Secondary CD5+ Diffuse Large B-Cell Lymphoma Not Associated With Transformation of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (Richter Syndrome)

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Key Words: CD5; Diffuse large B-cell lymphoma; Secondary disease; Immunohistochemistry; Non–Richter syndrome

Abstract

Few cases of secondary CD5+ diffuse large B-cell lymphoma (DLBCL) that are not Richter syndrome have been reported previously. We report 9 cases of non–Richter syndrome secondary CD5+ DLBCL. Among 529 cases of DLBCL, 38 (7.2%) were CD5+ DLBCL, including 9 of secondary CD5+ DLBCL. Five cases gained CD5 expression during the clinical course of DLBCL (group 1). Three cases showed transformation from CD5– low-grade B-cell lymphoma to CD5+ DLBCL (group 2). The remaining case showed coexistence of CD5+ DLBCL and CD5+ follicular lymphoma. The clonal relationships of CD5– and CD5+ tumors were confirmed in all 4 available cases. Cases of secondary CD5+ DLBCL that were not Richter syndrome were classifiable into 3 groups. Groups 1 and 2 showed the gain of CD5 during the clinical course or transformation of the tumors, suggesting that CD5 expression is closely associated with the progression of B-cell lymphoma.

Diffuse large B-cell lymphoma (DLBCL) is the largest and most widely heterogeneous category of aggressive lymphomas.1 CD5 expression in DLBCL is currently a focus of clinical and pathologic interest. Richter syndrome, transformation of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), is a well-known form of secondary CD5+ DLBCL, but few cases of secondary CD5+ DLBCL that are not Richter syndrome have been reported previously.

The CD5 molecule is a 67-kDa glycoprotein that is expressed by most T cells and a subset of B cells.2 Among mature B-cell neoplasms, most cases of CLL/SLL express CD5 and often undergo transformation to CD5+ DLBCL, so-called Richter syndrome. CD5 is also expressed in most cases of mantle cell lymphoma, but less frequently in DLBCL and intravascular large B-cell lymphoma and in only a small proportion of cases of extranodal marginal zone B-cell lymphoma,7,8 Burkitt lymphoma,9 follicular lymphoma (FL),10-14 splenic marginal zone B-cell lymphoma,15 and primary effusion lymphoma.16 It is speculated that these neoplasms could switch or transform to CD5+ DLBCL, although few reports have indicated that CD5+ FL can transform to CD5+ DLBCL.13,14 Moreover, apart from 1 case reported previously by our group,17 transformation of CD5– low-grade B-cell lymphoma to CD5+ DLBCL seems to be rare.

Matolcsy et al3 highlighted the phenomenon of de novo evolution of CD5 expression in DLBCL that is not a result of transformation, suggesting that such DLBCL is genotypically distinct from Richter syndrome–associated DLBCL. Yamaguchi et al5 reported that CD5+ DLBCL accounted for approximately 10% of de novo DLBCL cases and that CD5 was a marker of poor prognosis in de novo DLBCL. They indicated that patients with CD5+ DLBCL showed an
older age distribution, a female predominance, poor performance status, a higher level of serum lactate dehydrogenase, advanced stage, a tendency to have more than 1 extranodal site and B symptom, and a higher International Prognostic Index score than patients with CD5− DLBCL. However, there has been no report of secondary CD5+ DLBCL gaining CD5 expression during the clinical course. Herein we report 9 cases of secondary CD5+ DLBCL that were not Richter syndrome.

Materials and Methods

Patient Selection

We reviewed the pathology archives of the National Cancer Center Hospital, Tokyo, Japan, for the period between 2002 and 2007. The total number of patients with DLBCL with or without a low-grade B-cell lymphoma component was 529, and the total number of specimens available was 728 (1-5 per case). Clinical information was extracted from the medical records, and the Ann Arbor system was used for staging.

Morphologic Review

The materials were fixed in 10% neutral-buffered formalin overnight, embedded in paraffin, cut into sections 4 µm thick, and stained with H&E for routine histologic evaluation. All specimens were reviewed by 3 pathologists (A.M.M., H.T., and Y.M.) to confirm that the morphologic characteristics fulfilled the criteria for DLBCL in the 2001 World Health Organization classification of lymphoid neoplasms.1 When a low-grade B-cell lymphoma component was detected in previous or synchronous specimens, its histologic features were also evaluated. DLBCL was subclassified as the centroblastic, anaplastic, immunoblastic, or T-cell/histiocyte-rich variant. The presence of intravascular involvement, like that seen in intravascular large B-cell lymphoma, was also evaluated.

Immunohistochemical Studies, In Situ Hybridization, Flow Cytometry, and Interface Fluorescence In Situ Hybridization

We performed immunohistochemical analysis on formalin-fixed paraffin-embedded tissue samples by using a panel of monoclonal and polyclonal antibodies. Sections 4 µm thick were cut from each paraffin block, deparaffinized, and incubated at 121°C in citrate buffer, pH 6.0, for 10 minutes for antigen retrieval. Antibodies included those against the following antigens: CD3 (PS1, ×25; Novoceastra, Newcastle upon Tyne, England), CD5 (4C7, ×50; Novoceastra), CD10 (56C6, ×50; Novoceastra), CD20 (L26, ×100; DAKO, Glostrup, Denmark), CD23 (1B12, ×100; Novoceastra), bcl-6 (polyclonal, ×50; DAKO, Glostrup, Japan), cyclin D1 (SP4, ×25; Nichirei, Tokyo), MUM1 (MUM1p, ×50; DAKO, Kyoto), and p53 (DO7, ×100; DAKO, Glostrup), using an autostainer with the standard polymer (DAKO Autostainer Plus, Glostrup, for CD3, CD5, CD10, CD23, and cyclin D1) or the labeled streptavidin-biotin method (BioGenex Autostainer, San Ramon, CA, for CD20 and p53) or manually by the standard avidin-biotin complex method (bcl-6 and MUM1).

Immunohistochemical analysis for CD3, CD20, and CD5 was performed in all DLBCLs. Immunoreactivity for CD5 was judged positive if more than 20% of the tumor cells were stained. In all specimens of CD5− and CD5+ lymphomas of secondary CD5+ DLBCLs, CD5 was restained by the avidin-biotin complex method simultaneously, and the reproducibility of CD5 expression was confirmed. When a DLBCL had a CD5+ phenotype, the cyclin D1− phenotype was examined. The CD5− DLBCL or CD5− low-grade B-cell lymphoma and CD5+ DLBCL components were stained for CD10, bcl-6, MUM1, CD23, and p53, as well as for CD3, CD20, CD5, and cyclin D1.

To classify each case as having a germinal center B-cell (GCB) phenotype or a non-GCB phenotype, a panel of 3 antigens (CD10, bcl-6, and MUM1) was used according to the protocol reported by Hans et al.15 All immunohistochemical specimens were judged by one of us (A.M.M.) and confirmed by 2 others (H.T. and Y.M.). In situ hybridization (ISH) with Epstein-Barr virus-encoded RNA (EBER-1) probes (DAKO, Glostrup) was performed to detect possible EBV infection.

Flow cytometry was performed on a Beckman Coulter Epics XL-MCL instrument with System II software (Beckman Coulter, Fullerton, CA). Cells were stained with fluorescein isothiocyanate–labeled antibodies against CD20 (B-Ly1, DAKO, Glostrup) and phycoerythrin-labeled CD5 (DK23, DAKO, Glostrup). The total population of viable cells was gated using forward and right scatter. Double positivity of CD5 and CD20 was defined as 15% or more of the population expressing both markers. The results of flow cytometry and immunohistochemical analysis were compared, and their degrees of agreement were examined.

Interface fluorescence ISH analysis was optional and performed on sections 4 µm thick cut from each paraffin block. Judgment of the fusion gene was performed as described previously.19 Dual-color LSI IGH Spectrum Green/LSI BCL2 Spectrum Orange Dual Fusion Translocation Probes (Vysis, Downers Grove, IL) were used to detect IGH/BCL2 fusion.

Polymerase Chain Reaction and Sequencing

DNA was extracted from paraffin-embedded tissue sections by using the DNA Micro Kit (Qiagen, Tokyo). To amplify the rearranged immunoglobulin heavy chain variable region gene, CDR3, we performed seminested polymerase chain reaction (PCR) using primers directed at consensus sequences of framework 3 (Fr3A, 5′-ACA CGG C(C/T)(G/C) TGT ATT ACT GT-3′) of the variable region and common sequence of the joining region (LJH, 5′-TGA GGA GAC
GGT GAC C-3’ and VLJH, 5’-GTG ACC AGG GTN CCT TGG CCC CAG-3’). All PCR reactions were performed in 20-µL total volumes under standard conditions using LA Taq polymerase (Takara Bio, Shiga, Japan).

The amplified products were electrophoresed on 3% polyacrylamide gels. In cases without a monoclonal band, PCR products were purified by using Microcon YM-100 (Millipore, Bedford, MA). PCR amplification was performed by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and automated fluorescent sequencing was performed on an ABI prism 310-Avant Genetic Analyzer (Applied Biosystems).

Results

Characteristics of Patients With Non–Richter Syndrome Secondary CD5+ DLBCL

There were 38 cases of CD5+ DLBCL (7.2%), among which 9 cases of secondary CD5+ DLBCL were identified (9/38 [24%]). None of the cases was Richter syndrome.

Clinical information is summarized in Table I. The patients comprised 6 men and 3 women, ranging in age from 24 to 76 years with a median age of 63 years. Six patients had stage I or II disease, and 3 had stage III or IV disease at initial diagnosis. All patients received chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone or other types of regimen). The 5-year overall survival from initial diagnosis was 80%.

Three Histologic Groups of Secondary CD5+ DLBCL

The 9 cases of non–Richter syndrome secondary CD5+ DLBCL were classifiable into 3 groups. In the 8 cases constituting group 1 (gained CD5 expression during the clinical course of DLBCL, 5 cases) and group 2 (showed transformation from CD5− low-grade B-cell lymphoma to CD5+ DLBCL, 3 cases), the biopsy sites of CD5− B-cell non-Hodgkin lymphoma at initial diagnosis were lymph node (2), Waldeyer ring (1), and extranodal sites (5, 1 each in breast, bone marrow, maxillary sinus, orbit, and small intestine). The biopsy sites of the CD5+ DLBCLs were lymph node (5), tonsil (1), and extranodal sites (2, 1 each in skin and soft tissue). The single tumor constituting group 3 (case 9) was obtained by immunohistochemical analysis.

Table I
Patient Characteristics in 9 Cases of Secondary CD5+ Diffuse Large B-Cell Lymphoma

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>Months After Diagnosis</th>
<th>Histologic Type</th>
<th>Biopsy Site</th>
<th>PS</th>
<th>Stage</th>
<th>IPI</th>
<th>LDH (U/L)</th>
<th>EN</th>
<th>B Symptoms</th>
<th>Postbiopsy Therapy/Response</th>
<th>Follow-up (mo)/Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/62</td>
<td>0</td>
<td>DLBCL</td>
<td>Breast</td>
<td>0</td>
<td>I</td>
<td>LI</td>
<td>506</td>
<td>0</td>
<td></td>
<td>CHOP ×6 and RT, 40 Gy/CR</td>
<td>58/AWD</td>
</tr>
<tr>
<td>37</td>
<td>DLBCL</td>
<td></td>
<td>Cervical LN</td>
<td>0</td>
<td>II</td>
<td></td>
<td>146</td>
<td>1</td>
<td></td>
<td>R ×1 and ESHAP ×3</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>DLBCL</td>
<td></td>
<td>Inguinal LN</td>
<td>0</td>
<td>II</td>
<td></td>
<td>146</td>
<td>1</td>
<td></td>
<td>R ×1 and ESHAP ×3</td>
<td></td>
</tr>
<tr>
<td>2/F/63</td>
<td>7</td>
<td>DLBCL</td>
<td>Cervical LN</td>
<td>0</td>
<td>II</td>
<td></td>
<td>127</td>
<td>1</td>
<td></td>
<td>R ×4, CHOP ×6, and RT, 40 Gy/CR</td>
<td>4/AWD</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>Cervical LN</td>
<td>0</td>
<td>II</td>
<td></td>
<td>127</td>
<td>1</td>
<td></td>
<td>IT, RT at 30 Gy, R ×5, and IVAC ×3/PR</td>
<td>24/DOD</td>
</tr>
<tr>
<td>3/M/24</td>
<td>0</td>
<td>DLBCL</td>
<td>Soft tissue</td>
<td>0</td>
<td>I</td>
<td>LI</td>
<td>209</td>
<td>0</td>
<td></td>
<td>R-ChOP ×6 and RT, 40 Gy/CR</td>
<td>19/AWD</td>
</tr>
<tr>
<td>8</td>
<td>DLBCL</td>
<td></td>
<td>Waldeyer ring</td>
<td>0</td>
<td>II</td>
<td></td>
<td>209</td>
<td>0</td>
<td></td>
<td>Auto-PBSCT</td>
<td></td>
</tr>
<tr>
<td>4/M/63</td>
<td>0</td>
<td>DLBCL</td>
<td>Cervical LN</td>
<td>0</td>
<td>I</td>
<td></td>
<td>305</td>
<td>2</td>
<td></td>
<td>R ×8/CR</td>
<td>77/AWD</td>
</tr>
<tr>
<td>15</td>
<td>DLBCL</td>
<td></td>
<td>Inguinal LN</td>
<td>0</td>
<td>IV</td>
<td></td>
<td>305</td>
<td>2</td>
<td></td>
<td>EPOCH ×4/CR</td>
<td></td>
</tr>
<tr>
<td>5/M/66</td>
<td>0</td>
<td>DLBCL</td>
<td>Abdominal LN</td>
<td>0</td>
<td>IV</td>
<td></td>
<td>305</td>
<td>2</td>
<td></td>
<td>EPOCH ×4/CR</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>DLBCL</td>
<td></td>
<td>Prostate</td>
<td>0</td>
<td>I</td>
<td></td>
<td>150</td>
<td>1</td>
<td></td>
<td>R ×3, and ESHAP ×3</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>DLBCL</td>
<td></td>
<td>Skin</td>
<td>0</td>
<td>II</td>
<td></td>
<td>150</td>
<td>1</td>
<td></td>
<td>R ×3, and ESHAP ×3</td>
<td></td>
</tr>
<tr>
<td>6/M/47</td>
<td>0</td>
<td>FL, grade 2</td>
<td>Small intestine</td>
<td>0</td>
<td>II</td>
<td></td>
<td>439</td>
<td>2</td>
<td></td>
<td>ESHAP ×3 + R ×8, R-ICE ×4</td>
<td>123/DOD</td>
</tr>
<tr>
<td>75</td>
<td>DLBCL</td>
<td></td>
<td>Tonsil</td>
<td>0</td>
<td>I</td>
<td></td>
<td>439</td>
<td>2</td>
<td></td>
<td>R ×1, C-MOPP ×14, and VDS</td>
<td>57/AWD</td>
</tr>
<tr>
<td>56</td>
<td>MALT lymphoma</td>
<td></td>
<td>Orbit</td>
<td>0</td>
<td>I</td>
<td></td>
<td>439</td>
<td>2</td>
<td></td>
<td>C-MOPP ×6/PR</td>
<td></td>
</tr>
<tr>
<td>8/F/76</td>
<td>0</td>
<td>LPL</td>
<td>BM</td>
<td>2</td>
<td>IV</td>
<td></td>
<td>750</td>
<td>1</td>
<td></td>
<td>CHOP ×2/PR; R-ESHAP ×1/PR</td>
<td>5/AWD</td>
</tr>
<tr>
<td>0</td>
<td>DLBCL</td>
<td></td>
<td>Cervical LN</td>
<td>0</td>
<td>I</td>
<td></td>
<td>750</td>
<td>1</td>
<td></td>
<td>R ×8, CHOP ×3, RT, and 30 Gy/CR</td>
<td>2/AWD</td>
</tr>
<tr>
<td>9/M/27</td>
<td>0</td>
<td>DLBCL</td>
<td>Cervical LN</td>
<td>0</td>
<td>I</td>
<td></td>
<td>750</td>
<td>1</td>
<td></td>
<td>R ×8, CHOP ×3, RT, and 30 Gy/CR</td>
<td>2/AWD</td>
</tr>
</tbody>
</table>

AWD, alive with disease; AWOD, alive without disease; BM, bone marrow; BMT, bone marrow transplantation; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone, and procarbazine; CODOX-M, cyclophosphamide, doxorubicin, and high-dose methotrexate; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; DOD, died of disease; EN, number of extranodal sites; EPOCH, doxorubicin, vincristine, etoposide, cyclophosphamide, and prednisone; ESHAP, etoposide, carboplatin, cytarabine, and methylprednisolone; FL, follicular lymphoma; H, high; HI, high intermediate; ICE, ifosfamide, carboplatin, and etoposide; IPI, International Prognostic Index; IT, intrathecal methotrexate and prednisolone; IVAC, ifosfamide, etoposide, and high-dose cytarabine; L, low; LDH, serum lactate dehydrogenase; LI, low intermediate; LN, lymph node; LPL, lymphoplasmacytic lymphoma; MALT, mucosa-associated lymphoid tissue; NA, not available; NC, no change; PBSCT, peripheral blood stem cell transplantation; PD, progressive disease; PR, partial remission; PS, performance status; R, rituximab; RT, radiotherapy; VDS, vindesine, doxorubicin, and prednisone.

Table I is continued on the next page.

* By immunohistochemical analysis.
† The reference range for all cases except case 5 is 119-229 U/L, for case 5, it is 260-420 U/L. Values are given in conventional units; to convert to Système International units (µkat/L), multiply by 0.0167.

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from a lymph node. Table 1 and Table 2 summarize the clinicopathologic findings.

The 5 cases in group 1 gained CD5 during the clinical course of DLBCL (Image 1). CD5– DLBCL was the initial diagnosis presentation, and CD5 expression was 0% by immunohistochemical analysis. CD5+ DLBCL was the diagnosis at relapse, 7, 8, 15, 27, and 37 months after the initial diagnosis of CD5– DLBCL, respectively.

The 3 cases in group 2 gained CD5 throughout transformation of CD5– low-grade B-cell lymphoma to DLBCL. The initial diagnoses were CD5– FL grade 2, CD5– mucosa-associated lymphoid tissue (MALT) lymphoma, and CD5– lymphoplasmacytic lymphoma (LPL), and they transformed to CD5+ DLBCL. The case that showed transformation of CD5– FL to CD5+ DLBCL has been reported previously by our group. Although this FL case had a CD10+/CD5– phenotype in an FL component and a CD10–/CD5+ phenotype in a DLBCL component, *IGH/BCL2* fusion was detected in both components, suggesting that transformation to CD5+ DLBCL had occurred. The patient with CD5– MALT lymphoma had stage IV disease at initial diagnosis, and, after 56 months, CD5+ DLBCL was diagnosed in an abdominal LN.

In the LPL case (Image 2), multiple lymphoid cell aggregates with plasmacytoid differentiation were detected in a bone marrow aspiration specimen. This was diagnosed as low-grade B-cell lymphoma with plasmacytoid differentiation having a cytoplasmic IgM+ phenotype. The tumor extended to the general lymph nodes and bone marrow but involved no other sites, including the peripheral blood or spleen. The serum IgM level was high at 895 mg/dL. On the basis of this information, we diagnosed the disease as LPL. There was synchronous CD5+ DLBCL in the cervical lymph node.

The case constituting group 3 showed coexistence and gradual shift of CD5+/CD10+ FL grade 3b to CD5+/CD10+ DLBCL in the cervical lymph node.

### Immunohistochemical Studies, ISH, and Clonality Analysis for Secondary CD5+ DLBCL

All specimens were negative for cyclin D1 by immunohistochemical analysis. CD10 and CD23 were both positive.

#### Table 2

<table>
<thead>
<tr>
<th>Case No./Histologic Subtype</th>
<th>Cyclin D1</th>
<th>CD10</th>
<th>CD23</th>
<th>bel-6</th>
<th>MUM1</th>
<th>GCB/Non-GCB</th>
<th>p53</th>
<th>EBER-1 ISH</th>
<th>FISH</th>
<th>Clonality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  DLBCL, centroblastic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Related</td>
</tr>
<tr>
<td>2  DLBCL, centroblastic</td>
<td>+</td>
<td>(20)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>GCB</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>3  DLBCL, centroblastic</td>
<td>+</td>
<td>(80)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>4  DLBCL, centroblastic</td>
<td>+</td>
<td>(60)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5  FL, grade 2</td>
<td>+</td>
<td>(0)</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Related</td>
</tr>
<tr>
<td>6  DLBCL, centroblastic</td>
<td>+</td>
<td>(100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7  MALT lymphoma</td>
<td>+</td>
<td>(0)</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8  LPL/BM</td>
<td>+</td>
<td>(0)</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9  DLBCL, centroblastic</td>
<td>+</td>
<td>(100)</td>
<td>–</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
</tbody>
</table>

DLBCL, diffuse large B-cell lymphoma; EBER-1 ISH, Epstein-Barr virus–encoding RNA-1 in situ hybridization; FISH, interface fluorescence in situ hybridization; FL, follicular lymphoma; GCB, germinal center B-cell phenotype; LPL, lymphoplasmacytic lymphoma; MALT, mucosa-associated lymphoid tissue; ND, not done.

* By immunohistochemical analysis. Numbers in parentheses are the percentage of positive cells.

† By flow cytometry.

‡ Positive in the DLBCL and FL, grade 3b components.
only in case 9, composite CD5+ DLBCL and FL grade 3b. Bcl-6 and MUM1 were positive in 5 of 6 CD5+ DLBCLs, and 2 were classified as the GCB phenotype and 4 as the non-GCB phenotype. p53 expression was less than 10% in all 5 cases tested. EBER-1 ISH was negative in all 5 cases tested. In all specimens (CD5– DLBCL, CD5– low-grade B-cell lymphoma, and CD5+ DLBCL) from 9 cases of secondary CD5+ DLBCL, CD5 was restained in parallel by the avidin-biotin complex method, and reproducibility of the CD5 expression (positive or negative result) was confirmed in all of them.

Paraffin-embedded tissue sections of CD5– and CD5+ tumors were available in 6 cases, and a total of 12 samples was applied to molecular analysis. Monoclonal rearrangements of the CDR3 gene were detectable in 10 samples (83%), and sets of CD5– and CD5+ tumors were available for sequencing in 4 cases. The 2 tumors were clonally related in the 4 cases (Table 2).

**Immunohistochemical Studies for CD5 Expression in 30 Patients With DLBCL Undergoing Sequential Biopsies**

Transition of CD5 expression was examined by immunohistochemical analysis in 30 patients who underwent sequential biopsies among a total of 529 patients with DLBCL. Transition of CD5 expression was examined by immunohistochemical analysis in 30 patients who underwent sequential biopsies among a total of 529 patients with DLBCL. The number of sequential biopsies was 2 in 26 cases, 3 in 3 cases, and 5 in 1 case. The transition of CD5

**Image 1** (Case 4) Secondary CD5+ diffuse large B-cell lymphoma (DLBCL) derived from CD5– DLBCL. A and B, CD5– DLBCL in lymph node at initial diagnosis (A, H&E, ×400; B, CD5 by immunohistochemical analysis, ×400). C and D, CD5+ DLBCL in lymph node at relapse (C, H&E, ×400; D, CD5 by immunohistochemical analysis, ×400).
Image 2l (Case 8) Secondary CD5+ diffuse large B-cell lymphoma (DLBCL) derived from CD5− lymphoplasmacytic lymphoma (LPL). A, B, and C, Synchronous CD5− LPL in bone marrow (A, H&E, ×400; B, cytoplasmic IgM+ by immunohistochemical analysis, ×400; C, CD5 by immunohistochemical analysis, ×200). D and E, CD5+ DLBCL in lymph node (D, H&E, ×400; E, CD5 by immunohistochemical analysis, ×400) at initial diagnosis.
expression was −/− in 20 cases, −/+−/− in 2 cases, −−/−/−/− in 1 case, −/+ in 4 cases, −/+−/− in 1 case, and ++/+ in 2 cases; no case showed +/−.

Reproducibility of Data From Flow Cytometry and Immunohistochemical Studies in 89 DLBCLs

Among a total of 728 DLBCLs, flow cytometry results were available for 89 cases, and, therefore, the CD5 expression data obtained by immunohistochemical analysis and flow cytometry were compared. These results corresponded in 99% of the cases (88/89), including 83 cases with a CD5− phenotype and 5 cases with a CD5+ phenotype. The remaining case was CD5+ by immunohistochemical analysis and CD5− by flow cytometry.

Discussion

Among a total of 529 patients with DLBCL, 38 (7.2%) had CD5+ DLBCL. Probably because sequential biopsies were not infrequently performed at our institution, 9 cases (9/38 [24%]) of secondary CD5+ DLBCL were identified. All of them were non–Richter syndrome secondary CD5+ DLBCL. One of the most likely reasons for the lack of Richter syndrome cases in our series is that the frequency of CLL/SLL is very low in Japan compared with Western countries (1.3%).

The 9 cases of non–Richter syndrome secondary CD5+ DLBCL were classifiable into 3 groups. Five cases constituting group 1 gained CD5 during the clinical course of DLBCL, and corresponded to 18% of patients (5/28) with DLBCL in whom sequential biopsies showed CD5− at the first biopsy. Thus, if rebiopsies are performed more frequently at relapse, more secondary CD5+ DLBCLs might be detected. The 3 cases constituting group 2 gained CD5 at the time of transformation from CD5− low-grade B-cell lymphoma. Except for our 1 reported case, there has been no previous example of CD5− low-grade B-cell lymphoma transforming to CD5+ DLBCL. The sole case in group 3 showed a shift of CD5+ FL grade 3b to CD5− DLBCL. Recently, it has been reported that CD5+ FL can transform to DLBCL.

In the present study, we were unable to conclude that patients with non–Richter syndrome secondary CD5+ DLBCL had a poor outcome because the number of cases was small. However, group 1 and 2 tumors gained CD5 during the clinical course of CD5− DLBCL or transformation of CD5− low-grade B-cell lymphoma. Moreover, in 30 cases of DLBCL in which the patients underwent sequential biopsies, 5 showed a change of CD5 expression from negative to positive, but none changed from positive to negative. It was suggested that CD5 could be gained in association with tumor progression in DLBCL. This is in contrast with the fact that the B-chronic lymphocytic leukemia–specific markers CD5 and CD23 are frequently lost during transformation to DLBCL (Richter syndrome). If some cases of non–Richter-syndrome secondary CD5+ DLBCL in group 1 or 2 had been included in previously reported de novo CD5+ DLBCL, it would have been consistent with the role of CD5 as a progression marker in de novo CD5+ DLBCL.

The frequency of CD5+ DLBCLs among the total DLBCLs in the present series was 7.2%, which was lower than the figure of 10% reported by Yamaguchi et al. However, they performed immunohistochemical analysis on frozen tissue sections, whereas we used paraffin-embedded tissue sections, which could have led to false negativity. However, the high reproducibility of CD5 expression between flow cytometry and immunohistochemical analysis and between the polymer method and the avidin-biotin complex method of immunohistochemical analysis indicated that the CD5 expression data obtained by immunohistochemical analysis on paraffin-embedded tissues were not inferior to those obtained by flow cytometry. Therefore, we considered that the incidence of false negativity for detection of CD5 expression by immunohistochemical analysis on paraffin-embedded tissues was very low in DLBCLs.

Secondary CD5+ DLBCL was found to account for 24% of CD5+ DLBCL cases (9/38). Three groups of non–Richter syndrome secondary CD5+ DLBCL were identified and were shown to be derived from CD5− DLBCL, CD5− low-grade B-cell lymphoma, and CD5+ FL. In groups 1 and 2, CD5 was gained during the clinical course or transformation of tumors, suggesting that CD5 expression is closely associated with the progression of B-cell lymphoma.

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References


