Academic Pathology: Volume 7
DOI: 10.1177/2374289520901813
journals.sagepub.com/home/apc

SAGE

Educational Case: ALK-Negative Anaplastic Large Cell Lymphoma

Akira Satou, MD, PhD[1,2] and Andrew L. Feldman, MD[1]

The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.[1]

Keywords
anaplastic large cell lymphoma, classification of lymphomas, hematopathology, organ system pathology, pathology competencies, special studies, *TP63* rearrangement, white cell disorders

Received May 31, 2019. Received revised October 31, 2019. Accepted for publication December 27, 2019.

Primary Objective

Objective HWC3.3: Categories of Lymphoma. Compare and contrast low-grade or indolent lymphomas and high-grade or aggressive lymphomas with respect to underlying pathophysiology that yields specific morphologic features and clinical behavior.

Competency 2: Organ System Pathology; Topic HWC: Hematopathology-White Cell Disorders; Learning Goal 3: Classification of Leukemia and Lymphomas.

Secondary Objectives

Objective SP5.1: Special Studies. Describe the roles of immunohistochemistry, flow cytometry, cytogenetics, and molecular diagnostics in the diagnosis and classification of lymphoma and explain how, with examples, different techniques are most appropriate in diagnosis, staging, and management of disease.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic SP: Surgical Pathology; Learning Goal 5: Classification of Leukemia and Lymphomas.

Objective SP1.1: Obtaining the Specimen. Describe the procedures for obtaining a biopsy of a tissue lesion or mass in different sites, including superficial and deep soft tissues, solid organs, and tubular organs. Associate each procedure and specimen type with either cytology or surgical pathology and give examples of possible reasons and follow-up for false-negative biopsies.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic SP: Surgical Pathology; Learning Goal 1: Role in Diagnosis.

Patient Presentation

A 60-year-old male presents to the clinic with a lump in the left groin that he initially noticed about 4 weeks ago when he was in the shower. He reports that the lump has increased in size since then and has not been associated with any pain or tenderness. On several occasions over the last month, he has awoken at night with sweating and has felt achy and feverish at times,

[1] Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
[2] Department of Surgical Pathology, Aichi Medical University Hospital, Nagakute, Aichi, Japan

Corresponding Author:
Andrew L. Feldman, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA.
Email: feldman.andrew@mayo.edu

although he has not taken his temperature. These symptoms are relieved by acetaminophen. He is otherwise healthy except for mild hypertension controlled with diet and exercise. Review of systems is otherwise unremarkable.

Diagnostic Findings, Part 1

Vital signs are normal. Physical examination reveals a 2.5-cm firm, nontender, freely moveable mass consistent with a lymph node in the left inguinal region. No lymphadenopathy is noted elsewhere. No skin lesions are present. The remainder of the examination is unremarkable. Laboratory studies show lactase dehydrogenase 289 units/L (normal 100-190 units/L). Chest X-ray is unremarkable.

Questions/Discussion Points, Part 1

What Is the Differential Diagnosis of Lymphadenopathy in Adult Patients, and What Are the Risk Factors for Malignancy?

Lymphadenopathy is defined as lymph nodes that are greater than 1 cm in size. Etiologies of lymphadenopathy include malignancies, infections, autoimmune disorders, miscellaneous and unusual conditions, and iatrogenic causes. The most common underlying malignancies include lymphoma, leukemia, multiple myeloma, and metastatic carcinoma. Infections include bacterial infections, viral infections, tuberculosis, syphilis, and toxoplasmosis. Autoimmune disorders associated with lymphadenopathy include systemic lupus erythematosus, Still disease, Sjögren syndrome, and rheumatoid arthritis. Miscellaneous and unusual conditions are varied but include Castleman disease, Kikuchi lymphadenitis, Kimura disease, IgG4-related disease, and sarcoidosis. Iatrogenic causes chiefly include medications and serum sickness. The most likely cause of lymphadenopathy can often be identified by history and physical examination alone.

Only 1.1% of cases of unexplained lymphadenopathies are due to underlying malignancy. Risk factors for malignancy include older age, long duration of lymphadenopathy (greater than 4-6 weeks), generalized lymphadenopathy, male sex, supraclavicular location of lymphadenopathy, and systemic symptoms, including fever, night sweats, and weight loss. Age is a particularly important factor: Malignancy is identified in 4% of cases of unexplained lymphadenopathy in patients aged 40 years and older but in only 0.4% in patients younger than 40 years.[2]

What Procedures Can Be Performed to Obtain a Specimen for the Evaluation of Lymphadenopathy?

The most common procedures for tissue evaluation of lymphadenopathy are fine-needle aspiration (FNA), needle core biopsy, and excisional biopsy. Fine-needle aspiration uses a narrow-gauge needle to sample cells for cytology and has the advantage of being the least invasive means to obtain diagnostic material from lymph nodes. In some situations, sampling can be performed endoscopically and may be guided by imaging methods such ultrasound or computed tomography. In addition to cytologic preparations, the material obtained can be assessed by ancillary techniques such as flow cytometry, genetic testing, and microbiologic culture. Immunohistochemical stains can be performed on cell block material. The major limitations of FNA are the total amount of material obtained and the inability to appreciate tissue architecture. The latter is particularly important in the diagnosis and classification of lymphomas. Needle core biopsies utilize a larger bore needle that is more invasive than FNA, but deep anatomic sites can still often be reached with imaging guidance, and more overall tissue can be obtained. Fine-needle aspiration is sometimes performed during the same procedure for cytological preparations and ancillary testing such as flow cytometry. Although appreciation of overall lymph node architecture remains limited, needle core biopsy is often sufficient for definitive diagnosis and classification of lymphoma. Excisional lymph node biopsy affords the most comprehensive diagnostic evaluation of lymphadenopathy but is the most invasive and may not be feasible in critical anatomic sites or in high-risk patients. False-negative results from FNA and needle core biopsies can occur if inadequate lesional tissue is sampled. Even when lesional tissue is present, it may not reflect heterogeneity in the underlying process, such as the presence of more than 1 tumor grade. Pathologists should request a larger biopsy if FNA or needle core biopsy material cannot be adequately evaluated to guide clinical management, or if discussion with the clinician suggests a discrepancy between the pathologic diagnosis and the clinical presentation.

Diagnostic Findings, Part 2

Because of the clinical suspicion of lymphoma and the easily accessible location of the lymph node, an excisional biopsy rather than an FNA or needle core biopsy was performed. Histologically, the lymph node architecture is effaced by a diffuse proliferation of atypical mononuclear cells (Figure 1A). On high-power magnification, the cells are medium to large in size and have ovoid or irregular nuclei. Some of the cells have eccentric, kidney-shaped nuclei, the so-called "hallmark cells" (Figure 1B). Immunohistochemical stains were performed. The atypical cells are diffusely and strongly positive for CD30 (Figure 2A) and negative for anaplastic lymphoma kinase (ALK; Figure 2B). They were also positive for CD2 and negative for CD3, CD4, CD8, CD15, CD20, CD45, PAX5, T-cell-restricted intracellular antigen-1 (TIA-1), and granzyme B (not shown).

Questions/Discussion Points, Part 2

What Is the Diagnosis Based on Pathological Examination of the Lesion and Correlation With Clinical Findings?

Based on the histologic and immunohistochemical features of the tumor and the clinical presentation, the diagnosis is

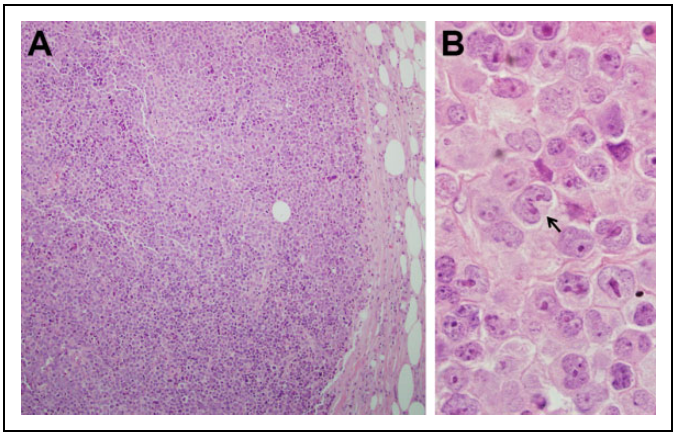

Figure 1. Histological findings of the left inguinal lymph node. A, A low-magnification image shows the lymph node architecture to be effaced by a diffuse proliferation of atypical mononuclear cells (hematoxylin and eosin [H&E], ×100). B, At higher magnification, medium-sized to large atypical lymphocytes are seen with ovoid or irregular nuclei, including some cells with eccentric, kidney-shaped nuclei (the so-called "hallmark cells," arrow; H&E, ×1000).

ALK-negative anaplastic large cell lymphoma (ALCL). Classic Hodgkin lymphoma (CHL) is another malignant lymphoma containing large neoplastic CD30-positive cells. However, CHL more typically shows scattered neoplastic cells in a mixed inflammatory background rather than sheets of tumor cells as in this case. In addition, although immunophenotypic features may vary, CHL often co-expresses CD15 and the B-cell transcription factor PAX5 and is less commonly positive for pan-T-cell markers such as CD2.

ALCLs comprise a group of T-cell lymphomas with common pathologic features but with varying genetics and clinical features. The tumor cells are typically large, and hallmark cells should be present at least focally for a diagnosis of ALCL. These cells have eccentric horseshoe-shaped or kidney-shaped nuclei and a prominent Golgi zone. Immunophenotyping is mandatory for diagnosis. By definition, all ALCLs express CD30, a type 1 transmembrane protein expressed by activated lymphocytes that is also known as tumor necrosis factor receptor superfamily 8 (TNFRSF8). CD30 is evaluated by flow cytometry in some centers, but the mainstay of phenotyping is immunohistochemistry. In addition to CD30, immunohistochemistry should also evaluate a range of T- and B-cell antigens and often cytotoxic markers. Although ALCLs are of T-cell origin, most cases show loss of several T-cell antigens or sometimes all T-cell antigens ("null" cell type). Immunohistochemistry for ALK should also be performed, as described below. *ALK* translocations also may be detected using fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction, or next-generation sequencing approaches. Molecular studies to detect clonal T-cell receptor gene rearrangements may be helpful in some cases, but often are not required and may be negative in a subset of ALCLs.

Describe the Classification of Anaplastic Large Cell Lymphoma

According to the World Health Organization (WHO) classification, ALCLs are classified based on clinical presentation (systemic or localized) and the expression of ALK.[3] ALK-positive ALCLs express ALK fusion proteins as a result of chromosomal rearrangements involving the *ALK* gene on chromosome 2p23. ALK-negative ALCLs show similar morphologic and phenotypic features, but *ALK* rearrangements and ALK fusion proteins are absent. Both diseases typically present with advanced stage disease (stage III-IV) and B symptoms (including fever, night sweats, and weight loss). Extranodal involvement is common; involved sites may include bone and/or bone marrow, liver, skin, and soft tissue. ALK-positive ALCL occurs most commonly in children and young adults, whereas ALK-negative ALCL occurs in adults, with a peak incidence between 40 and 65 years. Patients with ALK-positive ALCL typically respond well to chemotherapy, and the long-term survival rate is 80%.[4] The prognosis of ALK-negative ALCL is poorer, even after adjusting for age.

While systemic ALK-negative ALCL is considered an aggressive lymphoma, the WHO also recognizes 2 localized forms of ALK-negative ALCL, primary cutaneous ALCL and breast implant–associated ALCL, that typically have more indolent clinical behavior.[5,6] However, ALCL is not graded cytologically, and cases with aggressive and indolent clinical behavior may have similar cytologic features. In general, there is not a formal grading system for T-cell lymphomas. This is in distinction to B-cell lymphomas, where some entities such as follicular lymphoma have specific cytologic grading criteria, and other entities are specifically designated as high grade. Some more indolent lymphoid neoplasms carry the designation "lymphoproliferative disorder" rather than "lymphoma," such as indolent T-cell lymphoproliferative disorder of the gastrointestinal tract.

What Additional Tests Could Be Done to Predict the Clinical Course of ALK-negative ALCL?

FISH studies to evaluate chromosomal rearrangements involving the *DUSP22/IRF4* locus on 6p25.3 and *TP63* on 3q28 help predict the clinical course of ALK-negative ALCL. These rearrangements occur in about 30% and 8% of systemic ALK-negative ALCLs, respectively.[7] They usually occur singly but rarely occur concurrently; they have not been reported in ALK-positive ALCL. *DUSP22*-rearranged ALCLs are associated with favorable prognosis similar to those of ALK-positive ALCL (5-year overall survival up to 90%), while *TP63*-rearranged ALCLs demonstrate aggressive clinical behavior and have very poor outcomes (5-year overall survival, 17%). "Triple-negative" ALCLs lacking rearrangements of *ALK*, *DUSP22*, and *TP63* have an intermediate prognosis. In the present case, immunohistochemistry for p63 was positive (Figure 3A). Immunohistochemistry for p63 is highly sensitive for *TP63* rearrangement and is a useful screening test but is not

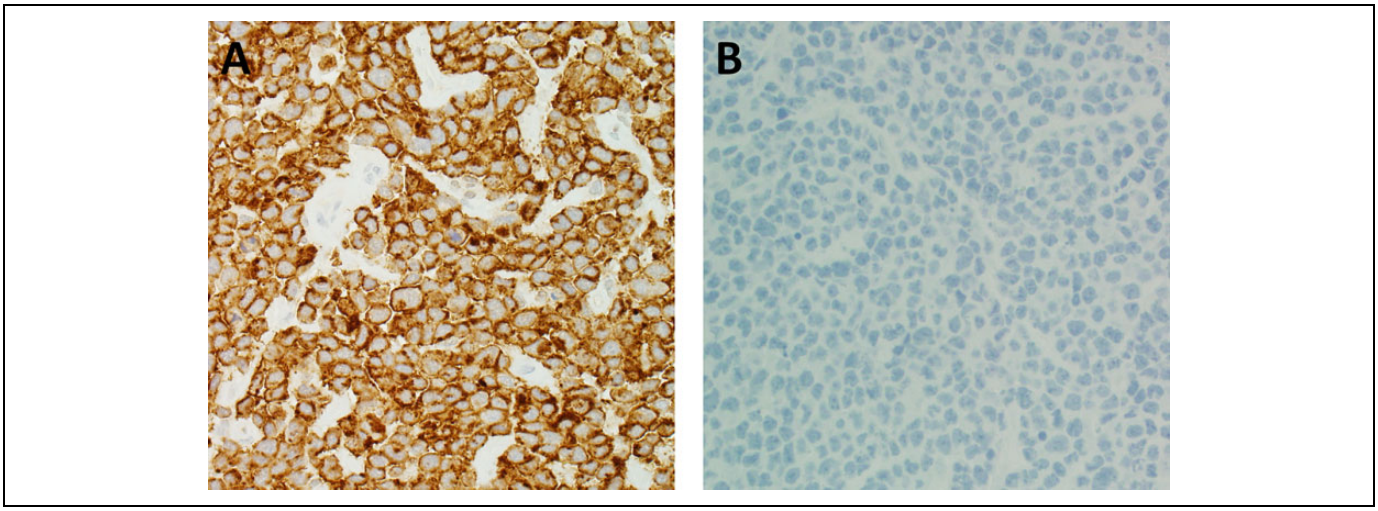

Figure 2. A, The tumor cells are diffusely and strongly positive for CD30 by immunohistochemistry (×400). B, The tumor cells are negative for ALK by immunohistochemistry (×400). ALK indicates anaplastic lymphoma kinase.

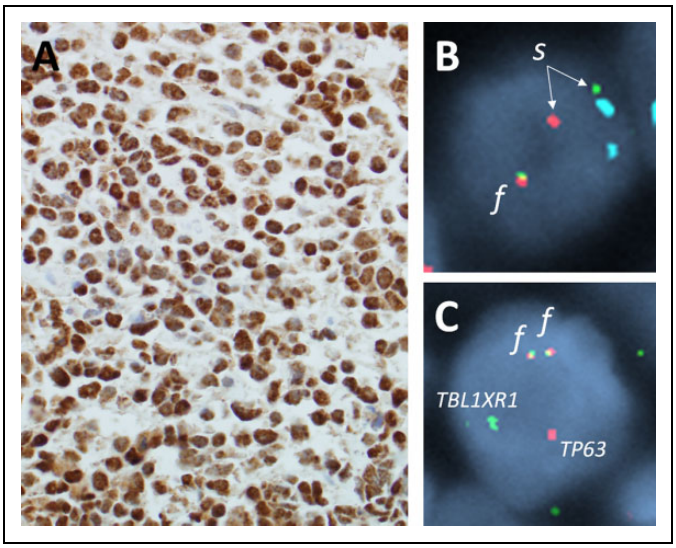

Figure 3. A, The tumor cells are positive for p63 by immunohistochemistry (×400). B, Interphase fluorescence in situ hybridization (FISH) using a break apart probe for the *TP63* locus on 3q28 shows evidence of a *TP63* rearrangement (×600). A single cell nucleus is shown (dark blue). There is one normal red–green fusion signal (*f*). The other allele shows separation of the 2 probes (*s*, arrows). The 2 aqua signals represent the centromeres. C, FISH using a dual fusion probe to *TP63* (red) and *TBL1XR1* on 3q26 (green) shows evidence of a *TBL1XR1-TP63* fusion (×600). The 2 fusion signals (*f*) are attributable to an inversion on chromosome 3, inv(3)(q26q28). *TBL1XR1* is the most common fusion partner identified in cases of *TP63* rearrangement.

specific; when positive, the presence of a *TP63* rearrangement must be confirmed by another method such as FISH[8] as it was in this case (Figure 3B and C). Based on this result, this patient would be predicted to have an aggressive clinical course and a poor probability of long-term survival. The patient received combination chemotherapy with cyclophosphamide, vincristine, Adriamycin, and prednisolone (CHOP), a standard regimen for systemic ALCL. However, he developed progressive lymphadenopathy at multiple sites, as well as liver and bone involvement, and died 1 year after diagnosis.

Teaching Points

- Anaplastic large cell lymphomas are malignant T-cell lymphomas characterized by the presence of distinct morphologic features, including hallmark cells and the expression of CD30; ancillary studies including immunohistochemistry as well as clinical correlation are necessary for accurate diagnosis and classification.
- Anaplastic large cell lymphomas are classified by the WHO based on clinical presentation (systemic or localized) and the expression of ALK.
- Systemic ALCLs include ALK-positive ALCL and ALK-negative ALCL; both tend to present at advanced stage and may involve extranodal sites.
- ALK-positive ALCL occurs mostly in young patients and has a relatively good prognosis, whereas ALK-negative ALCL occurs in older adults and overall has a poorer prognosis.
- Localized ALCLs include primary cutaneous ALCL and breast implant–associated ALCL.
- Among ALK-negative ALCLs, those with *DUSP22* rearrangements have a relatively favorable prognosis, similar to that of ALK-positive ALCL.
- ALK-negative ALCLs with *TP63* rearrangements ALCLs show aggressive clinical behavior and very poor outcomes.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Andrew L. Feldman https://orcid.org/0000-0001-5009-4808

References

1. Knollmann-Ritschel BEC, Regula DP, Borowitz MJ, Conran R, Prystowsky MB. Pathology competencies for medical education and educational cases. *Acad Pathol*. 2017:4. doi:10.1177/2374289517715040.
2. Gaddey HL, Riegel AM. Unexplained lymphadenopathy: evaluation and differential diagnosis. *Am Fam Physician*. 2016;94:896-903.
3. Swerdlow S, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. In: *WHO Series on Histological and Genetic Typing Of Human Tumours*. Vol 2. 4th ed. Geneva, Switzerland: International Agency for Research on Cancer/World Health Organization. 2017:395-396; 413-422.
4. Gascoyne RD, Aoun P, Wu D, et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood*. 1999;93:3913-3921.
5. Hapgood G, Pickles T, Sehn LH, et al. Outcome of primary cutaneous anaplastic large cell lymphoma: a 20-year British Columbia Cancer Agency experience. *Br J Haematol*. 2017;176:234-240.
6. Oishi N, Miranda RN, Feldman AL. Genetics of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). *Aesthet Surg J*. 2019;39(Supplement_1):S14-S20.
7. Parrilla Castellar ER, Jaffe ES, Said JW, et al. ALK-negative anaplastic large cell lymphoma is a genetically heterogeneous disease with widely disparate clinical outcomes. *Blood*. 2014;124:1473-1480.
8. Wang X, Boddicker RL, Dasari S, et al. Expression of p63 protein in anaplastic large cell lymphoma: implications for genetic subtyping. *Hum Pathol*. 2017;64:19-27.