Case Reports:
CD20 Positive T-cell Lymphoma/Leukemia: A Rare Entity with Potential Diagnostic Pitfalls

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Abstract. Mature T-cell neoplasms are relatively uncommon, accounting for approximately 10% of all non-Hodgkin lymphomas. This category of hematopoietic neoplasms is clinically aggressive and shows a poor response to therapy and shortened survival. The antigen CD20 has long been thought to be a specific marker for B-cell lineage and has been used to help differentiate T-cell and B-cell neoplasms. We present two cases of a rare subset of T-cell leukemia/lymphoma having a unique immunophenotype, both being CD20+. The significance of CD20 antigen in T-cell lymphomas is yet to be determined, but may allow treatment with novel therapeutic agents (eg, rituximab, a recombinant anti-CD20 monoclonal antibody).

Keywords: CD20 antigen, lymphoma, leukemia, rituximab

Introduction

The classification of lymphomas and leukemias is based on clinical presentation, morphology, immunohistochemistry, and genetics. Many of the individual B-cell neoplasms show characteristic morphologic features and the majority of B-cell lymphomas show reproducible profiles of CD marker expression, particularly CD19 and CD20 [1]. Subclassification within the B-cell neoplasms can be determined by the presence of additional surface antigenic markers either by immunohistochemical staining or by immunophenotyping using flow cytometry. For example, follicular lymphomas express CD10 but are negative for CD5, while mantle zone lymphomas are CD5 positive but negative for CD10. Using these markers, along with a series of others, it is possible to limit the differential diagnosis quickly.

In contrast to B-cell neoplasms, the cells in malignant T-cell lesions show greater morphologic diversity, not only between distinct entities but also among the cells within the same disease process. In a similar fashion, certain immunohistochemical markers may be closely associated with particular disease entities, but they are not disease-specific and great variation of antigenic expression is seen even within two lesions with a common diagnosis.

A marker that is commonly used in the investigation of lymphomas and leukemias is the antigen CD20. This marker has long been thought to be specific for B-cells, both benign and malignant. As a result it has been used to help differentiate T-cell and B-cell neoplasms. However, rare cases have been reported in the literature that show T-cell lymphomas/leukemias expressing CD20 [2-9]. We report the morphologic, histologic, immunohistochemical, immunophenotypic, and molecular analysis of two such cases and cytogenetic analysis in one case along with a review of the literature. One of our cases is a CD20 positive adult T-cell lymphoma/leukemia (ATLL), an entity not previously reported.

Case Reports

Case 1. The patient was a 45-yr-old male with chronic renal failure who was admitted with a recent history of lethargy and mental confusion. He was found to be hypercalcemic (24 mg/dl) and...
was treated with fluids and panidrinate. Physical examination disclosed prominent lymphadenopathy and splenomegaly. A cervical lymph node biopsy showed effacement of the normal nodal architecture with a diffuse infiltrate of small- to medium-sized lymphocytes with irregular nuclear contours and prominent nucleioli in many cells (Fig. 1). Mitotic activity was moderate. Immunohistochemical evaluation showed strong staining of the neoplastic cells for CD3, CD5, CD43, and CD20. These same cells were negative for CD10, CD23, and CD30 (Table 1). Flow cytometry identified a population of lymphocytes that was positive for CD45, CD2, CD3(dim), CD7(dim), CD4, CD5, CD20, CD25(dim), and FMC7. These same cells were negative for CD8. Cytoplasmic/nuclear markers for these cells were positive for cCD3(bright), but negative for TdT. Southern blot analysis of the neoplastic cells demonstrated a clonal population of T-cells expressing the beta TCR clonality. No clonal rearrangement of the immunoglobulin heavy chain was detected. Polymerase chain reaction (PCR) for the detection of Human T-cell Lymphotrophic Virus (HTLV) 1 and 2 was positive. A definitive diagnosis of Adult T-cell Leukemia/Lymphoma (ATLL) with aberrant CD20 expression was made.

The patient refused further treatment. His serum calcium level decreased during the next seven days and he was discharged to home. He came two days later to the emergency department with a fever and a white blood count of 24,000 cells/mm$^3$ (65% lymphocytes with atypical forms present). He had a urinary tract infection and sepsis. Serologic studies for HIV were negative. He died within 48 hr.

Case 2. The patient was an 84-yr-old male who noticed a lump in his neck in February 2005. The lump did not respond to antibiotic therapy. An excisional lymph node biopsy was interpreted as a peripheral T-cell lymphoma not otherwise specified (NOS). As shown in Fig. 2, the lymph node architecture is nearly effaced with a diffuse, monomorphic, atypical, and immature cell population predominantly in an interfollicular pattern. The large neoplastic cells have 1-3 eosinophilic nucleioli and there is focal, but not zonal, necrosis with a high mitotic rate. The lesion extends into the perinodal soft tissue and 3 residual germinal centers are surrounded by the neoplastic infiltrate. Immunohistochemical staining shows bright CD20 staining within the benign germinal centers as well as in scattered cells within the neoplastic infiltrate. In addition, the neoplastic cells show weak-moderate CD 20 positive membrane staining. There is bright CD3 and CD5 staining of the neoplastic cells and scattered positive staining within the benign germinal centers.

The neoplastic cells show 30-50% positivity with Ki-67 staining and are negative for CD30 (Table 1). Flow cytometry showed a population of cells that were positive for CD2,CD3, CD4, CD5, CD25, CD38, CD45, and CD52. The same cells

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Table 1. Immunohistochemical staining of the lymphomas (ND = not done).

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<th>CD20</th>
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<td>Case 2</td>
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<td>30-50%+</td>
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Fig. 1 (page 265). Panel A (upper left). There is complete effacement of the normal nodal architecture in the cervical node biopsy shown. (H&E). A diffuse infiltrate of small to medium sized lymphocytes is present. Panel B (upper right). At higher magnification these cells show irregular nuclear borders and have variable amounts of cytoplasm. Mitotic figures are scattered throughout the lesion and vasculature is prominent. Panel C (lower left). On immunohistochemical staining, essentially all cells are positive for CD3. Panel D (lower right). These same cells also show strong staining for CD20 consistent with a T-cell lymphoma with aberrant CD20 expression.

Fig. 2 (page 265). Panel A (left). The cervical node biopsy shows a marked expansion of the interfollicular area by a diffuse large-sized monomorphic lymphoid population. Many of these cells have multiple nuclei. Residual follicles are scattered throughout the specimen. Panel B (middle). Essentially all cells show positive staining for CD20 antigen; however, the intensity varies from strong staining of the follicular structures to dim intensity for the interfollicular regions. Panel C (right). CD3 positivity on immunostaining confirms the marked T-cell expansion of the interfollicular zone. The residual normal follicles do not stain except for scattered positive cells. These results confirm the dim CD20 positivity of the T-cell lymphoma.
CD20 positive T-cell lymphoma/leukemia

Fig. 1. See legend on page 264.

Fig. 2. See legend on page 264.
were dim positive for CD20 and negative for CD7. Cytogenetic analysis of 20 metaphase cells showed the following: 45,X,-Y[6]/46,XY[14]. TCR gene analysis by RT-PCR showed a monoclonal population with TCR gamma clonality.

A computed tomographic (CT) scan in March 2005 showed a 3.9 cm mesenteric mass. Follow-up CT scans of the chest and neck in April 2005 showed multiple enlarged lymph nodes bilaterally in the neck and in the right axilla, mediastinum, and right hilum. A bone marrow biopsy in May 2005 showed no evidence of lymphoma involvement and normal molecular and cytogenetic studies.

The patient was initially treated for his Stage III disease early in May 2005 with rituximab and a CHOP regimen with the substitution of liposomal doxorubicin for conventional doxorubicin. In June 2005, he was admitted multiple times for abdominal pain and constipation. An abdominal CT scan showed enlargement of the mesenteric mass first seen in March to a greatest dimension of 9.6 cm. He was then started on palliative chemotherapy of cytoxan, vincristine, and prednisone of which he received two cycles. Due to lack of response, the chemotherapy treatment was discontinued. Palliative radiotherapy was instituted, to which he had an excellent initial response. His declining physical condition caused him to miss numerous appointments and the treatment was stopped in August 2005. He died eight days later.

Discussion.

Mature T-cell lymphomas are relatively rare, accounting for approximately 10% of non-Hodgkins lymphomas worldwide. They are more commonly seen in persons of Asian or Native American descent. It is critical that B-cell and T-cell lymphomas be differentiated from one another, as T-cell lymphomas are more aggressive, are treated with different chemotherapeutic agents, and have a much poorer response. The World Health Organization’s (WHO) Classification of Hematopoietic and Lymphoid Tumors stresses that immunophenotyping be an integral part of this process [1]. It is well known that B-cell lymphomas may co-express T-cell antigens (eg, CD43). It has also been shown that up to 40% of precursor T-cell neoplasms may show lineage infidelity by expressing B-cell markers such as CD79 and that many may even express myeloid markers such as CD13 and CD33 [4,8,10]. However, mature T-cell neoplasms are generally considered to express only T-cell associated antigens.

We describe two cases of CD20 positive T-cell lymphomas. In both cases, immunohistochemistry showed the neoplastic cells to be positive for CD3, CD5, and CD20. Flow cytometry confirmed these results as well as demonstrating these cells to mark with other T-cell specific antigens, including CD4. Southern blot analysis of Case 1 showed a monoclonal rearrangement of the TCR beta gene and no rearrangement of the Immunoglobulin Heavy Chain (IHC) gene, while RT-PCR analysis of Case 2 showed monoclonal rearrangement of the TCR gamma gene. PCR analysis for the presence of HTLV-1 and 2 was positive in Case 1.

Historically, CD20 has been regarded as a specific B-cell marker and has been used to distinguish B-cell from T-cell neoplasms. However, a small subset of non-neoplastic T-cells express CD20 weakly in disease-free individuals [11]. Two populations of CD20 positive cells can be demonstrated among all lymphocytes: bright CD20 and dim CD20. The bright CD20 cells show other markers consistent with normal B-cells while the dim CD20 cells are marked as T-cells. Two-thirds of the dim CD20 T-cells are CD8 positive while one-third are CD4 positive. This population of T-cells has a tendency to express the \( \gamma \delta \) TCR gene.

Although rare, CD20 expression in T-cell lymphoma/leukemia has been reported in 21 cases over the past two decades [2-9]. In 9 cases, TCR rearrangement studies were done, and all but one of these demonstrated a clonal population. The ninth case demonstrated only germline arrangement of the TCR gene. Of the 21 previously reported cases, 6 were CD8 positive, 3 were CD4 positive, one showed dual CD4/CD8 expression, one lacked expression of either marker, and detection of either antigen was not reported in 10 cases. Both of our cases were CD4 positive.

It is unknown whether CD20 positive T-cell lymphomas represent an aberrant expression of CD20 or are a malignant transformation of the
minor subpopulation of normal CD20 positive T-cells previously discussed. In a report by Sun et al [3], lymphoma cells showed dim CD20 positivity in the lymph node, but were CD20 negative in skin metastases. One explanation for this was that CD20 was not an integral part of the lymphoma cells and was lost as the tumor progressed. A similar observation of loss of CD20 antigen was made by Kitamura et al [4] with a CD20 positive small bowel T-cell lymphoma, where metastatic tumor to the liver did not express CD20. These reports make definitive determination of the cell of origin of these lymphomas impossible at this time.

In the work-up of lymphomatous neoplasms, the use of ancillary studies such as flow cytometry has become an invaluable part of narrowing the differential diagnosis. Sun et al [3] point out that flow cytometry analysis is useful in making the distinction between B and T-cell lymphomas in a couple of ways. First, CD19 is a common and reliable B-cell marker. However, it is not available as an immunohistochemical stain in every laboratory, in which case it may only be demonstrated by flow cytometry. Second, the CD20 positive T-cell lymphomas tend to be CD5 bright and CD20 dim, while CD5 positive B-cell lymphomas tend to be CD5 dim and CD20 bright. This difference of staining intensity may be difficult to appreciate under the microscope, but is much more evident with flow cytometry.

There are cases in which flow cytometry cannot be used, most often due to inadequate sample size or cell viability, or is simply inconclusive. As a result, the diagnosis depends on light microscopic findings including the immunohistochemical staining pattern. A limited immunohistochemical panel can lead to erroneous diagnoses. Two typical markers used for T-cells and B-cells are CD3 and CD20, respectively. Algino et al [12] explain that with such a small panel, a CD5 positive B-cell neoplasm could be missed and, likewise, a CD20 positive T-cell lymphoma could be misdiagnosed. Only with a more extensive panel can this issue be clarified. Similar conclusions were drawn in two other reports [4,10]. Current recommendations would be the use of CD20 and CD79a as B-cell markers and CD3 and CD5 as T-cell markers.

Although many B- and T-cell neoplasms can be correctly diagnosed using morphology and a limited immunohistochemical panel alone, a multidisciplinary approach that includes flow cytometry, cytogentic studies, fluorescent in-situ hybridization (FISH) probes, and molecular studies is required to define these neoplasms more completely.

References