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A Tissue Counterpart to Monoclonal B-Cell Lymphocytosis

Clinical and Pathologic Features

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Context.—B-cell clones discovered in tissue biopsies, without overt lymphoma, may represent a tissue counterpart to peripheral blood monoclonal B-cell lymphocytosis (MBL), herein termed tMBL.

Objective.—To characterize the clinicopathologic features of tMBL.

Design.—During a 10-year period, we retrospectively identified non–bone marrow/peripheral blood cases with monotypic B cells detected by tissue-based flow cytometry, but without an identifiable lymphomatous infiltrate on routine histopathology. We excluded cases with prior diagnosis of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma or MBL.

Results.—Fifty-four cases were identified (35 lymph node, 3 splenic, and 16 soft tissue/viscera). Forty-six cases were CLL-type, 2 were atypical CLL, and 6 were non-CLL. tMBL was detectable by immunohistochemistry in 14 cases were CLL-type, 2 were atypical CLL, and 6 were non-CLL. Concurrent blood flow cytometry, available in 10 cases, showed 4 with low-count MBL (3 CLL-type, 1 with non-CLL-type), 5 with high-count MBL (all CLL-type), and 1 case negative for clonal population. With median follow-up of 51 months, 2 patients had progression of disease (CLL, 68.7 months; and diffuse large B-cell lymphoma, 5.9 months). Patients with IHC-detectable tMBL had increased monoclonal B cells per total lymphocyte events (P = .01), morphologic evidence of bone marrow involvement (P = .04), higher white blood cell count (P = .02), and increased absolute lymphocyte count (P = .02).

Conclusions.—tMBL spans an immunophenotypic spectrum similar to MBL, is detectable by immunohistochemistry in a minority of cases (often CLL immunophenotype), and is likely systemic in most cases. Development of overt lymphoma is uncommon but may occur, warranting clinical follow-up.

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containing either small lymphocytic lymphoma (SLL) or monotypic B cells with a CLL phenotype who also were known to have an MBL in blood (<5 × 10^9/L). They found that presence of proliferation centers and lymph node size greater than 1.5 cm was associated with progression or need for active treatment. The authors suggested that small lymph nodes with a CLL-phenotype clone without proliferation centers might represent the tissue counterpart of MBL. Thus, whether a true tMBL exists (in the absence of MBL) and what the features might be is an open question. The 2016 update of the WHO classification further expanded upon the MBL subtypes mentioned by Marti et al in 2005. In addition to CLL-type, ACLL-type was introduced and refers to MBL expressing CD5, CD19, bright CD20, while CD23 can show positivity or negativity. Non-CLL-type was defined by expression of CD19 and CD20, dim or absent CD5, absence of CD10, and bright surface immunoglobulin. The update included specification of low-count (<0.5 × 10^9/L) and high-count (>0.5 × 10^9/L) CLL-type MBL with no or finite risk of progression, respectively, as well as the concept that non-CLL-type was associated with a risk of splenic marginal zone lymphoma. How all these new categories relate to tMBL remains unclear. The goal of our study was to characterize the clinicopathologic features of incidental tMBLs identified in tissue at our institution.

MATERIALS AND METHODS

Case Selection and Clinical Data Review

The study was conducted with local institutional review board approval. The Pathology Department archives were searched for all cases reviewed between 2009–2019 with FC analysis demonstrating a monotypic B-cell population in which a corresponding lymphomatous infiltrate was not evident by re-review of the routine hematoxylin-eosin (H&E)–stained sections. Flow cytometry was performed in-house on 31 cases (6-color FC, 0.04% sensitivity; BD FACSCanto, BD Biosciences-US) or was performed at outside reference laboratories (all remaining cases). In these latter cases, the FC report, including relevant reported population percentages, were reviewed. Of these, 2 cases had primary dot plots available for review. A case was determined as positive for tMBL on tissue-based flow cytometry (TBFC) using criteria set forth by Marti et al in 2005 for the definitions of MBL, and included overall k:λ ratio >3:1 or <0.3:1, or greater than 25% of B cells lacking or expressing low level surface immunoglobulin, or a disease-specific immunophenotype. In all cases, histology and immunostaining were re-reviewed. Presence of a proliferation center was by definition exclusionary. Of note, an overt lymphoma could be present in the biopsy if the tMBL population differed in phenotype from the overt lymphoma (composite overt lymphoma with tMBL). Patients with (1) a history of CLL/SLL, (2) PB finding of MBL before the tissue biopsy in question, or (3) patients lacking TBFC confirmation of the tMBL were also excluded. All available clinical, molecular (IGH and IGK gene rearrangement PCR [polymerase chain reaction] assays), flow cytometric information related to the sample, and accompanying bone marrow biopsies (if performed) were reviewed.

Morphologic and Immunophenotypic Review

All H&E and immunohistochemical (IHC) stains were examined for the following features: preservation of normal lymph node architecture (with special attention to lymph node sinuses and parafollicular areas), presence of proliferation centers (for exclusion of SLL), immunophenotype, and presence of additional malignancy (both lymphoid and other). If a bone marrow biopsy was performed as a result of, or in association with, the tMBL in question, it was re-reviewed.

At a minimum, all cases had IHC stains for CD3, CD5, CD19, CD20, lymphoid enhancer-binding factor 1 (LEF1), and cyclin-D1. In cases with a clinical history of a CD10⁺ lymphoma, immunostaining with CD10, BCL2, and BCL6 was also performed.

Follow-Up and Statistical Analysis

Progression was defined as development of new non-Hodgkin B-cell lymphoma detected by clinical, histopathologic, or flow cytometric means after the date of diagnosis of tMBL. The immunophenotype of the B cell of progression, and whether it matched the identified tMBL, was recorded. Dates and treatment type were recorded. Overall survival (OS) from the time of tMBL diagnosis was determined for all patients.

Statistical analyses included Mann-Whitney test, Fisher exact test, χ² test for trend, and Kaplan-Meier analysis with log-rank (Mantel-Cox) test and were calculated with Prism software package, version 8 (GraphPad Software Inc, La Jolla, California).

RESULTS

Clinical and Laboratory Features

Fifty-four cases were identified; these included 35 lymph node biopsies, 3 splenectomies, and 16 soft tissue/other viscera biopsies. The median age at diagnosis of the tMBL was 65 years (average, 66.35 years; range, 37–88 years). Twenty-nine of the patients were male and 25 female (M:F, 1.2:1). Clinical and laboratory data are summarized in Table 1.

The major indication for biopsy was lymphadenopathy (19 cases). Thirteen patients had a history of prior malignancy. These included 2 cases with history of small B-cell lymphoma (1 follicular lymphoma, 1 marginal zone lymphoma), 2 with history of diffuse large B-cell lymphoma (DLBCL), 1 with history of classic Hodgkin lymphoma, 1 with history of T-cell lymphoma, and 7 with history of carcinoma.

Of the 3 cases derived from splenectomies, splenomegaly was present in 1 patient, secondary to hereditary sphero- cytosis. The splenectomy was therapeutic and an NCLL tMBL was detected. The indication for splenectomy for the other 2 cases was suspicious lesions of uncertain significance seen on imaging performed for evaluation of cytopenias. One patient had a history of prior CD10⁺ DLBCL in remission and had a CD10⁻ NCLL tMBL, while the other patient had a CLL-type tMBL. In all cases, histology of spleen was unremarkable and tMBL was undetectable by IHC. Thus, these 3 cases of tMBL do not appear to represent overt splenic lymphomas.

Complete blood cell count data were reviewed in all cases and the median white blood cell and absolute lymphocyte counts were 7.73 × 10^9/L (range, 2.74–16.4) and 2.29 × 10^9/L (range, 0.3–7.8), respectively. In the single case with an absolute lymphocytosis with count above 5 × 10^9/L, peripheral blood flow cytometry (PBFC) confirmed monotypic B cells quantified as 0.390 × 10^9/L cells/L, and the tMBL detected in this case was NCLL-type. Concurrent serum protein electrophoresis data were available in 23 cases. Three cases demonstrated an identifiable M-protein (immunoglobulin [Ig] Mκ, IgGκ, and IgGκ), none of which matched the light chain associated with the identified tMBL. Immunoglobulin gene rearrangement studies were performed in 6 cases, and in all cases a clone was detected. In 2 of these 6 cases there was composite tMBL with another lymphoma, and a biclonal pattern was detected in both. Hepatitis C serology was available in 28 cases with only 1 positive case (3.6%). This was a patient with CLL-type tMBL identified on a liver core biopsy specimen.

Tissue Monoclonal B-Cell Lymphocytosis—Habermehl et al
Tissue-Based Flow Cytometry Findings

Forty-six cases of tMBL were classified as CLL-type, with 2 classified as ACLL, and 6 classified as NCLL. Eighteen cases were monotypic for $\kappa$ light chain, 22 for $\lambda$, and 14 were surface immunoglobulin negative. TBFC list-mode data were available for 31 cases, allowing detailed analysis of lymphocyte subsets, and are summarized in Figure 1. In general, tMBL made up a minority of total lymphocyte events with a median of 16% of total lymphocyte events, while a median of 42% of B-cell events was associated with monotypic cells. In 1 case, tMBL made up nearly 100% of B-cell events and was detectable by IHC. However, this case represented a minority of overall lymphocyte events and was not noticeable on review of the H&E section alone.

Histopathologic and IHC Features

The most common H&E finding on biopsy was reactive changes in 32 of the 54 cases (59%). In lymph nodes, reactive changes always included paracortical hyperplasia, mild sinus histiocytosis, and follicular hyperplasia. If in soft tissue, reactive changes included collections of small lymphocytes, with rare plasma cells and granulocytic elements. Granulomatous inflammation was never present.

### Table 1. Clinical and Laboratory Features of tMBL, by Immunohistochemistry (IHC) Detection Status

<table>
<thead>
<tr>
<th></th>
<th>All Cases (n = 54)</th>
<th>tMBL Detectable by IHC (n = 14)</th>
<th>tMBL Undetectable by IHC (n = 40)</th>
<th>Statistical Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>65 (37–88)</td>
<td>63.5 (49–78)</td>
<td>66.5 (37–88)</td>
<td>$P = .31$</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>29:25</td>
<td>5:9</td>
<td>24:16</td>
<td>$P = .13$</td>
</tr>
<tr>
<td>Indication for excision/biopsy</td>
<td></td>
<td></td>
<td></td>
<td>$P = .58$</td>
</tr>
<tr>
<td>Nonspecific lymphadenopathy</td>
<td>41% (19/46)</td>
<td>42% (5/12)</td>
<td>34% (4/12)</td>
<td></td>
</tr>
<tr>
<td>Staging/surveillance</td>
<td>28% (13/46)</td>
<td>42% (5/12)</td>
<td>24% (8/34)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>16% (7/46)</td>
<td>8% (1/12)</td>
<td>18% (6/34)</td>
<td></td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>2% (1/46)</td>
<td>8% (1/12)</td>
<td>0% (0/34)</td>
<td></td>
</tr>
<tr>
<td>Solid tumor</td>
<td>11% (5/46)</td>
<td>25% (3/12)</td>
<td>6% (2/34)</td>
<td></td>
</tr>
<tr>
<td>Localized mass</td>
<td>17% (8/46)</td>
<td>0% (0/12)</td>
<td>24% (8/34)</td>
<td></td>
</tr>
<tr>
<td>Management of benign disease</td>
<td>13% (6/46)</td>
<td>25% (3/12)</td>
<td>8% (3/34)</td>
<td></td>
</tr>
<tr>
<td>Phenotype of tMBL, No. of cases</td>
<td></td>
<td></td>
<td></td>
<td>$P = .08$</td>
</tr>
<tr>
<td>CLL-type</td>
<td>85% (46/54)</td>
<td>100% (14/14)</td>
<td>80% (32/40)</td>
<td></td>
</tr>
<tr>
<td>ACLL-Type</td>
<td>4% (2/54)</td>
<td>0% (0/14)</td>
<td>5% (2/40)</td>
<td></td>
</tr>
<tr>
<td>NCLL-type</td>
<td>11% (6/54)</td>
<td>0% (0/14)</td>
<td>15% (6/40)</td>
<td></td>
</tr>
<tr>
<td>Light chain type, n</td>
<td></td>
<td></td>
<td></td>
<td>$P = .10$</td>
</tr>
<tr>
<td>$\kappa$, $\lambda$, surface negative</td>
<td>18, 22, 14</td>
<td>4, 3, 7</td>
<td>14, 19, 7</td>
<td></td>
</tr>
<tr>
<td>WBC, median (range), $10^3$/L</td>
<td>7.73 (2.74–16.4)</td>
<td>9.17 (5–16.4)</td>
<td>7.41 (2.74–16.24)</td>
<td>$P = .02$</td>
</tr>
<tr>
<td>ALC, median (range), $10^3$/L</td>
<td>2.29 (0.3–7.8)</td>
<td>3.15 (0.95–4.97)</td>
<td>1.57 (0.3–7.8)</td>
<td>$P = .02$</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>23% (3/13)</td>
<td>100% (2/2)</td>
<td>9% (1/11)</td>
<td>$P = .04$</td>
</tr>
<tr>
<td>Hepatitis C status positive</td>
<td>4% (1/28)</td>
<td>12.5% (1/8)</td>
<td>0% (0/20)</td>
<td>$P = .29$</td>
</tr>
<tr>
<td>History of prior malignancy, n</td>
<td>24% (13/54)</td>
<td>29% (4/14)</td>
<td>23% (9/40)</td>
<td>$P = .72$</td>
</tr>
<tr>
<td>Cases with hematologic progression</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>$P = .49$</td>
</tr>
<tr>
<td>Follow-up, median (range), mo</td>
<td>51 (3–158.1)</td>
<td>81.8 (3.2–158.1)</td>
<td>36.8 (3–106.2)</td>
<td>$P = .22$</td>
</tr>
<tr>
<td>5-y overall survival</td>
<td>86% (37/43)</td>
<td>100% (12/12)</td>
<td>81% (25/31)</td>
<td>$P = .06$</td>
</tr>
</tbody>
</table>

Abbreviations: ACLL, atypical CLL; ALC, absolute lymphocyte count; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NCLL, non-CLL; tMBL, tissue monoclonal B-cell lymphocytosis; WBC, white blood cell count.

All significance determined by Mann-Whitney U test, Fisher exact test, or $\chi^2$ test for trend as appropriate. Bold values denote statistical significance.

a A single case demonstrated ALC >$5 \times 10^3$/L; this case was tMBL NCLL-type, and peripheral blood flow estimated circulating monotypic B cells at 0.390 $\times 10^3$/L.

b One case (undetectable by IHC) progressed to DLBCL 5.9 months after tMBL, while the other case progressed to CLL 68.7 months after tMBL (immunoglobulin light chain was identical in tMBL and progression in both instances).

**Figure 1.** Tissue monoclonal B-cell lymphocytosis (tMBL) involvement as a percentage of total lymphocyte and total B-cell events by flow cytometry, in relation to immunohistochemistry (IHC) detection status. Percentage involvement of total lymphocyte events was significantly higher in cases detectable by IHC. Percentage involvement of B-cell events by tMBL was higher in cases detectable by IHC; however, it did not reach statistical significance.
Composite low-grade or aggressive lymphoid neoplasms, in addition to tMBL, were identified in 12 of 54 (22%) and 9 of 54 (17%) cases, respectively. The associated neoplasm included DLBCL (5), follicular lymphoma (10), marginal zone lymphoma (1), Hodgkin lymphoma (3), mycosis fungoides (1), and peripheral T-cell lymphoma (1). Additionally, composite metastatic carcinoma was seen in a single case.

To address concerns of potential low-level involvement by a prior or undiagnosed low-grade lymphoma other than CLL being mistakenly called a tMBL, we investigated all 12 cases where the patient had either a prior or concurrent diagnosis of follicular lymphoma (11 cases) or marginal zone lymphoma (1 case). In all these cases, the identified tMBL was of CLL phenotype.

Immunohistochemistry was performed in all cases and cyclin-D1 was absent in all cases. An abnormal B-cell population consistent with the TBFC phenotype was identified by IHC in 14 of the 54 cases (26%)—all CLL-type tMBL. The pattern of IHC infiltration was the same across all 14 cases and was characterized as nodular aggregates of CD20+ B cells located in the paracortical/interfollicular areas if from a lymph node specimen (Figure 2, A through G). In all cases tMBL with IHC detectability was always focal, with less than 10% of lymph node area. In nonnodal tissues, there were simply loose clusters of CD20+ B cells.

Peripheral Blood Flow Cytometry Findings

Ten cases had concurrent PBFC and 9 had detectable MBL clones. Of these, 8 cases were CLL-type and 1 case was NCLL-type MBL. No cases of undiagnosed CLL were found. The median clonal B-cell count in PB was 0.55 × 10^3/μL. We had an even distribution of high- and low-count MBL. In all PB MBL cases, the detected immunophenotype was the same as the detected tMBL. The single case negative by PBFC was characterized as NCLL-type tMBL by TBFC. A summary of all PBFC data can be found in Table 2.

Figure 2. Reactive lymph node involved by tissue monoclonal B-cell lymphocytosis (tMBL). A, On routine stains, lymph node demonstrates heterogeneous small lymphocytes with patent sinuses containing histiocytes as well as a degree of paracortical hyperplasia. Subsequent immunostains for CD3 (B), CD5 (C), CD20 (D), and lymphoid enhancer-binding factor 1 (LEF1, E) are subtle, but highlight presence of chronic lymphocytic leukemia–type (CLL-type) tMBL with slight nodular pattern. Tissue-based flow cytometry performed on the specimen demonstrated an abnormal B-cell population, shown in green, which expressed CD5 and dim CD20 (F), dim surface κ (G), as well as CD19 and CD23 (not shown). Immunohistochemistry (IHC) for cyclin-D1 is negative (not shown). Most cases remain undetectable after IHC (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×400 [B through E]).
Bone Marrow Findings

We defined histologic evidence of marrow involvement as any abnormal lymphocytic infiltrate detectable by morphology on H&E. Thirteen patients had a bone marrow biopsy as part of the workup of the tMBL. Three biopsies demonstrated an abnormal lymphoid infiltrate in an interstitial nodular pattern that occupied 5% to 30% of marrow cellularity, all confirmed via IHC, with 2 of 3 additionally confirmed via FC (FC not performed in the third case). Cytologically, aggregates consisted almost exclusively of small lymphocytes with clumped chromatin and scant cytoplasm. No cases demonstrated proliferation centers. No infiltrate was seen in the remaining 10 bone marrow biopsies, either by H&E or IHC.

Clinical Follow-Up

Of the 54 cases, 43 had clinical follow-up data available. With a median time of 51 months (range, 3–158.1 months), the 5-year OS was 86% (37 of 43). There were 6 deaths during our follow-up and for these patients, the range of time from diagnosis of tMBL to time of death was 5.6 to 102.4 months with a median of 45.2 months. Two patients had progression of disease (CLL-type tMBL to CLL, 68.7 months; and NCLL-type tMBL to DLBCL, 5.9 months), and 2 died with progression of disease (CLL-type tMBL to CLL, 68.7 months; and NCLL-type tMBL to DLBCL, 5.9 months), and 2 died with progression of disease (CLL-type tMBL to CLL, 68.7 months; and NCLL-type tMBL to DLBCL, 5.9 months), and 2 died with progression of disease (CLL-type tMBL to CLL, 68.7 months; and NCLL-type tMBL to DLBCL, 5.9 months). The predominant management modality resulting from detection of tMBL was “watchful waiting” (19 cases) and in 6 cases, rituximab therapy alone resulted from tMBL detection. A significant subset of patients (12 cases) received some type of chemotherapy or immunochemotherapy as a result of alternate malignancy that would likely have an effect on the detected tMBL (ie, Rituximab–Cyclophosphamide, doxorubicin hydrochloride [Hydroxycamycin], vincristine sulfate [Oncovin], Prednisone [R-CHOP]). Treatment details are described in Table 3.

Comparison of IHC Detectability

The 14 patients with tMBL detectable by IHC were more likely to have higher white blood cell count ($P = .02$) and absolute lymphocyte count ($P = .02$) at the time of diagnosis than those without IHC-detectable tMBL (Table 1). In addition, tMBL detectable by IHC was more likely to have a larger percentage of monoclonal B cells per total lymphocyte events ($P = .01$; Figure 1). Median percentage involvement of B-cell events by tMBL was higher in cases detectable by IHC; however, this did not reach statistical significance. There was no significant association between ability to detect tMBL by IHC and the number of clonal B cells in PB (Table 2), or between IHC detection status and history of malignancy, prior lymphoma, or concurrent lymphoma. Lastly, patients with tMBL detectable by IHC were more likely to have morphologic evidence of involvement in an associated bone marrow biopsy ($P = .04$; Table 1). The 5-year OS was 86% (37 of 43 cases; 81% or 25 of 31 cases for IHC undetectable versus 100% or 12 of 12 cases for IHC detectable). Kaplan–Meier analysis with log-rank testing showed a trend for shorter OS for patients with IHC-undetectable tMBL ($P = .06$, log-rank test; Figure 3, A). Because all NCLL tMBLs were present in this IHC-undetectable group, we further compared OS of the NCLL tMBL group to the combined CLL and ACLL tMBL group in an exploratory analysis. This showed a shorter OS in the NCLL group ($P = .01$, log-rank test; Figure 3, B).
excluded cases with prior history of CLL/SLL and any case with histologic evidence of CLL/SLL on H&E. This might allow us to better capture the clinical relevance of incidental tMBL.

Readers may note that lymphadenopathy and organomegaly are usually exclusion criteria for MBL, and wonder how this criterion applies to tMBL. The 2016 WHO, in direct reference to work done by Gibson et al,8 states that “nodal infiltrate by CLL-type cells without proliferation centers in individuals without lymphadenopathy >1.5cm on CT... may constitute a nodal equivalent of MBL rather than SLL.”9 The inference is that tMBL could exist within a spectrum of mild to moderate lymph node enlargement—and our findings support the observation as well.

As with blood-based MBL, tMBL can be subcategorized by phenotype into CLL, ACLL, and NCLL types. Immunohistochemistry does allow one to detect these populations morphologically in a minority of cases (26% or 14 of 54 in this study), either by abnormal location of B-cell–rich infiltrates (supported by FC evidence of a B-cell clone) or abnormal immunophenotype such as CD5 expression in B-cell collections. The patterns of lymph node involvement are subtle interfollicular nodular infiltrates and ill-defined paracortical expansions of B cells. In soft tissues, there is a pattern of chronic inflammation with a slightly expansive loose cluster of small lymphocytes that does not allow overt lymphoma diagnosis.

Given that tMBL was detected initially by FC, it is often a small clone with no obvious routine histologic correlate. Similarly, detecting composite tMBL and lymphoma was essentially related to finding 2 separate clonal populations by flow. Absent flow cytometric studies, it is possible one might detect a small distinct lymphoproliferative disorder, if given a big enough tissue area for assessment (nearly impossible on a needle biopsy), and diligent observation with our suggested panel of IHC stains. Importantly, we found that IHC detection status was significantly associated with other pathologic features that likely relate to disease burden, including higher percentages of monoclonal cells detected by TBFC, higher white blood cell/absolute lymphocyte count at the time of diagnosis, and bone marrow involvement. We identified marrow involvement in 3 of 14 cases (all CLL-type), involving a range of 5% to 30% of marrow cellularity. Given that we excluded all cases with known history of CLL or evidence of CLL/SLL on initial histology before diagnosis of tMBL, we would not expect our findings to show greater than 30% marrow involvement (a finding typically associated with CLL), and our findings are in line with current literature.10

We demonstrate low level of involvement of lymphocytes by clonal B cells in our TBFC (median 16% of total lymphocyte events), confirming that clonal infiltrate is a minor component and difficult to diagnose in many cases without the assistance of TBFC. Concurrent PBFC was available in 10 cases, and 9 of these were positive for an MBL, with the same immunophenotype identified by TBFC. One could consider whether the tMBL cells detected in cases that had no obvious infiltrate by IHC were due to blood contamination. We think this highly unlikely since tissue samples are submitted in saline, transferred to Roswell Park Memorial Institute (RPMI) growth medium for transport to the laboratory, and then transferred to fresh RPMI medium for processing. Thus, the cells detected were resident in the tissue itself and it is not unreasonable to think that pathologists using a poorly sensitive method such as IHC visualization would not be able to find individual or very small collections of CLL-phenotype tissue-based MBL cells.

Combined with our cases demonstrating bone marrow involvement, we surmise that tMBL is a systemic disease at detection and is not limited to the tissue. It is worth noting that in no instance did a reflex PBFC result in a circulating monoclonal B-cell count greater than 5 x 10^9/L (upgrade to CLL). This suggests that in a patient without known CLL, it is unlikely that a patient with tMBL has undiagnosed CLL. This finding makes intuitive sense, considering that MBL identified in PB is estimated to be 100 times more frequent than CLL.11 Additionally, while a patient with tMBL likely has MBL on further workup of the blood, we show no association of IHC detection status on eventual high-count or low-count MBL classification.

That CLL-type tMBL was the only detected subtype in soft tissue is intriguing and could reflect biologic differences in homing between ACLL– or NCLL-type cells and CLL-type cells. Though the finding was not significant (P = .054), we surmise this could become a significant finding with greater power added to the study (for further information of these cases see supplemental digital content, Supplemental Table 1). The tissue microenvironment may be important in the growth and expansion of CLL clones. For example, one study found that the lymph node microenvironment may activate BAFF/ APRIL (B cell–activating factor/a proliferation-inducing li-
CXCR4, the latter of which is known to increase tissue localization and survival of CLL-type B cells. Rawstron and others compared homing mechanisms of MBL to CLL and found that CLL-type MBL showed decreased expression of homing markers CXCR5 and CD62L. Literature comparing tissue homing mechanisms of CLL to other low-grade B-cell lymphomas, or other MBL subtypes, remains extremely scant. It seems this topic, in particular, could be a subject for future investigation.

As with CLL, ongoing studies demonstrate a subset of MBL patients with poorer clinical outcomes peripherally related to disease burden, such as infection. Although this is a retrospective study with limited follow-up (median, 51 months), we did not observe new onset or frequent infections from the time of tMBL identification.

We document the relatively indolent course of tMBL, with only 2 cases of lymphoma “progression.” In both cases the patients had no prior diagnosis of lymphoma, underwent a “watch and wait” management approach, and the progressions had the same TBFC phenotype and light chain as the detected tMBL. In 1 case, progression to DLBCL occurred quickly (5.9 months). It is possible that the detected tMBL represented the DLBCL at a low level owing to undersampling for histology assessment, and may not indicate true progression. The biopsy sample for this case was an ultrasound-guided lymph node sample that was fragmented, with few pieces preset for evaluation. The second progression to CLL occurred far later (68.7 months) and, interestingly, was in the cohort of tMBL detectable by IHC. This likely represented progression as can be seen in blood-based MBL. Unfortunately, we were unable to formally confirm clonal relationship in these cases owing to lack of suitable tissue for molecular studies. Indeed, there were a total of 6 cases with a history of lymphoma before diagnosis of tMBL. Although in every case the tMBL was of a different immunophenotype from the prior diagnosed lymphoma, we cannot exclude clonal relationship owing to lack of available tissue for further molecular analysis.

Only 10 of our cases had concurrent PBFC, again due to the limitations of a retrospective study. However, it does appear that tMBL is systemic (albeit low tumor burden), since almost all tested patients had PB MBL. Lastly, the trend for shorter OS in IHC-undetectable cases is intriguing (Figure 3). In an exploratory analysis we found that this difference in OS was associated with type of tMBL, with NCLL tMBL faring poorly compared to CLL and A CLL types. We also noted that there was no significant difference in the rate of malignancy before diagnosis of tMBL between NCLL and CLL/A CLL subgroups as a causative explanation. In the end, we recognize that this potential association is preliminary, should be interpreted carefully, and involves a heterogeneous patient population that underwent varying management strategies. Thus, we recommend that further study is warranted to confirm this finding.

If tMBL is detected, what is the appropriate workup? Our findings suggest characterization via IHC on the biopsy, since detection is associated with higher tumor burden and can confirm phenotypic subtype and formally exclude mantle cell lymphoma. We recommend an antibody panel consisting of CD3, CD5, CD20, LEF1, cyclin-D1, and possibly SKY-box transcription factor 11 (SOX11). The current consensus is that CLL prognostic marker assays are of limited utility and should not be performed in PB MBL and this is reasonable for tMBL as well. Bone marrow biopsy is also not recommended unless diagnostic uncertainty remains or there are unexplained cytopenias. However, follow-up PBFC should be performed, as these patients will likely have PB MBL, which could be categorized according to current WHO 2016 criteria and provide clinically relevant information, such as excluding CLL and also informing follow-up strategy in case of high- or low-count MBL detection. Finally, it may be prudent to closely follow all NCLL tMBL cases owing to the apparent shorter OS in this group.

Lastly, a final consideration is whether or not this entity warrants a category within upcoming editions of the WHO. We think the answer to this question is “yes,” as it would then formally recognize the phenomenon of tMBL and give pathologists a category to place the finding, as well as backing for a recommendation to check PB.

**References**


Tissue Monoclonal B-Cell Lymphocytosis—Habermehl et al

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