Clinicopathologic Features of CD5-Positive Nodal Marginal Zone Lymphoma

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ABSTRACT

Objectives: To describe the clinicopathologic findings of seven patients with CD5-positive nodal marginal zone lymphoma (NMZL).

Methods: We searched cases of NMZL over a 10-year interval and identified seven cases of CD5-positive NMZL. The clinical, histologic, and immunophenotypic findings of this group were reviewed, and the frequency of dissemination in this group was compared with that of 66 patients with CD5-negative NMZL.

Results: Other than CD5 expression, the histologic and immunophenotypic findings were typical of NMZL. Six (86%) of seven patients had lymphadenopathy above and below the diaphragm, and all six patients assessed had bone marrow involvement. In the CD5-negative group, 28 (42%) patients had lymphadenopathy above and below the diaphragm, and 36 (55%) had bone marrow involvement (P = .045 and P = .037, respectively). Six of seven patients were alive at last follow-up, with a median follow-up of 32 months (3-154 months).

Conclusions: CD5 expression in NMZL correlates with a higher frequency of dissemination, but patients have an indolent clinical course and excellent overall survival.

Nodal marginal zone lymphoma (NMZL) was initially described and designated as monocytoid B-cell lymphoma by Sheibani and colleagues1 in 1986 based on their observation that the neoplastic cells resembled, in part, monocytoid B cells as seen in pathologic states, such as infection by Toxoplasma gondii. Subsequently, the term nodal marginal zone lymphoma was proposed to emphasize the morphologic and immunophenotypic similarity between NMZL and marginal zone lymphomas involving extranodal sites and the spleen.2 NMZL is currently defined in the World Health Organization (WHO) classification as a primary nodal B-cell lymphoma that morphologically resembles extranodal or splenic marginal zone lymphoma but without evidence of extranodal or splenic disease.3 Studies have suggested that NMZL has a distinctive gene expression profile that distinguishes it from other marginal zone lymphomas.4,5 NMZL is uncommon, accounting for less than 2% of all lymphomas.3,6

The current WHO classification system does not include immunophenotype in the definition of NMZL.3 However, NMZL typically has a nonspecific B-cell immunophenotype, positive for monotypic immunoglobulin and pan-B-cell markers and usually negative for CD5, CD10, CD23, BCL-6, and cyclin D1. CD5 is a membrane glycoprotein that is normally present on T-cell subsets as well as memory B cells and appears to have an effect that contributes to B-cell survival.7 The physiological functions of CD5 in humans, however, remain incompletely understood. Others have reported that CD5 expression can occur in a subset of marginal zone lymphomas and may correlate with dissemination of disease. Ferry et al8 described three cases of CD5-positive extranodal marginal zone lymphoma of mucosa-associated lymphoid
tissue (MALT) type and proposed that CD5 expression is a marker of dissemination and bone marrow involvement. Subsequent case studies and small series also have suggested that patients with CD5-positive MALT lymphoma more often present with disseminated disease than patients with CD5-negative MALT lymphoma.9,10 Similarly, CD5 expression in splenic marginal zone lymphoma has been documented and reported to be associated with an increased propensity for peripheral blood and bone marrow involvement.11,12 Other reports have described small subsets of cases of CD5-positive extranodal or splenic marginal zone lymphoma.13-19 In contrast, fewer cases of CD5-positive NMZL have been reported in the literature, mostly as case reports or as subsets of larger series.20-25 In this study, we have collected the largest case series to date of patients with CD5-positive NMZL to more completely describe the clinicopathologic features of this disease as well as assess the impact of CD5 expression on disease dissemination and prognosis.

Materials and Methods

Case Selection and Morphologic Review

We searched the archives of our hospitals from January 1, 2000, through June 30, 2010, for cases of B-cell lymphoma that were positive for CD5 by immunohistochemistry, flow cytometric immunophenotyping, or both methods. We then selected cases of NMZL as defined using the criteria of the 2008 WHO classification.3 Clinical staging was based on the Ann Arbor system. The workup included history and physical examination; computerized tomographic scans of the neck, thorax, and abdomen; and upper gastrointestinal endoscopy in a subset of patients to exclude the presence of extranodal disease. Six of seven patients underwent bone marrow aspiration and biopsy; one patient declined examination. Clinical and laboratory data were obtained by review of medical records. The study was conducted according to an MD Anderson Cancer Center institutional review board–approved protocol.

We reviewed H&E-stained slides prepared from formalin-fixed, paraffin-embedded tissue sections from the diagnostic lymph node biopsy specimens of each patient. In six patients, Wright-Giemsa–stained bone marrow aspirate smears and touch imprints were reviewed, and 500-cell differential counts were performed. In addition, H&E-stained slides of bone marrow aspirate clot and biopsy specimens were reviewed.

Immunophenotypic and Cytogenetic Analysis

Flow cytometric immunophenotypic analysis was performed on cell suspensions of lymph node, bone marrow aspirate, and peripheral blood specimens using a FACScan instrument (Becton-Dickinson Biosciences, San Jose, CA) as described previously.26 The lymphocyte population was gated using right angle side scatter and CD45 expression. The panel of monoclonal antibodies included reagents specific for CD3, CD5, CD10, CD19, CD20, CD22, CD23, CD38, CD43, FMC-7, and immunoglobulin κ and λ light chains (Becton-Dickinson).

Immunohistochemical analysis was performed using formalin-fixed, paraffin-embedded tissue sections; an avidin-biotin-peroxidase complex method; and an automated immunostainer (Ventana Biotech, Tucson, AZ), as described previously.27 All tissue sections underwent heat-induced antigen retrieval. The antibodies used included reagents specific for CD3 (Dako, Carpinteria, CA), CD5 (Labvision/Neomarkers, Fremont, CA), CD10 (Novocastra/Vision Biosystem, Newcastle upon Tyne, UK), CD20 (Dako), PAX-5 (Transduction Labs, San Diego, CA), BCL-2 (Novocastra/Vision Biosystem), BCL-6 (Dako), SOX11 (Cell Marque, Rocklin, CA), and cyclin D1 (SP4, Labvision/Neomarkers). CD5 expression was evaluated by comparing the pattern and intensity of staining with antibodies specific for CD3, CD5, and CD20. Neoplastic B cells typically expressed CD5 in a membranous pattern and with dimmer staining intensity compared with reactive T cells.

The results of conventional cytogenetics were available in four of six patients who underwent bone marrow aspiration and biopsy. Conventional cytogenetic analysis was performed on metaphase cells prepared from bone marrow aspirate specimens using standard techniques.28 Giemsa-banded metaphases were analyzed and the results were reported using the International System for Human Cytogenetic Nomenclature (2013).29

Comparison With CD5-Negative NMZL Patients

We performed a second search of our hospital archives from January 1, 2000, through June 30, 2010, for CD5-negative cases of NMZL. Clinical and radiographic data were obtained from the medical record. Clinical staging and workup were similar to that performed for the patients with CD5-positive NMZL. Cases that showed evidence of extranodal or splenic disease at any point in the clinical course were excluded.

We identified 91 cases of CD5-negative NMZL. There were 34 men and 47 women, with a median age of 60 years (range, 21-87 years). Staging information was available for 66 patients. Frequency of bone marrow involvement and presence of lymphadenopathy above and below the diaphragm were compared with the CD5-positive NMZL group. This comparison was performed using a two-tailed Fisher exact test using GraphPad Prism software (version 6.01, GraphPad Software, La Jolla, CA). Findings with \( P \leq .05 \) were considered statistically significant.
Results

Clinical Findings

We identified seven cases of CD5-positive NMZL representing 8.6% of all cases of NMZL diagnosed at our institution during the study interval. There were five women and two men, with a median age of 49 years (range, 36-80 years).

Table 1. Lymph node excisional biopsy specimens were obtained from the cervical (n = 4), axillary (n = 2), and gastreoploic (n = 1) regions. No patients had extranodal sites of disease or splenomegaly. One patient had B-type symptoms and one patient had concurrent hepatitis C infection. At the time of presentation, all seven patients had disseminated disease. Specifically, six patients had widespread lymphadenopathy with bone marrow involvement.

Laboratory data were reviewed from all seven patients.

Table 2. A complete blood count was performed in all patients and was abnormal in three: one (case 4) had a low leukocyte count of 1.5 × 10^9/L (normal range, 4-11 × 10^9/L), one (case 7) had a low hemoglobin of 9.7 g/dL (normal range, 12-14 g/dL) and a low platelet count of 91 × 10^9/L (normal range, 150-440 × 10^9/L), and a third patient (case 3) had leukocytosis (29.7 × 10^9/L) with an absolute lymphocytosis value of 25.3 × 10^9/L. One patient had a serum paraprotein of immunoglobulin G (IgG) λ type. The serum β₂-microglobulin level was elevated in three of five patients assessed. No patients had an elevated serum lactate dehydrogenase level or evidence of hepatitis B or human immunodeficiency virus infection.

Morphologic Findings

In all cases, the lymph node architecture was partially or near totally replaced by lymphoma that surrounded reactive follicles with active germinal centers. Four cases showed prominent follicular colonization. Mitotic figures were infrequent in all cases. Cytologically, the neoplastic cells were composed of small lymphocytes with slightly irregular nuclear contours, condensed chromatin, and moderate cytoplasm interspersed with scattered large cells with vesicular nuclear chromatin. Four cases had substantial numbers of monocytoïd cells, and two tumors exhibited prominent plasmacytoid differentiation, including one neoplasm that had clusters of plasma cells containing abundant intracytoplasmic immunoglobulin (Russell bodies). Prominent follicular colonization was also present in this case. Both cases with plasmacytoid differentiation also had small lymphocytes and monocytoïd cells. None of the cases showed proliferation centers.

The bone marrow was involved in all six patients assessed. In the aspirate smears, the lymphoid cells were predominantly of small to medium size with round to slightly irregular nuclear contours and scant cytoplasm. In biopsy specimens, the infiltrate was composed predominantly of

### Table 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age, y</th>
<th>Initial Site</th>
<th>LAD</th>
<th>BM</th>
<th>B</th>
<th>Ann Arbor Stage</th>
<th>HCV</th>
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<td>Axillary</td>
<td>MC</td>
<td>Yes</td>
<td>No</td>
<td>IVA</td>
<td>No</td>
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<td>MC</td>
<td>NP</td>
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<td>IVA</td>
<td>No</td>
</tr>
<tr>
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<td>F/55</td>
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<td>MC</td>
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<td>No</td>
<td>IVA</td>
<td>No</td>
</tr>
<tr>
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<td>F/80</td>
<td>Axillary</td>
<td>MC</td>
<td>Yes</td>
<td>No</td>
<td>IV</td>
<td>No</td>
</tr>
<tr>
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<td>F/49</td>
<td>Cervical</td>
<td>MC</td>
<td>No</td>
<td>No</td>
<td>IVA</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>M/37</td>
<td>Cervical</td>
<td>MC</td>
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<td>No</td>
<td>IVA</td>
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</tr>
<tr>
<td>7</td>
<td>M/43</td>
<td>Gastroepiloic</td>
<td>MC</td>
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<td>Yes</td>
<td>IVA</td>
<td>Yes</td>
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</table>

B, presence of B symptoms (fever, weight loss, and night sweats); BM, bone marrow involvement; HCV, hepatitis C virus; LAD, lymphadenopathy; MC, multicompartmental, above and below the diaphragm; NP, testing not performed.

### Table 2

<table>
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<tr>
<th>Case No.</th>
<th>WBC, × 10^9/L</th>
<th>Hb, g/dL</th>
<th>Plt, × 10^9/L</th>
<th>Lymph, × 10^9/L</th>
<th>Paraprotein</th>
<th>LDH, U/L</th>
<th>β₂M, mg/L</th>
<th>BM, %</th>
<th>BM (Pattern)</th>
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<tr>
<td>1</td>
<td>6.9</td>
<td>14.1</td>
<td>244</td>
<td>3.4</td>
<td>IgG λ</td>
<td>584</td>
<td>2.8</td>
<td>5</td>
<td>Interstitial</td>
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<tr>
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<td>13.8</td>
<td>298</td>
<td>0.8</td>
<td>NP</td>
<td>140</td>
<td>NP</td>
<td>NP</td>
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<tr>
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<td>256</td>
<td>25.3</td>
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<td>612</td>
<td>2.5</td>
<td>20</td>
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</tr>
<tr>
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<td>14.5</td>
<td>180</td>
<td>0.8</td>
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<td>2.4</td>
<td>5</td>
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<tr>
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<td>4.7</td>
<td>9.7</td>
<td>91</td>
<td>0.8</td>
<td>None</td>
<td>393</td>
<td>NP</td>
<td>15</td>
<td>Nodular</td>
</tr>
</tbody>
</table>

β₂M, β₂-microglobulin (reference range, 0.7-1.8 mg/L); BM, bone marrow involvement; Hb, hemoglobin (reference range, 14.0-18.0 g/dL, for men; 12.0-16.0 g/dL, for women); LDH, lactate dehydrogenase (reference range, 313-618 U/L); Lymph, absolute lymphocyte count (reference range, 1.00-4.00 × 10^9/L); NP, testing not performed; Plt, platelet (reference, 140-440 × 10^9/L); WBC, white blood cells (reference range, 4.0-11.0 × 10^9/L).
Image 1 A, Lymph node involved by CD5-positive nodal marginal zone lymphoma (H&E; ×200). B, High-power view with centrocyte-like cells, monocytoid cells, and scattered large cells (H&E; ×1,000). C, The lymphoma cells are brightly positive for CD20 (immunohistochemistry with hematoxylin counterstain; ×400). D, The lymphoma cells coexpress CD5 (immunohistochemistry with hematoxylin counterstain; ×400). E, The lymphoma cells are negative for CD3 (immunohistochemistry with hematoxylin counterstain; ×400).
small lymphoid cells in an interstitial (n = 3) or nodular (n = 3) pattern. The extent of involvement in the bone marrow ranged from approximately 5% to 20% of the medullary space (Table 2).

**Immunophenotypic and Cytogenetic Findings**

All seven cases were assessed by immunohistochemical analysis [Table 3]. All cases were positive for CD5 and CD20, and all cases assessed were also positive for BCL-2 (5/5) and PAX5 (1/1). In a subset of tumors, the neoplastic cells were positive for CD43 (2/4) and were dimly positive for BCL-6 (2/3). The neoplastic cells in all cases were negative for cyclin D1 (0/7), CD23 (0/6), SOX11 (0/5), and CD10 (0/4).

Flow cytometric immunophenotyping was performed on cell suspensions of lymph node specimens from five patients [Table 4]. The neoplastic cells in all cases were positive for monoclonal surface immunoglobulin light chain (three k and two l), CD5, CD19, CD20 (moderate to bright), and FMC-7 (n = 3). One of three cases assessed was dimly CD23 positive. The neoplastic cells were negative for CD10 in two cases assessed. Flow cytometric immunophenotyping was performed on six positive bone marrow aspirate samples as well as four concurrent peripheral blood samples and showed similar results. All samples showed a monoclonal B-cell population with aberrant expression of CD5. Conventional cytogenetic analysis performed on morphologically involved bone marrow aspirate specimens from four patients showed a diploid karyotype.

**Therapy and Clinical Outcome**

Treatment and follow-up information were available for all seven patients [Table 5]. The median follow-up was 32 months (range, 3-154 months), and six of seven patients were alive at last follow-up. Two patients were observed without specific therapy and were alive with disease at last follow-up. Of the three patients who received rituximab alone, two achieved complete remission and one was alive with disease. Two patients received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy: one was alive with disease and one died of complications from chemotherapy.

We compared the seven patients with CD5-positive NMZL with a group of 66 patients with CD5-negative NMZL. In the CD5-positive group, six (86%) of seven patients had...
lymphadenopathy above and below the diaphragm, and all six patients assessed had bone marrow involvement. In contrast, in the CD5-negative group, 28 (42%) of 66 patients had lymphadenopathy above and below the diaphragm as judged by radiologic imaging studies, and 36 (55%) of 66 had bone marrow involvement. These differences between the CD5-positive and CD5-negative NMZL patients were statistically significant ($P = .045$ and $P = .037$, respectively).

**Discussion**

Assessment of CD5 expression is a useful tool in the workup of B-cell lymphomas. CD5 is characteristically expressed in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma, whereas other B-cell lymphomas are usually CD5 negative. However, in addition to NMZL, CD5 expression has been reported in subsets of splenic marginal zone lymphoma, extranodal marginal zone lymphoma of MALT type (MALT lymphoma), lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, follicular lymphoma, and diffuse large B-cell lymphoma. However, very few cases of CD5-positive NMZL have been reported, and the clinicopathologic features of such cases have not been fully described in the literature.

In this study, we report seven cases of CD5-positive NMZL. Other than expression of CD5, each case had histologic and immunophenotypic features typical of NMZL. Based on the total number of NMZL cases at our two institutions, CD5 was expressed in 8.6% of all NMZL cases. However, since not all cases of NMZL at our institutions have been assessed by flow cytometry, a more sensitive method for detecting CD5 expression by neoplastic B cells, it is possible that the frequency of CD5 expression could be higher. Based on our findings, the frequency of CD5 expression in NMZL appears to be higher than in MALT lymphoma, reported in less than 5% of cases, but is lower than CD5 expression in splenic marginal zone lymphoma, in which CD5 can be expressed dimly in approximately 20% of cases. The clinical importance of CD5 expression in patients with extranodal and splenic marginal zone lymphomas has been addressed by others. CD5 expression in both extranodal and splenic marginal zone lymphomas has been reported to correlate with an increased risk of disseminated disease. However, the relationship between CD5 expression and disease dissemination has not been shown previously for patients with NMZL. Here we show that patients with CD5-positive NMZL have a higher frequency of advanced-stage disease and bone marrow involvement at presentation compared with patients with CD5-negative NMZL. The frequency of dissemination in CD5-positive NMZL patients in this study is higher than what has been reported for patients with NMZL in the literature. In recent case series, 17% to 77% of patients with NMZL had disseminated disease. Bone marrow involvement in patients with NMZL has been reported to range from 21% to 62% of patients.

The role of CD5 expression in lymphoma remains incompletely understood, as does the physiologic significance of CD5 expression by human B cells in general. While CD5 is characteristically associated with naïve and memory B cells in mice, CD5 expression can be found at low and variable frequencies in all B-cell subsets in adults. CD5 expression is also somewhat labile and influenced by the microenvironment, as others have indicated that multiple disease sites in an individual patient can show differences in CD5 expression. CD5 expression has been shown to be a negative regulator of the immune system as well as a possible marker of antigenic exposure; however, the role of these functions on disease dissemination remains obscure.

Subsets of human CD5-positive B cells are thought to represent B1 cells. B1 cells are innate-like B cells arising from an antigen-independent pathway that is distinct from conventional B-cell (B2) development. These cells have been associated with autoantibody production, T-cell regulation, and autoimmune disease. Compared with B2 cells, B1 cells have been shown to have a unique activation pattern. Rather than clonal expansion, these cells rely on rapid tissue migration and redistribution to achieve cell accumulation at sites of immune stimulation. It is possible that a relationship exists between B1 cells and CD5-positive NMZL and that the unique activation features of B1 cells contribute to disease dissemination. It is also possible that CD5 may simply be a marker of another, as yet undefined, process that increases the risk of dissemination in marginal zone lymphomas.

Despite the high frequency of disseminated disease, CD5 expression does not appear to have an impact on prognosis. The overall survival in our cohort was high, with six (86%) of seven patients alive at last follow-up, although follow-up was short for some patients. One patient died of complications from R-CHOP chemotherapy, two patients achieved complete remission, and the remaining patients were alive with disease. Although the treatment regimens were nonuniform, the overall clinical picture seems to be that of an indolent disease. As the only patient death was from complications of R-CHOP therapy, it seems that this group of patients may best benefit from more conservative management.

The expression of CD5 in these cases of NMZL raised the differential diagnosis of well-known CD5-positive B-cell neoplasms, such as CLL/SLL and mantle cell lymphoma, as well as less common cases of CD5-positive low-grade B-cell lymphoma, such as follicular lymphoma and CD5-positive lymphoplasmacytic lymphoma/Waldenström macroglobulinemia. Unlike CLL/SLL, the tumors we describe lacked proliferation centers, the large cells did not resemble...
paraimmunoblasts, and surface immunoglobulin and CD20 were expressed brightly.21,22 Unlike mantle cell lymphoma, the neoplastic cells in these cases had monocytoid features, follicular colonization, and plasmacytic differentiation, features unusual in mantle cell lymphoma. In addition, the neoplastic cells were negative for cyclin D1. The morphologic findings and absence of SOX11, assessed in five of seven cases, argue against the possibility of cyclin D1–negative mantle cell lymphoma.39 Unlike follicular lymphoma, the tumors we report did not form follicles and were negative for CD10. Two of six cases were dimly positive for BCL-6; however, dim BCL-6 expression in NMZL has been reported by others in a subset of cases.30,40 While it is possible that BCL-6 expression is another facet of the immunophenotypic heterogeneity of NMZL, its significance remains unclear. Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia is excluded in all cases since only one patient had a paraprotein that was of the IgG type.41 Lymphoplasmacytic lymphoma could be considered in the two cases with plasmacytic differentiation (cases 4 and 6). However, both of these cases also contained morphologic features consistent with NMZL, including monocytoid morphology and a marginal zone/perifollicular pattern of infiltration.

In summary, our findings indicate that CD5 expression occurs in a significant subset of NMZL, almost 10%. Other than CD5 expression, these cases have morphologic and immunophenotypic features similar to cases of conventional NMZL. Patients with CD5-positive NMZL appear to have a higher frequency of disease dissemination and bone marrow involvement. The clinical course of these patients, however, is indolent, and overall survival appears to be excellent.

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References


