

ALK-positive Large B-cell Lymphoma: Report of Two Cases and Review of the Literature

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Summary

Anaplastic lymphoma kinase positive large B-cell lymphoma (ALK+ LBCL) is a rare variant of large B-cell lymphoma, but it rarely expresses B-lineage antigens (e.g CD20, CD79a) while showing an abnormal immunophenotype, such as positive for ALK, EMA, CD38, CD138. Only 55 cases of ALK+ LBCL have been reported so far in the published literature [1]. We present two additional cases of ALK+ LBCL, which were sent to our department for consultation from other hospitals. One was initially diagnosed as metastatic poorly differentiated carcinoma and the other was not able to be diagnosed. The two cases were finally diagnosed as "ALK+ LBCL" at our department of pathology (see below). The chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) was given, respectively. Case 1 was given radiation therapy after chemotherapy, and case 2 wasn't. Case 1, followed up for 16 months, was free of disease. Case 2 died at 8 months after the diagnosis.

Due to the abnormal immunophenotype and rareness, the diagnosis of ALK+ LBCL is often difficult, even being misdiagnosed as carcinoma or plasma cell neoplasm. However, the treatment for these diseases is quite different from each another. Avoiding misdiagnosis of ALK+ LBCL is of great clinic importance. In this report, we describe the clinical, morphological, immunohistochemical and cytogenetic features of the two ALK+ LBCL cases, reviewed 55 published cases, and compared the features with those of literature-reported cases. We came to the conclusions: ① ALK+ LBCL could affect non-adult and adult with a bimodal age distribution, with an average age of 12.5 years in the non-adult, and 43.5 years in the adult. ② ALK+ LBCL is a potential diagnostic pitfall for pathologists. Its diagnosis clues are that neoplastic cells are immunoblastic or/and plasmacytoid with prominent central nucleoli, positive for ALK, EMA and some markers of late (plasma cell-like) B-cell differentiation, like CD138, VS38 and immunoglobulins, negative for B-cell markers like CD20 and CD79a. ③ The investigation of involved genetic abnormality (CLTC-ALK fusion, or NPM-ALK rearrangement) contributes to confirm ALK+ LBCL. ④ ALK+ LBCL has highly aggressive biobehavior and poor response to standard therapies. More patient case collection and prospective studies are needed.

Case Descriptions

Case 1

A 25-year old man presented a mass on the right neck that had been noticed for about one month. Physical examination showed the mass of right neck was 1.5×1.2×1.0cm in size with a tender. No fever was reported. Hematologic studies, lactate dehydrogenase and serum protein electrophoresis were all within normal limits. An excisional biopsy of the mass was done, and the diagnosis of ALK+ LBCL was finally made (see below). Bone marrow biopsy, computed tomography (CT), and magnetic resonance imaging (MRI) of the thorax and abdomen were performed. The results showed bone marrow was negative for involvement by lymphoma, and no additional mass lesion or lymphadenopathy was detected anywhere else in the body. The disease was in stage I. The patient underwent six cycles of CHOP chemotherapy followed by right neck irradiation. Follow-up imaging studies showed no evidence of recurrence. At 16 months, he was free of disease.

Case 2

A previously healthy 32-year old man complained of a rapidly enlarging tender lump on his left neck with a low fever for about 2 months. Physical examination showed he had superficial lymphadenopathies in his axillary and inguinal areas besides the mass of left neck. CT imaging showed no other mass or lymphadenopathy in anywhere else in the body. The range of the lymphadenopathies in

size is from 1.5cm to 3.0cm in the maximum diameter. An excisional biopsy of the neck mass was done, and the diagnosis of ALK+ LBCL was finally made (see below). A bone marrow biopsy was followed, and no involvement by lymphoma was found. Other laboratory tests showed no distinct abnormalities. The disease was in stage III. The patient underwent six cycles of CHOP chemotherapy therapy, and died at 8 months after diagnosis.

The clinical features of the two patients are summarized in [Table 1](#).

Materials and Methods

Preparation of specimen

The two cases were sent to our department for consultation

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from other hospitals with the samples of hematoxylin and eosin(HE) slides and corresponding paraffin blocks. The submitted paraffin-embedded tissue blocks were respectively cut into 3µm, 4µm, and 6µm sections for HE staining, Immunohistochemical staining, and DNA extraction, respectively. Additionally, the tumor tissue of case 1 from the submitted paraffin-embedded tissue blocks was cut 6 sections in 10µm thickness for RNA extraction. Clinical and laboratory data for each of the two patients were obtained through physician interview and medical chart review.

Immunohistochemical analysis

Immunohistochemical analysis was performed for the two cases on 4µm formalin-fixed, paraffin-embedded tissue sections using EnVision (Dako, Denmark) two-step method. Briefly, the sections were deparaffinized with xylene and rehydrated using graded ethanol concentrations. After heat-induced antigen retrieval in 0.01 mol/L citrate buffer (pH6.0), the slides were incubated with primary antibodies (Dako) LCA, ALK1, EMA, CD57, IgA, CD138, VS38C, CD3, CD45RO, CD20, CD79a, CD68, and CD30 (Table 2) at 4°C overnight. The next day, the sections were washed with phosphate buffered saline three times, incubated with the EnVision reagent (Dako) at room temperature for 30 minutes, visualized with 3,3'-diaminobenzidine-chromagen solution and finally counterstained with hematoxylin (Sigma). Appropriate positive and negative tissue control samples were used with each run.

Clone rearrangement of IgH gene by DNA PCR

Five 6µm paraffin-embedded sections of tumor tissues of the two cases were deparaffinized. The 6µm sections were lightly stained with hematoxylin for microdissection. The microdissections were performed under a dissection microscope with a scalpel. Tumor cells should account for at least 80% of the total cells isolated. The microdissected

tissues were transferred directly into an Eppendorf tube with 200µl cell lysis buffer (0.5 mol/L Tris-HCl, 20 mmol/L EDTA, 10 mmol/L NaCl, 10 g/L SDS, 0.5 g/L Proteinase K). The subsequent DNA extraction was performed according to the protocol of the DNA extraction kit (Qiagen). PCR amplification was performed with the condition described by Tan et al. [2] employing commercially available PCR-based kits (InVivoScribe Technologies, San Diego, CA). No template DNA was used as negative control. PCR products were analyzed by electrophoresis using 1.0% agarose gels, stained with ethidium bromide.

RNA extraction and RT-PCR sequencing

Total RNA of case 1 was extracted from tumor tissue using Trizol reagent (Invitrogen Life Technologies) as described previously [3-5]. RNAs extracted from the t(2;5)-positive SU-DHL-1 and Karpas299 cell line were used as positive controls, while DEPC water and RNA from proper negative tissue (normal lymph node) were used as negative controls. Reverse transcription of RNA into cDNA was performed by incubating one µg RNA (purified by DNase digestion using TURBO DNase from Ambion), one µL of random primer (Promega, USA), and 200 U of reverse transcriptase (Promega, USA) in a 25 µL reaction volume at 37°C for one hour. One µL cDNA was then submitted to PCR amplification. To assess the quality of cDNA, the transcript of a housekeeping gene PGK was simultaneously detected as an internal control. PCR reaction was performed using specific primers (*CLTC F-GAAGGAGTACTTGACAAAGGTGGAT*; *ALK R-CGGAGCTTGCTCAGCTTGTA*). Information regarding the primers, their sequences and annealing temperatures were previously described. The optimized thermal cycling condition for ALK mRNA and ALK-associated fusion gene amplification consisted of an initial denaturation step at 95°C for 10 minutes and then 42 cycles of 94°C for 30 seconds, 57°C/60°C for 30 seconds, and 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. The presence of PCR products was tested using 2% agarose gels, compared with a 100 bp DNA marker. After the bands were clearly observed and the sized was determined, the products were purified. Sequencing was performed on the ABI Prism 3730 Sequence Detector System. *ALK* and *CLT* structures were obtained from Ensembl (www.ensembl.org).

Results

The histologic findings in the two cases were similar. They showed a diffusely infiltrating growth pattern, but focally arranged in nested and gland-like construction in case 1. Neoplasm cells exhibited distinct immunoblast-like morphologic features with regular uniform big round nuclei containing large central nucleoli and moderate lightly basophilic cytoplasm. Both of the cases presented similar immunophenotypic profiles, the tumor cells were positive for LCA, ALK1, IgA, CD138, CD38, VS38c, CD57, EMA, whereas negative for CD20, CD79a, CD3, CD45RO, CD30 and CD68. With regard to ALK protein, its expression in the two cases was restricted to the cytoplasm that showed a fine granular cytoplasmic-staining pattern ALK expression (Figure 1). The rearrangement of the clone IgH gene was found in the two cases, and ALK-CLTC fusion was identified by direct sequencing in case 1 (Figure 2). (Test of ALK-CLTC fusion was not performed in case 2).

Discussion

ALK+ LBCL was originally described by Delsol et al. [3] in 1997. This lymphoma was identified due to its characteristic lack of CD30 expression in an otherwise large series of classical T-/null cell ALK-positive anaplastic large cell lymphomas (ALCL) [4]. It showed very

Case no.	Sex/age (years)	Tissue sampled	Staging bone marrow	Stage of disease	Therapy	Present clinical status
1	M/25	Right neck lymph node	Negative	I	CHOP and left neck XRT	Alive at 16 months after diagnosis
2	M/32	Left neck lymph node	Negative	III	CHOP	died at 8 months after diagnosis

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; XRT, radiation therapy.

Table1: Clinical features of the two patients with ALK+ LBCL.

Antibody	Dilution	Source
LCA	1:100	Dako
ALK1	1:10	Dako
EMA	1:200	Dako
CD57	1:100	Dako
IgA	1:100	Dako
CD138	1:50	Dako
VS38c	1:10	Dako
CD3	1:100	Dako
CD45RO	1:50	Dako
CD20	1:100	Dako
CD79a	1:200	Dako
CD68	1:100	Dako
CD30	1:100	Dako

Table 2: Antibodies used for immunohistochemical analysis.

aggressive behavior, high relapse rate and little response to standard regimens [5]. For the time being, this rare tumor had been recognized as “Diffuse large B-cell lymphoma with expression of full-length ALK” by WHO classification of lymphomas (3th edition) [6]. The disease was defined as an entity, and termed “ALK positive large B-cell lymphoma” in the latest WHO classification of lymphomas (4th edition) [7]. This lymphoma often exhibited a sinusoidal growth pattern and was composed of monomorphic large immunoblast-like cells with round pale nuclei containing large central nucleoli and abundant cytoplasm, or showed plasmablastic differentiation. Atypical multinucleated neoplastic giant cells might be seen [8,9]. It was revealed to derive from

B cells based on expression of monotypic light chain [8], but exhibited a unique immunophenotypic profile characterized by cytoplasmic, granular ALK reactivity consistently [10] with expression of EMA, plasmacytic markers (e.g CD38, CD138), and variable expression of CD4 and CD57, while often lacking expression of B-lineage (e.g CD20, CD79a), T-lineage (e.g CD2, CD3) markers and CD30. Genetic studies showed that the majority of ALK+ LBCL cases were characterized by the CLTC-ALK fusion, and a small minority had the NPM-ALK rearrangement [11]. Although ALK+ LBCL is one of B cell lymphomas, it expresses EMA and plasma cell markers (CD38, CD138, VS38c) instead of expressing CD20 and CD79a, which may be mistaken as

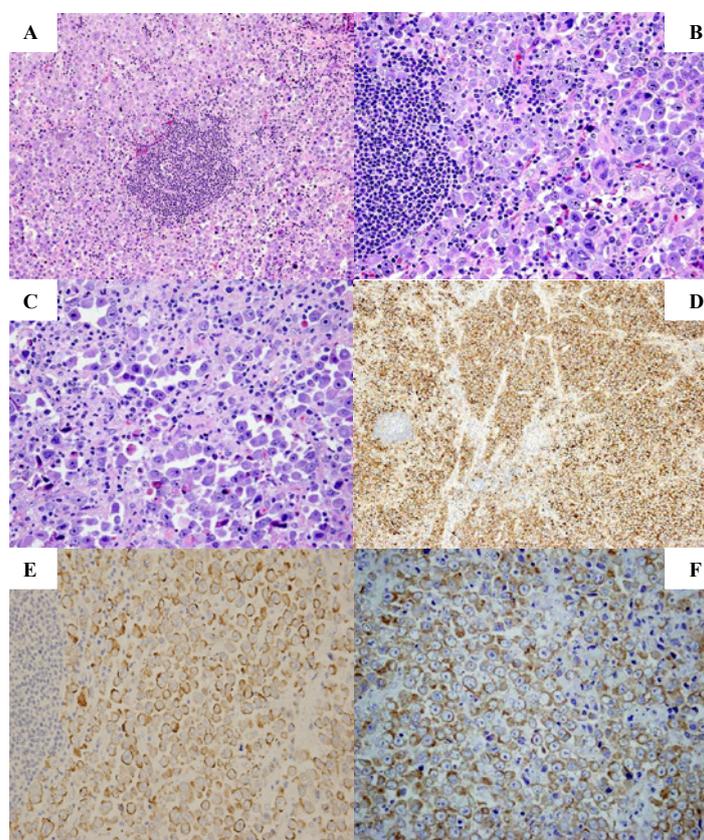


Figure 1: Diffuse proliferation of tumor cells with round regular nuclei and single central eosinophilic nucleoli, and moderate amounts of basiphilic cytoplasm. Gland-like arrangement of tumor cells was locally seen in C (A, HE 100×; B, HE 400×; C, HE 400×). Immunostains of EMA(D), VS38c(E), ALK(F) (EnVision two-step: D, 100×; E, 200×; F, 400×).

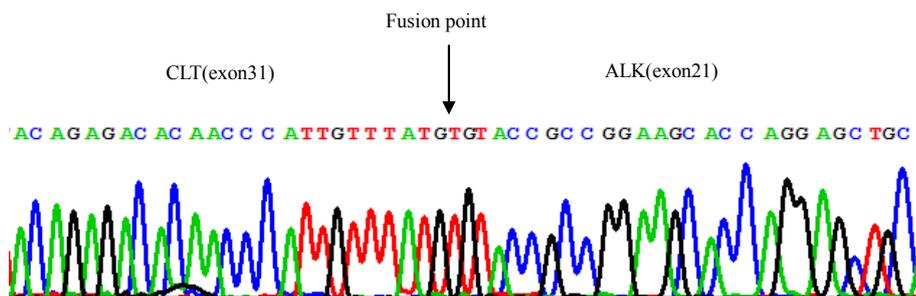


Figure 2: The sequence of the translocation fusion point. Arrow indicates the corresponding fusion point.

metastatic poorly differentiated carcinoma or plasma cell neoplasm. It is a potential challenge for pathologists to make the diagnosis due to the abnormal immunophenotypes as well as infrequency of the entity.

Clinically, the average age of the reported ALK+ LBCL in the literature was 37.9 years, ranging from 9 to 72 years of age with a bimodal age distribution, with an average age of 12.5 years in the non-adult and an average age of 43.5 years in the adult. The male to female ratio was about 3:1. Data on primary sites of presentation were available in 51 cases. Twenty-seven (52.9%) were exclusively nodal in origin. The lymph node was the commonest primary site of involvement, particularly cervical and mediastinal areas. The remaining cases (47.1%) had some extranodal component, including bone (n=9), liver and spleen (n=4), head and neck (n=4), gastrointestinal tract (n=3), and others (n=8) including bone marrow, CNS, gonads and muscle. Both of our patients were male. One was 25 years and the other was 32 years. The sites of involvement were both located in the lymph nodes.

Microscopically, ALK+ LBCL is characteristic of immunoblastic/plasmablastic microscopical appearance with round nuclei, prominent single central nucleoli, and abundant cytoplasm. Sometimes, the neoplastic cells demonstrate ample eosinophilic cytoplasm, somewhat resembling epithelioid appearance. This lymphoma often shows a sinusoidal growth pattern. In this report, both of the presented cases mainly showed a diffusely infiltrating pattern instead of sinusoidal growth. However, the neoplastic cells in case 1 showed focally nested and gland-like arrangement. In terms of morphology, differential diagnosis of ALK+ LBCL should include anaplastic variant of DLBCL, plasmablastic lymphoma, plasmablastic myeloma, and metastatic poorly differentiated carcinomas.

Immunohistochemically, this lymphoma does rarely express the usual B-lineage (e.g. CD 19, CD20, CD79a) or T-lineage markers (e.g. CD2, CD3, CD7), demonstrating the “null” phenotype with ALK expression and EMA, which strongly suggests an anaplastic large cell lymphoma (ALCL). ALK+ LBCL, however, expresses plasmacytic differentiation markers, such as CD138, CD38, and VS38c besides ALK and EMA.

ALK+ LBCL presents 100% positivity for plasmacytic differentiation markers like CD138, VS38c and MUM. EMA was expressed in 97% of the cases. B-cell related antigens such as CD20 and CD79a were expressed in ALK+ LBCL in 11% and 18%, respectively. These observations support the inference that ALK+ LBCL is derived from post-germinal B-cell lymphocytes that have undergone class switching and plasmacytic differentiation. Additionally, expression of monotypic cytoplasmic light chain occurred in 85% of all cases. Based on these findings, ALK+ LBCL falls into the category of non-GC DLBCL [5,12]. In our study, both cases exhibited immunophenotypic profiles similar to those in the published literature. Table 3 shows immunohistochemical features of 55 cases of ALK+ LBCL reported in the literature.

Genetically, the most frequent ALK gene rearrangement was clathrin-ALK in 75% cases; however 17% corresponded to NPM-ALK fusion [11,13,14]. The ALK gene is located on chromosome 2p23 and it can be translocated to either the clathrin gene locus located on chromosome 17q23 or to the NPM1 gene located on chromosome 5q35, constituting the clathrin-ALK or NPM-ALK fusion products, respectively [11,12,15]. In the literature, the clone rearrangement of immunoglobulin heavy chain (IgH) gene was investigated by PCR in 20 studied cases of ALK+ LBCL, and the rearrangement was found in

	Number studied	Number positive/weak	%
Immunohistochemistry			
ALK	55	55	100
Cytoplasmic		48	87.3
Nuclear		6	10.9
Other		1	1.8
VS38c/CD138/MUM1	44	44	100
EMA	42	41	97.6
CD45	31	23/2	80.6
CD4	45	12/5	37.8
CD57	24	3/5	33
Perforin	24	2	8
CD20	49	4/1	10.2
CD79a	48	7/2	18.8
CD30	50	5	10
EBV	17	0	0
HHV8	2	0	0

ALK – anaplastic lymphoma kinase; EMA – epithelial membrane antigen; EBV – Epstein Barr virus; HHV8 – human herpesvirus 8

Table 3: Immunohistochemical features of 55 cases of ALK-LBCL reported in the literature.

Molecular studies			
ALK gene rearrangement	25	25	100
Clathrin/ALK		19	76
Nucleophosmin/ALK		4	16
Other rearrangements		2	8
IgH gene rearrangement	20	17	85

IgH – immunoglobulin heavy chain

Table 4: Genetic feature of investigated cases of ALK+ LBCL reported in the literature.

17 of the 20 cases (85%), which confirmed the B-cell lineage of this disorder. In this report, the fusion in case 1 was studied employing analysis of direct sequencing, and CLTC-ALK fusion was found, which was identical to those reported previously [10,12,15-17]. Additionally, the rearrangement of the IgH gene was detected in both cases. Table 4 shows genetic features of investigated cases of ALK+ LBCL reported in the literature.

Prognostically, the clinical course of ALK+ LBCL was aggressive with primary refractory disease and high relapse rates. Its prognosis depended largely on clinical stage [10]. The classical CHOP regimen appeared insufficient to treat this condition, which indicates newer, more intensive therapies will be needed, even though some cases could have prolonged survival times as the authors described in the article [10]. In this report, both patients were treated with six cycles of CHOP chemotherapy. Case 1, in stage I and simultaneously treated with radiation therapy, was alive at 16 months after diagnosis. Another patient, in stage III, treated with CHOP chemotherapy, died at 8 months after diagnosis.

We believe that a combination of chemotherapy and radiotherapy could prolong the survival time, while stage of disease could be the

most important prognosis factor. To improve understanding of ALK+ LBCL and develop newer therapeutic techniques for its treatment, more collection of patient cases and prospective studies are needed.

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