Occurrence of multiple myeloma after fludarabine treatment of a chronic lymphocytic leukemia: evidence of a biclonal derivation and clinical response to autologous stem cell transplantation

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The occurrence of multiple myeloma (MM) with another distinct B-cell lymphoproliferative neoplasm has been occasionally reported in the literature. The clonal relationship between MM and other lymphoid tumors occurring in the same patient is still a matter of debate. Here we report the immunologic and molecular characterization of a patient who developed overt MM after treatment with fludarabine for a previous diagnosis of chronic lymphocytic leukemia (CLL).

Case report

In 1992, a 49-year-old man was diagnosed in our center as having stage II (B) CLL with small peripheral lymphadenopathies, a palpable spleen and a white blood cell count of 13×10⁹/L with 70% lymphocytes; hemoglobin concentration and platelet count were normal. Peripheral blood immunophenotyping revealed a monoclonal B lymphocyte population expressing IgD and IgM surface immunoglobulins as well as CD19, CD20, CD5 and CD23 surface markers. Bone marrow biopsy revealed a 70% interstitial and nodular infiltration of mature small B-lymphocytes. The patient was observed without therapy for four years.

One year after diagnosis, a double M-component was identified at immunoelectrophoresis as IgDκ and κ free chains. Bence-Jones (BJ) proteinuria was checked but never found; the gammapathy remained stable but the serum concentration of polyclonal Ig, which was slightly below the normal range at the moment of CLL recognition, progressively reduced over the following 3 years. In 1996, because of enlargement of the lymphadenopathies, the patient was treated with six courses of fludarabine, which induced a complete clinical remission of the CLL. The peripheral lymphocyte count was 1.7×10⁹/L, although the circulating lymphocytes still had a phenotype consistent with CLL. At that time, a BJ proteinuria (κ light chain) amounting to 1.2 g/24 hours was identified. Bone marrow biopsy performed at the same time identified an excess of large, atypical and nucleolated plasma cells (PC) (30% of the total cellularity) in addition to the 40% infiltration of mature B-lymphocytes related to the pre-existing CLL. A critical review of the prior marrow biopsies identified that some plasma cells were already present before starting fludarabine treatment, but that...
the percentage was inferior to 5% and still consistent with the diagnosis of LLC.

During the following year, the patient developed overt myeloma characterized by an increasing amount of Bence-Jones proteinuria which reached 4 g/day and the appearance of a lytic lesion in the left humerus on X-ray; the double M component was unchanged at immunoelectrophoresis and hypogammaglobulinemia was severe. The patient was started on treatment with 4 monthly courses of VAD (vincristine, adriamycin, dexamethasone) followed by treatment intensification with high-dose busulfan (16 mg/kg) and melphalan (120 mg/m²) with autologous hematopoietic progenitor rescue. Progenitors were collected from peripheral blood after high-dose (7 g/m²) cyclophosphamide plus G-CSF and positively selected using a CD34+ MoAb and an avidin-biotin immunoaffinity device (Ceprate CellPro). Neutrophil counts >1 × 10^9/L were reached after 14 days, while megakaryocytic engraftment was slow and still incomplete. (platelets 80 × 10^9/L at the latest follow-up, 18 months after transplantation).

After high-dose treatment, all clinical, cytological and immunophenotypic evidence of CLL disappeared. The response of the M M component was partial because a small amount of monoclonal IgDκ plasma cells were always detectable in the marrow and there was a persistence of a minimal amount of Bence-Jones proteinuria, while IgD and κ M-components disappeared on serum immunoelectrophoresis. The most important laboratory and histologic data of this patient during the different steps of the treatment are summarized in Table 1.

To establish the clonal relationship of CLL and M M cells, immunologic and molecular studies were carried out in 1996, at the end of fludarabine treatment, when the coexistence of the two malignancies was clinically evident and we could clearly distinguish marrow infiltration by both lymphocytes and plasma cells. Using the APAAP immunocytochemical technique, the immunophenotype of the lymphocytes was IgMλ, while the PC, which could be distinguished morphologically from the lymphocytes, were positive for IgD and κ, but negative for IgM and λ.

Moreover, we examined the pattern of Ig gene rearrangement in the patient’s peripheral blood and bone marrow aspirate, using standard Southern analysis and molecular hybridization with a human 32P-labeled JH-specific probe, as previously reported.

![Figure 1. Representative results of the clonal analysis of immunoglobulin heavy-chain (Igh) gene configuration of bone marrow (BM) and peripheral blood (PB) tumor samples. Genomic DNA was digested with HindIII and subjected to Southern blot hybridization with a DNA probe representative of the JH locus. U-937, a monoblastic cell line was used as a control of the germline configuration of Igh. The bone marrow sample showed two different monoclonal rearrangements. The peripheral blood sample displayed one monoclonal rearrangement. The monoclonal rearrangement of Igh identified in the peripheral blood is identical to one of the two monoclonal rearrangements shown by the bone marrow sample. The bone marrow sample and the peripheral blood sample displayed both the germline band and the rearranged bands, consistent with the presence of contaminating normal cells.](image-url)
tional MM developed a few months to several years (up to 19 years) after CLL diagnosis\(^1\)\(^2\)\(^3\)\(^4\) and usually determined the therapeutic decisions and the prognosis of the patient. In most cases of concurrent MM and CLL, death supervened after a few months because of disease progression or infection. In the patient described in this study, an overt MM with BJ 

proteanuria, atypical marrow plasma cell infiltration and bone lytic lesions developed 4 years after CLL diagnosis. However, an IgD gammapathy had been recognized soon after CLL diagnosis. Intriguingly, the IgD gammapathy had a different light chain to that shown by the CLL lymphocytes, raising the possibility of the concurrence of two separate lymphoid clones.

In our patient, progression toward MM followed fludarabine treatment, which, conversely, was effective against CLL. We are unable to establish whether the growth of the MM clone after fludarabine treatment occurred because of chance alone or whether it was promoted by the immunosuppression due to this nucleoside analog.\(^5\)\(^6\) In fact, in a separate patient, the development of a MM after experimental IL-4 immunotherapy of CLL\(^7\) suggested a possible biological role of this cytokine which has multiple effects in vitro on immunologic and hemopoietic cells, including stimulation of B-cell growth and maturation.\(^8\) This issue deserves further investigation, since the role of CLL chemotherapy on MM growth is not known because most patients affected by both diseases have not been treated for CLL.

Whereas most patients reported in the literature had an unfavorable outcome due to MM, our patient was submitted to high-dose treatment with autologous CD34\(^+\) selected progenitor cells transplantation achieving partial response demonstrated by a reduction of the micromolecular proteinuria and of the plasma cell infiltration. IgD and \(\kappa\) components disappeared on serum immunoelectrophoresis, probably reflecting the fact that chemotherapeutic drugs could select a marrow plasma cell clone unable to excrete the entire Ig. Also, no detectable CLL lymphocytes were detectable in the bone marrow and peripheral blood. Although the role of high-dose treatment and selection of CD34\(^+\) blood progenitors is still matters of debate in the management of these B-cell lymphoproliferative diseases,\(^9\)\(^10\) both disorders in our case responded, probably reflecting a favorable effect obtained either by myelodestructive treatment or by the purged graft.

The clonal relationship between CLL and MM was studied in our patient with both immunologic studies and Ig gene rearrangement analysis. By immunophenotyping, monoclonal B-lymphocytes stained with \(\lambda\) chains whereas PC stained with \(\kappa\) chains, suggesting that the two diseases arose from different clones. The finding of two cell subsets expressing different heavy and/or light chains cannot be considered definitive evidence of biclonality because of the possibility of a switch-over of heavy chain Ig isotype and/or light chains. A more definitive marker of clonality is the study of the genetic structure by Southern blotting. The Ig heavy chain rearrangement analysis performed on the bone marrow revealed the presence of two distinct tumor clones, one of which was also present in the peripheral blood. Overall, these results suggest that, in this patient, CLL and MM originated from different B-cell progenitors. This finding is consistent with similar observations reported in the literature.\(^6\)\(^9\)

In conclusion, our study suggests that the MM developed in our patient after fludarabine treatment for a previously diagnosed CLL derived from a separate clone and that both disorders were responsive to a CD34\(^+\) selected ASCT.

Contributions and Acknowledgments

FP collected the clinical data and wrote the paper. GG and DC performed the laboratory analyses, interpreted the biological data and collaborated in writing the paper. FZ contributed to the clinical data. RF and MB critically reviewed the concepts and the conclusions of the study. The criteria for the order of the authors’ names are based on their contribution as delineated above. The last name is that of the chief of the Department in which the study was performed.

Funding

This work is supported in part by grants from Treviso AIL (Associazione Italiana Leucemie) and AIL 30 ore per la vita, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received March 15, 2000; accepted May 6, 2000.

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