Bone Marrow Histology in Monoclonal B-Cell Lymphocytosis Shows Various B-Cell Infiltration Patterns

Ulla Randen, MD,1,2 Anne M. Tierens, MD, PhD,1 Geir E. Tjønnfjord, MD, PhD,2,3 and Jan Delabie, MD, PhD1,2

Key Words: Monoclonal B-cell lymphocytosis; MBL; CLL; Bone marrow histology

DOI: 10.1309/AJCPPHSUQM8XBJH7

Monoclonal B-cell lymphocytosis (MBL) was first recognized as an indolent variant of chronic lymphocytic leukemia (CLL) in patients with Rai stage 0 disease. The patients showed no disease progression, even after a period of more than 24 years.1 A similar indolent form of CLL was later described in otherwise healthy family members of patients with CLL and in cohort studies of outpatients.2-5 The condition has since been called MBL, and its natural course and genetics have been studied extensively.6 MBL is now defined as less than $5 \times 10^9/L$ monoclonal B cells in the blood of otherwise healthy patients. Large population-based screening studies have revealed that the prevalence of MBL increases with age, with a frequency of up to 14% at an age of more than 62 years.7-9 Virtually all patients with clinically evident CLL had a preceding MBL, whereas the risk for patients with CLL-like MBL to develop leukemia is about 1% per year.7,8 MBL likely arises through chronic stimulation of a limited B-cell repertoire in older individuals. Thus, it is a phenomenon associated with senescence of the lymphoid system.10 MBL shows genetic changes such as 13q14 deletion, trisomy 12, and deletion of 17p and 11q, as also seen in CLL.7 CD38 and ZAP-70 expression, VH gene usage, and cytogenetic abnormalities have not allowed prediction of the transition of MBL to CLL, but B-cell counts, especially low-count MBL, have been associated with a very low risk of progression to CLL.11,12 In addition to the more frequent CLL-like immunophenotype of MBL, characterized by CD5 and CD23 expression and weak expression of CD20 and immunoglobulins, an atypical CLL-like phenotype with moderate or strong CD20 and immunoglobulin expression is also recognized. Furthermore, MBL with a non–CLL-like phenotype, characterized...
by the absence of CD5 expression, has also been described.\textsuperscript{5,6} In view of the frequency of MBL in the general population, MBL is often seen in combination with other diseases, including primary nonmalignant as well as malignant hematologic disorders, nonhematologic malignancies, and certain infectious diseases. Hitherto, there have been no systematic studies of bone marrow infiltration, including bone marrow histologic findings, in patients with MBL despite its high frequency in the population. We therefore investigated whether MBL involved the bone marrow and, if so, what its histology was like and whether it could be differentiated from bone marrow involvement by lymphoma. We studied the bone marrow histology in 26 patients with well-documented MBL.

Materials and Methods

Patient Selection

Patients were selected from the database of the Department of Pathology, Oslo University Hospital, Oslo, Norway. Only patients with an established MBL diagnosed between 2008 and 2011 and from whom a trephine biopsy was procured at the time of MBL diagnosis were selected for this study. All patients with non-Hodgkin lymphoma were excluded from the study, with the exception of 2 cases in which lymphoma involvement was easy to distinguish from MBL by morphology and immunohistochemistry. In total, 26 patients were retrieved from the database, including 18 men and 8 women with a mean age 70 years (range, 50-94 years). The patients underwent bone marrow examination as part of the clinical workup of their initial symptoms or primary disease. In 17 patients who presented with nonspecific symptoms such as fatigue, weight loss, or fever, no disease was eventually diagnosed. Chronic myeloproliferative disease was diagnosed in 4 patients and myelodysplastic syndrome in 1 patient. Three patients had a plasma cell neoplasia, 1 had a primary cutaneous follicular lymphoma, and 1 had a hepatosplenic T-cell lymphoma. One patient had renal cell carcinoma. A bone marrow aspirate had also been procured in all 26 patients.

Flow Cytometry

Flow cytometry analyses were performed on blood and bone marrow samples from all 26 patients. Samples were anticoagulated with heparin. Between 0.5 and 1 × 10\textsuperscript{6} cells were stained for surface antigens. For samples analyzed before 2011, a 4-color analysis\textsuperscript{13} was performed with the following antibody combinations labeled with fluorescein isothiocyanate (FITC)/phycoerythrin (Pe)/peridinin-chlorophyll cyanine 5.5 (PercPCy5.5)/allophycocyanin (APC): (1) CD20/CD5/CD19/CD43, (2) FMC7/CD23/CD19/CD5, (3) Igk/Ig\textsubscript{l}/CD20/CD19, (4) CD22/CD24/CD19/CD34, and (5) cyBcl2/CD10/CD19/CD38. From 2011, an 8-color flow cytometry analysis\textsuperscript{14} was used with the following antibody combinations labeled with Pacific Blue/e450 (PB/e450), Krome Orange (KO), FITC/Pe/PercPCy5.5/phycoerythrin cyanine 7 (PeCy7)/APC/APC Hilite\textsubscript{7}, or APC/cyanine 7 (APC\textsubscript{7}/cy7): (1) CD20+CD4/CD45/CDS+Igk/CDS6+Ig\textsubscript{k}/CD5/CD19+TCR\textsubscript{g}/CD38 and (2) CD20/CD45/CD23/CD10/CD79b/CD19/CD200/CD34. All antibodies for the 4-color panels were purchased from Becton-Dickinson (San Jose, CA) except anti-FMC7, CD22, CD23, and anti-Bcl2, which were purchased from DAKO (Glostrup, Denmark). For the 8-color panels, CD56, CD5, CD3, and 79b were purchased from Becton-Dickinson; CD23 from DAKO; CD200 from eBioscience (San Diego, CA); CD8, κ, and λ from Cytognos (Salamanca, Spain); and the rest from Beckman Coulter (Brea, CA). Flow cytometry analysis was performed on a FACScalibur or LSRII instrument (Becton-Dickinson) using CellQuest Pro and FACSDiva software (Becton-Dickinson), respectively.

Immunohistochemistry

The primary antibodies used for immunohistoenotypic analysis of the lymphoid infiltrates were anti-CD45, anti-CD20, anti-Bcl6, anti-Mum1, anti-IgA, anti-IgD, anti-IgG, anti-IgM, and anti-Ki67 (all from DAKO); anti-CD10, anti-Bcl2, anti-CD21, anti-CD23, anti-CD5, anti-CD43, and anti-cyclin D1 (all from Novocastra Laboratories, Newcastle upon Tyne, UK); anti-PAX5 (Becton-Dickinson); and anti-CD3 (Thermo Fisher Scientific, Fremont, CA). For all antibodies, heat-induced epitope retrieval was performed in a microwave oven by heating the slides for 5 minutes at 750 W and subsequently for 15 minutes at 500 W in retrieval buffer. The detection system EnVision (DAKO) was used for all antibodies. The stain was developed with 3,3’-diaminobenzidine and H\textsubscript{2}O\textsubscript{2}, and the slides were counterstained with hematoxylin. The immunostaining was performed in a DAKO Autostainer (DAKO) according to the instructions of the manufacturer.

Results

Flow cytometry analysis revealed a CLL-, an atypical CLL-, or a non–CLL-like immunophenotype MBL in 11, 5, and 10 patients, respectively Table II. Monoclonal B cells were also detected in the bone marrow samples of all 26 patients. The immunophenotypes of the cells in the bone marrow were identical to those of the respective blood samples.

The trephine biopsy specimens showed no evidence of abnormal lymphoid infiltration in 6 of 26 patients. Infiltration
of the bone marrow by small B cells was found in 20 patients. Three infiltration patterns were discerned: 7 patients showed small interstitial foci, varying from 1 to 5, with monotonous small lymphoid cells with a rounded nucleus. At the periphery of these foci, cells infiltrated between fat cells in small rows (Image 1A-D). Eight patients showed more rounded lymphoid foci, varying from 1 to 9, without interstitial infiltration between fat cells at the periphery. These lymphoid foci consisted of small lymphoid cells with a slightly more irregular nuclear contour, as well as a few scattered immunoblasts and histiocytic cells (Image 1E-H). Centrally located tiny germinal centers could occasionally be observed (Image 1H). The latter were highlighted by CD21 staining. Finally, 5 patients showed a discrete diffuse lymphocytosis that was only discerned upon immunohistochemical staining for CD20. Diffuse B-cell lymphocytosis in patients with MBL could be distinguished from the presence of hematogones by the homogeneous small size of B cells in the former and by the variable small to medium-large size in the latter. In addition, hematogones showed a variable CD20 expression in contrast to the homogeneous CD20 expression seen with MBL. Intrasinusoidal infiltration was not seen in any of the cases.

Of interest, there was a correlation between the infiltration pattern and the immunophenotype of MBL (Table 1). The interstitial pattern was exclusively seen in patients with MBL with a CLL-like or an atypical CLL-like immunophenotype. The 2 other patterns were most commonly seen in non–CLL-like MBL, although these patterns were also observed in 3 patients with a CLL-like or an atypical CLL-like MBL.

### Discussion

With the extensive use of highly sensitive multiparameter flow cytometry analyses of blood and bone marrow samples, MBL is increasingly being detected. The etiology of MBL is not clear, but it is hypothesized that it results from chronic and persistent antigen stimulation. CLL-like and atypical CLL-like MBL shows genetic changes such as chromosome 13q deletion, trisomy 12, and even chromosome 17p deletions as well as stereotyped immunoglobulin receptors as seen in CLL, adding to the argument that MBL is a precursor state of CLL. Less is known about non–CLL-like MBL, although genetic changes are also seen, albeit different from those
demonstrated in CLL-like MBL. Whether non-CLL-like MBL is a precursor state for non-Hodgkin lymphoma—in particular, marginal zone lymphoma or lymphoplasmacytic lymphoma and perhaps also diffuse large B-cell lymphoma, as the immunophenotype and genetic changes seem to indicate—remains to be demonstrated. Whether and how MBL affects the bone marrow was hitherto poorly described except for 1 study published as an abstract. However, knowledge about bone marrow infiltration in MBL is of potential interest to avoid diagnosis of marrow infiltration with lymphoma. We therefore studied trephine biopsy specimens by histology and immunohistochemistry in 26 individuals with established MBL. We reviewed the flow cytometry findings in peripheral blood and bone marrow in all patients.

Histology and immunohistochemistry highlighted abnormal lymphoid infiltration in the bone marrow of 20 patients, displaying patterns that were indistinguishable from limited infiltration by CLL or marginal zone lymphoma. CLL or small lymphocytic lymphoma shows nodular or diffuse non-paratrabecular interstitial infiltration with monotonous small lymphoid cells variably admixed with paraimmunoblasts. Occasionally, more limited focal patchy interstitial...
infiltrates are seen in CLL, indistinguishable from the pattern of infiltration reported here for MBL with a CLL-like or an atypical CLL-like immunophenotype.\textsuperscript{20} Marginal zone lymphoma in the bone marrow most frequently shows focal, more rounded nonparatrabecular infiltrates with small lymphoid cells often admixed with some immunoblasts and histiocytic cells as well as follicular dendritic cells.\textsuperscript{20,21} This is also one of the patterns seen in MBL with a non–CLL-like immunophenotype, although this pattern could occasionally be observed in MBL with a CLL-like and an atypical CLL-like immunophenotype. Of interest, diffuse but limited B-cell infiltration was almost exclusively seen in MBL with a non–CLL-like immunophenotype. This infiltration pattern is distinct from the diffuse but intrasinusoidal infiltration pattern often seen in splenic marginal zone lymphoma.\textsuperscript{20} Intrasinusoidal infiltration was not seen in any of the MBL cases.

There was perfect agreement between the immunophenotype of MBL in peripheral blood and the monoclonal B cells in the bone marrow. In addition, immunohistochemical findings were also in agreement with the immunophenotypes detected by flow cytometry. This supports the finding...
that lymphoid infiltration seen in bone marrow is the tissue counterpart of MBL.

The frequent involvement of bone marrow in patients with MBL displaying an infiltration pattern and immunophenotype that is indistinguishable from that of limited infiltration with lymphoma, especially CLL or marginal zone lymphoma, is of diagnostic importance. Our results indicate that a final diagnosis of lymphoma in the bone marrow should not be made in the absence of an established lymphoma diagnosis or in the absence of adequate clinical information. Whether the patient with limited lymphoid infiltration in the bone marrow, originally taken as part of the investigation for other diseases, should be screened for lymphoma and how extensive this screening should be is a matter of discussion. Noninvasive investigations with abdominal ultrasound and chest x-ray in addition to a complete blood cell count, whenever these have not been performed as part of the investigations for the patient’s primary disease, are most likely indicated, as recommended for MBL.10,22

Whether the extent of bone marrow infiltration with MBL correlates with increased progression toward overt clinical disease is of interest but still needs to be studied. This question may be answered by studying a larger cohort of patients with a longer follow-up time.

From the 1Department of Pathology and the 2Department of Hematology, Oslo University Hospital, Oslo, Norway; and 3Institute of Clinical Medicine, University of Oslo, Oslo, Norway.

This study was supported by a grant from the Norwegian Cancer Society.

Address reprint requests to Dr Randen: Dept. of Pathology, Oslo University Hospital, Ullernchausséen 70, 0310 Oslo, Norway; e-mail: uranden@ous-hf.no.

References


© American Society for Clinical Pathology