Indolent B-cell lymphomas include follicular lymphoma (FL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and marginal zone lymphomas (MZLs). They are a diverse group of disorders with different clinical, morphological, immunophenotypic and genetic features. However, because of several histological similarities, such as in cell size and nodular structure, it may sometimes be difficult to differentiate them and to make a definitive diagnosis. In this review article, we summarize the histopathology of indolent B-cell neoplasms excluding FL and including hairy cell leukemia, and briefly mention recent genetic findings useful for their differential diagnosis. In addition, a provisional subtype of low-grade B-cell lymphoma, “prolymphocytic/paraimmunoblastic lymphoma”, is described. [J Clin Exp Hematop 54(1): 11-22, 2014]

**Keywords:** indolent B-cell neoplasms, lymphoma, pathology

**INTRODUCTION**

Indolent B-cell lymphomas show relatively good prognosis, cause few symptoms such as weight loss and fever, and exhibit a chronic course and slow progression. Histologically, they show small to medium-sized cell proliferation, including follicular lymphoma (FL), marginal zone lymphomas (MZLs) and chronic lymphocyte leukemia/small lymphocytic lymphoma (CLL/SLL), according to the World Health Organization classification published in 2008 (Table 1).\(^1\) Because of several historical similarities, such as cell size and nodular structure, reaching a definitive diagnosis may be difficult. Therefore, for this goal, immunohistochemistry and chromosomal analysis are important. This review article summarizes the morphology, immunophenotype and other latest findings on each subtype of indolent B-cell neoplasms except for FL (Table 2).

**CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA**

CLL and SLL are considered clinical variants of the same disease, with leukemic cases being referred to as CLL and non-leukemic cases being referred to as SLL. CLL/SLL is rarer in Asia than in the West,\(^2\) and it occurs more frequently in middle-aged and elderly individuals with a slightly higher incidence in men (M:F = 1.5-2:1). The majority of cases of CLL/SLL are asymptomatic. Painless lymph node swelling is the most common symptom,\(^3\) whereas hepatosplenomegaly, thrombocytopenia and anemia sometimes occur. In most cases, SLL is diagnosed after it has already infiltrated the bone marrow and the disease is at an advanced stage.

Histologically, CLL/SLL shows the proliferation of small B lymphocytes characterized by CD5 and CD23 expression. It infiltrates areas such as the peripheral blood, bone marrow, lymph nodes and spleen. The architecture is usually effaced in the affected lymph nodes. The pattern of infiltration is diffuse, with scattered proliferation centers (Fig. 1A). Some cases spare the lymph follicles and exhibit paracortical proliferation. The majority of proliferating cells are small lymphocytes with round nuclei, less cytoplasm and a high nuclear/cytoplasmic ratio (Fig. 1B). In addition to small lymphocytes, proliferation centers contain varying degrees of small to medium-sized prolymphocytes and medium-sized to large paraimmunoblasts with round to oval nuclei and central nucleoli (Fig. 1C). Dutcher bodies and plasma cell differentiation are not evident. Infiltration occurs in both red and white pulps of the spleen, but is more prominent in the white pulp. Proliferation centers develop but are usually less prominent
than those in the lymph nodes. Three patterns of infiltration are observed in the bone marrow: nodular, interstitial and diffuse. Some researchers reported that cases with the diffuse pattern had the worst prognosis. The proliferation centers are not as prominent as those in the nodal lesions.

CLL/SLL typically expresses pan-B-cell antigens such as CD20, CD22, CD79a and the surface immunoglobulin (sIg). CD5, CD23 and CD43 are also positive. The expressions of CD10, FMC7 and cyclin D1 are negative. The proliferation centers are not as prominent as those in the nodal lesions.

Table 1. Low-grade mature B-cell neoplasm of WHO classification

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>CD5</th>
<th>CD10</th>
<th>CD20</th>
<th>CD23</th>
<th>CD43</th>
<th>BCL6</th>
<th>MUM1</th>
<th>Cyclin D1</th>
<th>Annexin A1</th>
<th>LEF1</th>
<th>Characteristic genetic findings</th>
</tr>
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<tbody>
<tr>
<td>FL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>t(14;18) (q32;q21), BCL6 translocation trisomy 3, t(11;18)(q21;q21)</td>
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<tr>
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<td>-</td>
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<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>+**</td>
<td>-</td>
<td>-</td>
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</tr>
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<tr>
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<td>-</td>
<td>+</td>
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<td></td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>+**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>MYD88 L265P trisomy12, del (11p22-23), del (13q14), del (17p13), del (6p21)</td>
</tr>
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<td>CLL/SLL</td>
<td>+</td>
<td>dim+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+****</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>-</td>
<td>t(2;6)(p11.2;p25), t(6;22)(p5;p11.2)</td>
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<td>HCL</td>
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<td>-/+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>dim or focally+</td>
<td>+</td>
<td>NA BRAF V600E</td>
</tr>
</tbody>
</table>

Reviewed entities in this article are underlined.

Table 2. Summary of immunohistological markers and characteristic genetic findings

CLL/SLL typically expresses pan-B-cell antigens such as CD20, CD22, CD79a and the surface immunoglobulin (sIg). CD5, CD23 and CD43 are also positive. The expressions of CD10, FMC7 and cyclin D1 are negative. The proliferation centers are not as prominent as those in the nodal lesions.

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Lymphoid-enhancer-binding factor 1 (LEF1), a WNT/β-catenin signaling mediator, was recently shown to be characteristically positive in CLL/SLL, including in CD5-negative cases. LEF1 may be positive in grade 3 FL and diffuse large B-cell lymphoma (DLBCL), whereas it is negative in mantle cell lymphoma (MCL), MZLs and grade 1-2 FL. Furthermore, SOX11 has been shown to be positive specifically in MCL. Thus, these new markers can be used in the differential diagnosis.

CLL/SLL sometimes transforms into other subtypes. Approximately 2-8% of cases develop into high-grade B-cell lymphomas such as DLBCL (Richter syndrome). The clinical characteristics of Richter syndrome include systemic symptoms such as fever and weight loss, sudden deterioration of the patient’s condition, rapid enlargement of the lymph nodes and extranodal infiltration (i.e., hepatosplenomegaly). Richter syndrome has a poor prognosis, with a median survival time of < 1 year. CLL/SLL is classified into 2 groups according to the presence of somatic mutations in the immunoglobulin heavy chain variable region (IGVH); furthermore, 73% of the cases with clonally related Richter syndrome showed unmutated IGVH, whereas IGVH of 4/5 unrelated cases was mutated. Approximately 0.5% of cases are complicated by Hodgkin transformation, which is considered to be a variant of Richter syndrome. Clinically, the prognosis is poor, similar to that in cases that progress to DLBCL. Reed-Sternberg cells and Hodgkin cells exhibit immunophenotypes that are typical of classical Hodgkin lymphoma, and in many cases, the cells are positive for Epstein-Barr virus.

The prognostic factors include the presence of somatic hypermutations (SHMs) in IGVH, expressions of ZAP70 and CD38, and deletions of 11q22-23, 17p and 13q14.3. SHMs in the IGVH often strongly influence prognosis, with
unmutated cases resulting in rapid disease progression and poor prognosis. Even in mutated cases, those with VH3-21 have poor prognosis. ZAP70 or CD38 expression is a poorer prognostic factor. Deletions of 11q22-23 and 17p are associated with poor prognosis, whereas a 13q14.3 deletion usually results in a good prognosis.

**SPLENIC MARGINAL ZONE LYMPHOMA**

Splenic marginal zone lymphoma (SMZL) infiltrates the spleen, bone marrow and peripheral blood. It comprises 1-2% of all lymphomas and usually occurs in the elderly. Most cases present with marked splenomegaly as well as autoimmune thrombocytopenia and anemia. In SMZL, peripheral lymph nodes and other mucosa-associated lymphoid tissue (MALT) are not usually infiltrated, except for the splenic hilar lymph nodes. M protein is observed in approximately 1/3 of cases, but it does not usually cause marked hyperviscosity or hypergammaglobulinemia. The diagnosis is usually made from splenectomy samples.

Splenic lesions are characterized by their micronodular pattern, in which the lymph follicles (white pulps) increase in number and size (Fig. 2). The representative follicle exhibits a dual-layer structure, while small lymphocytes form a mantle zone-like structure around a small regressed germinal center. The marginal zone, which is present outside of the mantle zone and expands externally, contains a mixture of marginal zone cells with abundant cytoplasm as well as large cells that resemble centroblasts and immunoblasts. Disease progression is often characterized by infiltration of the red pulp, while the main site of proliferation becomes unclear.

Characteristic histopathological features of infiltration occur in other areas, such as the bone marrow, peripheral blood and splenic hilar lymph nodes. These histopathological features, together with clinical findings, often aid in the correct diagnosis. In terms of bone marrow infiltration, small lymphoma cells in the sinuses or between bone trabeculae may be difficult to recognize morphologically, but can be identified by anti-CD20 immunohistochemistry (Fig. 2D). Although peripheral blood infiltration does not occur as frequently as bone marrow infiltration, small lymphoma cells are often found in the peripheral blood, and in some cases, lymphoma cells show hairy cell morphology. Infiltration into the splenic hilar lymph nodes usually exhibits a micronodular pattern formed of small lymphoma cells as well as expanding interfollicular regions.

**NOTCH2** mutations were recently shown to be almost specific to SMZL. The mutations occur in approximately 20% of SMZL cases and are not detected in other low-grade B-cell lymphomas except for non-splenic MZLs. Therefore, they can be useful for differentiating cases of low-grade B-cell lymphoma that occur along with splenomegaly.
HAIRY CELL LEUKEMIA

Hairy cell leukemia (HCL) is a rare B-cell leukemia that shows proliferation of leukemic cells characterized by oval nuclei and abundant cytoplasm with capilliform protuberances. HCL comprises approximately 2% of leukemia cases and often occurs in middle-aged men. The bone marrow and spleen (red pulp) are also involved. Infiltration into the liver is common and may also occur in the skin. Lymph node swelling does not generally occur.

Histologically, the leukemic cells localize to the red pulp and the white pulp is usually atrophic. The splenic cords are filled with leukemic cells (Fig. 3A). “Red blood cell lakes” that are surrounded by leukemic cells are scattered. Typical lymph node lesions show a paracortical pattern. Reticular fibrosis often results in the inability to aspirate bone marrow; thus, bone marrow biopsy is useful for the diagnosis of HCL. Tumor cells infiltrate bone marrow to varying degrees, from mottled infiltration at early stages to diffuse infiltration at advanced stages. The cytomorphology is small to medium-sized cells with round to oval-shaped centrally located nuclei. The cytoplasm is abundant and the intercellular borders are distinct, presenting the so-called “fried-egg” morphology (Fig. 3).

Immunophenotypically, pan-B-cell antibodies and FMC7 are positive, whereas CD5, CD10 and CD23 are negative. Furthermore, strong expression of CD20 and CD22 is observed. Positive immunostaining for CD103, CD11c, CD25, CD123, TRAP, DBA.44, annexin A1 and T-bet is characteristic of HCL. Co-expression of TRAP and DBA.44 is a characteristic finding, but caution is needed because some other B-cell neoplasms may be positive for them. In B-cell neoplasms, annexin A1 is highly specific to HCL. Cyclin D1 expression is dim + or focally +, although the cytogenetic rearrangement t(11;14)(q13;q32) is not seen. Some cases are partially CD10-positive. Recent studies have shown that the BRAF mutation (V600E) can be utilized as a specific molecular marker for HCL. A monoclonal antibody specific to BRAF with V600E mutation (clone VE1) is available (Fig. 3).
LYMPHOPLASMACYTIC LYMPHOMA

Lymphoplasmacytic lymphoma (LPL) consists of a mixture of cells that differentiate into varying degrees of plasma cells, including small lymphocytes, lymphoplasmacytoid cells and plasma cells. Approximately 5% of B-cell neoplasms are LPL. They mainly infiltrate the bone marrow, but lesions in lymph nodes as well as extranodal lesions such as those in the spleen, liver and peripheral blood are also seen. Clinically, LPL is usually accompanied by IgM-type M proteinemia (Waldenström macroglobulinemia).46-48

Nodular and diffuse infiltrations are histopathologically evident in the bone marrow (Fig. 4). Similar to MALT lymphoma, neoplastic plasma cells and lymphoplasmacytoid cells may harbor Dutcher bodies. In lymph nodes, although the sinuses remain, diffuse proliferation in the interfollicular area is observed.

The MYD88 L265P mutation has recently been reported to occur specifically in Waldenström macroglobulinemia and not in MZLs, CLL or other low-grade B-cell neoplasms that involve the bone marrow.49-52 It was sometimes difficult to differentiate LPL from other low-grade B-cell neoplasms that showed plasmacytoid differentiation and M proteinemia. In such cases, a diagnosis of small B-cell lymphoma with plasmacytic differentiation could often not be avoided. However, the identification of the MYD88 L265P mutation will help with the differentiation of LPL from other low-grade B-cell neoplasms.

EXTRANODAL MARGINAL ZONE LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

Marginal zone B cells are the normal counterpart of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Infiltration occurs in a variety of extranodal organs, with digestive organs accounting for half. Gastric involvement affects 85% of digestive tract cases.53,54 Other organs may include the lung (14%), head and neck (14%), ocular adnexa (12%), skin (11%) and thyroid gland (4%).3 Most cases are localized (stages 1-2). Although some cases exhibit infiltration into multiple organs involving the bone marrow and lymph nodes, the long-term prognosis is perceived to be relatively good.55,56 Moreover, plasmacytoid differentiation is seen in many cases, and M proteinemia appears in 1/3 of cases.57

MALT lymphoma presents a variety of histological findings (Fig. 5A-5G), such as the marginal zone, the outer layer of the mantle zone, being the main site of infiltration; the development of small to medium-sized centrocyte-like or monocytoid cell infiltrates in the marginal zone; cases that show plasmacytoid cells with intranuclear pseudoinclusion of...
immunoglobulin (Dutcher body) or acidophilic spheres in the cytoplasm (Russell body); infiltration of lymphoma cells into the epithelium, forming lymphoepithelial lesions; and infiltration and proliferation within germinal centers (follicular colonization) that make it difficult to distinguish MALT lymphoma with prominent colonization from FL. Large cells such as centroblastoid cells and immunoblastoid cells are seen in varying proportions. A case with sheet proliferation of large cells inside MALT lymphoma should be diagnosed as DLBCL transformed from MALT lymphoma.

Chromosomal abnormalities include t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q2;21) and t(3;14)(p14;q32); each rearrangement triggers expression of API2-MALT1 chimeric protein or deregulated expression of BCL10, MALT1 or FOXP1, respectively. Although trisomy 3 or 18 is not specific to this condition, it is often observed in MALT lymphoma. Such chromosomal translocations and the frequencies of trisomies differ according to the primary organ, with t(11;18)(q21; q21) found mainly in the lung and stomach, t(14;18)(q21;21) in the ocular adnexa and salivary glands, and t(3;14)(p14;q32) in the thyroid gland, ocular adnexa and skin.58,59

NODAL MARGINAL ZONE LYMPHOMA

Nodal marginal zone lymphoma (NMZL) is characterized by the presence of a primary lesion in the lymph nodes and is a neoplastic counterpart of post-germinal center marginal zone cells. The diagnosis of NMZL is made only after other low-grade lymphomas are ruled out. The histopathological findings of NMZL overlap with those of other MZLs, including MALT lymphoma and SMZL, as well as LPL. Thus, it is essential to verify that extranodal lesions (apart from those in the bone marrow, liver and spleen) do not exist. However, NMZL can be difficult to distinguish from these lymphoma subtypes with secondary lymph node infiltration. Lymph node involvement of NMZL can be localized or systemic, and infiltration into the bone marrow or peripheral blood is rare.25,60,61 Plasmacytoid differentiation is seen, but M proteinemia is generally not.62

The histopathological features of NMZL resemble those of EMZL and SMZL. Lymphoma cells proliferate in a marginal zone pattern in the lymph nodes.63,64 Lymph follicles may be hyperplastic or atrophic and be colonized by lymphoma cells. The pattern of infiltration can be categorized into 3 variants.65 The “MALT type” is accompanied by the formation of reactive germinal centers, and its lymphoma cells usually exhibit monocytoid morphology with abundant cytoplasm (Fig. 5H). Detailed systemic examinations reveal the presence of MALT lymphoma in approximately half of these cases. The “splenic type” is characterized by nodular infiltration with or without a residual germinal center (Fig. 5I). The lymphoma cells of this type are weakly positive for IgD. The “splenic type” takes its name from the fact that its histological features resemble those of regional lymph node infiltration in SMZL. Cases that do not fit into either of the above types are designated as the “polymorphic type,” in which the follicles are often atrophied and colonized. In addition, some cases are accompanied by plasmacytoid differentiation and/or eosinophilic infiltration.

“PROLYMPHOCYTIC/PARAIMMUNOBLASTIC LYMPHOMA” (PPL)

A new disease entity of low-grade B-cell lymphoma has recently been proposed, which is characterized by prolymphocyte and paraimmunoblast proliferation, co-expression of MUM1 and BCL6, and reciprocal translocation of the IRF4 and immunoglobulin light chain genes.66 Reciprocal translocation of the IRF4 and immunoglobulin genes has been reported in myeloma67 and high-grade B-cell lymphomas,68 but not in low-grade B-cell lymphomas. To date, 4 cases have been identified, including 3 reported cases66 and one additional case, all of which showed no B symptoms such as fever, weight loss and night sweats. Bone marrow infiltration was
Indolent B-cell neoplasms other than FL

Fig. 5. Morphology of MALT lymphoma and NMZL. (5A) The lymphoma cells diffusely infiltrate the gastric lamina propria and grow around reactive follicles in a marginal zone distribution. (5B) The neoplastic cells are small to medium-sized, with slightly irregularly shaped nuclei and a moderate amount of pale to clear cytoplasm, so-called monocytoid cells. Scattered large cells are also present. (5C) The right half of the picture shows plasma cell differentiation. Note the plasma cells with intranuclear pseudo-inclusions of immunoglobulin (Dutcher bodies). (5D) LEL of the stomach. Epithelial invasion of lymphoma cells resulting in distortion and eosinophilic degeneration of gastric glandular epithelium. (5E) LEL of the thyroid. Thyroid follicles stuffed with lymphoma cells (“MALT ball” LEL). (5F, 5G) Follicular colonization. Neoplastic monocytoid cells infiltrate into reactive follicles (5F), and immunostaining for CD21 highlights partially disrupted follicular dendritic cell network (5G). (5H) NMZL, MALT type. In this variant, reactive hyperplastic follicles with mantle zone and germinal center are well preserved. The lymphoma cells with pale cytoplasm involve the perifollicular zone and extend out into the paracortex. (5I) NMZL, splenic type. A vague nodular pattern is seen. Neoplastic cells replace follicles with completely atrophic germinal centers.
Fig. 6. Morphology of “prolymphocytic/paraimmunoblastic lymphoma”. Small lymphocyte-predominant (6A-6D), prolymphocyte-predominant (6E, 6F) and paraimmunoblast-predominant (6G, 6H) cases. In the small lymphocyte-predominant case, small lymphocytes infiltrated with proliferation centers (6A, 6C). In some areas, diffuse proliferation of prolymphocytes was observed (6B, 6D). This feature is similar to that of “tumor-forming subtype of CLL/SLL”. The prolymphocyte-predominant case showed diffuse infiltration of prolymphocytes admixed with occasional paraimmunoblasts and small lymphocytes (6E, 6F). Prominent nodular paraimmunoblastic infiltration was observed with a background of prominent fibroblastic band formation (6G, 6H).
observed in 2 cases, but none of the 4 cases was leukemic. Histologically, cases of “PPL” show the disappearance of lymph node architecture and diffuse or nodular proliferation of small lymphocytes, prolymphocytes and paraimmunoblasts at various ratios. Therefore, 3 cytomorphological variants may be defined: small lymphocyte-predominant, prolymphocyte-predominant and paraimmunoblast-predominant (Fig. 6). Of the 4 cases above, 2 showed diffuse prolymphocytic-predominant proliferation like the tumor-forming subtype of CLL/SLL. One case showed paraimmunoblastic-predominant nodular proliferation against a background of prominent fibrosis. This might be considered grade 3 FL, but FDC networks were not detected by immunostaining. The final case presented with proliferation of small lymphocytes with the accompanying proliferation centers and resembled the histology of SLL. However, diffuse proliferation of the prolymphocytes was also observed. In immunostaining, the co-expression of IRF4/MUM1 and BCL6 (Fig. 7A, 7B) was characteristic of this condition, which is atypical in low-grade B-cell lymphomas including SLL. CD23 staining was positive, but IgM was strongly positive and IgD was negative, which are atypical for SLL. CD5 immunohistochemical staining was negative in all cases, but was dim + in 2 cases on flow cytometry. The Ki-67 labeling index was low (around 10%) (Fig. 7C). IGK was the translocation partner in 3 cases, whereas IGL was the partner in the remaining case. No cases involving the translocation of IGH have been identified to date. Well-known translocations such as BCL2, BCL6, CCND1 and MYC were not detected in any of these cases.

The examination of additional cases is necessary to differentiate “PPL” from SLL. The low-grade B-cell lymphoma cases with proliferation of prolymphocytes and paraimmunoblasts as well as the characteristic co-expression of MUM1 and BCL6 should be examined for the translocation of IRF4 and immunoglobulins.

**CONCLUSION**

In this review article, the features of primary indolent B-cell lymphomas are described, excluding FL but including HCL, with special focus on the histopathological features. A description has also been provided for a provisional subtype of low-grade B-cell lymphoma, which was initially referred to as “PPL.”

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SS and NT contributed equally to this article. The authors do not have any conflicts of interest to declare.

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**Fig. 7.** Immunohistochemistry of “prolymphocytic/paraimmunoblastic lymphoma”. The majority of lymphoma cells show nuclear staining for both IRF4/MUM1 (A) and BCL6 (B). Ki-67 labeling index is approximately 10% (C).
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