Histopathologic and Immunophenotypic Features of Childhood and Adult Anaplastic Large-Cell Lymphomas

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ABSTRACT

The t (2;5) (p23; q35) translocation associated with CD30-positive anaplastic large-cell lymphoma (ALCL) creates a hybrid gene encoding the chimeric nucleolar protein nucleophosmin-anaplastic lymphoma kinase (NFM-ALK) protein, which can be demonstrated by immunostaining with ALK1 monoclonal antibody. In this study, 40 specimens of ALCL from 6 pediatric, 34 adult patients, were immunostained with monoclonal antibodies against CD30 (Ber-H2), EMA, CD45 (LCA), CD3, CD20 (L26), CD15, and ALK1 antigens, and results were correlated with histopathologic features. The mean age of the pediatric and adult patients was 10-years and 38-years, respectively. ALK1 was positive in 14 cases (35%) representing 83% of pediatric and 26% of adult patients, statistically significantly higher in the pediatric group (p= 0.01). Considering the better prognosis attributed to cases with t (2;5), it is interesting to note that the percentage of ALK1-positive cases is significantly higher in pediatric patients with coexpression of EMA, compared to adults.

Key Words: Anaplastic large-cell lymphoma, ALK1, CD30, Epithelial membrane antigen.

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INTRODUCTION

Anaplastic large-cell lymphoma (ALCL) is a clinically and pathologically heterogeneous disease, first defined by Stein et al, and designated as "Ki-1 cell lymphoma", eventually entered the updated Kiel classification[1,2]. Nowadays, ALCL is accepted as a subgroup of T-cell nonHodgkin lymphomas (NHL) in both the Revised European-American (REAL) classification and World Health Organisation (WHO) classification of lymphoid neoplasms[3,4]. According to histologic features, common (pleomorphic and monomorphic),

small cell, Hodgkin lymphoma-related, lymphohistiocytic, and other less common variants have been described[5]. Clinically ALCL can be subdivided into different groups as systemic, primary cutaneous type, ALCL arising in HIV-positive patients, and ALCL occurring after another lymphoproliferative process, such as lymphomatoid papulosis, mycosis fungoides, and Hodgkin lymphoma.

A subset of ALCL harbour a genetic aberration usually the t (2;5) (p23; q35) translocation containing the anaplastic lymphoma kinase (ALK) gene at 2p23[6,7]. This translocation fuses part of the nucleophosmin (NPM) gene on chromosome 5q35 to a portion of the ALK receptor tyrosine kinase gene on chromosome 2p23, resulting in aberrant expression of the nucleolar protein nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) protein[8]. This translocation causes the dysregulation of the ALK gene by bringing it under the control of the more constitutively active NPM promoter and the activation of ALK receptor tyrosine kinase results in an unregulated mitogenic signal. Thus, the role of NPM-ALK protein in the aberrant phosphorylation of cellular elements, causing a direct oncogenic effect, is being questioned on the pathogenesis of ALCL. Three different antibodies, p80, ALK1, and ALKc can detect abnormal expression of ALK[9-11]. The monoclonal antibody, ALK1, recognises a formalin resistant epitope in both the 80-kD NPM-ALK chimeric and the 200-kD normal human ALK protein[10]. Such analysis may be of diagnostic importance, since ALCL cases with t (2;5) translocation are reported to have a good prognosis with appropriate treatment[12-15]. This report describes the histopathologic and immunophenotypical spectrum of ALCL on 6 pediatric and 34 adult ALCL cases, including its relation to ALK1 antibody besides the routine panel of antibodies against CD30, EMA, CD45, CD3, CD20, and CD15 antigens.

MATERIALS and METHODS

All cases diagnosed as ALCL at Ege University Faculty of Medicine during the last 10-year period (1991-2000) were retracted for the study, and histopathologic review and an immunohistochemical study was performed on 40 cases with available tissue specimens. The routine panel included antibodies against CD30, EMA, CD45, CD3, CD20, CD15 and ALK1 antigens; in selected cases, additional stains for CD45RO, cytokeratin, S-100, HMB-45, CD68, desmin, actin, vimentin, PLAP, and myeloperoxidase were also performed. The immunohistochemical procedure defined below was performed by using streptavidin-biotin-peroxidase (LSAB2 kit, Dako, Denmark) reaction with a series of negative controls.

Immunohistochemical Procedure

Briefly, after overnight incubation of 4 µm thick sections prepared on Poly-L-Lysine coated slides from formalin or B5-formalin fixed, paraffin embedded tissue sections (54 °C), the routine deparaffinization and rehydration steps were followed. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for five minutes and washed in distilled water. For antigen retrieval, sections were incubated in sodium citrate buffer (0.01 mol/L, 6.0 pH) for two 5 minute cycles in a household microwave oven (600 W). The sections prepared for ALK1 staining were treated with a third cycle in microwave oven. After cooling to room temperature, sections were washed twice with distilled water and once with TRIS buffer (TBS). Then, sections were incubated for 30 minutes at room temperature with primary antibodies, Ber-H2 (CD30), EMA, LCA (CD45), anti-CD3, L26 (CD20), and LeuM1 (CD15) (prediluted, Dako, Denmark), and ALK1 monoclonal antibody (David Y. Mason, Oxford, England). The incubation with primary antibodies was followed by sequential incubations for 30 minutes with biotinylated link antibody and peroxidase labelled streptavidin. A specific rabbit-anti-mouse IgM antibody was used as secondary antibody for sections stained for LeuM1. Between each step, sections were washed twice in Tris-buffered saline (TBS) solution for five minutes at room temperature. The final step for localisation of the peroxidase deposition was achieved by diaminobenzidine (Dako, Denmark) chromogenic reaction, followed by counterstaining with hematoxylin, dehydration, and mounting. Primary antibodies were omitted in negative controls and replaced by nonimmune serum.

Statistical Analysis

A multivariate analysis using Logistic Regression test and Pearson Correlation test was performed for the evaluation of the results, using SPSS for Windows 8.0.

RESULTS

A total of 40 cases of primary ALCL were diagnosed at our institution during the 10-year study period, representing 4% of all NHLs recorded and results are summarised on Table 1 and 2. The series was characterised by a bimodal age distribution in the third through fourth and seventh decades and the age range was 2 to 75 years; 34 patients were older than 17 years, with only six pediatric cases. The mean age of the study group was 34-years, 10-years in pediatric and 38-years in adult patients. Male to female ratio was 1.8. One fourth of the cases presented with primary nodal invovement and three cases were of primary cutaneous type.

The most common histopathologic finding was the infiltration of large cells with characteristic features, such as folded, indented, eccentrically located nuclei with partially dispersed chromatin and prominent nucleoli which were not inclusion-like as seen in Reed-Sternberg cells of Hodgkin lymphoma (Figure 1, 2A). These cases subtyped as pleomorphic ALCL, comprised 58% of our study group, 50% of childhood cases (n= 3) and 59% of adult patients (n= 20). The monomorphic variant with a relatively monotonous population of medium to large cells with infrequent giant cells formed 30% of all cases, 33% of pediatric (n= 2) and 29% of adult patients (n= 10). Other less common variants were two with sarcomatoid features, one small cell, one Hodgkin lymphoma-related and one lymphohistiocytic ALCL.

On immunohistochemical evaluation, all of the cases were positive for CD30 and the staining was mainly confined to cell membranes or Golgi zones (Figure 2B). Immunostaining with EMA, LCA, ALK1, CD15 was 68%, 48%, 35%, 10%, respectively, and 22 cases were of T-cell phenotype (55%) and 18 null-cell (45%).

In the ALK1-positive cases (35%), typically almost all the neoplastic cells revealed a strong staining reaction and this reaction was predominantly cytoplasmic with occasional nuclear labelling in some (Figure 2C). No staining was observed in neighbouring reactive lymphocytes and histiocytes. All of the 14 ALK1-positive cases coexpressed EMA, except one with T-cell phenotype (Table 2, case no. 26). Including the latter case, eight ALK1-positive cases were null-cell and six were T-cell. Considering only the EMA-positive 27 ALCL cases, 13 were ALK1-positive (48%). All the ALK1-positive cases presented with nodal involvement, except two pediatric extranodal cases, one presenting as a pelvic mass and the other showing involvement of a kidney.

Of the six cases representing the pediatric group and all were EMA-positive and five were ALK1-positive. The mean age of these ALK1 and EMA-positive cases was 9 (range 2 to 16). Only one of these five cases demonstrated a T-cell phenotype. Comparing with the adult cases where the ALK1 positivity is found as 26%, the frequency of ALK1 positivity in pediatric patients was statistically significantly higher representing 83% of the pediatric group (p= 0.01). EMA postivity was also statistically significantly higher in pediatric cases representing 62% and 100% of adult and pediatric cases, respectively (p= 0.04). The coexpression of ALK1 and EMA in the adult and pediatric ALCLs was 24% and 83%, respectively.

All of the four CD15-positive cases were ALK1-negative, and three coexpressed T-cell markers. Of the-

Table 1. Clinical, pathologic and immunologic features of pediatric ALCL cases

No	Age	Sex	Presentation	AKL1	CD30	EMA	LCA	CD3	CD20	CD15	Histology subtypes
1	16	M	Servical LN & lung	-	+	+	+	-	-	-	Pleomorphic
2	2	M	Servical LN	+	+	+	+	-	-	-	Pleomorphic
3	2,5	M	Kidney	+	+	+	-	+	-	-	Sarcomatoid
4	11	M	Pelvic mass	+	+	+	-	-	-	-	Pleomorphic
5	16	M	Servical LN	+	+	+	-	-	-	-	Monomorphic
6	13	F	Inguinal LN	+	+	+	-	-	-	-	Monomorphic

M: Male, F: Female, LN: Lymph node

Table 2. Clinical, pathologic and immunologic features of adult ALCL cases

No	Age	Sex	Presentation	ALK1	CD30	EMA	LCA	CD3	CD20	CD15	Histology subtypes
1	35	M	Servical LN	-	+	+	-	+	-	-	Monomorphic
2	40	F	Servical LN	+	+	+	-	-	-	-	Monomorphic
3	45	M	Servical LN	-	+	-	+	+	-	-	Pleomorphic
4	28	M	Supraclavicular LN	+	+	+	+	+	-	-	Monomorphic
5	39	F	Servical LN	+	+	+	+	+	-	-	Pleomorphic
6	27	M	Servical LN	-	+	+	-	+	-	+	Pleomorphic
7	35	M	Submandibular LN	-	+	-	-	-	-	-	Monomorphic
8	62	M	Stomach and LN	-	+	-	-	-	-	-	Pleomorphic
9	47	M	Abdominal LN	-	+	+	+	-	-	-	Pleomorphic
10	28	M	Servical LN	-	+	-	+	+	-	-	Pleomorphic
11	31	F	Inguinal LN	-	+	+	+	+	-	-	Pleomorphic
12	35	M	Servical LN	-	+	+	+	+	-	-	Monomorphic
13	52	F	Abdominal skin	-	+	-	+	+	-	-	Monomorphic
14	57	M	Servical LN	-	+	+	-	-	-	-	Pleomorphic
15	29	F	Servical LN	-	+	-	-	-	-	-	Pleomorphic
16	35	M	Servical LN	-	+	-	+	+	-	+	Pleomorphic
17	53	M	Mediastinal mass	-	+	+	-	+	-	-	Pleomorphic
18	62	F	Servical LN	-	+	+	-	+	-	-	Pleomorphic
19	43	M	Servical LN	+	+	+	-	+	-	-	Monomorphic
20	30	F	Axillary LN	-	+	-	-	+	-	-	Pleomorphic
21	38	M	Servical LN	-	+	+	+	+	-	+	Sarcomatoid
22	75	F	Inguinal LN	-	+	-	+	-	-	-	Monomorphic
23	41	M	Inguinal LN	+	+	+	+	-	-	-	HL-R
24	36	M	Servical LN	+	+	+	-	+	-	-	Lymphohistiocytic
25	21	F	Thymus & lung	-	+	-	+	+	-	-	Pleomorphic
26	67	F	Retroperitoneal LN	+	+	-	-	+	-	-	Pleomorphic
27	58	M	Inguinal LN	-	+	+	-	-	-	+	Pleomorphic
28	41	M	Forearm skin & LN	-	+	-	-	-	-	-	Monomorphic
29	42	F	Servical LN	+	+	+	+	-	-	-	Monomorphic
30	20	M	Servical LN	+	+	+	-	-	-	-	Pleomorphic
31	48	M	Axillary LN	-	+	+	-	+	-	-	Pleomorphic
32	48	F	Axillary LN	-	+	-	+	+	-	-	Small cell
33	33	F	Axillary skin	-	+	+	+	-	-	-	Pleomorphic
34	18	M	Perirectal mass	-	+	+	+	+	-	-	Pleomorphic

M: Male, F: Female, LN: Lymph node, HL-R: Hodgkin lymphoma-related

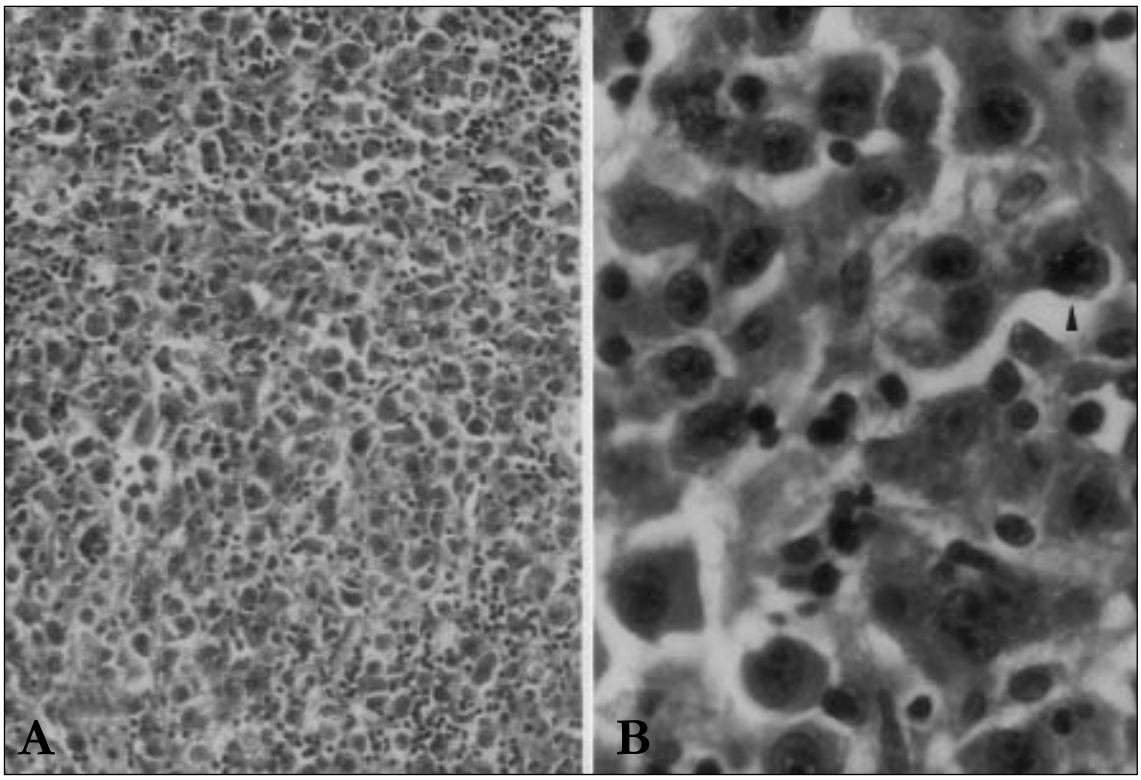

Figure 1. Anaplastic large cell lymphoma of the lymph node. A: The tumor cells are cohesive with abundant cytoplasm (H & E x 200). B: Large cells exhibit pleomorphic folded nuclei and prominent nucleoli, with frequent mitotic figures (arrowhead) (H & E x 1000).

se three cases, two were EMA-positive (Table 2, case no. 6, 21). The case with null-cell phenotype also presented EMA positivity (Table 2, case no. 27). The pediatric group did not express CD15.

DISCUSSION

ALCL accounts about 1% to 10% of NHLs in the general population and 4% of our series[10,16]. Approximately 10% to 20% of the pediatric lymphomas are reported as ALCL in different series and the incidence is lower accounting for 4% of our cases; meanwhile, the incidence among adult lymphomas is 4% within the limits of other series reporting 2% to 8%[17,18]. Children consist about 15% of our series, which is reported between 10% to 25%, thus the lower rate of occurrence among NHLs could be attributed to a higher incidence of other NHL subtypes[19]. The male to female ratio is 1.8 to 1 in our population with a male predominancy similar to other series[19-21]. Extranodal disease at diagnosis accounts for about 40% to 60% of ALCLs, 32% to 78% in children[5,19,21], and 25% to 59% in adults, which is about 25% in our patient group, especially higher in the pediatric cases[22-25].

Approximately 60% to 70% of ALCL cases are reported to have a T-cell phenotype and the ALCL series of this study presented a T-cell phenotype in 55%, the rest had no evidence of T-or B-cell associated antigen expression[5]. But, it has been postulated that many of the cases designated as null-cell ALCL, have T-cell receptor gene rearrangements, and most cases of T-or null-cell type coexpress EMA[26,27]. There are discrepancies regarding ALCL of B-cell phenotype, which was considered only in the modified Kiel scheme[2]. Recent evidence of a somatic mutation in the IgH V region genes of 90% of B-cell ALCLs suggests that the tumor cells of this entity originate from the germinal center or postgerminal center B-cells[28]. The statistically significant lack of detection of the t (2;5) translo-

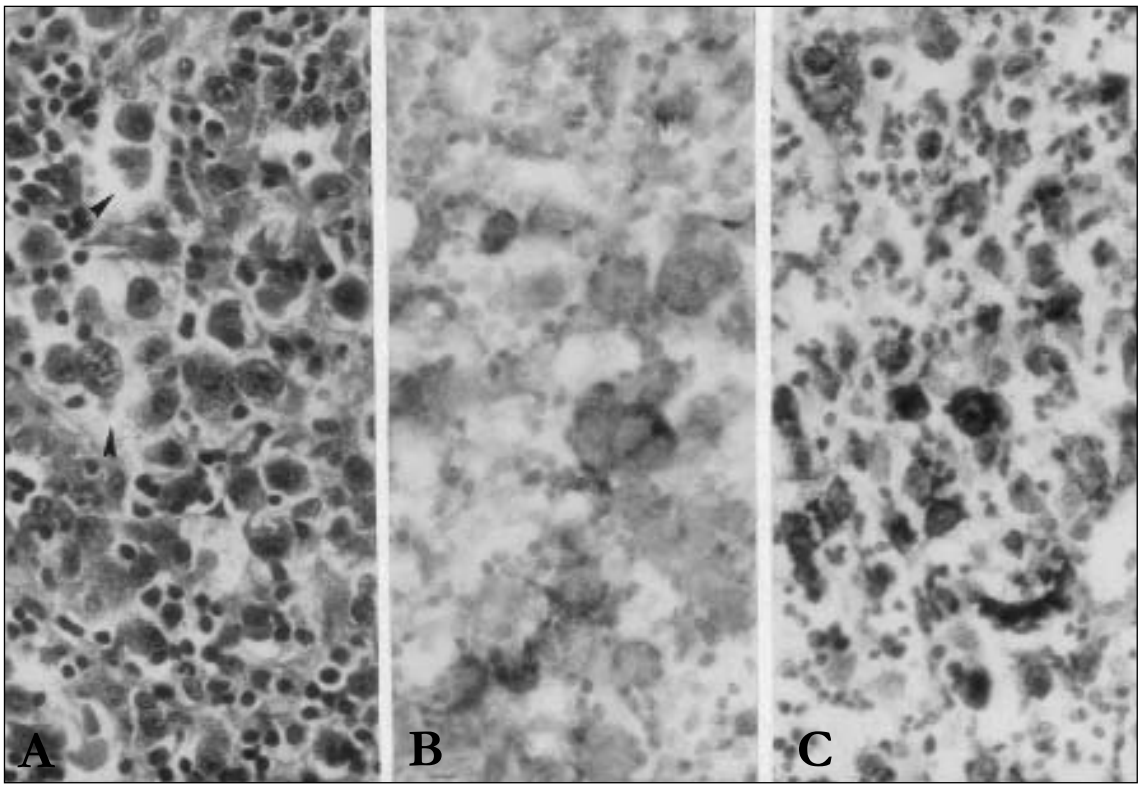

Figure 2. A: Sinusoidal infiltration of large tumor cells (arrowheads) (H & E x 400). B: The CD30 (Ber-H2) positivity in large cells (paraffin immunoperoxidase with DAB x 400). C: The nuclear and cytoplasmic reactivity of large cells with ALK1 (paraffin immunoperoxidase with DAB x 400).

cation, together with its' similarity to B-cell large cell lymphomas on clinical grounds have placed this entity within the large B-cell NHL category and only T- or null-cell phenotypes are now recognised as ALCL in both the REAL and WHO classifications[15,3,4].

The Hodgkin lymphoma-related ALCL, created as a provisional entity in the REAL classification, has been a subject of controversy and it is still not clear whether it's a specific entity or a heterogeneous group, representing the grey zone between Hodgkin lymphoma and ALCL[3,10-12,14,29-35]. A sheet-like growth pattern, sinus involvement, a high mitotic rate, lack of inclusion-like nucleoli, and admixed inflammatory cells may be used as histopathologic criteria favoring ALCL[5]. Still some comment that majority of Hodgkin lymphoma related ALCL are atypical Hodgkin lymphoma rich in neoplastic cells and the WHO classification project has been considered to include these cases under Hodgkin lymphomas with a proposed designation as ALCL-like Hodgkin lymphoma[33,4,5]. Presence of ALK1, EMA, LCA, and/or T-cell antigens in tumor cells are used as immunohistochemical clues favoring the diagnosis of ALCL; in contrast, expression of CD15 and the lack of EMA, LCA, and T-cell antigens supports the diagnosis of Hodgkin lymphoma. Cases with nodular growth, fibrous bands and capsular thickening were diagnosed as ALCL-Hodgkin's related in the presence of EMA and/or LCA positivity and T-cell phenotype[5,12]. Despite these criteria, most cases with features of ALCL and Hodgkin lymphoma are reported to be CD15-positive and p80 or ALK1-negative[36]. Approximately 15% to 20% of ALCLs in general are reported to express CD15 and this corresponds to 10% of the cases in this study and only one adult patient with nodular sclerosis-like pattern coexpressed ALK1, CD30, EMA, and LCA in the absence of CD15 (Table 2, case no. 23)[5].

The common type, representing 83% of pediatric

and 88% of adult ALCL cases in this study, is reported to consist of 33% to 60% of all ALCL cases, with pleomorphic subtype in two-thirds and monomorphic in the rest[16,22,24]. Pleomorphic and monomorphic histology represented 58% and 30% of our cases, respectively. The ALK1 positivity of pediatric and adult ALCL cases of common type was 80% and 23%, respectively. Though the lymphohistiocytic type is reported to occur mainly in young patients with favorable response to chemotherapy, there was only one adult patient in this series expressing ALK1[11,37]. Some studies on this subtype have stated that these cases must be included among peripheral T-cell lymphomas, positivity of ALK protein and detection of t (2;5) translocation in the majority support the relationship with ALCL[11,37,38].

The product of t (2;5) has been demonstrated in approximately 30% to 60% of ALCLs, ranging between 13% and 80%, by various methods such as Southern blot analysis, reverse transcriptase polymerase chain reaction, DNA polymerase chain reaction, and immunohistochemistry with antibodies p80 and ALK1 against the ALK domain of the fusion protein[5-12,14,15,29-33,36]. Correlation with clinical features has shown a higher degree of ALK gene dysregulation in younger patients and better survival[31,39]. ALK1 positivity of the adult and pediatric population in the study group is 26% and 83%, respectively, statistically significantly higher in the pediatric group (p= 0.01). When ALK gene dysregulation is correlated with histologic subtypes, more than 80% of monomorphic, and 30% to 50% of pleomorphic ALCL, and less than 10% of Hodgkin lymphoma-related ALCL have an evidence of t (2;5)[30,36]. The ALK1 positivity of the monomorphic and pleomorphic subtypes were much lower in our series, representing 50% and 22%, respectively. Comparison of the pediatric and adult groups revealed ALK positivity in 100% and 40% of monomorphic, 67% and 15% of pleomorphic subtypes, respectively (p= 0.002). Correlation of evidence of ALK gene dysregulation with immunophenotypes, reveals that 40% to 65% of T-or null-cell, 5% to 15% of B-cell, and less than 10% of CD15-positive cases express ALK; and over 70% of ALCLs with ALK gene expression coexpress EMA[5,12,31,36,40]. None of the CD15-positive cases coexpressed ALK1 protein in our series, meanwhile all of the ALK1-positive cases coexpressed EMA, except one (93%).

These results suggest that ALK1 positivity of ALCLs occur more often in younger patient population, nodal presentation, T-or null-cell phenotype, common (particularly monomorphic) morphology, and EMA coexpression; and the ALK1-positive pediatric ALCLs seem to be a homogenous group with common morphologic and phenotypic features.

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