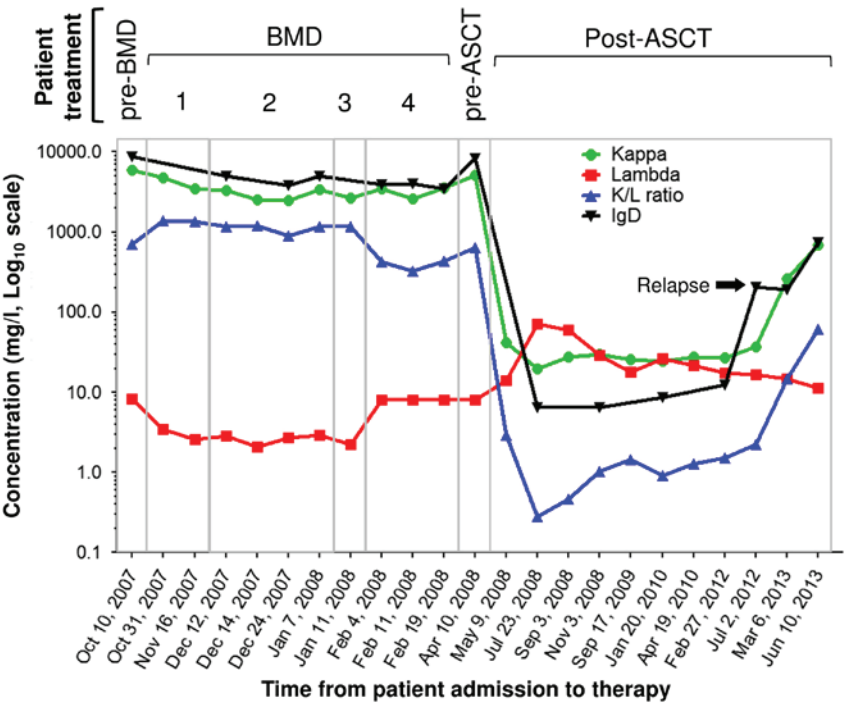


Figure 3. Graphical representation of serum free light chains, κ/λ ratio and IgD during the overall monitoring phase of the IgD-κ multiple myeloma patient. The plot depicts all collected data in the course of the monitoring of the patient, from October 2007 to June 2013. The numbers of cycles of bortezomib, Myocet® and dexamethasone chemotherapy are indicated. The black arrow represents the initiation of the relapse phase. ASCT, autologous stem cell transplant; BMD, bortezomib, Myocet® and dexamethasone chemotherapy; Ig, immunoglobulin.

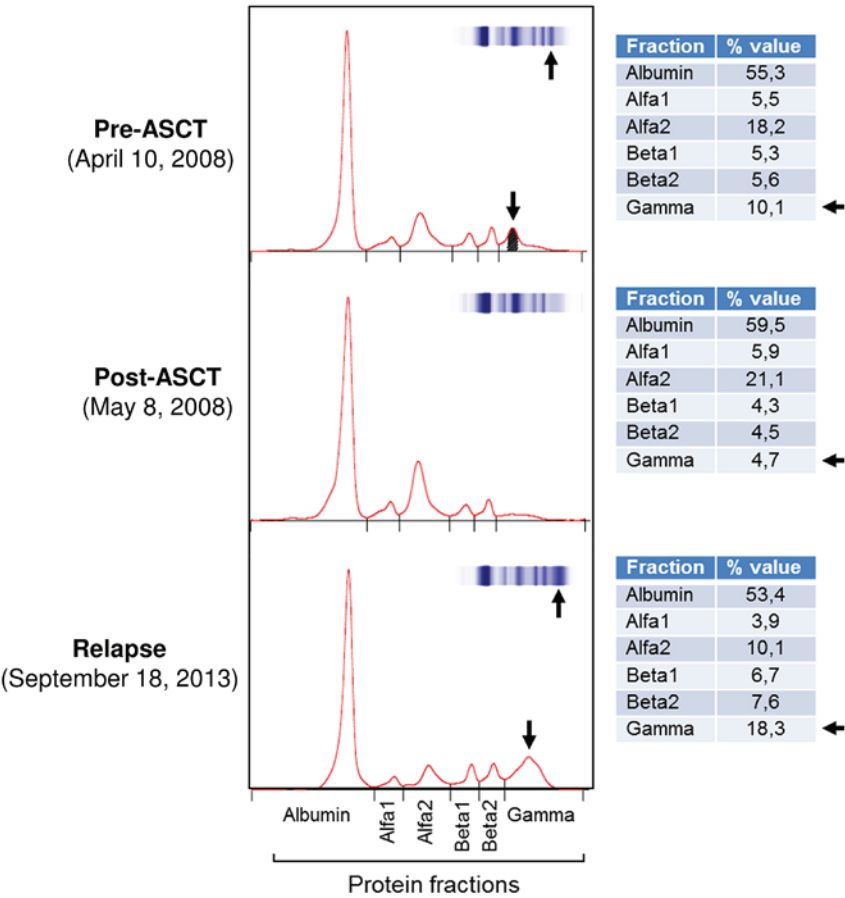


Figure 4. SPE profiles during the autologous stem cell transplant and relapse phases of the immunoglobulin D-κ disease. Serum samples were collected from the patient at the indicated timepoints and SPE was then performed in order to quantify the indicated protein fractions. The tables flanking each SPE panel present the percentage values of the indicated protein fractions. The black arrows depict the monoclonal component observed within the gamma region of the indicated serum SPE profiles and protein fraction tables. ASCT, autologous stem cell transplant; SPE, serum protein electrophoresis.

first ASCT performed in April 2008 (Fig. 2), in addition to evaluation of other classical serological parameters, including $\beta 2$ M, lactate dehydrogenase, Ca and hemoglobin). FLC/IgD parameters were also evaluated for the whole duration of post-ASCT and disease relapse (Fig. 2 and Table I).

In July 2012, after 4 years of ASCT, the levels of IgD first exhibited a substantial increase above the normal range (Fig. 3 and Table I), and 8 months later, in March 2013, the levels of κ -sFLC exhibited a further significant increase (Fig. 3). Due to this κ -sFLC increase, the presence of an IgD- κ MM was further confirmed by sIFE and urine IFE (Fig. 1B).

Accordingly, in view of a possible relapse, the patient agreed to undergo a bone marrow aspirate in March 2013, evidencing a PC population (4% of bone marrow cells) with the typical phenotypic hallmarks of CD38⁺ CD138⁺ CD28⁺ CD56⁺ CD117⁺ CD19⁻ CD45⁻ cells, including the presence of neoplastic PCs with κ light chains restriction, as indicated by the flow cytometric analysis performed at relapse (Fig. 1A). In addition, SPE was performed, which further supported the recurrence of a monoclonal peak inside the γ region. This recurrence was also confirmed by the percentage values of the γ regions from the SPE and IFE profiles (Fig. 4). Indeed, the high γ fraction value depicted in the pre-ASCT phase (10.1%, Fig. 4) reflects the values observed at relapse (18.3%, Fig. 4); there are similarly high values compared with post-ASCT (4.7%, Fig. 4).

Upon another chemotherapy line, the patient underwent a second ASCT, and is currently monitored by measurement of sFLC and total heavy chain IgD.

Discussion

The present study reports the follow-up of a patient with IgD- κ MM that requires a careful approach and monitoring.

The patient refused bone marrow aspirate in various occasions, even under suspicion of relapse; thus, the patient had to be followed up by means of sFLC and total heavy chain IgD measurements for monitoring MRD. The sFLC values over the four BMD cycles were generally constant, indicating the unresponsiveness of the patient. Compared with pre-ASCT levels, the total IgD and light chain values displayed a post-ASCT decrease, including the FLC levels. This was further confirmed by the disappearance of the κ -sFLC in the post-ASCT IFE. In addition, the total heavy chain IgD and κ -sFLC values observed in July 2012 suggested a potential relapse of the disease, since a consequent relapse in March 2013 supported by the relative sIFE values was observed. These findings were also confirmed by the flow cytometric analysis performed at relapse, which demonstrated unbalanced expression levels of κ/λ chains, also displaying the presence of neoplastic PCs. Lastly, the SPE profiles of the patient appeared to be compatible with the overall diagnostic evaluation of the disease evolution, and provided further confirmation to the reliability of this unusual IgD- κ MM patient follow-up.

These data clearly suggest a coherent outcome of heavy chain and FLC levels alongside the diagnostic profile, and demonstrate its effectiveness as an early marker for diagnosis by monitoring the MRD and relapse of IgD- κ MM.

Furthermore, in the present and peculiar case study, the availability of a rapid, quantitative and non-invasive test has been revealed of high significance in the monitoring of IgD- κ MM patients, particularly when bone marrow aspirate analysis is not available. In addition, evaluation of serum total heavy chain IgD could represent another useful clinical tool when employed in specialized centers where IgD MM cases could be better characterized and monitored (9,11). Finally, the present conclusions appears to be in line with the recent International Myeloma Working Group statements for MM patients that are not eligible for ASCT (12).

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