

Case Report

ALK-positive anaplastic large cell lymphoma: a clinicopathological analysis of two cases

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Abstract: ALK-positive anaplastic large cell lymphoma (ALCL) is a rare type of non-Hodgkin lymphoma. To investigate the clinical and pathological characteristics of ALCL, We presented two cases of ALCL. The two cases occurred in subaxillary and supraclavicular lymph nodes respectively. Histological analysis showed that the tumors typically consist of large lymphoid cells with pleomorphic morphology and horseshoe-like nuclei. Immunohistochemically, the tumor cells were positive for ALK, CD3, CD4, CD30, CD45RA and EMA, but negative for CD15, CD20, CD45RO, CD56, CD68, CD79a, CD138 and cytokeratins. Ki67 index was 50%-60%. Follow-up showed both patients died 2 and 9 months after the diagnosis, respectively. The two cases showed that ALK+ ALCL was a highly-progressive non-Hodgkin's lymphoma and should be distinguished from other large cell malignancies.

Keywords: Anaplastic large cell lymphoma, anaplastic lymphoma kinase, translocation

Introduction

Anaplastic large cell lymphoma (ALCL) is a rare type of mature T-cell tumors, comprising three pathologically similar but clinically distinct entities: systemic ALK-positive ALCL, systemic ALK-negative ALCL, and primary cutaneous ALCL. Histologically, Most of ALCL are consists of large lymphoid cells with pleomorphic or horse-shoe-shaped nuclei [1-3]. ALCL may occur in both adults and children, and typically presents a characteristic chromosomal translocation, t(2;5)(p23;35), which fuses the anaplastic lymphoma kinase (ALK) gene on chromosome 2 with the nucleophosmin (NPM) gene on chromosome 5 and results in a NPM-ALK fusion protein, ALK overexpression and enhanced tyrosine kinase activity [2]. As targeted therapy of ALK inhibitor Crizotinib has been used in ALCL treatment, the detection of ALK gene translocation plays an important role in the pathologic diagnosis and therapeutic consideration [4, 5]. Recently, two cases of ALK positive ALCL, occurring in extranodal sites with systemic involvement, were diagnosed in our department with combined histopathological examination and immunohistochemical staining.

Case presentations

Case 1

A 73-year-old male presented with a mass in his left axilla for 4 months with episodic fevers, joint pain, skin rash and hoarseness. The patient had a history of psoriasis for more than ten years. Physical examination showed mild fever (38.0°C), facial flushing, skin pachulosis with scattered red rashes, speckles and lichenoid reaction. Multiple superficial lymph nodes (cervical, supraclavicular, inguinal, and left subaxillary) were enlarged; Liver and spleen were normal in size. Complete blood count and liver function panel were within normal limits. EBV, HPV and HBV tests were negative. Abdominal ultrasonic examination revealed that the liver and spleen were in normal size and no enlarged lymph nodes were detected in retroperitoneal region. Endoscopy of the stomach and duodenum were unremarkable. Renal function analysis and serum protein electrophoresis were normal. The left enlarged cervical lymph node was biopsied. The tissue was fixed in 10% buffered formalin for 16 hours in room temperature, and embedded in paraffin. Successive 4 µm sec-

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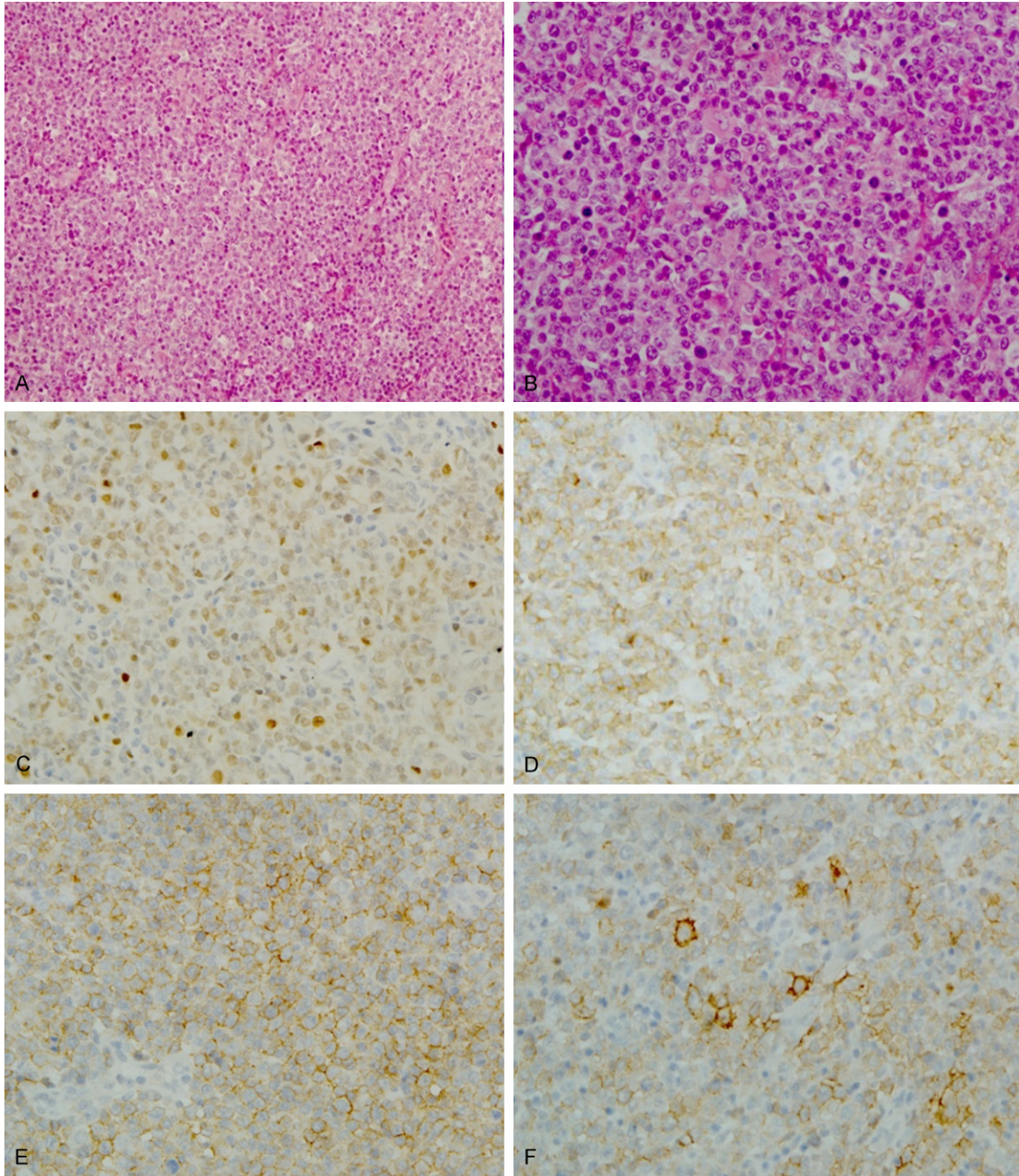


Figure 1. Histopathological appearances and immunohistochemical findings of ALK positive anaplastic large cell lymphoma (Case 1). A. The tumor consists of diffuse large cell infiltration and proliferating venules (HE, original magnification, $\times 200$); B. The predominant population of tumor cells show irregular nuclei with eccentric kidney-shaped morphology (HE, original magnification, $\times 400$); C. The malignant cells are positive for ALK with nucleic and cytoplasmic staining (Immunohistochemistry MaxVision, original magnification, $\times 400$); D. The tumor cells show membranous staining of CD3 (Immunohistochemistry MaxVision, original magnification, $\times 400$); E. The most tumor cells are positive for CD4 (Immunohistochemistry MaxVision, original magnification, $\times 400$); F. Some of large tumor cells are positive for CD30 (Immunohistochemistry MaxVision, original magnification, $\times 400$).

tions were cut and stained with hematoxylin and eosin and immunohistochemistry (MaxVision method) (Maixin, Fuzhou, China). Mouse monoclonal antibodies (ALK1, CD3,

CD4, CD5, CD15, CD20, CD30, CD43, CD45, CD45RO, CD56, CD79a, cytokeratin 7 and AE1/AE3) (Santa Cruz, CA, USA) were used in this study. Microscopic examination revealed that

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the lymph node structure was destroyed by a heterogeneous infiltrate of large pleomorphic cells with abundant pale cytoplasm, vesicular chromatin and prominent nucleoli (**Figure 1A** and **1B**). The large atypical cells were prominent in a background of small lymphocytes, macrophages, neutrophils and frequent apoptotic cells. Immunohistochemical staining showed the large atypical cells were positive for ALK, CD3, CD4, CD5, CD30, CD43 and CD45RA (**Figure 1C-F**), but negative for CD15, CD20, CD45RO, CD56, CD79a, cytokeratin 7 and AE1/AE3. According to WHO lymphoma classification of 2008, a diagnosis of anaplastic large cell lymphoma, ALK-positive was rendered.

Staging included a whole body positron emission tomography/computed tomography (PET/CT), which demonstrated abnormal sized but mildly hypermetabolic lymph nodes scattered throughout the bilateral cervical, axillary, mediastinal and inguinal lymph node chains, along with foci of abnormal metabolic activity in upper abdominal mesentery, gastrocolic ligament and subpleural consolidation. No areas of abnormal hypermetabolism were detected in the gastrointestinal tract. Bone marrow biopsies showed mildly hypocellular and no involvement of the neoplastic cells. Serum LDH was 412 IU/L and β -2 microglobulin was 5.12 μ g/L. Patient was stratified under high risk group by International Prognostic Index (IPI) score of 5. The patient condition became stable after three cycles of CTOP, CEOP and CMOP chemotherapy regimens were subjected but the lymph nodes had no changes in size. Before the fourth cycle of chemotherapy, the patient ran a fever with increased size of lymph nodes and loss of appetite; LDH rose to 588 U/L, HGB 79 g/L, serum creatinine 383 μ mol/L and urea nitrogen 22.24 mmol/L. Three days after CEOP treatment, the patient died of systemic failure. It took only nine months for this patient from the confirmed diagnosis to death.

Case 2

A 39-year-old Chinese man visited our hospital with a four-month history of abdominal distension, complaining of weak enforcement and slight fever. The patient had no nausea, vomiting, diarrhea and swelling at extremities. Physician at the local hospital treated the patient as peritonitis. Physical examination showed the patient had fever of 39.2°C and left

supraclavicular lymphadenopathy (2.5×2.2×1.5 cm). There were no other superficial lymph node enlargements. Abdominal bulge was observed with shifting dullness. X-ray chest radiography had no positive findings. Laboratory tests showed an increased white blood cell count (including 80% neutrophils, 18% lymphocytes and 6% monocytes), elevated erythrocyte sedimentation rate (100 mm/h; normal value, <20 mm/h), and higher C-reactive protein level (10.0 mg/dL; normal value, <0.30 ml/dL). Other biochemical and serologic parameters, except lactate dehydrogenase 288 U/L, were within normal limits. Abdominal ultrasonic checkup showed that multiple masses located around the pancreas and adjacent hepatic hilum, sized from 1.5 cm to 8.25 cm in diameter; fluid sonolucent area was also detected within the abdominal cavity. Bone marrow biopsies were normal. The supraclavicular lymph node was biopsied. The tissue was fixed in 10% buffered formalin for 16 hours in room temperature, and embedded in paraffin. Successive 4 μ m sections were cut and stained with hematoxylin and eosin and immunohistochemistry (MaxVision method) (Maixin, Fuzhou, China). Mouse monoclonal antibodies (ALK, CD3, CD4, CD5, CD15, CD20, CD30, CD43, CD45, CD45RO, CD56, CD79a, cytokeratin 7 and AE1/AE3) (Santa Cruz, CA, USA) were used in this study.

HE staining showed infiltration of atypical large lymphoid cells with prominent nucleoli and mitotic figures (**Figure 2A** and **2B**). Immunohistochemical staining revealed the atypical cells were positive for CD3, CD30, CD45, CD45RO, EMA and ALK, negative for CD20, CD34, CD79a. Ki-67 labeling index was about 60% (**Figure 2C-F**). Human T-cell lymphotropic virus (HTLV) antibody was negative. ALK-positive ALCL was diagnosed. The ascetic fluid was greyish in color and turbidity and Rivalta test was negative. The patient was given systemic chemotherapy (CTOP regimen). However, persistent high fever and rapid systemic dissemination occurred and the patient died of multiple organ failure nine weeks later after his first visit to this hospital. No autopsy was performed.

Discussion

ALCL is more common in male, involving both nodal and extranodal sites (such as bone, intestine, muscle, liver, and spleen, etc.).

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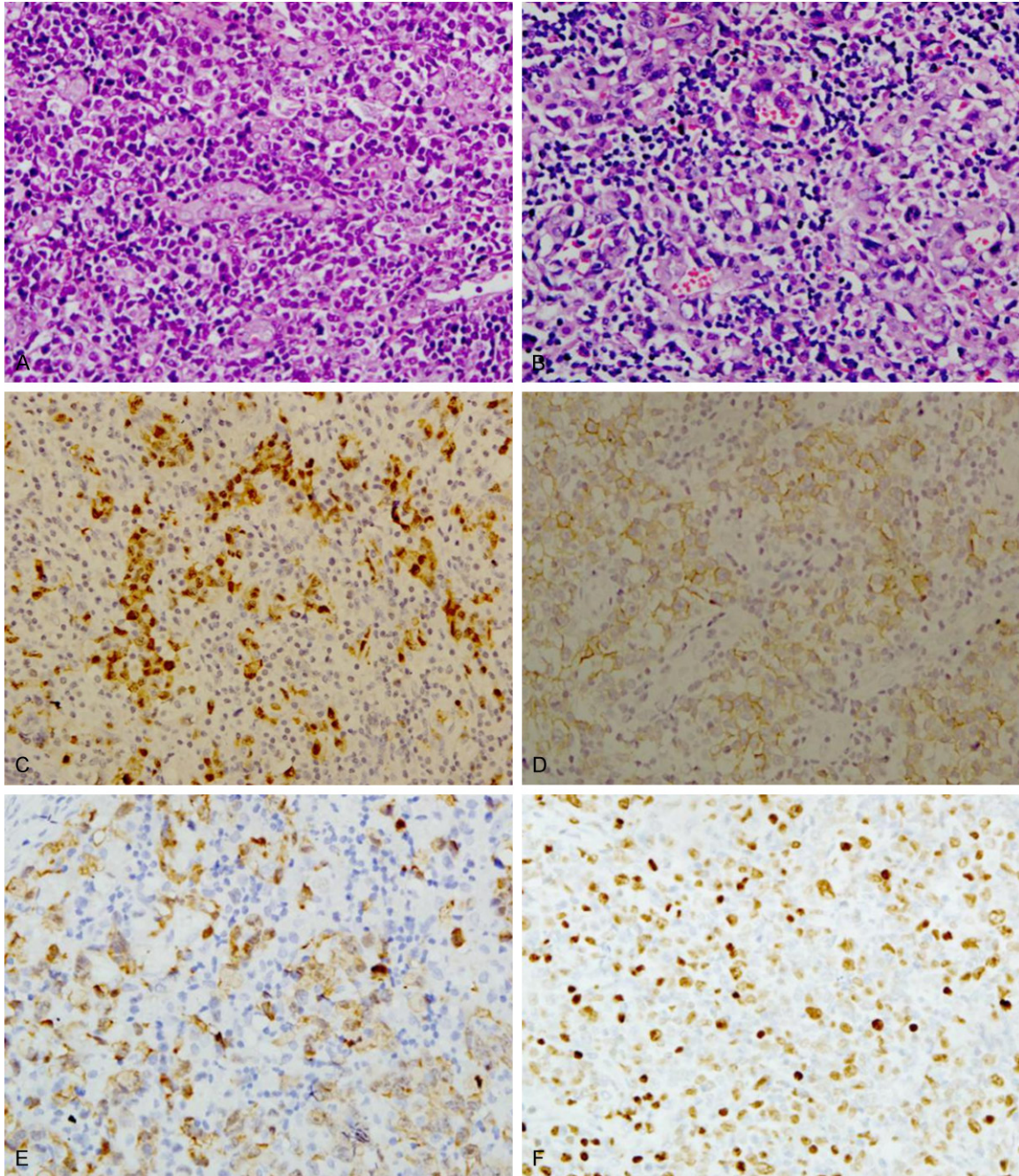


Figure 2. Histopathological appearances and immunohistochemical findings of ALK positive anaplastic large cell lymphoma (Case 2). A. The tumor consists of diffuse large cells with abundant eosinophilic cytoplasm and proliferating venules (HE, original magnification, $\times 400$); B. The tumor cells show highly atypical nuclei with eccentric oval, kidney-shaped morphology and condensed staining with small lymphoid cell background (HE, original magnification, $\times 400$); C. The most malignant cells are strongly positive for ALK with nucleic and cytoplasmic staining (Immunohistochemistry MaxVision, original magnification, $\times 400$); D. The most tumor cells show positive staining of CD30 (Immunohistochemistry MaxVision, original magnification, $\times 400$); E. The most tumor cells are positive for EMA (Immunohistochemistry MaxVision, original magnification, $\times 400$); F. Ki-67 labeling index of large tumor cells is more than 50% (Immunohistochemistry MaxVision, original magnification, $\times 400$).

Clinically, the patients were usually found at an advanced stage, typically associated with

weight loss, fevers and night sweats (B symptoms).

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The diagnosis of ALK+ ALCL requires the pathological examination of any affected nodal or extranodal tissue where the tumor is found, such as the cervical node, the liver or bone in the case of systemic ALCL [7, 8]. For the case of cutaneous ALCL, a skin excision is recommended [9, 10]. To make a diagnosis under the WHO classification, it emphasizes the identification of “hallmark” cells and immunopositivity for CD30 [1]. Integration of those data with clinical presentation is crucial for final classification and management of patients.

The WHO classification acknowledges the recognition of large cells with irregular nuclei and abundant eosinophilic cytoplasm. The morphologic features need to be combined with immunophenotypic evidence that tumor cells are originated from T lymphocytes, such as the expression of immunologic markers CD3 or CD4, but always it is required the expression of CD30 (also known as Ki-1) in all the neoplastic cells. Systemic ALCL usually expresses the anaplastic lymphoma kinase (ALK), and the other types of ALCL do not express ALK [1, 11, 12, 16]. The hallmark cells are of medium size and feature abundant cytoplasm (which may be clear, amphophilic or eosinophilic), kidney-shaped nuclei, and a paranuclear eosinophilic region. By definition, on histological examination, hallmark cells are always present. Although they are not present in large numbers, they are usually located around blood vessels. Both our cases are satisfied with the diagnostic standards of ALK+ ALCL.

Another useful marker which helps to differentiate this lesion from Hodgkin lymphoma is clusterin. The tumor cells have a Golgi staining pattern (hence paranuclear staining), which is characteristic of this lymphoma. The cells are also typically positive for a subset of markers of T-cell lineage. However, as with other T-cell lymphomas, they are usually negative for the pan T-cell marker CD3. Occasional examples are of null (neither T nor B) cell type. These lymphomas show immunopositivity for ALK protein in 70% of cases. They are also typically positive for EMA. In contrast to many B-cell anaplastic CD30 positive lymphomas, they are negative for markers of Epstein-Barr virus (EBV) [1].

The majority of cases of ALCLs contain a clonal rearrangement of the T-cell receptors, which may be identified using PCR techniques, such

as TCR- β or - γ complex primers Biomed-2 system. The chromosomal translocation in ALCL cases is most commonly involved in the nucleophosmin gene on chromosome 5 and is characterized by t(2;5)(p23;q35) [2, 11, 14]. The product of this fusion gene may be identified by immunohistochemistry using antibody to ALK protein and in situ hybridization can also be used for detection of this chromosomal translocation [13].

As the appearance of the cohesive tumor cells, nest-like growth pattern within lymph nodes and positivity for EMA may mimic metastatic carcinoma, it is important to include markers for cytokeratins in any diagnostic panel (these will be negative in the case of anaplastic large cell lymphoma). Other mimics include CD30-positive B-cell lymphoma with pleomorphic cells and Hodgkin lymphomas. These are identified by their expression of the markers of B-cell lineage and frequent presence of EBV infection. Primary cutaneous T-cell lymphoma may also be reactive for CD30; it could be excluded by its anatomic distribution [10]. ALK expression may also be noted in some cases of large-cell B-cell lymphoma and occasionally in acinar rhabdomyosarcomas.

Treatment in adults is typically anthracycline-based, with autologous stem cell transplantation in relapsed disease. The anti-CD30 immunoconjugate Brentuximab Vedotin and the specific ALK inhibitor Crizotinib are changing the treatment paradigm in ALK-positive ALCL. Both agents have showed encouraging responses in relapsed ALCL [4, 5]. It remains to be expected how these novel agents are used, but it is very possible that they may improve overall responses and survival in both children and adults.

The long-term overall survival of ALCL patients is approximately 70-90% in children and over 70% in adults. Staging systems and prognostic risk factors are different in both childhood and adult ALCL patients. No particular risk factor has been clearly identified for ALCL. Overall, the prognosis of ALK+ ALCL is remarkably better than other T-cell lymphomas. The IPI and the PIT scores could in general predict survival in patients with ALK+ ALCL. But, unfortunately, these two patients were dead shortly after the diagnosis, this might be related to the advanced stage of disease and unresponsive to chemotherapy.

Disclosure of conflict of interest

None.

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