Case Study

Simultaneous Presentation of Waldenström Macroglobulinemia and Multiple Myeloma: Multidisciplinary Diagnosis, Treatment and 30-Month Follow-up

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Waldenström macroglobulinemia and multiple myeloma are mature B-cell neoplasms deriving from post-germinal cells at different stages of differentiation. The simultaneous presentation of Waldenström macroglobulinemia and multiple myeloma in the same patient is a very rare phenomenon and, so far, only two cases have been described. We report the case of a 75-year Caucasian female patient, with a silent clinical history, who presented with anemia and two different monoclonal proteins (IgMϰ and IgGϰ). The trephine biopsy showed the presence of a dual population, represented by small lymphoplasmacytoid cells and by plasma cells, which infiltrated the bone marrow with a clearly different pattern. Both immunohistochemistry and flow cytometry demonstrated the biclonal origin such neoplastic cells, since lymphoplasmacytoid cells resulted IgMϰ while plasma cells were IgGϰ. This biclonal pattern was further confirmed by the demonstration of a different *IgH* gene rearrangement of the two neoplasms. The patient was treated with bortezomib, dexamethasone and rituximab, achieving partial remission of both Waldenström macroglobulinemia and multiple myeloma. After a 30-month follow-up, she is in stable disease. Multiple myeloma has been described in association with other indolent B-cell neoplasms, mostly chronic lymphocytic leukemia, while Waldenström macroglobulinemia can be followed by diffuse large B-cell lymphoma in some instances, after chemotherapy. The association of Waldenström macroglobulinemia and multiple myeloma seems to be very rare. Our study shows that an integrated diagnostic work-up is very useful in such cases, with an interesting role for flow cytometry. 〔*J Clin Exp Hematop 53(1): 29-36, 2013*〕

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INTRODUCTION

Waldenström macroglobulinemia (WM) is a hematologic neoplasm characterized by a monoclonal lymphoplasmacytic expansion, with a variable plasma cell component and accompanied by a serum monoclonal immunoglobulin M (IgM).[1] It is an infrequent disease, accounting for approximately 2% of all hematologic malignant neoplasms, and shows a 2.8% frequency of association with other hematologic diseases. In these instances, acute myeloid leukemia and diffuse large B-cell lymphoma can occur, always being therapy-related.[2]

So far, an association of WM with multiple myeloma (MM) has been reported in only two cases,[3,4] and in a single case the evolution of WM into an aggressive form of IgM-positive multiple myeloma has been described.[5]

On the other hand, MM is a more frequent disease and is characterized by the proliferation of a single clone of plasma cells derived from B cells in the bone marrow and, in most cases, by the production of a monoclonal protein such as IgG, IgA or light chain only.[6] Compared to WM, the association of MM with another B-cell lymphoproliferative disease has been reported in a higher number of cases. The simultaneous occurrence of MM and chronic lymphocytic leukemia (CLL) seems to be the most frequent event. Sometimes, CLL is discovered incidentally in patients with newly-diagnosed MM,[7-9] while in other cases MM develops during the evolution of CLL.[7,9-12] In turn, CLL may also occur during the evolution of MM.[13]

The coexistence of MM with B-cell diseases other than CLL seems to be rarer, and occasional cases of metachronous or synchronous occurrence of MM with follicular

lymphoma,[14] malignant renal lymphoma,[15] MALT lymphoma,[16] and mantle cell lymphoma[17] have been reported.

Therefore, because of the rarity of a combined presentation of WM and MM, we describe a case of a patient who presented with severe anemia, two different monoclonal proteins (IgMϰ and IgGϰ) and evidence of simultaneous occurrence of IgGϰ MM and WM. Diagnosis was made by combination of morphology with immunohistochemistry, flow cytometry and *IgH* gene rearrangement analysis. In the present paper we present the diagnostic work-up, results of therapy, a 30-month follow-up.

CASE REPORT

A Caucasian 75-year-olf female, with a silent clinical history, presented with anemia-related symptoms (fatigue and effort dyspnea). A complete blood count showed: Hb 9.3 g/dL; white blood cell $5,71 \times 10^9$/L (differential: neutrophil 55%, lymphocyte 35%, monocyte 7%, eosinophil 2.5%, and basophil 0.5%); platelets 271×10^9/L. Chemistries showed: plasmatic protein 9.6 g/dL, β_2-microglobulin 8.5 mg/L (normal range < 2), IgG 3,250 mg/dL, IgA 55 mg/dL, and IgM 1,380 mg/dL. Serum protein electrophoresis showed γ-globulins 46.3% and an altered γ-globulin area morphology, with the apparent presence of two spikes (Fig. 1). Calcium concentrations were normal as well as creatinine concentrations. Serum immunofixation demonstrated the presence of two monoclonal proteins, one identified as IgGϰ, the other as IgMϰ. Urinary immunofixation was negative for monoclonal proteins. X-ray skeletal survey showed a few lytic areas involving femurs. No involvement of lymph nodes nor hepato-splenomegaly were found by whole body computed tomography and ultrasound sonography.

The patient underwent bone marrow evaluation by myeloaspirate and trephine biopsy. Samples from myeloaspirate were subjected to light microscope observation, flow cytometry, molecular tests for *IgH* gene rearrangement, karyotype
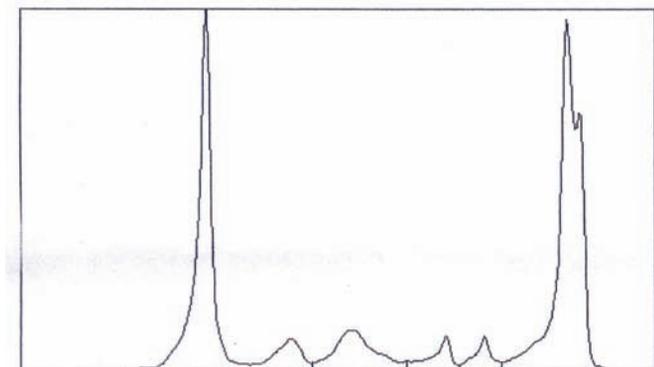
and fluorescent *in situ* hybridization (FISH) tests. Smears were stained by May-Grünwald-Giemsa and showed conspicuous infiltration (70%) by lymphocytes and lymphoplasmacytoid cells, and the concomitant presence of plasma cells, which accounted for 10% of cellularity (Fig. 2).

Flow cytometric immunophenotyping of bone marrow samples was performed using a FacsCanto II cytometer (Becton Dickinson) equipped with three lasers (405, 488 and 633 nm). Samples (50 μL) were stained with fluorochrome-conjugated monoclonal antibodies (MoAbs) specific for the following antigens: CD5, CD8, CD10, CD11c, CD16, CD19, CD20, CD22, CD23, CD25, CD27, CD38, CD45, CD56, CD81, CD103, CD117, CD138, (purchased from Becton Dickinson), rabbit F(ab')$_2$ polyclonal antibodies directed to γ and μ immunoglobulin heavy chains and ϰ and λ immunoglobulin light chains (purchased from Dako). A multiparametric method with either seven or eight fluorescences was used for each tube, associating MoAbs conjugated with FITC, PE, PerCP-Cy5.5, PE-Cy.7, APC, APC-Cy.7, AmCyan and Pacific Blue. Lymphocytes were gated using CD45 expression and right angle scatter, and at least 100,000 events/tube were acquired. A second gate included CD19$^+$ events and was used to analyze the expression of the other markers. Plasma cells were identified by the CD38/CD138 combination, acquiring 500,000 events/tube. A second gate was set to analyze events with homogeneous forward scatter and side scatter (SSC) properties, and was used to analyzed the expression of the other markers eventually associated with plasma cells (CD10, CD20, CD27, CD56, CD81, and CD117).[18,19]

Immunophenotyping showed infiltration by two neoplastic clones. The former was predominant (60%) and consisted
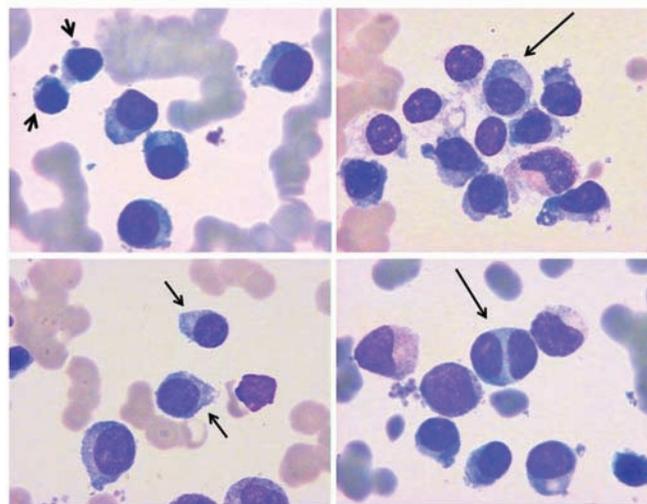
Fig. 2. Myeloaspirate at diagnosis showing infiltration by lymphocytes, plasmacytoid lymphocytes and plasma cells ($\times$1,000). Some plasma cells (*the longest arrows*), plasmacytoid lymphocytes (*intermediate arrows*) and lymphocytes (*the shortest arrows*) are shown.

Fig. 1. Altered morphology of serum protein electrophoresis. The gamma region shows two different spikes.

of B lymphocytes (CD19^{+} and CD20^{+}) with restriction for μ heavy chains and $\varkappa$ light chains, and without CD27 expression (Fig. 3). The latter was represented by atypical plasma cells (CD38^{+}, CD138^{+}, CD19^{-}, and CD45^{-}), which accounted for 10% of cellularity and were negative for CD10, CD20, CD27, CD56, CD81, and CD117, with restriction for cytoplasmic γ heavy chains and cytoplasmic $\varkappa$ light chains (Fig. 4A-4E). As a final result, neoplastic lymphocytes and MM plasma cells were found in different areas of CD45/SSC dot-plot (Fig. 4F). In our samples plasma cells belonging to the neoplastic clone of WM were not found, since the entire CD38^{+}/CD138^{+} cell population displayed features consisting with the MM pheno-
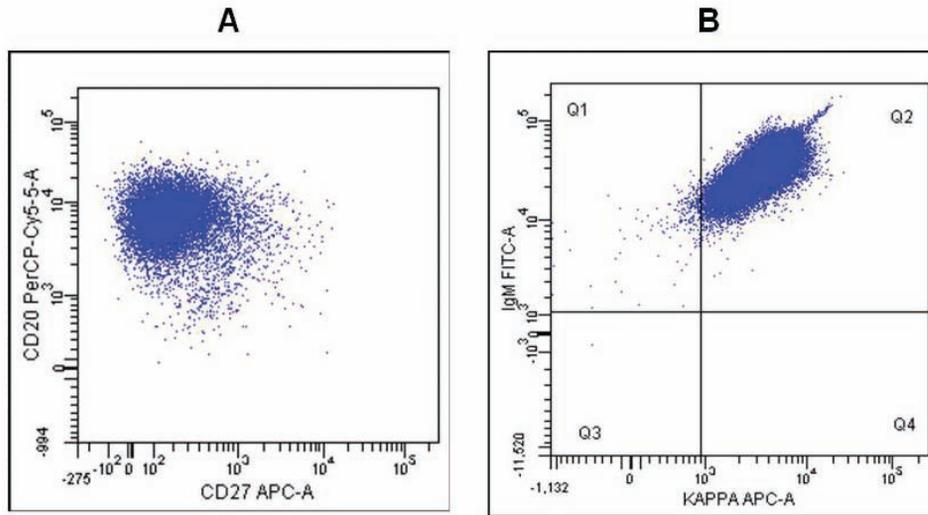
Fig. 3. Immunophenotyping of bone marrow lymphocytes after CD19 gating. B-cells show bright expression of CD20, negativity for CD27 (*3A*), and restriction for μ heavy chain and $\varkappa$ light chain (*3B*).
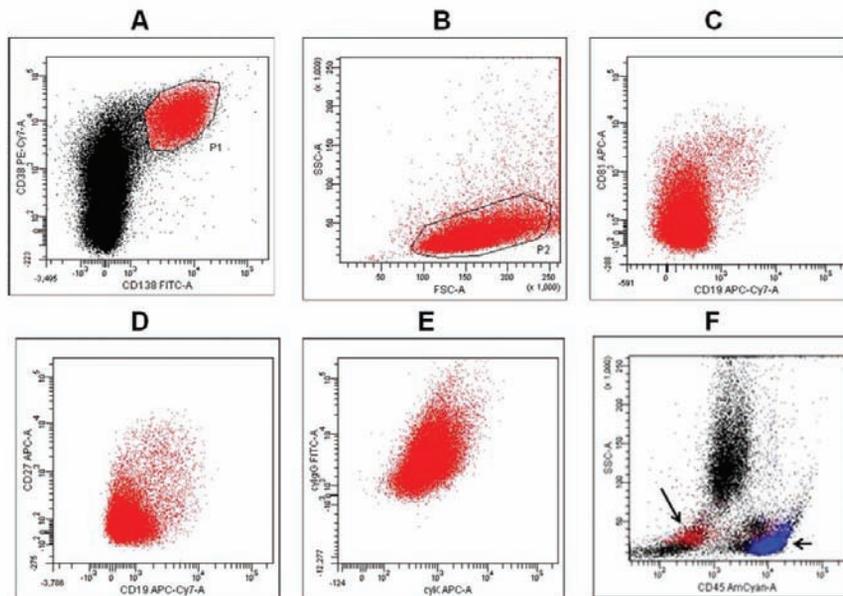
Fig. 4. Immunophenotyping of bone marrow plasma cells. (*4A*) CD38/CD138 gating. (*4B*) forward scatter/side scattergating. (*4C-4E*) Abnormal phenotype (negative for CD19, CD27, and CD81) and restriction for γ heavy chain and $\varkappa$ light chain. (*4F*) multiple myeloma plasma cells (*long arrow, red dots*) and Waldenström macroglobulinemia lymphocytes (*short arrow, blue dots*) occupy different areas of CD45/side scatter dot-plot.

type.

Bone marrow trephines were fixed in Myelodec® reagent A (Bio-Optica) for 2 hr, decalcified, embedded in paraffin, and cut into 3-5 μm sections. Morphological evaluations were carried out on hematoxylin-eosin, Giemsa, and Gordon-Sweet for reticulin-stained sections. Immunohistochemical stainings were performed using a peroxidase-based system including antibodies specific for CD3/PS1, CD5/4C7, CD20/L6, CD23/1B12, CD138/B-A38, bcl2/100-D5, bcl6/PG-B6p, and cyclin-Dl (DSC-6), and polyclonal antibodies directed to immunoglobulin γ and μ heavy chains, and ϰ and λ light chains. A BenchMark automated Slide Stainer (Ventana) was used.

The trephine biopsy showed a typical packed marrow with very high cellularity (90%) (Fig. 5A) and with diffuse/interstitial infiltrate of two morphologically distinct cell populations. The intertrabecular spaces were mostly filled by small B cells (Fig. 5B), which were about 70% of cellularity. These neoplastic cells showed lymphoplasmacytoid morphology with numerous Dutcher bodies, and were associated with small plasma cells; both lymphoid cells and plasma cells showed immunoreactivity for μ heavy chain (Fig. 5C).

A distinct plasma cell population, accounting for about 15% of cellularity, was found and consisted of CD138+ plasma cells, which were CD20- (Fig. 5D), showed restriction for cytoplasmic λ heavy chains (Fig. 5E) and were organized in small clusters. Both γ chain+ plasma cells and μ chain+ lymphoplasmacytoid cells stainedmonotypically for ϰ light chains (Fig. 5F).

IgH gene rearrangement analysis was performed on DNA extracted from bone marrow mononuclear cells separated by Ficoll/ Hypaque. Fluorescent polymerase chain reaction (PCR) was performed using VH region primer and CDR3/JH consensus primers 5' labeled with 6-FAM fluorochromes.[20,21] The PCR products were tested by capillary electrophoresis on an automatic sequencer (ABI PRISM 3100) and analysed with GeneMapper 4.0 software, obtaining accurate sizing and quantification of the peak areas. This allows accurate predic-

tion of the equivalence point of PCR products with $10^{-4}-10^{-5}$ sensitivity of molecular assays. The samples were also analysed for FR1 region to confirm the specific rearrangements. Reaction mixtures were prepared using the same JH consensus primer, with seven VH region family-specific primers.[22]
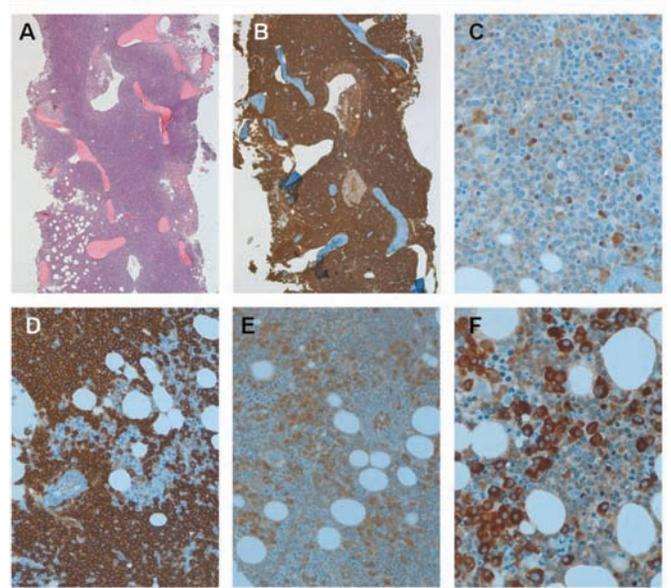
Fig. 5. Histology and immunohistochemical analysis of the bone marrow biopsy at diagnosis. (**5A**) Hematoxylin-eosin stain: markedly increase cellularity. ×40. (**5B**) CD20 immunostain: the intertrabecular spaces are mostly filled by small B cells. ×40. (**5C**) IgM immunostain: the neoplastic cells show lymphoplasmacytoid morphology with numerous Dutcher bodies, and are associated with small plasma cells; both lymphoid cells and small plasma cells show immunoreactivity for μ heavy chain. ×50. (**5D**) The CD20 immunostaining is patchy, moth-eaten. Larger plasma cells, which are CD20-negative, tend to form aggregates or clusters. ×250. (**5E**) IgG immunostain: plasma cells express γ heavy chain. ×250. (**5F**) ϰ light chain immunostaining: plasma cells and lymphoplasmacytoid cells both stain monotypically for mlight chain. ×250.
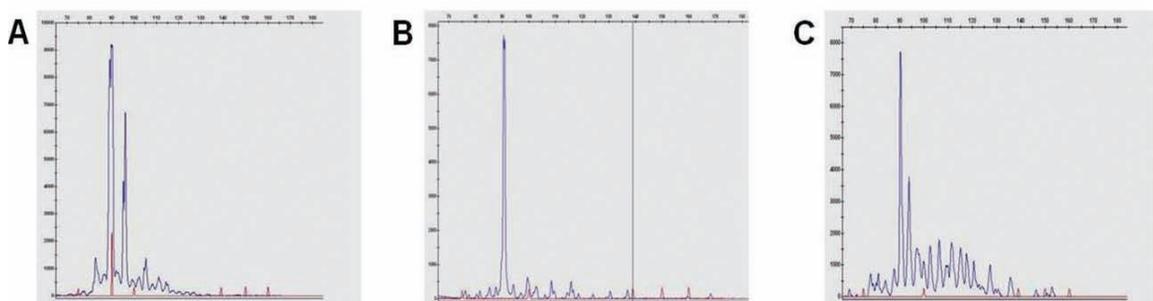
Fig. 6. Polymerase chain reaction assays for *IgH* gene rearrangement. (**6A**) Two different clonal rearrangements can be seen at diagnosis. (**6B**) A single clonal rearrangement is observed at the end of therapy. (**6C**) The second clonal rearrangement re-appears at the end of 30-month follow-up.

PCR products were assessed by electrophoresis on a 2,5% agarose gel (about 350 bp). Two different monoclonal rearrangements of IgH were detected by both PCR (Fig. 6A) and gel electrophoresis (not shown). Unfortunately, it was not possible to study light immunoglobulin gene rearrangement.

Karyotyping, carried out by conventional G-banding methods, showed normal 46,XX, and FISH with probes for 17p53, 14q32 and 13q14.3 resulted negative. At the end of workup, a final diagnosis of WM associated with IgGκ MM was made.

Our patient underwent therapy with combination of bortezomib, dexamethasone and rituximab, according to the following schedule: bortezomib, i.v., weekly at 1.6 mg/m² on days 1, 8, 15, and 28 days × 8 cycles; rituximab, 375 mg/m² weekly on cycles 1 and 4; dexamethasone, 20 mg on the day of and on the day after bortezomib. Because of severe reactions, the second rituximab course was not entirely administered.

As a result, a progressive reduction of total proteinemia, IgG and IgM levels was observed (Fig. 7), along with progressive increase in Hb values. At the end of treatment, complete response of MM (i.e. no M-protein detected in serum or urine by immunofixation for a minimum of 6 weeks and fewer than 5% plasma cells in bone marrow, absence of phenotypically abnormal bone marrow plasmacells) according

to the guide-lines published by the Haemato-oncology Task Force of the British Committee for Standards in Haematology (BCSH) and UK Myeloma Forum,[23] was obtained.

As far as the WM component is concerned, partial response was obtained, according to response criteria by the Third International Workshop on Waldenström's macroglobulinemia,[24] with IgM⁺ immunofixation, IgM 370 mg/dL, persistence of 30% of IgM/κ⁺ lymphocytes (bone marrow biopsy).

Protidogram showed a mild, single monoclonal band of γ-globulins, and the PCR assay form *IgH* gene rearrangement showed a unique clonal band (Fig. 6B). At the time of submission (30-mon follow-up) of the present paper, IgM levels were 400 mg/dL, but recurrence of MM was observed with immunofixation positive for IgG/κ and 1,810 mg/dL IgG. Protidogram appeared very similar to that of presentation (not shown). The new histological examination of bone marrow showed 30% neoplastic lymphocytes and 10% clonal plasma cells. Immunophenotyping showed a low amount (20%) of clonal lymphocytes along with 3% immunophenotypically abnormal and clonal plasma cells (Fig. 8). PCR for *IgH* gene rearrangement was again characterized by two distinct clonal peaks (Fig. 6C). Therefore, after a 30-mon follow-up, a good control of disease was achieved, with our patient feeling well.

DISCUSSION

The association, either synchronous or metachronous, of two different B-cell lymphoproliferative diseases can be detected in few cases and CLL seems the most frequent disease which can occur with MM. This phenomenon is very interesting because the clonal relationship of MM and a concomitant B-cell disease is a matter of debate. In few instances, a derivation from a single B-cell precursor has been suggested by the demonstration of identical *IgH* gene rearrangements, but opposite results were found in most cases and biclonal origin prevails.[25]

Interesting epidemiologic studies carried out in the past
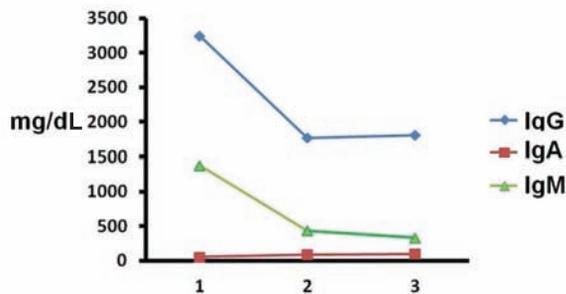
Fig. 7. IgG, IgA and IgM behavior at diagnosis (*1*), after therapy (*2*) and at the end of follow-up (*3*).
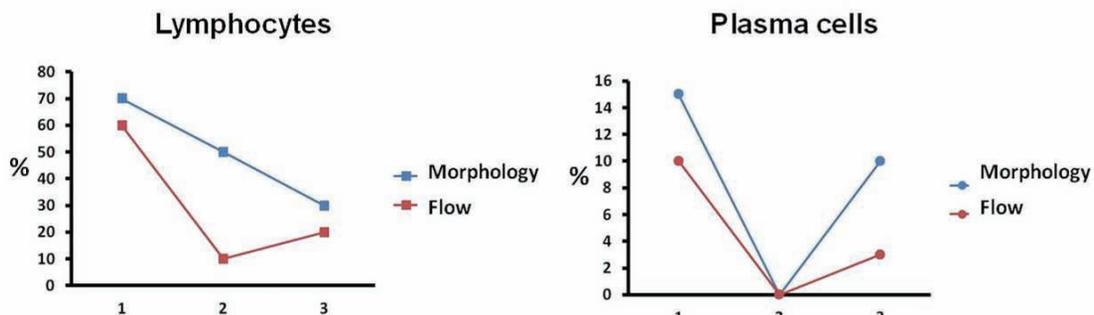
Fig. 8. Percentages of bone marrow lymphocytes and plasma cells measured by morphology (trephine biopsy) and flow cytometry at diagnosis (*1*), after therapy (*2*), and at the end of follow-up (*3*).

have shown that patients with MM, enrolled in the Swedish Cancer Registry, had a slight increase in standardized incidence ratio for non-Hodgkin lymphomas in the period 1 to 9 years after diagnosis.[26] A similar study carried out in Japan between 1962 and 1996 did not show any significant incidence of association of MM and lymphoma, either synchronous or metachronous.[27] Thus, the detection of non-Hodgkin lymphoma at the time of diagnosis of MM should be considered an infrequent event and represents an interesting biological phenomenon.

As far as WM is concerned, its association with MM seems to be a very rare phenomenon and only two cases have been reported so far. In the first one, described by Fine *et al*. in 1981[3] and concerning a patient with biclonal gammopathy IgMϰ and IgGλ, MM was detected in bone marrow sections and WM in lymph node aspirations. Flow cytometric and molecular assays were not available. In the second case, describedin 2012 by Wang E, *et al*.,[4] a patient developed a sequential development of WM and plasmablastic IgA multiple myeloma over a 10-year period of untreated IgM gammopathy. Another, but very rare possibility, is represented by evolution of WM into an IgM-producing MM.[5]

In our case, an old female without a significant clinical history presented with anemia-related symptoms and with the presence of two monoclonal proteins, IgMϰ and IgGϰ, which were suggestive of twodifferent neoplastic clones. Flow cytometry, carried out by a wide MoAb panel able to detect both plasmacells and lymphocytes, showed the co-existence of two different abnormal clones. B-lymphocytes (CD19+ and CD20+) showed restriction for γ heavy chain and for ϰ light chain, and resulted to be CD27-negative. Plasma cells, identified by the classical CD138+/CD38+ combination, were characterized by an abnormal phenotype (being CD19−, CD27−, and CD81−) and by positivity for intracytoplasmic γ heavy chain and ϰ light chain.

Morphology and immunohistochemistry, carried out on sections obtained from trephine biopsy, confirmed the peculiar association of WM and MM. Infiltration by lymphocytes and lymphoplasmacytoid cells, attributable to WM, showed a diffuse/interstitial pattern which characterizes a high percentage of WM cases,[28] while the neoplastic plasma cell population attributable to MM infiltrated the bone marrow in a different way, focally forming confluent aggregates. It is known that plasma cell in WM are usually morphologically mature and are generally mixed with the predominant lymphocytic population. Small perisinusoidal clusters can sometimes be found, but their infiltration patter is quite different from that shown by MM plasma cells.[1]

The biclonal nature of these populations was further supported by immunohistochemistry because, although both MM and WM were restricted to ϰ light chain, a divergent positivity for μ and γ heavy chains was found. In addition, the molecular analysis of IgH gene confirmed the presence of two distinct clonal rearrangements, clearly deriving from two different neoplastic clones. Both immunophenotypic and morphologic findings were in agreement with the initial diagnostic suspect of an association of MM and WM. The above findings seem to exclude a common clonal origin of the two neoplastic clones, and are consistent with a distinct clonal progenitor.

WM is likely to develop from a post-germinal memory-like B cell that has undergone somatic hypermutation but not isotype switching, and is able to acquire a lymphoplasmacytoid phenotype and IgM secretory capacity.[29,30] In addition, the neoplastic B-cells of WM show the phenotype of CD27− memory B-cells. The absence of CD27 on the surface of B-cells seems to be a typical feature of WM and is likely to depend on shedding from cell surface, since the neoplastic burden has been found to correlate with soluble CD27 concentrations.[31] Lymphoplasmacytoidcells of WM retain expression of mature B-cell markers, such as CD20 and surface IgM,[32] which are normally lost in healthy plasmablasts and plasma cells. On the contrary, myelomatous plasma cells are CD19− and/or CD45−, with rare exceptions, and often lack CD27 and CD81. Other B-cell-associated markers (CD10 and CD20) are positive in very low percentage of cases.[18,33]

Although both MM and WM tend to occur in the elderly, compared to MM, WM is rare. In fact, data from the US Surveillance, Epidemiology and End Results (SEER) Registry estimate the incidence of WM to be 0.36 per 100,000 persons-year,[34] a rate that is less than one-tenth that of MM (6 per 100,000 in Europe).[35] Therefore, the probability that the two diseases could occur simultaneously is very low and seems the justify the rarity of such an event.

We chose to treat our patient with a schedule which included bortezomib, dexamethasone and rituximab. Unfortunately, rituximab was not tolerated, and its administration was incomplete. Nevertheless, the efficacy of therapy was good and an acceptable control of both WM and MM was achieved. Indeed, the combination of weekly bortezomib and rituximab exhibited significant activity in untreated patients with WM,[36] and the association of bortezomib and dexamethasone has been employed as first line therapy in patients with MM.[37,38] Therefore, we used a hybrid therapy schedule in an attempt to control both WM and MM.

Another interesting aspect of our observation is represented by the application of flow cytometry in the diagnostic approach of such a clinical case. Some interesting papers have shown that the neoplastic clone of WM can be identified by multiparameter flow cytometry, despite the lack of a specific immunophenotype.[32,39] We found that the combination of IgM clonality and lack of CD27 seems to improve diagnosis and in our case such interpretation was found to correlate with morphology. Indeed, very recent observations[40] demonstrate that in WM flow cytometry yields results comparable to morphology with immunohistochemistry and that using both

techniques in parallel improves diagnosis.

In conclusion, the current contribution shows that rare cases of co-existence of MM and WM may occur and that an integrated diagnostic approach, based on the simultaneous usage of morphology, and flow cytometry and molecular assays is able to facilitate the recognition of such peculiar disease association. The therapeutic schedule used in our patient, which and allowed to obtain a good control of both WM and MM, might be used in further cases.

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