

# Triple-hit lymphoma

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We report a case of a triple-hit lymphoma in a 72-year-old man. This lymphoma was diagnosed using morphology, flow cytometry, immunohistochemistry, and cytogenetics. Since many triple-hit lymphomas have not been documented in the literature, it is important to bring attention to this entity, as this lymphoma has different prognostic and therapeutic implications than other diffuse large B-cell lymphomas, thus making a correct and early diagnosis important.

Chromosomal translocations are biologic and diagnostic hallmarks of disease in many B-cell lymphomas. There is a unique subset, the so-called “double-hit lymphomas,” that are defined by a chromosomal breakpoint affecting the *MYC*/8q24 locus in combination with another recurrent breakpoint, usually at (14;18)(q32;q21) involving *BCL-2*. This led to a new category of lymphomas in the 2008 World Health Organization (WHO) classification: “B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL).” Double-hit lymphomas are associated with a poor prognosis. An even more uncommon entity is the “triple-hit lymphoma.” The exact incidence is unknown, and the WHO has not, as of yet, classified it specifically. Triple-hit lymphomas also have morphologic, phenotypic, and genetic features intermediate between DLBCL and BL. However, the characteristic cytogenetic abnormalities involve chromosomal rearrangements of *c-MYC*, *BCL-2*, and *BCL-6* genes. The clinical implication of correctly diagnosing this entity is significant, as triple-hit lymphomas also have a much worse prognosis than either DLBCL or BL alone, and therapeutic options are different. We report a case of a patient presenting with fever, fatigue, night sweats, and an inguinal mass that was biopsied to reveal a DLBCL of germinal center origin on first impression. Because of a high proliferative index, cytogenetics were obtained, which showed chromosomal rearrangements consistent with a diagnosis of triple-hit lymphoma.

## CASE REPORT

A 72-year-old African American man with previous chronic gout and hypertension was admitted to Baylor University Medical Center at Dallas due to weight loss of 25 pounds, fatigue, and failure to thrive. He was found to have an enlarging inguinal mass on the left side measuring 7 cm, which had been

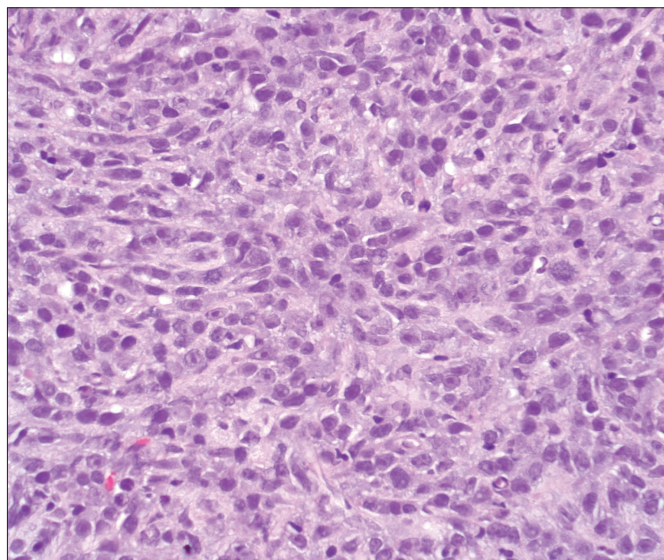


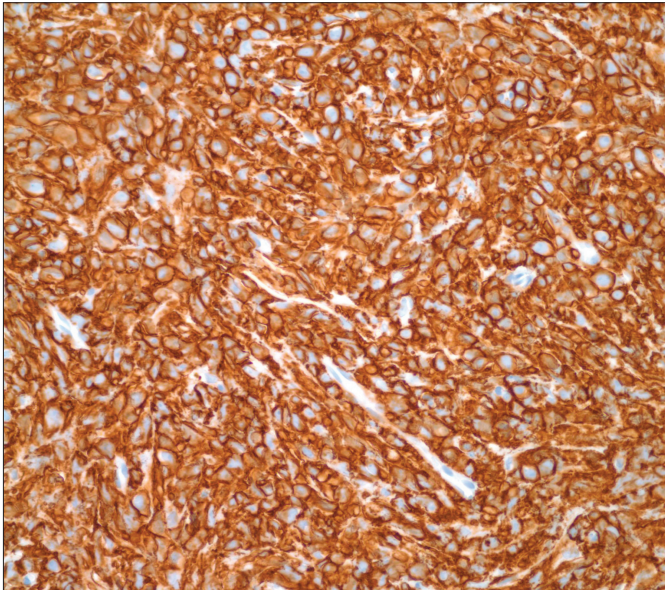
Figure 1. Large cell lymphoma (hematoxylin and eosin,  $\times 500$ ).

present for 2 months. His lactate dehydrogenase was 882 U/L (reference range, 100–190); beta-2-microglobulin, 5.56 ng/mL (reference range, 0.60–2.29); ferritin, 926 ng/mL (reference range, 22–322); hemoglobin, 8.5 g/dL; and hematocrit, 24.9%. Radiographic imaging disclosed bilateral kidney masses, as well as a mass in the left lobe of the liver. His hospital course was complicated by acute renal failure and urinary tract infection treated with antibiotics and intravenous fluids.

The inguinal mass was biopsied. Sections showed a diffuse infiltrate consisting of sheets of discohesive medium and large cells with large nuclei, irregular nuclear membranes, and prominent nucleoli with no architectural pattern (Figures 1 and 2). Flow cytometry showed a 62% population of large clonal B cells expressing CD10, CD19, CD20, CD45, and Kappa, but not CD5. Immunohistochemistry showed CD45, BCL-2, BCL-6, CD10, CD20, and CD79a positivity and CD30, MUM1, EBER, and CD34 negativity. Ki-67 (proliferation index) was very high (>90%) (Figure 2).

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**Figure 2.** CD20 immunostain indicating B-cell lymphoma (CD20 immunoperoxidase stain  $\times 400$ ).

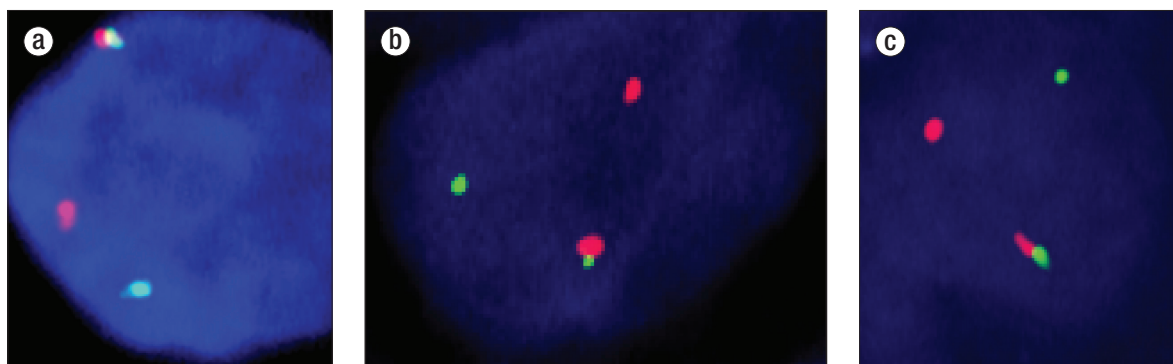
The morphology, immunochemistry stains, and flow cytometry results supported a diagnosis of a lymphoma with features intermediate between DLBCL and BL. Cytogenetic analysis was performed with fluorescence in situ hybridization to reveal gene rearrangements of *BCL-2* (18q21), *BCL-6* (3q27), and *c-MYC* (8q24) genes (Figure 3). These features are consistent with a triple-hit lymphoma. A bone marrow biopsy was negative. A positron emission tomography scan revealed masses in the left inguinal region, both kidneys, and the left lobe of the liver. The patient was discharged to another facility in stable condition for treatment and management options. On follow-up we were told that the patient was treated with rituximab and hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone) alternately with rituximab, methotrexate, and cytarabine. Apparently the patient had an initial response but the tumor recurred, and he died about 6 months following the initial diagnosis.

## DISCUSSION

B-cell lymphomas commonly have chromosomal gene rearrangements (1). For example, rearrangement of the *BCL-2* or

*c-MYC* gene with the immunoglobulin gene (*IG*) can result in follicular lymphoma or BL, respectively. However, to have two gene rearrangements (double-hit lymphomas) is uncommon (2). Translocation of the *BCL-2* gene on chromosome 18q21 results in constant inactivation of apoptosis (3). Translocation of the *c-MYC* gene on chromosome 8q24 results in constant cell proliferation (3). These double-hit lymphomas are associated with an aggressive clinical course, with poor response to treatment, complex karyotypes, and pathologic features of DLBCL and BL (4). These pathological features include a “starry sky” appearance with a high MiB-1 (which is consistent with BL), but with larger cells with irregular nuclei and more prominent nucleoli (which is consistent with DLBCL). The incidence is estimated to be approximately 2% of all B-cell lymphomas (5). The median survival time is reported to be about 5 months, significantly shorter than for either DLBCL or BL (6).

Triple-hit lymphomas have been infrequently reported, with only a small number of case reports noted. These lymphomas are rare and the exact incidence is unknown. The Mitelman lymphoma database in 2009 reported only eight triple-hit lymphomas out of 796 lymphomas containing a *BCL-6* gene rearrangement (7). Triple-hit lymphomas are defined similar to double-hit lymphomas as having morphologic, biologic, and cytogenetic properties similar to both DLBCL and BL, but possessing three, instead of two, gene rearrangements: *c-MYC*, *BCL-2*, and *BCL-6* genes (7). They are also associated with a more aggressive clinical course, as these lymphomas have a propensity to spread to extranodal sites, including the bone marrow and central nervous system (8). Because of the more complicated clinical course and gene rearrangements, the standard chemotherapy used for DLBCL or BL is ineffective (8). The survival rate for these lymphomas has been reported to be about 4 months, shorter than for DLBCL, BL, and double-hit lymphoma (8). One of the challenges is how to recognize these lymphomas and order the appropriate molecular studies so that aggressive treatment may be started. We are routinely ordering molecular studies to detect *BCL-2*, *BCL-6*, and *c-MYC* rearrangements on any B-cell lymphoma with features intermediate between DLBCL and BL, any lymphoma with a Ki-67 proliferation index  $>90\%$ , and any DLBCL that has recurred and is refractory to therapy.



**Figure 3.** Fluorescence in situ hybridization showing (a) *BCL-2*, (b) *BCL-6*, and (c) *c-MYC* gene rearrangements as a break-apart probe with two separate signals (red and green). A normal cell, for comparison, has the red and green signals connected.



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## Avocations



Photo © Amanullah Khan, MD, PhD. Dr. Khan (e-mail: aman1963@gmail.com) is an oncologist on the medical staff of Baylor Medical Center at McKinney.