Composite B-Cell and T-Cell Non-Hodgkin Lymphoma of the Tibia

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Abstract

We report a unique case of de novo composite lymphoma in the tibia of a 35-year-old man who presented with increasingly frequent and intense pain in the right upper leg. He was otherwise healthy without significant medical history. A plain radiograph of the right leg showed a permeative lesion with alternating areas of radiolucency and radiodensity in the upper third of the tibia. Magnetic resonance imaging showed a large, heterogeneous enhancing lesion involving the medullary and cortical bone of the proximal tibia with cortical disruption and extension into the adjacent soft tissue. A biopsy showed sheets and clusters of large cells, punctuated by clusters of small, irregular lymphocytes. Flow cytometry and immunohistochemical analysis showed composite lymphoma: diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell non-Hodgkin lymphoma with predominantly small cell morphologic features. The DLBCL expressed CD19, CD20, CD79a, CD5, CD10, CD23, CD38, CD117, bcl-2, and bcl-6, with monotypic expression of immunoglobulin κ light chain. The T cells expressed CD2, CD3, CD5, CD7, and CD8, with partial loss of CD4. Clonal rearrangement of T-cell receptor γ chain gene was found. Neither the large B cells nor the small T cells expressed Epstein-Barr virus–encoded RNA. Physical examination and immunologic studies showed no evidence of lymphadenopathy, organomegaly, or other mass lesions in the body. No peripheral lymphocytosis or bone marrow involvement was present.

A composite lymphoma is the rare occurrence of 2 or more distinct lymphoma types at the same anatomic site. The combination might include a Hodgkin lymphoma with a B-cell or a T-cell non-Hodgkin lymphoma (NHL), a B-cell NHL with a T-cell NHL, or 2 distinct B-cell or T-cell NHLs at the same anatomic site. Most of the reported cases included a combination of a classic Hodgkin lymphoma with an NHL, usually of the B-cell type.1-4 The presence of 2 distinct B-cell NHLs in the same tissue or organ also has been reported and might represent clonal evolution, in at least some cases.5,6 The occurrence of a B-cell and a T-cell NHL, however, is a rare and enigmatic phenomenon. Most of the reported cases of composite B-cell and T-cell NHL included cases of cutaneous T-cell lymphoma (mycosis fungoides) with B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia, either lymphoma type antedating the other.7,8 Simultaneous occurrence of a B-cell and a T-cell NHL, other than a cutaneous T-cell lymphoma, also has been reported, including cases of an angiocentric T-cell lymphoma with a diffuse large B-cell lymphoma (DLBCL)10,11 and other peripheral T-cell NHLs with low grade B-cell NHLs.8 We describe a very unusual and hitherto unreported case of a primary composite lymphoma that arose in the upper tibia and was composed of a diffuse large B-cell NHL and a peripheral T-cell NHL with predominantly small cell morphologic features.

Case Report

A 35-year-old man with no significant medical history was evaluated for intolerable pain in his right leg. He described the onset of pain some 15 years ago with intermittent episodes,
but the intensity and frequency of the pain increased only about 3 or 4 years ago. He did not complain of any other symptoms, specifically no fevers, weight loss, or night sweats.

Physical examination of the right leg showed a swollen knee with exquisite tenderness at the upper end of the tibia. The rest of the physical examination was normal with no evidence of lymphadenopathy or splenomegaly. A plain radiograph of the right leg showed a permeative lesion with alternating areas of radiolucency and radiodensity in the upper third of the tibia (metaphysis and proximal diaphysis) and a small effusion in the knee joint. The femur and fibula appeared normal. Magnetic resonance imaging showed a large and heterogeneous enhancing lesion involving the medullary and cortical bone of the proximal tibia with cortical disruption and extension into the adjacent soft tissue. Technetium 99m scanning of the tibia showed abnormally increased uptake in the proximal tibia consistent with the magnetic resonance imaging findings. Computed tomography scans of the chest, abdomen, and pelvis did not show any enlargement of the lymph nodes, spleen, or liver. Results of all pertinent laboratory tests were normal, including WBC count (8,100/µL [8.1 × 10⁹/L] with a normal differential count), hemoglobin concentration (14.0 g/dL [140 g/L]), platelet count (305 × 10³/µL [305 × 10⁹/L]), and lactate dehydrogenase level (186 U/L). A curettage biopsy of the tibial lesion was performed.

Materials and Methods

Morphologic Examination

The biopsy specimens were fixed in 10% buffered formaldehyde and embedded in paraffin, and 5-µm sections were cut and stained with H&E for light microscopic examination.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue samples as previously described. Antibodies were used against the following antigens: CD1a, CD3, CD5, CD10, CD20, CD30, CD43, CD45, CD68, CD79a, CD99, bcl-2, bcl-6, terminal deoxynucleotidyl transferase (TdT), myeloperoxidase, IgG, IgM, immunoglobulin κ and λ light chains, cytokeratin, S-100, neuron-specific enolase, and vimentin.

Flow Cytometric Analysis

Fresh tissue samples were transported immediately in RPMI solution to the flow cytometry laboratory, and cells were released by teasing followed by filtration of separated cells. Mononuclear cells were stained with various combinations of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, phycoerythrin–cyanin-5 (PC5)-, or phycoerythrin–Texas red (ECD)-labeled monoclonal antibodies against the following antigens: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, CD34, CD38, CD45, CD117, HLA-DR, and immunoglobulin κ and λ light chains. The combinations included κ-FITC/λ-PE/CD19-ECD/CD45-PC5, CD5-FITC/CD23-PE/CD19-ECD/CD45-PC5, CD38-FITC/CD10-PE/CD19-ECD/CD45-PC5, CD3-FITC/CD20-PE/CD19-ECD/CD45-PC5, CD2-FITC/CD7-PE/CD3- ECD/CD45-PC5, CD38-FITC/CD117-PE/CD45-ECD, and CD8-FITC/CD3-PE/CD3-ECD/CD45-PC5. Intracytoplasmic evaluations for bcl-2 and intranuclear detection of TdT also were performed using permeabilization techniques.

Four-color flow cytometric immunophenotyping was performed on a cytometer (Coulter XL Epic, Beckman Coulter, Miami, FL) by collecting 10,000 ungated list-mode events, selecting an appropriate gate on the combination of CD45 and side scatter, and analyzing cells within the most appropriate gate. An antigen was considered positively expressed when at least 25% of the gated cells expressed that antigen. B-cell clonality was defined when the immunoglobulin κ/immunoglobulin λ light chain ratio was more than 4:1 or less than 0.5:1 as described previously. Isotype controls were run in all cases. All antibodies were purchased from Beckman Coulter except for bcl-2, which was obtained from DAKO (Carpinteria, CA), and used according to the manufacturers’ guidelines.
T-Cell and Immunoglobulin Heavy Chain Receptor Gene Rearrangements

The T-cell receptor γ chain gene assay was performed as described by Lawnicki and coworkers. Data were analyzed using GeneScan software (Perkin Elmer Applied Biosystems, Foster City, CA). Only clonal peaks with a height greater than 2 times the maximum height of the background polyclonal distribution in duplicate assays were interpreted as positive. The clonality analysis of the immunoglobulin heavy chain gene was performed as described by Lozano et al.

In Situ Hybridization for Epstein-Barr Virus–Encoded RNA

In situ hybridization was performed on paraffin-embedded sections according to the manufacturer’s instructions using a Peptide Nucleic Acid ISH Detection kit and Epstein-Barr Virus–encoded RNA peptide nucleic acid probe labeled with FITC (DAKO).

Results

Light Microscopic Examination

The biopsy specimen showed replacement of the normal architecture of the bone and marrow by a tumor composed of a dual population of cells. About 40% to 50% of the tumor was composed of sheets of large cells with moderate to abundant amounts of pale cytoplasm and large nuclei with distinct nucleoli. Mitoses were frequent. Sheets of large cells were often punctuated by clusters of small cells with scant cytoplasm and irregular to cleaved nuclei without prominent nucleoli. In some areas, sheets of these small cells predominated, whereas in others, small cells often were intermixed with large cells. Areas of necrosis also were seen.
Immunohistochemical Analysis

The large cells stained positively for CD20, CD79a, CD10, CD38, bcl-2, and bcl-6, whereas the small lymphocytes stained positively for CD3, CD5, CD43, and CD99. The staining pattern was mutually exclusive between large and small cells. The large cells showed weak and focal staining for CD45 (leukocyte common antigen). None of the tumor cells showed any staining for cytokeratin (AE1/AE3), CD1a, CD30, TdT, neuron-specific enolase, S-100, and myeloperoxidase. Vimentin was positive in all tumor cells.

Flow Cytometric Analysis

Flow cytometric analysis of the tumor showed a dual population of cells, including a population of large cells (41% of gated cells) that showed dim expression of CD45 and a population of small cells (47% of gated cells) with bright expression of CD45. The large cells showed expression of B-cell markers CD19 and CD20 with coexpression of CD5 (56%), CD10 (49%), CD23 (58%), CD38 (46%), CD117 (87% on CD38+ cells), and bcl-2 (96% on CD19+ cells), with monotypic surface expression of immunoglobulin κ light chain (κ/λ = ~10:1). The small lymphocytes expressed CD2 (92%), CD3 (93%), CD5 (90%), CD7 (92%), and CD8 (40%) with partial loss of CD4. Neither large nor small lymphocytes showed significant expression of CD34 or TdT.

Gene Rearrangement Studies by Polymerase Chain Reaction

Clonal T-cell receptor γ chain gene rearrangement was detected by polymerase chain reaction. No clonal rearrangement, however, could be detected for immunoglobulin heavy chain by DNA amplification using consensus primers for heavy chain gene variable (framework III) and joining regions. The DNA could not be amplified adequately using consensus primers for variable (framework II) and joining regions.
regions. The analytic sensitivity of the assay was only about 70% with this technique.

**In Situ Hybridization for Epstein-Barr Virus–Encoded RNA**

The test result was negative in large and small lymphocytes.

**Discussion**

We have described a rare case of a composite lymphoma composed of a DLBCL and a peripheral T-cell lymphoma with predominantly small cell morphologic features. The lymphoma is unique among other reported cases because it arose primarily in the tibia without evidence of a B-cell or a T-cell NHL elsewhere.

Many other features of this lymphoma merit discussion. The coexpression of CD5, CD10, and CD23 among B-cell NHLs is an unusual phenomenon, reported only in rare cases of DLBCL and less commonly in follicular lymphomas. CD5, a T cell–associated antigen, is expressed by a subset of prefollicular B cells and is absent from follicular germinal center B cells, which express CD10. Rare cases of mantle cell lymphoma and follicular lymphoma exist that express both CD5 and CD10. Simultaneous expression of CD5, CD10, and CD23 in B-cell lymphomas, however, is very rare. No single normal B-cell population is known to express CD5, CD10, and CD23 together. Thus, this immunophenotype in lymphomas is the result of aberrant expression of at least 1 antigen. DLBCLs do not show a consistent immunophenotype. Most cases lack expression of CD5, CD10, or CD23; a small percentage express CD10 and an even smaller number of cases...
express CD5 or CD23. Thus, the expression of CD5, CD10, and CD23 in this lymphoma represents an unusual phenotype.

The expression of bcl-6 in large B-cells (DLBCL) indicates a follicle center cell origin of this component of the composite lymphoma.\(^1\) Extranodal follicular lymphomas and DLBCL of follicle center cell origin occur in sites such as skin, thyroid, intestine, orbit, and others. These sites can show benign follicular lymphoid hyperplasia, and, hence, the development of follicular lymphoma or DLBCL of follicle center origin is conceivable and might represent clonal expansion of a resident transformed follicle center cell.

The expression of CD117 (c-kit) in this lymphoma also is unusual. CD117 is a class III receptor tyrosine kinase and has an important role in the differentiation of immature hematopoietic and lymphoid cells.\(^2\) Its normal expression is limited to immature myeloid cells, T-lymphoid blasts, and a subset of natural killer cells but not B cells. The expression of CD117 in large B-lymphoma cells in this case represents aberrant expression of this antigen. This phenomenon is observed rarely. Bravo et al\(^3\) described only 1 case of B-cell NHL with t(14;18) that showed high expression of CD117. Although only rarely reported, evaluation of CD117 expression in B-cell neoplasms might identify a subset of lymphomas that express this antigen and might benefit from anti-CD117 antibodies.

A composite lymphoma is defined as the presence of at least 2 distinct lymphoma types at the same anatomic site and might represent a chance occurrence or a de novo lymphoma with 2 clones (divergent clones evolved from an initial single neoplastic clone or 2 clones developed from 2 independent mutational hits in separate cells). It is likely that some cases represent the first scenario of chance coexistence and might more aptly be called collision composite lymphoma, whereas the others belong to the latter category and truly signify a composite lymphoma.

Although the term composite lymphoma is used without qualifiers to include both categories, it might be useful to distinguish the two categories. This term was first coined by Custer\(^4\) in 1954 to describe a lymphoma with more than one histologic pattern in 2 organs or within the same organ. It now is accepted generally that this term be limited to 2 or more distinct lymphoma patterns in the same tissue. The presence of 2 distinct lymphoma types in different organs might be classified more correctly as synchronous lymphomas rather than composite lymphoma. Also, the report by Custer\(^4\) predates the development of commercially available antibodies, and it is unclear whether the case described by Custer\(^4\) indeed was true composite lymphoma or transformation of a preexisting lymphoma.

In 1977, Kim et al\(^5\) expanded the definition of a composite lymphoma to include cases of Hodgkin lymphoma with NHL. Several subsequent reports, however, indiscriminately used this term to include cases of transformation of a preexisting lymphoma.\(^6\) Cases of usual transformations such as Richter transformations in small lymphocytic lymphoma and large cell transformation in follicular lymphomas must not be included under the rubric of composite lymphoma because transformed lymphomas pathogenetically represent a clonal evolution and generally pursue an aggressive and rapid course, whereas a de novo composite lymphoma might not exhibit an aggressive clinical course.

We prefer to differentiate among various groups described under this term to identify true de novo composite lymphoma from cases that represent unusual transformations and development of unrelated lymphomas to convey pathogenetic information and the clinical significance of these cases. The development of a distinct but clonally related lymphoma arising in an anatomic site already involved by a lymphoma, such as Burkitt lymphoma evolving in a follicular lymphoma, might be termed more appropriately transformation composite lymphoma. A case reported by Tsang et al\(^7\) of a mantle cell lymphoma arising in a patient with follicular lymphoma might be included under this category.

Clonally unrelated lymphomas that arise asynchronously in different sites but happen to involve the same anatomic site at some point during the disease might be referred to as collision composite lymphomas because they conceptually are different from de novo composite lymphoma, and each lymphoma type bears its own prognosis. Unlike transformation composite lymphoma, each component of a collision composite lymphoma also involves a separate anatomic site, such as small lymphocytic lymphoma in the bone marrow and a T-cell lymphoma of the skin. Several reported cases fall under this group.\(^8\)\(^9\)\(^25\) It is in this context that we would like to redefine a de novo composite lymphoma as follows: a lymphoma that is composed of 2 or more distinct lymphoma types, each with its own clone arising at the same anatomic site and clinically identified at the same time, without any history of lymphoma elsewhere in the body. This narrow definition of de novo composite lymphoma excludes cases of collision composite lymphoma and transformation composite lymphoma and ensures that each category conveys its own prognosis and defines a distinct clinicopathologic scenario.

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