Multifocal Mantle Cell Lymphoma In Situ in the Setting of a Composite Lymphoma

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ABSTRACT

Objectives: Mantle cell lymphoma in situ (MCLIS) consists of immunophenotypically defined but histologically inapparent neoplastic cells restricted to narrow mantle zones, without expansion or invasion beyond the mantle zone. We report a unique case of MCLIS associated with a much more manifest nodal marginal zone lymphoma (MZL) in an inguinal lymph node, porta hepatitis lymph node, and bone marrow.

Methods: Biopsies from all three locations were evaluated using standard H&E-stained sections, immunohistochemistry, flow cytometry, metaphase cytogenetics, and/or fluorescence in situ hybridization (FISH).

Results: This case is unique for three reasons. First, the histologically covert mantle cell lymphoma was multifocal, detected in all three locations using one or more of flow cytometry, immunohistochemistry, cytogenetics, and FISH. Second, the MCLIS was always accompanied by a more histologically dominant MZL. Third, where evaluable, it did not grow in an appreciable mantle zone distribution, presumably due to destruction of the normal nodal architecture by the neoplastic MZL cells and the resulting absence of recognizable follicles and mantle zones.

Conclusions: This unique case provides new insight into the pathogenesis of MCLIS.
zone. The latter pattern is often seen in lymph nodes that are involved by overt MCL in other areas. Although other similar morphologically inconspicuous early clonal lymphoid lesions such as monoclonal gammapathy of undetermined significance (MGUS) and high-count monoclonal B lymphocytosis (MBL) are known to be precursor states for plasma cell myeloma and chronic lymphocytic leukemia (CLL), respectively, it is still unclear whether FLIS and MCLIS can transform into overt lymphoproliferative disorders.2,4-8

A composite lymphoma is defined as the simultaneous presence of two morphologically and immunophenotypically different lymphomas that may or may not be clonally distinct.9-11 In the past 20 years, of more than 150 reported cases of composite lymphoma, 32 have included MCL. Most of these cases were described in case reports,11-23 and a few others were reported in small case series.24-26 As described in a recent review of composite lymphoma involving MCL,10 the second component of these lesions was most often FL (nine cases), followed by CLL and Hodgkin lymphoma (five cases each). Two cases of composite marginal zone lymphoma (MZL) and MCL have been reported previously.16,18 Only one of these consisted of a nodal MZL.16

Here we report an unusual case of multifocal MCLIS that was associated with a much more manifest nodal MZL. To our knowledge, a similar case has not been reported previously.

Case Report

A 58-year-old man with recently diagnosed IgM κ MGUS sought treatment for left inguinal lymphadenopathy. He underwent diagnostic biopsy of a left inguinal lymph node. The lymph node was submitted to one of us (A.B.) in consultation, and it revealed MZL. There was no evidence of any extranodal or mucosal disease, and the spleen was not enlarged. Review of his medical record showed that he had undergone two other recent biopsies, one of the bone marrow and another of a porta hepatis node. These were subsequently also submitted for review. The bone marrow biopsy was performed 3 months prior to the inguinal lymph node biopsy to evaluate a duodenal polyp revealed multiple enlarged hypoechoic lymph nodes. More than 1 year after initial diagnosis, the patient is on rituximab maintenance therapy for the MZL, with most recent imaging showing a complete response.

Materials and Methods

Immunohistochemical Studies

Immunohistochemical studies were performed at both the referring institution, St Luke’s Hospital (CD3, CD5, CD10, CD20, CD23, cyclin D1, BCL6, and BCL2), and the Hospital of the University of Pennsylvania (CD138, MUM1, κ and λ light chain, SOX11, IgD, and Ki-67). The following antibodies and their clones (in parentheses) were obtained from Ventana Medical Systems (Tucson, AZ) and Leica Biosystems (Buffalo Grove, IL): CD3 (LN10), CD5 (4C7), CD10 (56C6), CD20 (MJ1), CD23 (1B12), cyclin...
D1 (SP4-R), BCL6 (LN22), BCL2 (BCL2/100/D), CD138 (MI15), MUM1 (MUMp), SOX11 (MRQ-58), and Ki-67 (MIB1). Antibodies used for κ light chain, λ light chain, and IgG were polyclonal. Immunostaining was performed on the Leica Bond (Leica Biosystems) and Ventana BenchMark XT (Ventana Medical Systems) using standard procedures per the manufacturer’s instructions. All immunostains were counterstained with bluing and hematoxylin.

Flow Cytometry

A six-color flow cytometric analysis was performed on whole lymph node and bone marrow specimens that were anticoagulated with heparin. Then, 100 μL of tissue containing 1 to 5 × 10^6 cells were stained for surface antigens, using monoclonal antibodies. The monoclonal antibody cocktails included λ-FITC/κ-PE/CD5-PerCP/Cy5.5/CD20-APC/CD10-PECy7, λ-FITC/κ-PE/CD19-PerCP/Cy5.5/CD11c-APC/CD38-PECy7, and FMC7-FITC/CD5-PE/CD19-PerCP/Cy5.5/CD23-APC/CD10-PECy7. Flow cytometry analysis was performed on a BD FACS Cantos II (BD Biosciences, San Jose, CA), using DeNovo FCS Express software (De Novo Software, Glendale, CA).

Metaphase Cytogenetics Analysis

Cells were cultured in RPMI 1640 with fetal bovine serum for 24 hours. They were then stimulated with CpG-oligonucleotide DSP30 and interleukin 2 for 72 hours. Harvesting, G-banding, and chromosome analyses were performed according to standard procedures. Chromosomes were classified and abnormalities were described according to the International System for Human Cytogenetic Nomenclature.

Fluorescence In Situ Hybridization

Standard methods with dual-fusion DNA probes for IGH and CCND1 (Vysis, Downers Grove, IL; MetaSystems, Waltham, MA) were used, as previously described. 29,30

Polymerase Chain Reaction Analysis

Polymerase chain reaction (PCR) was performed with an IGH assay using FR2 and FR3 primers (InvivoScribe Technologies, San Diego, CA), according to the manufacturer’s protocol. 31 The PCR products were analyzed by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Results

Left Inguinal Lymph Node

Morphologic Findings

H&E-stained sections revealed a node in which the normal architecture was largely effaced by an infiltrate growing in a vaguely nodular to diffuse pattern Table 1 and Image 1A. The cells in this infiltrate were monotonous and small to medium in size with clumped chromatin, inconspicuous nuclei, and occasionally irregular nuclear contours Image 1B. Few mitotic figures were seen. Pseudofollicular proliferation centers were not seen. No expansion of large cells was noted. Review of the histology following the immunohistochemical studies failed to detect a histocytologic correlate of the clusters of CCND1+ cells seen immunohistochemically (see below).
Immunohistochemical Findings

Immunohistochemical studies of the inguinal lymph node revealed that the bulk of the cells were CD20+ BCL2+ B cells [Image 1C] that were negative for CD5, CD10, CD23, BCL6, CCND1, SOX11, IgD, and MUM1. Ki-67 expression ranged from less than 5% to 20% in some areas. In one region of the node, several small collections of CCND1+ cells (ranging from ~20 to ~100 cells per collection) were noted, accounting for less than 1% of the total infiltrate [Image 1D]. Given the small size of these clusters, it was difficult to ascertain whether they coexpressed CD5, SOX11, and/or IgD. Since the normal nodal architecture had been obliterated by the MZL, it was not possible to appreciate a specific mantle zone distribution of the CCND1+ cells.

Flow Cytometry

Flow cytometry demonstrated two clonal B-cell populations. The dominant population (~83%) displayed very dim surface λ light chain restriction (vs light chain negative) and was negative for CD5 and CD10. The minor population (~4%) was κ light chain restricted, dimly CD5 positive, and CD23 negative [Image 1E] and [Image 1F].

Cytogenetics

Not performed on this specimen.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) performed with dual-fusion CCND1 and IGH probes on an area of the lymph node enriched with CCND1+ cells failed to document the presence of cells harboring a t(11;14) translocation in an analysis of 200 nuclei.

PCR Analysis

IGH PCR performed on this lymph node showed a single dominant peak.

Bone Marrow

Morphologic Findings

H&E-stained sections revealed a normocellular marrow with trilineage hematopoiesis. Several large aggregates of small, mature lymphocytes without overt morphologic atypia accounted for approximately 15% of the cellularity (Table 1) [Image 2A]. There was a mild prominence of plasma cells (~5%).

Immunohistochemical Findings

Immunohistochemical studies revealed that the lymphoid aggregates were mostly composed of CD20+ B cells [Image 2B] with a few interspersed CD3+ T cells and CD138+ plasma cells. The plasma cells did not have a clear light chain...
bias. Rare (<1%) singly scattered CCND1+ cells within the B-cell aggregates were interpreted to reflect stromal cells.

**Flow Cytometry**

Flow cytometry demonstrated two distinct abnormal B-cell populations. The dominant population of CD20+ B lymphocytes (~52%) was negative for CD5 and CD10 and surface light chain negative. A lesser population of CD20+ B cells (~4%) was κ light chain restricted, CD5 (dim) positive, and CD23 negative. A minor (<1%) monoclonal light chain–restricted and brightly CD138-positive plasma cell population was also detected.

**Cytogenetics**

Metaphase cytogenetics on the marrow showed the karyotype to be 47,XY,+12[6]/46,XY,t(11;14)(q13;q32)[4]/46,XY[10] A minor (<1%) monoclonal κ light chain–restricted and brightly CD138-positive plasma cell population was also detected.

**FISH**

FISH performed with dual-color/dual-fusion CCND1 and IGH probes showed that 0.7% of cells harbored a CCND1-IGH fusion (normal <0.4%).

**PCR Analysis**

Not performed on this specimen.

**Porta Hepatis Node**

**Morphologic Findings**

A Diff-Quik stain of the fine-needle aspirate revealed a monomorphic population of small lymphocytes (Table 1).

**Flow Cytometry**

Flow cytometry revealed the presence of two abnormal CD20+ B-cell populations. The major population comprised approximately 76% of the cells and was negative for CD5, CD10, and CD23, without surface light chain expression. The minor population comprised around 3% of cells, was dimly CD5+ and negative for CD10 and CD23, and was κ light chain restricted. Immunohistochemistry, cytogenetics, FISH, and PCR were not performed on this specimen. Table 1 summarizes the findings in the above three specimens.

**Discussion**

In this case, we describe a patient with two lymph nodes and a bone marrow that showed a κ-restricted MCLIS associated with a much more manifest nodal MZL at all three sites. The histologically apparent MCL was detected in
all three locations through a variable combination of flow cytometry (three of three sites), immunohistochemistry (two of two sites), cytogenetics (one of one site), and FISH (one of two sites).

In MCLIS, clonal CCND1+ B cells grow in the mantle zones of otherwise reactive-appearing lymph nodes, but by definition neither cause expansion of the mantle zone nor invade into the interfollicular region or germinal center. They are not evident on H&E stains and are only revealed after performance of immunohistochemical stains such as CCND1 and CD5. In our case, histology revealed a lymph node effaced by MZL. Immunohistochemical studies revealed a second CCND1+ B-cell population that was not evident histologically, highly suggestive of MCLIS. This population was clonally distinct based on flow cytometry. However, the CCND1+ B cells were not detectable in mantle zones, almost certainly due to destruction of the nodal architecture by the neoplastic MZL cells and the resulting absence of recognizable follicles and mantle zones. Instead, they were found in small aggregates scattered throughout the lymph node. To our knowledge, this pattern has not been reported before, likely due to the rarity of both composite B-cell lymphomas and MCLIS.

Cytogenetic analysis of the bone marrow biopsy specimen performed 3 months earlier confirmed the presence of two distinct B-cell clones with different karyotypes. Interestingly, despite its typically lower analytic sensitivity than FISH, metaphase analysis revealed that 20% of dividing cells carried the t(11;14) translocation. This much larger proportion of MCL cells compared with the extremely low numbers detected by flow and FISH suggests an ex vivo growth advantage. It is also possible that their growth in vivo was suppressed by the more prevalent and dominant MZL cells. The failure of FISH and PCR to detect MCL cells with the t(11;14)/CCND1-IGH fusion and a distinct monoclonal IGH gene rearrangement, respectively, in the lymph node may have been due to technical reasons or overshadowing of the MCL-like clone by the dominant MZL clone.

To our knowledge, only two other reports have described composite lymphomas consisting of overt MCL and MZL. Three cases of MCLIS in association with a second small B-cell lymphoma have been described.
All three cases were of composite FL and MCLIS. The case reported here is unique for three reasons. First, the MCLIS was evident in three locales and was therefore multifocal. Second, the MCLIS was always accompanied by a more histologically dominant MZL. Third, where evaluable, it did not grow in an appreciable mantle zone distribution, presumably due to the lack of discernible mantle zones.

The presence of low numbers of cells harboring the t(11;14) translocation in the peripheral blood of healthy individuals is well known. One study showed that approximately 1% of individuals have circulating t(11;14)-positive cells, at a frequency of 2/10⁶ peripheral mononuclear cells, while another study found this prevalence to be 7%, at a frequency of 0.8 to 1.7/10⁶ peripheral mononuclear cells. This translocation was found to persist in the peripheral blood for up to 9 years after the initial assessment. Although these cells may be precursors to overt MCL, their persistence for almost a decade in one patient suggests that the t(11;14) translocation may not be sufficient for progression to malignancy. Our patient may have had MCL cells circulating in his peripheral blood, explaining why these cells were found in three separate areas. They may not have expanded into overt MCL due to a lack of sufficient additional genetic hits or an inability to expand once they had localized to the lymph nodes, due to suppression by the more dominant MZL. We were not able to test his peripheral blood for the t(11;14) translocation, since he had already started chemotherapy at the time of the consultation.

It is unknown whether MCLIS is a precursor lesion to MCL, respectively, the clinical implications of several other clonal hematologic disorders, including FLIS, MCLIS, and low-count MBL, have not been fully characterized. It is unclear whether patients with these lesions are at higher risk of developing overt FL, MCL, or CLL, respectively, compared with the general population. Given the incomplete understanding of FLIS and MCLIS, a 2010 workshop of the European Association for Hematopathology and the Society for Hematopathology suggested changing the names of FLIS and MCLIS to in situ MCL-like B cells of uncertain significance and in situ FL-like B cells of uncertain significance. The members of the workshop hoped that this change would reduce overdiagnosis and overtreatment of these lesions. Recent reviews have suggested closely monitoring but not treating patients with MCLIS, FLIS, and high-count MBL. A staging workup may also be warranted to rule out the presence of overt lymphoma elsewhere.

Of note, a small (<1%) monoclonal light chain–restricted plasma cell population was also detected in our patient’s bone marrow, consistent with his known MGUS. While it is tempting to speculate that this might be a facet of putative plasma cell differentiation by the MZL, this is clearly not the case given the light chain discordance. Rather, it suggests that the patient actually had three distinct B-cell clones.

In summary, this is the first reported case of a composite lymphoma consisting of multifocal MCLIS and nodal MZL. Both the MZL and the MCL clones were evident not only in the originally submitted lymph node but also in the previous bone marrow biopsy specimen and the porta hepatis node.
The multifocal nature of this case of MCLIS is particularly intriguing and provides new insight into the pathogenesis of this condition.

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References


38. Hirt C. Low prevalence of circulating t(11;14)(q13;q32)-positive cells in the peripheral blood of healthy individuals as detected by real-time quantitative PCR. *Blood*. 2004;104:904-905.
