

Mantle Cell Lymphoma, Blastoid Variant, Diagnosed on the Basis of Cytomorphology and Flow Cytometric Immunophenotyping of the Lymph Node Aspirate and Peripheral Blood

Mantle cell lymphoma, blastoid variant (B-MCL), is a very rare type of non-Hodgkin's lymphoma exhibiting an aggressive clinical course. We describe a case of B-MCL showing generalized lymphadenopathy and leukemic conversion in a 62-yr-old man. The case was diagnosed and subclassified as B-MCL on the basis of cytomorphology and immunophenotype. Microscopic examination of the peripheral blood (PB) showed a spectrum of cells ranging from small mature lymphocytes to medium- and large-sized lymphocytes with blast-like chromatin and prominent nucleoli. The lymphoma cells were monoclonal B cells with moderately intense surface IgM. They were CD5 positive, cyclin D1 positive, CD10 negative, and CD23 negative. The flow cytometric immunophenotyping and DNA ploidy analysis of the PB and material obtained by aspiration cytology supported the diagnosis of B-MCL. These findings underline the utility of aspiration cytology in diagnosing B-MCL when cytomorphologic examination is combined with flow cytometric analysis of immuno-phenotype and demonstration of proliferation markers.

Key Words : Lymphoma, Mantle-Cell; Blast Crisis; Leukemia; Cytology; Immunophenotyping; Antigens, CD5; Cyclin D1

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INTRODUCTION

Since its original description by Lennert in 1964, a variety of names were used for this neoplasm before the recent general acceptance of the term mantle cell lymphoma (MCL) (1). MCL is a neoplastic lymphoid proliferation related to the naive pregerminal center cells localized in the primary follicle and to the mature mantle zone of the secondary lymphoid follicles. MCL is a CD5 positive monoclonal B-cell malignancy and is strongly associated with the t(11;14)(q13;q32) translocation, which results in a rearrangement of the *bcl-1* locus and overexpression of PRAD1/cyclin D1 mRNA transcript and protein (2). At the time of diagnosis of MCL, this neoplasm frequently involves the bone marrow (BM) and peripheral blood (PB) (3).

A variant of MCL has been described. The blastoid variant (B-MCL) exhibits histologic transformation to a higher grade malignant lymphoma (4). This B-MCL exhibits a more aggressive clinical course than typical MCL. B-MCL can be problematic to diagnose correctly using morphologic examination alone. The neoplastic cells of B-MCL show larger and more pleomorphic nuclei with finely dispersed chromatin and increased mitotic figures. The cytomorphology of conventional MCL by fine needle aspiration (FNA) cytology and

PB has been described (5). However, the published literature describing the cytomorphologic feature of B-MCL diagnosed based on the lymph node (LN) FNA cytology is limited to only one case (6), and also the descriptions of morphological features of PB of B-MCL are very rare. We report a case of B-MCL diagnosed on the basis of cytomorphology and flow cytometric analysis of immunophenotype of a LN FNA cytology and PB in a 62-yr-old man with generalized lymphadenopathy and PB involvement. Surgical LN biopsy confirmed the diagnosis. The diagnosis was substantiated by the demonstration of *bcl-1* gene rearrangement in circulating mantle cell leukemic cells.

MATERIALS AND METHODS

Case

The patient was a previously healthy 62-yr-old man who had a 2-month history of epigastric fullness and generalized lymphadenopathy, most pronounced in both inguinal areas. Laboratory findings included the followings (reference ranges given in parentheses): hemoglobin level of 9.3 g/dL (14.0-18.0 g/dL) with normal red blood cell indices; normal platelet

count of $125 \times 10^9/\mu\text{L}$ ($130\text{--}400 \times 10^9/\mu\text{L}$); and a white blood cell count of $18.5 \times 10^9/\mu\text{L}$ ($4.8\text{--}10.8 \times 10^9/\mu\text{L}$) with an absolute lymphocytosis of $14.0 \times 10^9/\mu\text{L}$ ($0.9\text{--}2.9 \times 10^9/\mu\text{L}$). Seventy percent of the PB lymphocytes were designated "atypical lymphocytes or lymphoblast-like". Leukemic conversion was found in PB. The reticulocyte differential was 1.7% (0.49–1.87%). A whole body computerized axial tomography (CT) scan showed a markedly aggregated lymphadenopathy in retroperitoneum, celiac, peripancreatic, and portohepatic areas, and hepatosplenomegaly. Subsequent physical examination confirmed the hepatosplenomegaly. A FNA cytology of a 2.8 cm sized left inguinal LN was performed using a 23-gauge needle. Three separate aspirations were performed and the needle was rinsed in RPMI 1640 lymphoid culture medium in order to perform analysis of flow cytometric immunophenotyping and gene rearrangement studies. Air-dried and ethanol-fixed slides were made and stained using the Diff-Quick and Papanicolaou methods, respectively. A BM aspirate was obtained for cytogenetics in a sterile RPMI medium. A BM core biopsy was performed, and diffuse infiltration of malignant lymphoid cells was confirmed. Surgical LN biopsy confirmed the diagnosis. The Ann Arbor stage of the patient was determined as stage IV. The disease progressed very rapidly, and patient did not agree to receive a course of chemotherapy. He died 1 month after admission because of pleural effusion, ascites, and cardiac arrest. Autopsy was not performed.

Cyto- and histomorphological evaluation

Air-dried smears of the PB, BM aspirate, LN touch print, and LN aspirates were stained with Wright, Diff-Quick, or Papanicolaou. B5-fixed, paraffin-embedded, 5- μm sections of the LN, BM core biopsy were stained with hematoxylin-eosin and periodic acid-Schiff. For ultrastructural observation, PB mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. The pellet was washed in Hanks solution, fixed overnight at 4°C with phosphate-buffered 3% glutaraldehyde, post-fixed in 1% OsO_4 in phosphate buffer for 1 hr at 4°C, dehydrated in graded ethanol, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined under a JEM 2000 FXII electron microscope.

Immunophenotyping

Immunophenotyping of LN aspirate and PB was analyzed by 2-color flow cytometry on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.) for various antigens. The following commercially available monoclonal antibodies were used: CD4, CD5, CD8, CD10, and HLA-DR (Immune Source, Los Altos, CA, U.S.A.); CD3, CD7, CD16+56, CD19, CD20, CD21, CD22, CD23, Ig κ , and Ig λ (Becton Dickinson Immunocytometry Systems, San Jose, CA, U.S.A.); IgM, IgD, IgG, IgA, and IgE (Pharmin-

gen, San Diego, CA, U.S.A.). Dual staining was performed as follows: CD3/CD4, CD3/CD8, CD3/CD19, CD3/CD16+56, CD3/HLA-DR, CD5/HLA-DR, CD5/CD19, CD7/CD22, CD10/CD23, CD20/CD21, and Ig κ /Ig λ .

Also cyclin D1 (Santa-Cruz, CA, U.S.A.; R-124, 1:100) in LN biopsy was evaluated by immunohistochemistry.

DNA index and parameters, *bcl-1*/J_H junction and cytogenetic analysis

For DNA analysis, 10^6 cells of FNA cytology were washed twice with phosphate-buffered saline solution and fixed in 100% ethanol for 30 min at 4°C. After treatment with RNase for 40 min at 37°C the cells were stained with propidium iodide (50 mg/mL). Cell cycle analysis and DNA index were determined by FACSCalibur using calf thymocyte nuclei and chicken erythrocyte nuclei as controls.

DNA was extracted from LN aspirate by a standard phenol-chloroform ethanol precipitation technique. Polymerase chain reaction (PCR) analysis of the translocation involving the major translocation cluster of the *bcl-1* gene and the Ig heavy chain gene was performed using both primer pairs, *mcl-1*/J_H and *mcl-2*/J_H as previously described (7). The BM aspirate was analyzed for cytogenetic abnormalities by the trypsin-Giemsa banding method as previously described.

RESULTS

Cytomorphology of B-MCL by light microscope

LN FNA smears

The Diff-Quick and Papanicolaou-stained smears revealed a relatively uniform population of medium to large lymphoid cells. The nuclei were approximately three to four times larger than a mature, small lymphocytes nuclei. The nuclei had slightly irregular outlines and fine, evenly dispersed chromatin, and the nuclei were cleaved in some cells. Most of the nuclei contained 1 to 2 small nucleoli. The cytoplasm was scant and stained pale blue on the Diff-Quick stained smears. Mitoses were infrequently observed.

PB smears

The lymphocytes had a fairly pleomorphic appearance and consisted of a mixture of small, medium, and large cells. The cells had slightly irregular nuclear outlines and ranged from small lymphocytes with moderately coarse chromatin to medium-sized lymphocytes with blast-like fine chromatin. The predominant cells were medium-sized blast-like cells with visible amounts of cytoplasm, round nuclei, relatively fine chromatin and 1 to 2 prominent nucleoli. Some cells showed nuclear irregularities, and others showed cleaved nuclei (Fig. 1). The smaller atypical lymphocytes were reminiscent of those in leukemic phase of conventional MCL.

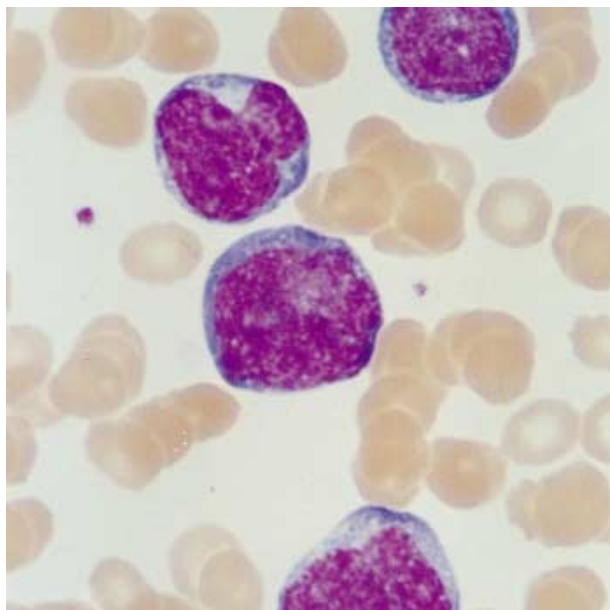


Fig. 1. Peripheral blood smear in leukemic mantle cell lymphoma, blastoid variant. The cells range from small lymphocytes to medium-sized blast-like cells with 1 to 2 nucleoli. Some cells show cleaved nucleus (Wright stain, $\times 1,000$).

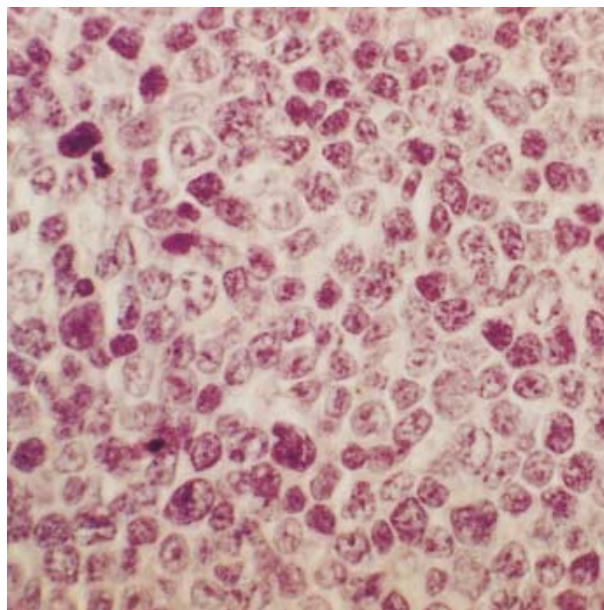


Fig. 2. Lymph node biopsy reveal the features of the pleomorphic malignant lymphoma cell showing medium and large cell with prominent nucleoli ($\times 400$).

BM aspiration and biopsy

The BM aspirate showed a markedly hypercellular marrow with decreased megakaryocytes. The normal hematopoietic cells were almost totally replaced by malignant lymphoid cells. Prussian blue staining showed slightly decreased storage of iron. Most significantly, the BM aspirate showed an increase in the number of small to medium and large-sized, slightly irregular lymphocytes. The infiltrative lymphoma cells were similar to those described in the PB, with numerous blast-like cells. The BM core and clot sections showed 30% cellularity with myeloid, erythroid, and megakaryocytic findings similar to those of the BM aspirate. The pattern of BM involvement was diffusely infiltrative. The lymphoid cells were small- to medium-sized and occasionally blast-like in the BM core biopsy.

LN biopsy

The LN was diffusely replaced by lymphoma cells with high mitotic rate (Fig. 2). The cells were small- to medium-sized with slightly irregular nuclear outlines, moderately finely dispersed blast-like chromatin, and small or inconspicuous nucleoli. No pseudofollicular proliferation centers, paraimmunoblasts, or large lymphoid cells were present. Scattered epithelioid histiocytes with granular eosinophilic cytoplasm did not contain nuclear debris.

Ultrastructural features

The PB of the present B-MCL case showed pleomorphic

appearance and consisted of a mixture of small, medium and large cells, and disclosed distinct ultrastructural features including lymphoblast-like cells with a large and 1 to 2 prominent nucleoli, high N/C ratio, and poorly developed cytoplasmic organelles, and cleaved or indented nucleus showing even heterochromatin distribution, absent or inconspicuous nucleolus, low N/C ratio, abundant mitochondria, and a well developed endoplasmic reticulum.

Immunophenotyping

Flow cytometric immunophenotypic analysis of the LN aspirate and PB identified a monoclonal population of malignant lymphoid B-cells with expression of CD19 (72%), CD20 (57%), CD22 (68%), IgM (55%), λ light chain (51%), and HLA-DR (70%), and aberrant expression of CD5 (76%), while CD10 and CD23 elicited no reaction. The coexpression of CD5/CD19 and CD5/HLA-DR revealed 57.3% and 60.4% respectively.

The immunohistochemistry for cyclin D1 protein was strong reactive in the nucleus of lymphoma cells in LN biopsy (Fig. 3)

Proliferation markers

Flow cytometric DNA ploidy analysis demonstrated diploid (31.6%) and aneuploid (68.4%) cell population with a DNA index of 1.35 and an S+G₂M fraction of 43.5%.

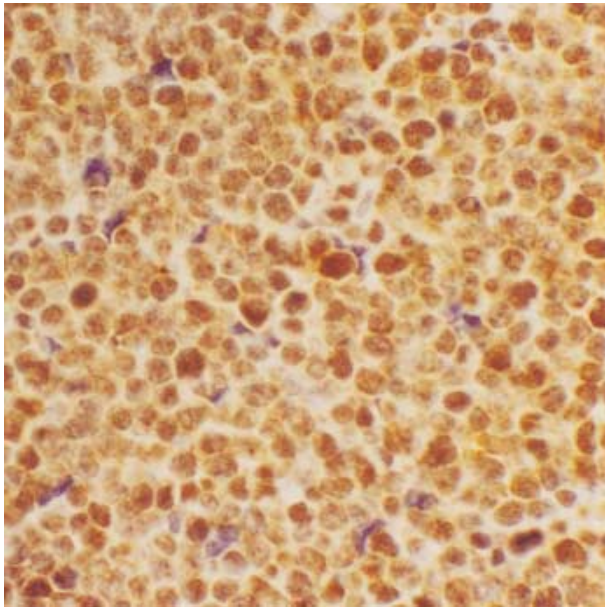


Fig. 3. Immunohistochemistry stain for the cyclin D1 protein show strong reactivity in the nucleus of lymphoma cell of lymph node biopsy ($\times 400$).

Gene rearrangement studies and chromosome analysis

PCR analysis revealed a clonal rearrangement of the *bcl-1*/Ig heavy chain fusion gene in DNA isolated from LN aspirate (Fig. 4). Cytogenetic analyses of 20 metaphases prepared from BM aspirates showed hypodiploid 44,X,-Y, and shared the following cytogenetic abnormalities: add(1)(q43),add(4)(q35),der(6)t(6;?)(q22;?),-9,-11,-13,-14,-15,+5mar[16]/46,XY[4].

DISCUSSION

MCL, formerly known as intermediately differentiated lymphocytic lymphoma (Rappaport classification), centrocytic lymphoma (Kiel classification), or mantle zone lymphoma, is now accepted as a distinct clinicopathologic entity of non-Hodgkin's lymphoma. The term MCL has become widely accepted and is used in the Revised European-American Classification of Lymphoid Neoplasm, first published in 1994 (8). MCL is generally recognized to represent about 2.5 to 5% of all non-Hodgkin's lymphoma in the U.S.A. and 1.5% in Korea (9).

Since its original description of B-MCL by Lardelli et al. (4), only few cases have been described in the literature. This B-MCL have been referred to by a number of names such as blastic, blastoid, large cell, lymphoblastoid, and pleomorphic. Histologic transformation has been reported in up to 17% of serially biopsied cases of MCL. B-MCL displays a distinctly more aggressive clinical course (4).

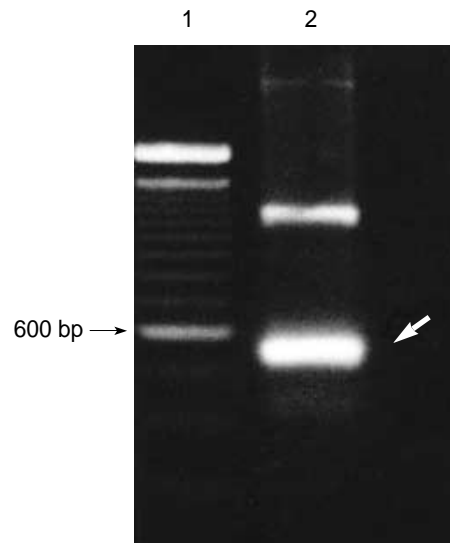


Fig. 4. Polymerase chain reaction analysis reveal a clonal rearrangement of the *bcl-1*/Ig heavy chain fusion gene in DNA isolated from lymph node aspirate. Lane 1. Molecular marker, *Hae* III-digested $\phi \times 174$ RF DNA fragment. Lane 2. Extracted DNA from lymph node aspirate.

While B-MCLs are uncommon lymphoid malignancies, this neoplasm is associated with some distinct cytomorphologic and immunophenotypic features (4). Conventional nodal MCL is characterized by a homogeneous population of small-to medium-sized lymphoid cells with scant pale cytoplasm, indented nuclei, dispersed chromatin, and inconspicuous nucleoli. Paraimmunoblasts and transformed cells are absent from conventional MCL, resulting in monotonous features. Cells of B-MCL exhibit a slightly larger size, from 1.5 to 3 times, blast-like morphologic features with more irregular nuclei, a more dispersed chromatic pattern, higher mitotic activity and more prominent nucleoli than those of conventional MCL (8). Furthermore, cells of B-MCL can be much less monotonous than those of common MCL, and thus may frequently be confused with lymphoblastic lymphoma or large cell lymphomas (4). The patient's clinical history can be very helpful in making this distinction, because patients with MCL are typically older adults, whereas patients with lymphoblastic lymphoma are typically young and present with mediastinal mass.

The FNA cytology of typical cases of MCL has been described (5), but the published literature describing the cytomorphologic features of B-MCL is limited to one case in which a primary diagnosis of MCL had already been established by surgical biopsy and the patients subsequently developed a pleural fluid or cerebrospinal fluid involvement, of which the cytologic evaluation confirmed B-MCL (6). Hughes et al. (6) demonstrated the first case of B-MCL diagnosed based on the FNA cytology combined with ancillary studies.

Immunophenotyping, cytogenetics, image analysis, and

molecular studies may be necessary to make a definitive diagnosis of MCL (6). Immunophenotypically, the conventional type and B-MCL is characterized by co-expression of CD5 and CD20 antigens, and usually lacking CD10 and CD23. The neoplastic cells have a monoclonal B-cell immunophenotype, almost always expressing surface IgM and IgD. The monoclonal λ light chain is expressed in about 60% of MCL. The MCL can be differentiated from chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) by the immunophenotypic marker, CD23 (10). Immunophenotypically, CLL expresses CD23 and weak intensity surface Ig and CD20, while mantle cell leukemic cells lack CD23 and expresses moderate to strong intensity surface Ig and CD20. If morphologic examination is combined with immunophenotypic and molecular analysis, more cases of B-MCL will be detected.

At the time of diagnosis, MCL frequently involves the PB and BM (3, 11). Neoplastic lymphoid (mantle cell leukemic) cells are found in PB smears of approximately one quarter to one third of patients with MCL using morphologic examination alone. Large studies of MCL have found a high proportion of cases with BM involvement from 53 to 93%. When confronted with a PB containing the blast-like cells, the differential diagnosis should include acute leukemia, prolymphocytic leukemia, and peripheralized large cell lymphoma. In B-MCL with PB involvement, the circulating lymphoma cells show slightly irregular nuclear contours, ranging from small cells with moderately coarse chromatin to medium-sized blast-like cells with finer blast-like chromatin than conventional type of leukemic phase of MCL.

Cells of acute lymphoblastic leukemia lack surface Ig and cyclin D1 expression, and express TdT; those of B-MCL express surface Ig, but not TdT. Large cell lymphoma cells rarely coexpress CD5. The leukemic cells of follicular center cell (FCC) lymphoma, small cleaved type, are smaller with coarse chromatin and nuclear cleft. The FCC lymphoma often expresses CD10 but lacks CD5, and is associated with a t(14;18). The PB smear in the mixed cell type of CLL shows less pleomorphism and rounded nuclei, however some cases of CLL may have irregular nuclear contours, making the morphological distinction from MCL.

It is difficult to diagnose patients with B-MCL correctly without immunophenotyping, and molecular analysis can substantiate the diagnosis. MCL is distinguished by a characteristic chromosomal translocation. A chromosomal translocation t(11;14) involving the Ig heavy chain gene locus (14q32) and the *bcl-1* locus (11q13) is seen in approximately 95% of MCL cases. This translocation has been detected in both conventional MCL and B-MCL, and results in an overexpression of the cyclin D1 protein, a cell-cycle protein that is not normally expressed at high levels in lymphoid cells. *Bcl-1* gene rearrangement and/or the t(11;14) translocation detected by either southern blot hybridization or PCR analysis, and tetraploid chromosomes may be more common in

B-MCL. Although our case did not exhibit the t(11;14) translocation in cytogenetic analysis, it should be noted that a *bcl-1* rearrangement and overexpression of cyclin D1 were present. While the t(11;14) seen in conventional MCL has also been reported in B-MCL, the full spectrum of cytogenetic abnormalities associated with B-MCL is not well-characterized because of the rarity of the B-MCL. B-MCL shows a higher proliferation rate, as shown by Ki-67 antigen expression, and has a higher incidence of p53 immunostaining than the conventional MCL (6).

The present case demonstrates that cytomorphology combined with flow cytometric analysis of immunophenotype and proliferation markers obtained by LN FNA cytology and PB is helpful to confirm B-MCL, which can be further substantiated by the demonstration of *bcl-1* gene rearrangement.

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