CD23 Expression in Mantle Cell Lymphoma: Clinicopathologic Features of 18 Cases

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Abstract

The distinction between small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and mantle cell lymphoma (MCL) has important clinical implications. Typically, SLL/CLL is CD23+, whereas MCL is CD23–. However, CD23 is expressed in a subset of MCLs, and the clinicopathologic features of patients with these neoplasms are not well described. We report 18 CD23+ MCLs, detected by flow cytometry in all cases (dim intensity, 16; bright intensity, 2), 5 (28%), also positive by immunohistochemical analysis. There were 13 men and 5 women (median age, 56 years), 5 of whom died (median survival, 46 months). Seventeen (94%) had bone marrow involvement. Lymphadenopathy (14 cases [78%]), splenomegaly (11 cases [61%]), and leukemic involvement (10 cases [56%]) were common. Five cases (28%) had blastoid morphologic features. The frequency of CD23 expression by MCL is method-dependent, being typically dim and most commonly detected by flow cytometry. In this small study group, bone marrow and leukemic involvement were relatively common.

The classification of small B-cell lymphoproliferative disorders is based on a combination of clinical, morphologic, immunophenotypic and cytogenetic parameters, as is described in the World Health Organization classification.[1] Immunophenotyping by flow cytometric or immunohistochemical analysis is particularly helpful in establishing the diagnosis because there is significant clinical and morphologic overlap between these diseases and immunophenotyping results are readily available. By contrast, conventional cytogenetics and fluorescence in situ hybridization (FISH) analysis for the t(11;14)(q13;q32), characteristic of MCL, require more time and may not be readily available to all practitioners.

CD23 is a low-affinity IgE Fc receptor and a marker of activated B cells. It is expressed weakly or is absent in resting mature peripheral blood and lymphoid tissue B cells.[2] Among CD5+ B-cell lymphoproliferative disorders, expression of the CD23 antigen is useful for distinguishing small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) from mantle cell lymphoma (MCL). The majority of SLL/CLL cases are CD23+, whereas MCL cases usually are CD23–. However, CD23 positivity can be observed in a subset of MCLs, and this finding can lead to diagnostic confusion.

The frequency of CD23 positivity in MCL is uncertain. In many reports, CD23 has been negative in MCL.[3-6] In reports that include CD23+ MCL cases, detected by flow cytometric or immunohistochemical analysis, the frequency of CD23 expression has ranged from 2% to 45%.[7-14] In these studies, CD23 positivity in MCL is discussed as a diagnostic pitfall, without description of the clinicopathologic features of these cases. Thus, potential differences between CD23– and CD23+ MCL cases are unknown. We describe the clinicopathologic

and immunophenotypic features of 18 cases of CD23+ MCL. In this group, CD23 expression was assessed by both flow cytometric and immunohistochemical analysis.

Materials and Methods

Case Selection

Data for 18 patients with MCL, evaluated at The M.D. Anderson Cancer Center, Houston, TX, from March 1996 to June 2003, were included in this study. The diagnosis of MCL was supported by a combination of morphologic and immunophenotypic features with cytogenetic data in a subset of cases. All MCL cases were CD23+ shown by immunophenotypic methods performed on bone marrow (BM), peripheral blood (PB), or lymph node (LN) specimens. Clinical information, including sex and age, physical findings, treatment history, and follow-up, were available for all cases.

Morphologic Evaluation

Available diagnostic material included LN, spleen, BM, and PB specimens that were retrieved from the files of our department. BM samples were available for all 18 patients, and other tissue specimens were available for 15 patients. In 1 case, the diagnosis of MCL was based on BM and PB specimens. The pattern of LN and BM involvement and cytologic features were assessed.

Immunophenotypic Studies

Flow cytometric immunophenotypic studies were performed at the time of initial evaluation at our institution on BM and PB specimens (7 cases), BM specimens (8 cases), an LN specimen (1 case), a PB specimen (1 case), or on all 3 specimen types (1 case). The samples were assessed using 3-color flow cytometric analysis and a FACScan (Becton Dickinson, San Jose, CA) instrument. Lymphocytes were gated for analysis using CD45 expression and right-angle light scatter as described previously.[3] Fluorescein isothiocyanate– and phycoerythrin-conjugated IgG1 and IgG2 antibodies were used as negative controls, and cursors were set to include more than 95% of events as negative. The panel of antibodies, conjugated to fluorescein isothiocyanate, phycoerythrin, or allophycocyanin, included reagents specific for CD3, CD5, CD19, CD20, CD23, FMC7, and immunoglobulin κ and λ light chains (Becton Dickinson).

Immunohistochemical stains for cyclin D1 (AM29, Zymed Laboratories, South San Francisco, CA) and CD23 (1B12, Novocastra Laboratories, Newcastle upon Tyne, England) were performed using formalin-fixed, paraffin-embedded tissue sections of LN (n = 3) and BM, the latter either aspirate clot (n = 12) or core biopsy (n = 3) specimens, as described previously.[11] Briefly, after deparaffinization and rehydration in graded alcohols and xylene, endogenous peroxidase was blocked with hydrogen peroxide. Heat-induced epitope retrieval was performed by heating slides in EDTA buffer at pH 8.0, using a Black and Decker (Towson, MD) Handy Steamer Plus, and then cooling for 20 minutes. Immunostaining was completed using the LSAB2 detection kit (DAKO, Carpinteria, CA), with appropriate positive and negative controls.

Cytogenetic Studies

Fourteen tumors were analyzed using conventional karyotypic analysis. Cells were placed in 10 mL of Ham F10 medium, with 20% fetal calf serum at a concentration of 2×10^6 to 4×10^6 nucleated cells per milliliter. The culture was incubated at 37°C for approximately 24 hours. Standard harvesting procedures were used. Colcemid (0.1 mL/10 mL) was added to the culture for 30 minutes at room temperature. For hypotonic treatment, a 0.075-mol/L concentration of potassium chloride was used for 30 minutes at room temperature. The fixation procedure consisted of 3 changes of methanol/glacial acetic acid (3:1), with a 10-minute interval between each change. A drying chamber (Thermotron Industries, Holland, MI) was used for slide preparation. Slides were placed in a 60°C oven overnight, followed by GTG banding. The karyotype reports were written using the International System for Human Cytogenetic Nomenclature (1995).[15]

FISH Studies

The commercially available LSI IGH/CCND1 dual-color, dual-fusion translocation probe (Vysis, Downers Grove, IL) was used, and FISH was performed using 5-µm-thick tissue sections of formalin-fixed, paraffin-embedded BM biopsy specimens.

Briefly, tissue sections were mounted on positively charged slides, air dried, and baked overnight at 60°C. Slides then were deparaffinized in Hemo-De (Fisher Scientific, Pittsburgh, PA) (3 cycles, at ambient temperature), dehydrated in 100% ethanol (room temperature), and air dried by placing the slides on a 45°C to 50°C slide warmer for 2 to 5 minutes. The slides then were immersed in 0.2N hydrogen chloride for 20 minutes, followed by washes (3 minutes each) in purified water and wash buffer (2× standard saline citrate [SSC], pH 7.0). Pretreatment consisted of immersing in sodium thiocyanate solution (Vysis) at 80°C for 30 minutes, again followed by washes in purified water and wash buffer. The tissue sections were digested in protease solution at 37°C for 10 minutes, washed in buffer, and dried on a slide warmer for 2 to 5 minutes. The slides were fixed in 10% formalin/phosphate-buffered saline for 10 minutes (at ambient temperature) and washed in buffer solution. Denaturation of the probe mixture and specimen were performed at 80°C (5 minutes) using the Vysis HYBrite hybridization

system. The probe mixture included 7 mL of LSI hybridization buffer, 1 mL of probe, and 2 mL of purified water. After an overnight hybridization at 37°C, the slides were washed in 2× SSC/0.1% NP-40 (Vysis) at 73°C for 2 minutes, rinsed in 2× SSC/0.1% NP-40 for 5 to 10 seconds at room temperature, air dried in darkness, and counterstained with 10 mL of 4,6-diamidino-2-phenylindole in antifade (DAPI). Hybridization signals were analyzed using a Zeiss Axioskop (Carl Zeiss Surgical, Thornwood, NY) equipped with appropriate filters. Images were captured using the Cyto Vision imaging system (Applied Imaging, Santa Clara, CA).

Results

Clinical Features

There were 13 men and 5 women with a median age of 56 years at the time of initial diagnosis (range, 44-72 years). The referral diagnoses included MCL (12 cases), B-cell SLL/CLL (3 cases), B-cell prolymphocytic leukemia (1 case), follicular small cleaved cell lymphoma (1 case), and diffuse large B-cell lymphoma vs MCL (1 case).

At diagnosis, 6 patients had diffuse or localized lymphadenopathy, 7 patients were found incidentally to have lymphocytosis during routine examination, 1 patient had lymphadenopathy and lymphocytosis, 1 patient had lymphadenopathy and splenomegaly, 1 patient had splenomegaly, 1 patient had a colon mass, and 1 patient underwent bone marrow aspiration and biopsy for assessment of thrombocytopenia. Nine (50%) of 18 patients received chemotherapy before referral to our hospital.

At our institution, 17 patients had lymphadenopathy (n = 6), splenomegaly (n = 3), or both (n = 8). One patient did not have lymphadenopathy or splenomegaly but recently had received treatment. One patient had undergone splenectomy before coming to our institution. Of the 14 patients who had lymphadenopathy, multiple sites usually were involved. Of the 11 patients with splenomegaly, the spleen size was reported as "palpable" or up to 22 cm inferior to the left costal margin.

Serum levels of β_2-microglobulin and lactate dehydrogenase were known for all 18 patients. The median β_2-microglobulin level was 3.3 µg/mL (281 nmol/L; reference range, 0.6-2.0 µg/mL [51-170 nmol/L]) with a range from 1.6 to 8.4 µg/mL (136-714 nmol/L); 16 patients (89%) had elevated levels. The median lactate dehydrogenase level was 458 U/L (reference range, 313-618 U/L) with a range from 75 to 1,319 U/L; 4 patients (22%) had elevated levels.

Peripheral Blood Findings

Ten (56%) of 18 cases had leukemic involvement, defined as a lymphocyte count of more than 5,000/µL (>5.0 × 10^9/L). In these 10 cases, the median WBC count was 60,300/µL (60.3 × 10^9/L; range, 16,500-196,000/µL [16.5-196.0 × 10^9/L]) with a median absolute lymphocyte count of 55,900/µL (55.9 × 10^9/L; range, 13,900-158,800/µL [13.9-158.8 × 10^9/L]).

In the remaining 8 patients, the median WBC count was 7,000 (7.0 × 10^9/L; range, 4,700-9,000/µL [4.7-9.0 × 10^9/L]) with a median absolute lymphocyte count of 1,800/µL (1.8 × 10^9/L; range, 180-4,300/µL [0.18-4.3 × 10^9/L]).

In 10 cases, peripheral blood smears were available for review, 7 from the leukemic group and 3 from the nonleukemic group. All 10 cases showed atypical lymphoid cells, morphologically consistent with MCL.

Morphologic Features

For 15 patients, LN, spleen, or extranodal specimens were available for review, including 13 LN, 3 spleen, 1 gallbladder, and 2 endoscopic colon biopsy specimens. Of the 13 LN specimens, 9 were obtained at the time of initial diagnosis and 4 were obtained 2 to 24 months after initial diagnosis.

The pattern of LN involvement included mantle zone and nodular (1 case), nodular (3 cases), nodular and diffuse (3 cases), and diffuse (6 cases). In 10 cases, the MCL had typical small cell features **Image 1**; 2 other cases were blastoid (1 classic and 1 pleomorphic variant). In addition, 1 LN with a diffuse pattern and small cell cytologic features had focal clusters of large cells, suggestive of focal blastoid MCL, pleomorphic variant.

Of 18 patients, 17 (94%) had BM involvement by MCL, representing 20% to 90% of the cellular elements. The patterns of involvement included nodular, interstitial, and diffuse and usually were found in multiple combinations. Fourteen cases had cytologic features of typical MCL on the BM aspirate smears. The neoplastic cells were predominantly small to intermediate size and had irregular nuclear contours, inconspicuous nucleoli, and scant cytoplasm. Three cases showed classic blastoid morphologic features in the BM specimens. The neoplastic cells were of intermediate size with fine chromatin, inconspicuous nucleoli, and scant cytoplasm. One BM case showed prolymphocytoid morphologic features on the aspirate smears as the cells were of intermediate size with prominent nucleoli.

Morphologic discordance between multiple biopsy sites was observed in 4 cases. Two cases that showed classic blastoid morphologic features in tissue specimens had BM involved by typical MCL. In a third case, an LN specimen showed typical morphologic features, but in the BM specimen, the tumor was prolymphocytoid. In the fourth case, the initial BM specimen showed typical MCL. However, 30 months later, the spleen and BM specimens showed blastoid MCL, pleomorphic variant.

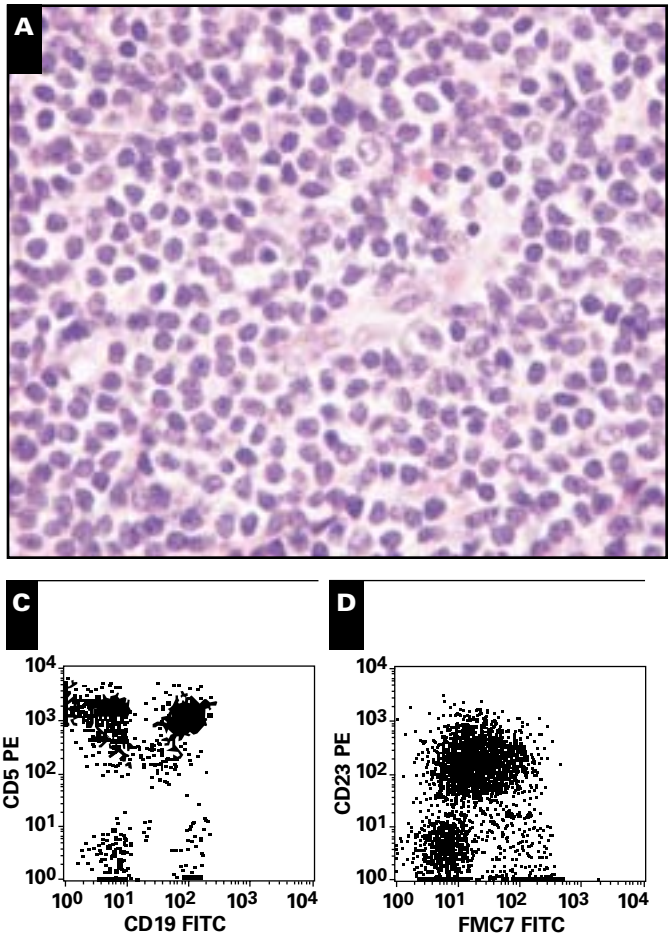

Image 1 CD23+ mantle cell lymphoma; cyclin D1+. **A**, Histologic sections of lymph node show that the neoplastic cells are small, with irregular nuclei and minimal cytoplasm (H&E, ×400). **B**, Approximately 50% of the neoplastic cells are CD23+, with variable intensity of staining (immunoperoxidase with hematoxylin counterstain, ×400). Flow cytometric analysis demonstrating expression of CD5 and CD19 (**C**) and expression of CD23 and dim FMC7 (**D**). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

Immunophenotypic Results

Flow cytometric immunophenotypic studies were performed in all cases and identified a CD5+ B-cell population with immunoglobulin light chain restriction (κ, 12 cases; λ, 6 cases). All cases were positive for CD19, CD20 (bright, 13 cases; dim, 2 cases; intensity not reported for 3 cases), and CD23 and were negative for CD3 and CD10 (Image 1) **Image 2**. Of 18 cases, 14 (78%) were FMC7+. CD23 expression was defined as bright (>$\log 10^2$ mean fluorescence intensity) in 2 cases and dim (<$\log 10^2$ mean fluorescence intensity) in 16 cases. In the 7 cases with flow cytometric results for both BM and PB specimens, no discordant results in antigen expression were identified.

With immunohistochemical methods, cyclin D1 was expressed in 17 (94%) of 18 cases assessed. The 1 case that lacked cyclin D1 expression carried the t(11;14). CD23 was positive in 5 (28%) of 18 cases assessed. In 4 cases, CD23 expression was weak and often detected focally, ranging from fewer than 5% to approximately 25% of neoplastic cells positive. One case was strongly CD23+.

Cytogenetic Results

Fourteen cases were analyzed using conventional cytogenetics, 3 of which also were analyzed by FISH, with clonal results in 9 cases. All 9 tumors showed the t(11;14)(q13;q32) with multiple additional karyotypic abnormalities **Table 1**. The 9 clonal cases included studies performed on BM (6 cases), LN (2 cases), and spleen and BM specimens (1 case).

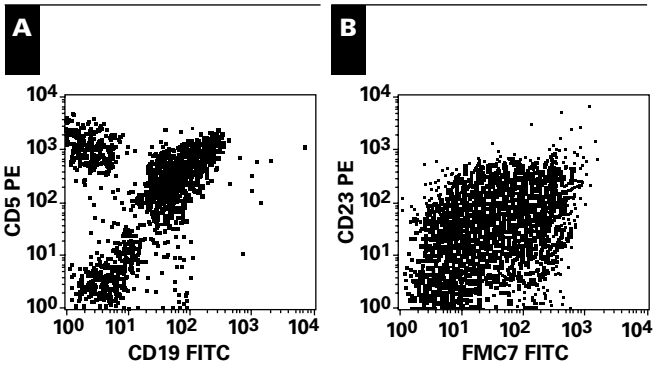

Image 2 CD23+ mantle cell lymphoma; cyclin D1–. Flow cytometric analysis demonstrating expression of CD5 and CD19 (**A**) and CD23 and FMC7 (**B**). This case had 1 metaphase with the t(11;14) detected by conventional cytogenetics. Fluorescence in situ hybridization analysis showed 54% of nuclei with the t(11;14). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

In 2 cases (cases 10 and 11), a single, abnormal metaphase was detected. In 1 case, the abnormality was del(14q); this case was shown immunohistochemically to be cyclin D1+. The second case had 1 metaphase with the following karyotype: 45,XY,–8,t(11;14)(q13;q32),add(12)(p11.2), add(18)(q23). However, FISH studies confirmed the presence of the t(11;14) in 54% of nuclei examined. This case was immunohistochemically negative for cyclin D1.

Three patients had a diploid karyotype. In these patients, the MCL was cyclin D1 positive and, in 1 case, FISH analysis confirmed the presence of a substantial number of nuclei with the t(11;14).

Follow-up Information

The median time of clinical follow-up was 52 months (range, 2-77 months). Eight patients were treated at our institution with fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone (Hyper CVXD) plus rituximab (anti-CD20). Eleven patients were treated in a nonuniform manner at other institutions. Five patients died. The median time from diagnosis until death was 46 months (range, 28-77 months).

Discussion

The frequency of CD23 expression in MCL, as reported in the literature, varies widely, ranging from 0% to 45% of cases. These results are summarized in **Table 2**.[6-8,10-14,16-27] As shown in Table 2, the method of detection is important for assessing the frequency of CD23 positivity. By using immunohistochemical methods in 7 studies, 4 (3.6%) of 110 MCL cases were positive for CD23.[8,10,16-18,24,25,27] By contrast, using flow cytometry in 9 studies, 21 (14.4%) of 146 MCL cases were CD23+, including in the study by Gong and colleagues,[14] in which 45% of MCL cases had dim CD23 expression.[6,7,11,14,19,20,24,25,27] Thus, the increased sensitivity of flow cytometry is needed to detect CD23 in most MCL cases, suggesting that CD23 positivity typically is dim.

In the present study, we compared flow cytometric analysis, which was performed as a part of the initial diagnostic evaluation, with immunohistochemical analysis in all cases. Only 5 cases positive by flow cytometric analysis were also positive immunohistochemically. In 4 of 5 cases, immunohistochemical detection of CD23 was weak and often detected focally. Similar results were reported by Tworek and colleagues.[24] Although the discrepancy between flow cytometric and immunohistochemical results also may be related to the type of fixative used before immunohistochemical analysis, as suggested by others,[25] all tissue specimens in the present study were fixed in formalin.

In addition to CD23 positivity, 4 (22%) of 18 cases in this study were FMC7–. FMC7 is another marker useful for distinguishing SLL/CLL from MCL, as SLL/CLL cases usually are negative and MCL cases usually are positive.[20] In fact, some have advocated using the combination of CD23 and FMC7 for differential diagnosis.[14,24] Thus, the potential for misdiagnosis of these MCL cases as SLL/CLL exists if flow cytometric results are not correlated with other findings. In our study, 1 of the 4 CD23+, FMC7– cases with leukemic involvement was misdiagnosed initially as SLL/CLL. However, all 4 cases had the t(11;14)(q13;q32), and cytogenetic results helped establish the correct diagnosis in the 1 misclassified case.

CD23, a 45,000-kd glycoprotein, is a low-affinity Fc receptor for IgE that is expressed by a subpopulation of normal resting B cells that express surface IgM and IgD.[28] CD23 also is overexpressed in SLL/CLL. However, whether CD23 has a role in the

Table 1
Summary of Abnormal Cytogenetic Findings

Case No.	Tissue	Cytomorphologic Features	Karyotype
1	BM	Typical	43-46,XY,+3,t(5;8)(q34;q13),add(6)(p22),add(7)(q36),–7,–9,t(11;14)(q13;q32),–17,–18,+20,–21,+mar[cp11]
2	BM	Blastoid	46,XY,+3,add(5)(p15),t(11;14)(q13;q32),–13[2]/46-48,XY,+3,add(5)(p15),–9,t(11;14)(q13;q32), –15,–17,–18,+19,+21,+1-6mar[cp15]
3	BM; spleen	Typical	46,XY,del(7)(q32),t(11;14)(q13;q32),add(16)(q24)[11]
4	LN	Blastoid	40-45,X,–Y,del(1)(p34.3p36.2),inv(1)(p22q21),–5,del(6)(q15q25),–8,t(11;14)(q13;q32), del(12)(q24.1q24.3),–18,add(22)(q13),+1-3mar[cp10]
5	BM	Typical	42-46,XX,add(1)(p36),add(3)(q29),–4,add(5)(p15),add(9)(p24),–11,add(11)(p15),+12,add(12)(q24), –13,–14,der(14)t(11;14)(q13;q32),–15,–17,–18,–20,–22,+3-8mar[cp18]
6	BM	Typical	42-46,XY,–2,der(10)t(2;10)(q21;q26),t(11;14)(q13;q32),+0-2mar[cp7]*
7	BM	Typical	46,XY,t(11;14)(q13;q32),add(22)(q13)[8]
8	LN	Typical	46,XX,t(11;14)(q13;q32),add(17)(p10)[17]
9	BM	Typical	46,XY,t(11;14)(q13;q32)[2]
10	BM	Blastoid	45,XY,–8,t(11;14)(q13;q32),add(12)(p11.2),add(18)(q23)[1]*
11	BM	Typical	46,XY,del14q[1]†

BM, bone marrow; LN, lymph node.
* Presence of t(11;14) confirmed by fluorescence in situ hybridization studies.
† This case was positive for cyclin D1.

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