CD5+ Follicular Lymphoma
A Clinicopathologic Study of Three Cases

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Key Words: Follicular lymphoma; CD5; t(14;18); bcl-2; IgH gene rearrangement; Immunohistochemistry; Flow cytometry

Abstract

Follicular lymphoma (FL) is a low-grade lymphoma that typically lacks CD5 antigen expression. We report 3 cases of FL with unusual expression of CD5. All cases showed histologic features of FL, including effaced nodal architecture, follicular growth pattern, and a spectrum of grades from 1 to 3 using World Health Organization criteria. In flow cytometric studies, all 3 cases showed a light chain–restricted, CD19+, CD20+ B-cell population coexpressing CD10 and low-level CD5. Immunohistochemical studies demonstrated an identical B-cell immunophenotype with weak expression of CD5 and coexpression of bcl-2 protein and the germinal center–associated markers, CD10 and bcl-6 protein. None of the cases showed expression of CD43, cyclin D1, or IgD. By molecular analysis, immunoglobulin heavy chain gene rearrangements were demonstrated in all 3 cases, and 2 of 3 cases had a t(14;18). These cases highlight the difficulty classifying these lymphomas by flow cytometric studies alone and emphasize the importance of recognizing FL in the differential diagnosis of CD5+ B-cell lymphomas.

Follicular lymphoma (FL) is the most common non-Hodgkin lymphoma in adults in the United States.1 In general, this indolent B-cell lymphoma has remained incurable for the majority of patients, despite its initial responsiveness to a variety of therapeutic modalities. The genetic hallmark of FL is the reciprocal t(14;18)(q32;q21), which is the most common chromosomal translocation in lymphoid malignant neoplasms and is identified in a majority of FL cases (85%-90%). The translocation is detectable in roughly 70% of cases by polymerase chain reaction (PCR) when primers for both the major breakpoint region (MBR) and the minor cluster region are used.2 The t(14;18) juxtaposes the bcl-2 oncogene with the immunoglobulin heavy chain (IgH) joining region, resulting in the constitutive overexpression of the antiapoptotic bcl-2 gene, a key early event in the oncogenesis of FL.3 Diagnostically, FL displays a growth pattern that recapitulates germinal center–like structures. It is composed of germinal center–derived B cells that display pan–B-cell markers (CD19, CD20, CD22, and CD79a) and monotypic surface light chains and frequently express the follicle center cell–associated antigens, CD10 and bcl-6 protein.4 However, FL generally is regarded as negative for expression of CD5, a feature that has a key role in the differential diagnosis of low-grade B-cell malignant neoplasms.5-11

The CD5 antigen is a 67-kd surface glycoprotein expressed on the vast majority of mature T cells.12 CD5 has been shown to have a role in thymocyte development and selection, T-cell activation, and T-cell–antigen-presenting cell interactions.13,14 CD5 also is expressed on a distinct subset of normal B cells, which are prominent early in life and particularly prevalent in fetal liver and spleen, as well as in cord blood.12
With age, their numbers decline. In lymphoid tissues, CD5+ cells are distributed primarily around germinal centers within follicular mantle zones. The function of CD5 in B cells is unknown. However, expression of CD5 may serve a role in B-cell activation, as CD5 has been shown to be associated with the immunoglobulin receptor complex and can be induced following mitogen stimulation in both normal and neoplastic B cells.\textsuperscript{15,16} CD5 expression also defines a distinct subset of B cells that produce low-affinity, polymorphic and autoreactive IgM antibodies in a T cell–independent manner. Antibody responses from CD5+ B cells often cross-react with a variety of bacterial antigens and are thought to be important in providing the first line of defense against invading pathogens early in life.\textsuperscript{12,16}

Among B-cell neoplasms, CD5 is expressed characteristically in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL).\textsuperscript{17,18} Reports have shown that CD5 expression is not limited to these B-cell neoplasms, but also can be found in small subsets of extranodal marginal zone B-cell lymphoma (MZBCL), de novo diffuse large B-cell lymphoma (DLBCL), and de novo Burkitt lymphoma.\textsuperscript{19-23}

Expression of CD5 also has been described in cases of follicular small cleaved cell (using the Working Formulation), nodular poorly differentiated lymphocytic (using the Rappaport classification), and centrocytic/centroblastic lymphoma (using the Kiel classification) with 1 report documenting CD5 expression in up to 50% of centrocytic/centroblastic lymphomas.\textsuperscript{19,24-26} These studies were performed before the widespread availability of diagnostic monoclonal antibody panels and the advent of routine flow cytometric and confirmatory molecular testing and, thus, included cases that were classified solely on the basis of histologic features. Since FL and MCL often share similar cytologic and architectural features, the distinction between these entities can be difficult on morphologic grounds and can be easily confused. Subsequent studies have failed to document CD5 expression in cases of FL, further supporting the contention that many of these initially reported cases may not have represented FL.\textsuperscript{6-10} However, a recent description of CD5 expression in 4 cases of FL with documented \textit{bcl-2} gene rearrangements by Tiesinga and colleagues\textsuperscript{27} confirms that a rare subset of FL can express CD5. In the present study, we describe the morphologic, immunophenotypic, and molecular features of 3 additional cases of FL with unusual expression of CD5. In contrast with the “floral” variant morphologic features reported by Tiesinga et al,\textsuperscript{27} our cases displayed classic histopathologic features of FL. We discuss the importance of recognizing this atypical immunophenotype and its diagnostic implications.

**Materials and Methods**

**Case Selection**

Three cases of CD5+ FL were retrieved from the surgical pathology archives and consultation files of the Section of Hematopathology, National Cancer Institute (NCI), National Institutes of Health, Bethesda, MD. Of the 3 patients, 2 (cases 1 and 2) were referred to the National Institutes of Health for treatment in approved research protocols of the NCI. The third (case 3) was referred in consultation from the Department of Pathology, University of Louisville, Louisville, KY. None of the patients had received treatment before our initial studies. In all 3 cases, flow cytometric analysis initially identified an unusual CD5+, CD10+, light chain–restricted B-cell lymphoma that necessitated additional studies for definitive lymphoma classification.

**Histologic Evaluation and Immunohistochemical Analysis**

Lymph node (n = 3) and bone marrow (n = 3) specimens were evaluated in formalin-fixed, paraffin-embedded (FFPE) and H&E-stained sections. Grading of FLs was based on the number of centroblasts per high-power field according to World Health Organization criteria.\textsuperscript{5} Immunohistochemical studies were performed on FFPE tissue sections with a panel of monoclonal and polyclonal antibodies \textbf{Table I} using heat-induced epitope retrieval techniques. In brief, following deparaffinization, the slides were placed in a microwavable pressure cooker containing 1.5 L of a 10-mmol/L concentration of citrate buffer (pH of 6.0) containing 0.1% Tween 20 (Sigma-Aldrich, Milwaukee, WI) and microwaved (model R4A80, Sharp Electronics, Rahway, NJ) for 40 minutes at 700 W. Immunohistochemical analysis was performed using an automated immunostainer (Ventana Medical Systems, Tucson, AZ) according to the manufacturer’s protocol with minor modifications. Primary antibody incubation was performed for 2 hours with antibody to bcl-2 protein; overnight with antibodies to CD5, CD10, bcl-6 protein, and cyclin D1; and for 32 minutes with the remaining antibodies. Predigestion with Protease 1 (Ventana Medical Systems) was performed for 8 minutes before incubation with antibody to CD23. Positive and negative controls were run with all cases and stained appropriately. The proliferative index as determined by MIB-1 immunostaining was estimated as a percentage of positive cells and scored from 1+ (0%-25% positive) to 4+ (75%-100% positive). Overexpression of p53 was defined as greater than 10% of cells positive, as previously described.\textsuperscript{28}

**Flow Cytometric Studies**

Flow cytometric studies were performed on cell suspensions from freshly resected lymph nodes in all cases.

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\textbf{Table I}
Lymphocytes were disaggregated from solid tissue by pushing tissue through a wire mesh with the plunger of a syringe and isolated using lymphocyte separation medium (BioWhittaker, Walkersville, MD). Cells were stained with various combinations of fluorescein isothiocyanate, phycoerythrin, or phycoerythrin-CY5–conjugated murine monoclonal or polyclonal antibodies (Table 1) using amounts of antibody recommended by the manufacturers, washed twice in phosphate-buffered saline containing 1% bovine serum albumin, and resuspended in phosphate-buffered saline containing 1% bovine serum albumin with 1% paraformaldehyde. Cells were analyzed by 3-color flow cytometry using a FACScan flow cytometer and CellQuest software (Becton Dickinson, San Jose, CA) by collecting at least 10,000 ungated list-mode events, gating on lymphocytes by forward vs side scatter, and analyzing cells in the appropriate lymphocyte gate. Cells stained with isotypic controls for IgG1 and IgG2 were used as controls. Flow cytometry for case 3 was performed at the University of Louisville using a similar method. Among 87 cases of histologically proven FL that were stained with a comprehensive lymphoma panel and analyzed at the NCI Flow Cytometry Laboratory during a 5-year period (January 1996 to December 2000), only 2 cases of FL coexpressed CD5 and CD20.

**IgH and bcl-2 Gene Rearrangement Studies**

FFPE tissue samples were sectioned on charged slides, deparaffinized with xylene, and hydrated before being scraped off the slides with a single-edge razor blade. The tissues were mixed with resin (Gene Releaser, Bioventures, Murfreesboro, TN) and preincubated in a thermocycler (Perkin Elmer 480, Perkin Elmer, Foster City, CA) according to the Gene Releaser protocol.

Amplification of IgH genes was performed using previously published consensus primer sequences against junctional (J) region (JH alpha, LJH, and VLJH) and framework determinants of the variable (V) region (VH or FR2A [framework 2]) of the IgH gene. To assess clonality of the IgH gene, PCR amplifications were performed on each sample using J region (JH alpha) and V region framework 3 (VH or FR3) consensus primers (ie, FR3-IgH PCR) for 36 cycles using conditions reported previously. Additional PCR amplification of case 2 samples was performed using a seminested procedure with J region (LJH) and V region framework 2 (FR2A) consensus primers for 35 cycles and J region (VJH) and V region framework 2 (FR2A) consensus primers for 25 cycles (ie, FR2-IgH PCR), according to the method of Ramasamy et al. Both FR3- and FR2-IgH PCR assays incorporated a modified hot-start PCR technique using the TaqStart antibody (Clontech, Palo Alto, CA). PCR products were separated by nondenaturing polyacrylamide gel electrophoresis (16% for FR3 and 10% for FR2) and visualized by UV irradiation after ethidium bromide treatment.

**Table 1**

**Antibodies Used for Immunophenotypic Analyses**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Leu-4*</td>
<td>M</td>
<td>Becton Dickinson, Mountain View, CA</td>
</tr>
<tr>
<td></td>
<td>Polyclonal†</td>
<td>1:200</td>
<td>DAKO, Carpinteria, CA</td>
</tr>
<tr>
<td>CD5</td>
<td>SFC12476G12*</td>
<td>1:50</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD10</td>
<td>J5*</td>
<td>M</td>
<td>Bedekman Coulter, Fullerton, CA</td>
</tr>
<tr>
<td></td>
<td>56C6†</td>
<td>1:20</td>
<td>Novocastra</td>
</tr>
<tr>
<td>CD19</td>
<td>Leu-12*</td>
<td>M</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>FMC-7</td>
<td>FMC-7*</td>
<td>M</td>
<td>DAKO</td>
</tr>
<tr>
<td>CD20</td>
<td>Leu-16*</td>
<td>M</td>
<td>Becton Dickinson</td>
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<tr>
<td></td>
<td>L26†</td>
<td>1:200</td>
<td>DAKO</td>
</tr>
<tr>
<td>CD23</td>
<td>Leu-20*</td>
<td>M</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td></td>
<td>Bu38†</td>
<td>1:200</td>
<td>The Binding Site, Birmingham, England</td>
</tr>
<tr>
<td>CD43</td>
<td>Leu-22*</td>
<td>1:50</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>IgM</td>
<td>Polyclonal*</td>
<td>1:15</td>
<td>Biosource International, Camarillo, CA</td>
</tr>
<tr>
<td>IgD</td>
<td>Polyclonal*</td>
<td>1:15</td>
<td>Biosource International</td>
</tr>
<tr>
<td></td>
<td>Polyclonal†</td>
<td>1:400</td>
<td>DAKO</td>
</tr>
<tr>
<td>IgA</td>
<td>Polyclonal†</td>
<td>1:15</td>
<td>Biosource International</td>
</tr>
<tr>
<td></td>
<td>IgG†</td>
<td>1:15</td>
<td>Beckman Coulter</td>
</tr>
<tr>
<td>kappa light chain</td>
<td>Polyclonal†</td>
<td>1:15</td>
<td>Beckman Coulter</td>
</tr>
<tr>
<td>lambda light chain</td>
<td>Polyclonal†</td>
<td>1:15</td>
<td>Beckman Coulter</td>
</tr>
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<td>bcl-2 protein</td>
<td>124†</td>
<td>1:20</td>
<td>DAKO</td>
</tr>
<tr>
<td>bcl-6 protein</td>
<td>PG-B6p†</td>
<td>1:20</td>
<td>DAKO</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>P2D11F11†</td>
<td>1:10</td>
<td>Novocastra</td>
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<tr>
<td>p53</td>
<td>DO7*</td>
<td>1:50</td>
<td>DAKO</td>
</tr>
<tr>
<td>MIB-1</td>
<td>MIB-1†</td>
<td>1:40</td>
<td>DAKO</td>
</tr>
</tbody>
</table>

M, antibodies were used according to the manufacturer’s recommendations

* Used in flow cytometric studies on fresh lymph node cell suspensions.

† Used in immunohistochemical studies on formalin-fixed, paraffin-embedded tissue sections.
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To assess for the presence of a t(14;18) involving the MBR, PCR amplifications were performed with bcl-2 and J region (JH alpha) primers according to previously published methods. Following 2% agarose gel electrophoresis, the amplified products were transferred to nylon membranes (Schleicher and Schuell, Keene, NH), cross-linked for 60 seconds using a UV cross-linker (Stratalinker, Stratagene, La Jolla, CA), and hybridized with a digoxigenin-labeled internal bcl-2–specific oligonucleotide probe (5'-CAACACAGAC-CCAGAGCCCTCCTGCCCTCCTTCCGCGGGGGC-3'). Bound probe was detected with an alkaline phosphatase–labeled, antidigoxigenin Fab fragment and CSPD as the chemiluminescence substrate according to the manufacturer's specifications (Boehringer-Mannheim, Indianapolis, IN).

Additional PCR assays were performed as controls for each sample using primers to a “housekeeping” gene, glyceraldehyde phosphate dehydrogenase, and the products were analyzed by 2% agarose gel electrophoresis and ethidium bromide staining. Negative controls included water and DNA extraction buffer.

**Results**

**Clinical and Pathologic Findings**

The clinical features for the 3 cases are summarized in **Table 2I.** The patients ranged in age from 32 to 55 years (median age, 44 years) and included 2 men and 1 woman. Lymph nodes from all 3 patients showed morphologic features of FL, including an effaced nodal architecture, predominantly follicular growth pattern, and a mixture of centrocytes and centroblasts **Image 1.** The lymph nodes showed a spectrum of grade 1 to 3 **Table 3I.**

Staging evaluation performed at diagnosis showed that 2 of 3 patients had advanced stage IV disease, including extensive lymph node and bone marrow involvement. Splenomegaly was noted in 1 of 3 patients.

**Flow Cytometric Findings**

Flow cytometric studies were performed on cell suspensions from freshly excised lymph nodes from all cases. All 3 B-cell neoplasms displayed a similar immunophenotypic profile; findings are summarized in Table 3. The B-cell origin of all 3 lymphomas was confirmed by flow cytometric analysis, which demonstrated bright expression of the B-cell lineage markers CD19 and CD20 (in all 3 cases) **Image 2A** and **Image 2B** and CD22 (in 2/2 cases). Evaluation of surface immunoglobulin expression showed bright monotypic light chain expression in all 3 cases, consistent with a clonal B-cell process. Two of 2 cases (cases 1 and 2) also expressed IgM heavy chains. All 3 cases expressed CD10 (Image 2A) **Image 2C**, suggesting a follicle center origin for these B-cell lymphomas. In case 3, the neoplastic B-cell population showed a spectrum of cell sizes, with many of the larger

<table>
<thead>
<tr>
<th>Case No./ Sex/Age(y)</th>
<th>Clinical Manifestations</th>
<th>Bone Marrow Involvement</th>
<th>Splenomegaly</th>
<th>Lymphadenopathy</th>
<th>Lymph Node Biopsy Site</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/32</td>
<td>Fatigue, night sweats, dull left upper quadrant pain</td>
<td>IV</td>
<td>Present</td>
<td>Present</td>
<td>Axillary, cervical, inguinal, periportal, celiac, retroperitoneal, pelvic</td>
<td>Axillary</td>
</tr>
<tr>
<td>2/F/55</td>
<td>Diffuse pruritic rash and enlarged right inguinal lymphadenopathy, unresponsive to antibiotic treatment</td>
<td>IV</td>
<td>Present</td>
<td>Absent</td>
<td>Supraclavicular, axillary, retroperitoneal, inguinal, mesenteric</td>
<td>Inguinal</td>
</tr>
<tr>
<td>3/M/46</td>
<td>Enlarged left inguinal lymph node</td>
<td>IA</td>
<td>Absent</td>
<td>Absent</td>
<td>Inguinal</td>
<td>Inguinal</td>
</tr>
</tbody>
</table>

* Age at initial examination.

| Table 3I | CD5+ Follicular Lymphomas: Summary of Pathologic Features

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Grade</th>
<th>% of CD19+ B-Cells Coexpressing CD5+</th>
<th>CD19⁺</th>
<th>CD20⁺</th>
<th>CD22⁺</th>
<th>CD10⁺</th>
<th>CD5⁺</th>
<th>CD23⁺</th>
<th>FMC-7⁺</th>
<th>bcl-6⁺</th>
<th>bcl-2⁺</th>
<th>IgD⁺</th>
<th>CD43⁺</th>
<th>Cyclin D1⁺</th>
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<tbody>
<tr>
<td>1</td>
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<td>98</td>
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<tr>
<td>2</td>
<td>2</td>
<td>97</td>
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<td></td>
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<td>3a</td>
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</tbody>
</table>

* Immunophenotypic analysis was performed by flow cytometric analysis.

† Immunophenotypic analysis was performed by immunohistochemistry studies.

‡ CD23 was detected in 2 of 3 cases by flow cytometry. However, CD23 expression could not be demonstrated by immunohistochemistry studies in any of the cases. In light of the greater sensitivity of flow cytometry detecting cell surface antigens, the reported CD23 expression reflects the flow cytometry results.
neoplastic B cells showing a lower level of CD10 expression. All 3 B-cell neoplasms also coexpressed low-level CD5 (Images 2B and 2C). The intensity of CD5 expression was less than that seen in normal T cells (Images 2B and 2C). In the light of the CD5 expression on these B-cell lymphomas, additional cell surface markers were performed and included CD23 and FMC-7, which can be particularly useful for the subclassification of CD5+ B-cell lymphomas. CD23 was coexpressed in 2 of 3 cases (cases 1 and 2). However, these 2 cases also coexpressed FMC-7. CD23 was not expressed in case 3, and FMC-7 was not evaluated in this case.

**Immunohistochemical Findings**

The results of immunohistochemical studies performed on FFPE lymph node sections are summarized in Table 3. Neoplastic follicles from all 3 cases were composed predominantly of CD20+ B cells Image 3A with varying numbers of CD3+ T cells Image 3B. Overexpression of the bcl-2 protein was observed in all 3 cases Image 3C. In case 3, bcl-2 protein expression was confined largely to the centrocytic population, with many of the centroblasts exhibiting dim to absent staining. Similar to flow cytometric studies, the neoplastic follicles showed weak expression of CD5 Image 3D and were highlighted by the expression of the follicle center cell–associated markers CD10 and bcl-6 protein. No evidence of CD43, IgD, or cyclin D1 expression was found in any of the B-cell lymphomas. CD23 expression was absent in all 3 cases but highlighted disrupted follicular dendritic cell meshworks within the neoplastic follicles.

The proliferative index was measured semiquantitatively by assessing MIB-1 antigen immunoreactivity. The proliferative rate ranged from 1+ (case 1, 0%-25% of cells positive) to 4+ (case 3, 75%-100% of cells positive) and corresponded to the histologic grade of each B-cell neoplasm (Table 3). Overexpression of the p53 protein was noted in 1 of 3 cases (case 2).

**Molecular Findings**

Molecular studies to assess clonality of the neoplastic B cells by IgH gene rearrangement analysis and to detect a t(14;18) involving the MBR are shown in Image 4 and summarized in Table 3. Clonal IgH gene rearrangements were detected in 2 of 3 cases (cases 1 and 2, Image 4) by FR3-IgH PCR. Clonality was confirmed in case 3 by PCR by FR2-IgH PCR (data not shown). PCR analysis identified bcl-2 gene rearrangements in 2 of 3 cases (cases 1 and 3, Image 4), confirming a t(14;18) involving the MBR. We did not perform bcl-2 gene rearrangement studies involving the minor cluster region.

**Discussion**

The expression of CD5 has been well studied in lymphoid malignant neoplasms. CD5 is expressed commonly in many T-cell lymphomas and characteristically is expressed in CLL/SLL and MCL. Until relatively recently, detection of the CD5 antigen in B-cell neoplasms has been problematic, as most CD5-specific monoclonal antibodies failed to detect low-level expression of CD5 in FFPE tissues, and, hence, most initial studies examining CD5 expression were restricted largely to immunohistochemical analysis of frozen tissue or flow cytometric analyses of cell suspensions. The recent availability of new CD5-specific monoclonal antibodies and the advent of heat-induced epitope retrieval techniques has permitted the examination of CD5 in archival tissues, further emphasizing the usefulness of the CD5 antigen in the differential diagnosis of non-Hodgkin B-cell lymphomas and specifically for distinguishing CLL/SLL and MCL from other B-cell neoplasms.
Studies have broadened the scope of B-cell lymphomas expressing CD5 to include infrequent cases of extranodal MZBCL, DLBCL, and Burkitt lymphoma. However, until a recent report by Tiesinga et al., the expression of CD5 had not been described in FL with a confirmed t(14;18). In the present article, we identified and reported the pathologic features of 3 additional cases of FL with unusual expression of CD5.

In our study, expression of CD5 in FL was a rare phenomenon. We identified only 2 cases of CD5+ FL among 87 cases of FL analyzed by flow cytometry during a 5-year period. Although flow cytometric results were available for 3 of 4 cases reported by Tiesinga et al., the incidence of CD5 positivity in their cases of FL was not reported. Although the cases in our study represented approximately 2% of all FL cases studied, a recent flow cytometric survey by Kaleem and colleagues showed no evidence of CD5 expression in 92 morphologically characterized FLs, suggesting that CD5 expression in FL overall may be even more infrequent than detected in our study.

Clinically, 2 of 3 patients had advanced disseminated disease with bone marrow involvement on staging, which is common for FL and similar to the advanced stage IV disease noted in 3 of 4 patients with CD5+ FL by Tiesinga et al. Morphologically, our cases of CD5+ FL were not histopathologically distinctive but resembled architecturally and cytologically classic CD5+ FL with a spectrum of grades from 1 to 3. Importantly, our cases showed none of the features of the floral variant noted by Tiesinga and colleagues, suggesting that CD5 expression in FL is not limited to the floral variant, but also can be seen in cases with classic nodular morphologic features.

All 3 of our cases initially were identified by flow cytometric analysis. However, definitive classification of these lymphomas was not possible based on these findings alone but required correlation with the results of immunohistochemical studies and molecular assays to determine a precise diagnosis. In flow cytometric studies, all 3 cases expressed the pan-B-cell markers CD19, CD20, and CD22 and showed light chain restriction, consistent with a clonal B-cell process. All 3 cases also coexpressed CD5 and CD10, an immunophenotype that rarely is observed in B-cell lymphomas. In fact, a recent flow cytometric study of 352 morphologically characterized lymphomas identified only 1 case of B-cell lymphoma that coexpressed CD5 and CD10, and this CD5+, CD10+ B-cell neoplasm subsequently was subclassified as an MCL based on cyclin D1 expression. In our cases, the concomitant expression of CD5 and CD10 raised the differential diagnosis of FL vs CLL/SLL or MCL. The relatively bright expression of light chains and CD20, observed in all 3 cases, is a feature not typically seen in classic CLL/SLL.

Evaluation of additional cell surface markers showed that 2 of 3 cases expressed both CD23 and FMC-7, and the third case was CD23−. Although CD23 and FMC-7 often are used to differentiate between CLL/SLL and MCL, they are not useful for the subclassification of FL because these antigens show a widely varied pattern of expression in FL.

Immunohistochemical studies were essential for confirming the diagnosis of FL. Expression of CD43 and IgD, markers commonly expressed on more than 90% of CLL/SLL and MCL, were consistently absent in our cases, arguing strongly against the diagnoses of CLL/SLL and
Moreover, the lack of cyclin D1 immunoreactivity argued further against a diagnosis of MCL. The presence of the germinal center–associated markers bcl-6 protein and CD10 in all 3 cases further supported the follicle center cell derivation of these CD5+ B-cell neoplasms. Overexpression of bcl-2 protein was present in all 3 cases and, although not specific for FL, has been well documented to be dysregulated and overexpressed in the majority of FLs. In case 3, the centroblast population showed decreased cytoplasmic expression of bcl-2 protein in comparison with adjacent centrocytes, a finding often observed in higher grade FL. Two cases of FL were CD23+ in flow cytometric studies but showed no CD23 expression in immunohistochemical analysis. The discordant expression of CD23 is difficult to reconcile and likely a result of the greater sensitivity of flow cytometric studies in detecting low-level antigens, particularly since CD23+ follicular dendritic meshworks were present in both of these cases.

Molecular analysis for IgH gene rearrangements confirmed clonality in all 3 cases. A t(14;18) involving the MBR was demonstrated in 2 of 3 cases and, thus, confirmed the diagnosis of FL. Failure to detect a t(14;18) in case 2 by PCR is not surprising, as PCR assays for the detection of t(14;18) using MBR-specific primer sets have high false-negative rates related to the use of alternate bcl-2 gene breakpoints.

In general, CD5 is the only marker that distinguished our cases of FL from their classic CD5– counterparts. In both flow cytometric and immunohistochemical analyses, CD5 expression...
was lower in intensity than that typically seen in the normal CD5+ T-cell population. In immunohistochemical studies, this low level of CD5 expression could easily have been dismissed as background staining, especially without previous knowledge of the flow cytometric results. Importantly, demonstration of CD5 expression using 2 separate techniques and 2 distinct CD5-specific monoclonal antibodies suggests that the CD5 expression seen in all 3 cases is not a result of nonspecific binding of monoclonal antibody to the neoplastic B cells.

The biologic significance of CD5 expression in these cases of FL is unclear. In some instances, the CD5 antigen may define a distinct subset of pre–germinal center B cells, which are thought to give rise to the neoplastic B cells in CLL/SLL and MCL. However, a pre–germinal center derivation of the neoplastic B cells in CD5+ FL is unlikely, particularly in the light of the concomitant expression of CD10 and bcl-6 protein, which suggests a follicle center cell origin. Alternatively, as a marker of activation, CD5 expression also could reflect the state of activation of the neoplastic B cells. The exact reason for the expression of CD5 in these cases is unclear but may be related to the activation state of the neoplastic cells. Importantly, the CD5 positivity in these cases of FL is unlikely to represent an effect of treatment, as none of the patients had received therapy before our initial studies.

The expression of CD5 has been associated with disseminated disease and aggressive behavior in some B-cell lymphomas. In extranodal MZBCLs, which typically manifest indolent and generally localized disease, unusual expression of CD5 has been reported by Ferry et al to identify a subset of lymphomas with disseminated and clinically aggressive disease. In addition, CD5 expression in de novo DLBCL also has been documented by Harada et al to correlate with a more aggressive clinical course with significantly decreased overall survival and more frequent bone marrow involvement when compared with CD5– de novo DLBCL. Yamaguchi et al showed a decreased failure-free survival in patients with CD5+ de novo DLBCL compared with their CD5– counterparts. Although these studies suggest that CD5 may be a marker of more aggressive disease, other studies have failed to support these findings. In our study, the clinical significance of CD5 expression is unclear as the number of cases is small and only limited clinical follow-up information was available. A larger cohort of patients with more extensive clinical follow-up is needed to adequately assess the clinical significance of CD5 expression.

We report 3 cases of FL with classic morphologic features, which unusually express CD5. Flow cytometric analysis was not sufficient to permit definitive classification of these lymphomas and required correlation with the immunohistochemical features and molecular findings. It is important to recognize that rare cases of FL can express CD5 and that FL must be considered in the differential diagnosis of CD5+ B-cell lymphomas.

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