Small B-Cell Neoplasms With Typical Mantle Cell Lymphoma Immunophenotypes Often Include Chronic Lymphocytic Leukemias

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Abstract

Mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) are CD5+ small B-cell neoplasms (SBCNs) with overlapping features. Flow cytometric immunophenotyping is often used to help differentiate CLL from MCL, and a characteristic CLL phenotype is considered essentially diagnostic. However, previous studies have not specifically examined how well a typical MCL immunophenotype distinguishes MCL from CLL. We identified 28 cases of SBCN with typical flow cytometry–determined MCL immunophenotypes consisting mostly of peripheral blood and bone marrow specimens. Fluorescence in situ hybridization analysis indicated that 57% (16/28) had t(11;14) translocations consistent with MCL, while 32% (9/28) lacked t(11;14) translocations but harbored other cytogenetic abnormalities commonly found in CLL. There were no significant morphologic or immunophenotypic differences between the t(11;14)-positive and t(11;14)-negative cases. Our findings suggest that many blood-based SBCNs with typical MCL immunophenotypes likely represent cases of phenotypically atypical CLL, which would have important clinical implications.

Accurate diagnosis of CD5+ small B-cell neoplasms has important prognostic and treatment-related implications. Most of these disorders consist of chronic lymphocytic leukemia (CLL) or mantle cell lymphoma (MCL).1,2 Patients with MCL usually have much shorter median survival times than patients with CLL, which often has an indolent clinical course, and they also receive different treatment.3,4 Distinguishing MCL from CLL can be complicated by overlapping morphologic features and similar clinical manifestations. Flow cytometric immunophenotyping has assumed an increasingly important role in the diagnosis of small B-cell neoplasms, especially when evaluating peripheral blood and bone marrow specimens. Besides CD5, CLL and MCL typically express the pan–B-cell antigens CD19 and CD20.1,2 Characteristically, CLL also expresses CD23 without FMC7 and shows dim expression of CD20 and dim surface immunoglobulin (sIg) expression, whereas MCL typically expresses bright CD20 and bright to moderate sIg, lacks CD23, and is FMC7+.5-10 Although the phenotypic features of CLL sometimes vary from the classic pattern, finding a characteristic CLL phenotype in a small B-cell neoplasm is considered essentially diagnostic.5,8 In cases with variant phenotypes such as those lacking CD23 and/or showing FMC7 positivity, accurate diagnosis often depends on testing for a t(11;14), which should be present in essentially all MCL cases and absent in CLL.11-13 Although finding a typical MCL phenotype by flow cytometry is thought to be relatively good for distinguishing MCL from CLL, this specific issue has not been evaluated in a rigorous manner. Previous studies related to this topic typically examined phenotypic differences between relatively small numbers of variably characterized cases of MCL and
CLL and, thus, would not be expected to identify rare cases of CLL that might have MCL phenotypes and/or were not focused on this question. However, previous studies have clearly shown that CLL can occasionally be CD23–, show FMC7 positivity, and express bright CD20 and/or bright sIg. In this study, we identified small B-cell neoplasms that had been submitted for flow cytometric analysis with typical MCL phenotypes and determined whether they represented MCL or cases of phenotypically atypical CLL using a chromosomal fluorescence in situ hybridization (FISH) panel for detection of t(11;14) and other cytogenetic abnormalities commonly found in CLL.

Materials and Methods

Specimens

We identified 28 cases of CD5+ small B-cell neoplasms with typical MCL immunophenotypes (bright CD20+, CD23–, FMC7+, and bright to moderate sIg) in the ARUP flow cytometry cryopreserved specimen database. The associated specimens were received in the flow cytometry laboratory between 2001 and 2007 and included peripheral blood (17 specimens), bone marrow (9 specimens), and tissue (7 specimens). Morphologic assessment was performed on Wright-stained smears or cytocentrifuged cell preparations made for the flow cytometry studies. The 18 reference cases with classic CLL phenotypes (dim CD20+, CD23+, FMC7–, dim sIg) were evaluated in our clinical flow cytometry laboratory during a 2-week period in August 2006, and signed out as “CD5+ B-cell lymphoproliferative disorders with a phenotype characteristic of CLL.” The specimens from 12 males and 6 females consisted of 17 peripheral blood specimens and 1 bone marrow specimen, containing a mean ± SD of 77% ± 20% CLL cells. Our research use of these specimens and information was approved by the University of Utah (Salt Lake City) Institutional Review Board (IRB No. 11905).

Flow Cytometry

Routine 4-color diagnostic flow cytometric studies were performed using an EPICS XL-MCL cytometer and EXPO32 software (Beckman Coulter, Miami, FL) and 5-color studies using an FC500 cytometer and CXP software (Beckman Coulter), as previously described. All cases were analyzed with antibodies against typical B-cell antigens (CD19, CD20, CD10, CD23, and FMC7), T-cell antigens (CD2, CD3, CD4, CD5, CD7, and CD8), CD16, and κ and λ light chains. Antibodies were used as recommended by the manufacturer (Beckman Coulter) and were conjugated with fluorescein isothiocyanate, phycoerythrin, phycoerythrin–Texas red, phycoerythrin-cyanin 5.1, or phycoerythrin-cyanin 7. Mean fluorescent intensity (MFI) values from 4-color studies of 13 MCL phenotype cases were converted to 5-color parameters using factors determined from evaluating 14 specimens by both methods. For CD20 expression to be labeled “bright” or non–CLL like, the MFI level had to be approximately 3 SD or more above the mean CD20 MFI level observed on the classic CLL phenotype cases. For sIg expression to be called “moderate to bright” or non–CLL like, the MFI level had to be approximately 1 SD or more above the mean κ or λ MFI level observed on the classic CLL phenotype cases.

Fluorescence In Situ Hybridization

Cryopreserved cells from each case were analyzed by interphase FISH using a set of probes specific for the t(11;14) translocation and the ATM (11q22.3-q23.1), chromosome 12 centromere, and the RB-1 (13q14), D13S25 (13q14.3), and p53 (17p13.1) loci (Vysis, Downers Grove, IL) as described. Briefly, each probe was analyzed separately for each sample. Hybridization and detection of signals were performed according to the manufacturer’s protocols. For each probe, 200 nuclei were evaluated. Peripheral blood samples from 20 people without hematologic diseases and with normal karyotypes were used as control samples. Results were considered abnormal if the percentage of nuclei with abnormal hybridization signals was greater than 3 SD from the mean.

Results

Determination of MCL vs CLL

We identified 33 cryopreserved samples, from 28 patients, that had clinical flow cytometry studies reporting a classic immunophenotype for MCL (CD5+, CD19+, bright CD20+, CD23–, FMC7+, and bright to moderate monotypic sIg). The majority were peripheral blood samples (17), 9 were bone marrow specimens, and 7 were tissue specimens. In 5 cases, 2 specimens were received from the same patient. These consisted of peripheral blood and bone marrow (3 cases) or tissue and bone marrow (2 cases). In these cases, both samples showed similar results by flow cytometry, and only 1 of the specimens was used for further analyses.

To determine which cases represented MCL and which might be phenotypically atypical CLL, FISH studies were performed. For classifying a case as MCL, the cryopreserved cells were required to contain a t(11;14) gene rearrangement. If a sample lacked this gene rearrangement but carried chromosomal abnormalities commonly detected by FISH in patients with CLL (trisomy 12, deletions for 13q14 [RB-1], 13q14.3 [D13S25], 17p13.1 [p53], and 11q22-23 [ATM]), the case was labeled atypical CLL. As indicated in Table I, 57% of patients (16/28) demonstrated a t(11;14) translocation.
consistent with MCL. Additional genetic abnormalities were also detected in 11 of these 16 cases, including monosomy 12 (2 cases), trisomy 12 (1 case), deletion 13q14 (6 cases), deletion p53 (3 cases), duplication p53 (1 case), deletion IgH (1 case), deletion 11q22 (2 cases), and duplication 11q22 (1 case). In contrast, 32% (9/28) of cases lacked a t(11;14) gene rearrangement but harbored various cytogenetic abnormalities commonly found in CLL. In 11% (3/28), FISH abnormalities were not detected, and these cases are not further described.

Clinical and Morphologic Features

The clinical features of the patients as separated by the FISH results are summarized in Table 2. The peripheral blood specimens all demonstrated lymphocytosis of small lymphocytes. In 5 of 15 peripheral blood cases, WBC and automated differential information was available, and the absolute lymphocyte values ranged from 10,100 to 85,900/µL (10.1-85.9 × 10⁹/L). In the remainder of cases, the lymphocyte count was estimated by visual inspection of peripheral blood smears and was placed into 3 categories: mild, 4,000 to 10,000/µL (4-10 × 10⁹/L); moderate, 10,000 to 50,000/µL (10-50 × 10⁹/L); and marked, more than 50,000/µL (50 × 10⁹/L). In general, MCL cases had higher levels of lymphocytosis than atypical CLL cases, with 4 of 8 MCL cases showing marked lymphocytosis vs 1 of 7 CLL cases. All cases, regardless of whether they were MCL or atypical CLL, demonstrated similar morphologic findings Image 1. The lymphocytes were predominantly small with scant cytoplasm and relatively round nuclear contours. Some cases had greater degrees of nuclear contour irregularity, although this feature

<table>
<thead>
<tr>
<th>Table 1</th>
<th>FISH Findings for Cases With MCL Immunophenotypes by Flow Cytometry*</th>
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<tbody>
<tr>
<td>t(11;14)</td>
<td>MCL</td>
</tr>
<tr>
<td>t(11;14) + others</td>
<td>11</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>0</td>
</tr>
<tr>
<td>del 13q14 (RB-1)</td>
<td>0</td>
</tr>
<tr>
<td>del 13q14.3 (D13S25)</td>
<td>0</td>
</tr>
<tr>
<td>Multiple CLL abnormalities</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
</tr>
</tbody>
</table>

CLL, chronic lymphocytic leukemia; FISH, fluorescence in situ hybridization; MCL, mantle cell lymphoma.
* MCL cases are defined as harboring the t(11;14); atypical CLL cases are those that lack t(11;14) but contain other abnormalities including trisomy 12, del 13q14, del 13q14.3, and del 17p13.1.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics of 25 Patients as Categorized by FISH Findings</th>
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<tbody>
<tr>
<td>Mean (range) age (y)</td>
<td>MCL</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>5/11</td>
</tr>
<tr>
<td>Sample type</td>
<td>PB, 8; BM, 4; tissue, 5; PF, 1</td>
</tr>
<tr>
<td>Degree of PB lymphocytosis†</td>
<td>↑↑↑, 4; ↑↑, 3; ↑, 1</td>
</tr>
</tbody>
</table>

BM, bone marrow; CLL, chronic lymphocytic leukemia; FISH, fluorescence in situ hybridization; MCL, mantle cell lymphoma; PB, peripheral blood; PF, pleural fluid.
† For 3 patients, there were 2 specimens.

The lymphocyte counts were as follows: ↑↑↑, >50,000/µL (50 × 10⁹/L); ↑↑, 10,000-50,000/µL (10-50 × 10⁹/L); ↑, 4,000-10,000/µL (4-10 × 10⁹/L).

Image 1 Representative peripheral blood smears of a case of atypical chronic lymphocytic leukemia (A) and a case of mantle cell lymphoma (B) demonstrate similar morphologic features with numerous small lymphocytes with round to slightly irregular nuclear contours (A and B, Wright, ×1,000).
was seen in atypical CLL and MCL cases. Smudge cells were occasionally seen but were not a prominent feature in any of the specimens.

**Immunophenotypic Features**

Review of the flow cytometric histograms confirmed immunophenotypic features typical of MCL (CD5+, CD19+, bright CD20+, CD23–, FMC7+, and bright to moderate monoclonal slg) in all cases **Image 2I**. To better evaluate the phenotypic features of these cases, MFI values for CD19, CD20, and slg expression were determined for the MCL and atypical CLL cases. In addition, comparative MFI data were also obtained for these markers from 18 classic CLL reference cases and 10 healthy control subjects. The average MFI values for CD20 expression levels were similar between the atypical CLL and MCL groups, both of which were near that observed for the healthy control subjects but much higher than the classic CLL phenotype group **Figure 1**. The average MFI values for κ and λ light chain expression were also similar for the MCL, atypical CLL, and healthy control groups, which were much higher than values observed for samples with the classic CLL phenotype. As expected, the intensity of CD19 expression was not appreciably different among all 4 groups.

**Discussion**

Small B-cell neoplasms that express CD5 without CD10 usually represent CLL or MCL but occasionally include other entities. Flow cytometric immunophenotyping is often used to help distinguish MCL from CLL, especially when analyzing fluid specimens such as peripheral blood or bone marrow aspirates. Numerous studies suggest that CD23 and FMC7 are relatively good discriminators, with MCL typically being CD23– and FMC7+ and CLL typically being CD23+ and FMC7–. Other markers frequently used to help discriminate MCL from CLL, although less reliable, include the intensity levels of CD20 and slg expression, with MCL showing bright CD20 and moderate to bright slg, whereas CLL typically has dim CD20 and dim slg expression. When deviations from the classic composite phenotypes occur, further workup is typically recommended to make a definitive diagnosis.

However, our study demonstrates that flow cytometric immunophenotyping cannot reliably discriminate between MCL and CLL even when a classic or typical MCL phenotype is encountered (bright CD20+, CD23–, FMC7+, and bright to moderate slg) and that an additional workup should always be performed before suggesting a diagnosis of MCL.

It may seem surprising that about one third of our cases with typical MCL phenotypes seemed to represent cases of phenotypically atypical CLL. It is unlikely, however, that any of our atypical CLL cases represented missed cases of MCL given the high sensitivity of FISH for the characteristic t(11;14) gene rearrangement, which can be detected in
approximately 97% of MCL cases. Although very rare cases of MCL may lack (11;14), these variant forms represent a very small percentage of MCL cases and would be unlikely to significantly affect our results. Insufficient numbers of neoplastic B cells could also explain a false-negative (11;14), but this was not a problem with our specimens because the tumor cell loads were relatively high, with a mean ± SD of 64% ± 23% of total leukocytes, and cytogenetic abnormalities were identified by FISH in all but 3 of the 28 initially included cases.

Our study also differed from previous reports in that we did not evaluate phenotypic differences between well-characterized cases of MCL and CLL but, instead, addressed the question of how specific a typical flow cytometry–derived mantle cell immunophenotype is for MCL. It is well known that CLL can show considerable phenotypic heterogeneity, with small numbers of cases lacking CD20, demonstrating FMC7 positivity and showing bright expression of CD20 and/or moderate to bright sIg expression. Although a typical MCL phenotype is unusual for CLL, our study highlights that it occurs and is found with reasonable frequency when evaluating blood and marrow specimens, which were the specimen types that included all of our MCL phenotype CLL cases. The higher incidence of CLL relative to MCL, especially in blood and marrow specimens, also increases the likelihood of finding CLL among the cases with typical MCL phenotypes.

Most CD5+ small B-cell neoplasms in our study that did not harbor (11;14) were found to instead contain cytogenetic abnormalities commonly found in CLL. Because these cytogenetic abnormalities do not usually appear in other small B-cell neoplasms besides MCL, it is reasonable to conclude that most, if not all, of these cases indeed represent cases of phenotypically atypical CLL. Of 28 cases initially included in our study, 3 with typical MCL phenotypes were not further evaluated because they did not display any of the genetic aberrations detected by our FISH panel. It is uncertain, therefore, whether these 3 cases represent atypical CLLs or other small B-cell neoplasms that do not normally express CD5 but occasionally can be CD5+, such as marginal zone lymphomas.

However, because the FISH panel we used detects genomic aberrations in only 75% to 80% of CLL cases, this still leaves a significant proportion of CLL cases that would not be identified by this method, which could easily represent phenotypically atypical CLLs. If these 3 cases are included with the atypical CLL group, then 43% of our MCL phenotype cases would represent phenotypically atypical CLLs. Careful review of the flow cytometric data did not reveal any definitive indicator or “tell” that a particular MCL phenotype case may represent atypical CLL. One atypical CLL case had a sIg MFI value that was within the range of classic CLL phenotypes, but this was still above our cutoff for moderate expression (1 SD above the classic CLL mean). Moreover, 1 of the MCL cases had a sIg MFI value that was slightly lower than the lowest atypical CLL case. There were also no identified morphologic or cytologic “tellings” that could reliably identify MCL phenotype cases as atypical CLLs. Indeed, the atypical CLL cases had lower lymphocytosis values than the MCL cases and also did not show increased cytologic atypia or increased numbers of smudge cells. Given these findings, our recommendation is that all peripheral blood and bone marrow flow cytometric cases with a classic MCL phenotype have additional FISH studies performed to confirm the presence of a (11;14) before considering a diagnosis of MCL. Although immunohistochemical staining for cyclin D1 can provide indirect evidence of a (11;14) for tissue specimens, current methods for evaluating cyclin D1 by flow cytometry are problematic and seem to lack sufficient sensitivity or specificity in distinguishing MCL from CLL.

In summary, our study highlights that finding a classic MCL phenotype using flow cytometry is not specific for MCL and only marginally reduces the possibility of atypical CLL from the differential diagnosis when evaluating small B-cell neoplasms in peripheral blood or bone marrow specimens. Even suggesting a diagnosis of MCL may be problematic without first evaluating for the possibility of a (11;14) because qualifier words that may be used are often dropped or overlooked when patients are evaluated by different physicians or other care providers. It is especially important that clinicians are not confused about a perceived “diagnosis” of MCL made by flow cytometric immunophenotyping because the treatments for CLL and MCL are very different.

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