

Clinicopathological Analysis of Systemic Anaplastic Large Cell Lymphoma

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Background : Several studies from western countries have reported variable prognoses for patients with systemic anaplastic large cell lymphoma (ALCL) depending strongly on the expression of anaplastic lymphoma kinase (ALK). However, no prognostic significance of ALK expression in Koreans was reported in a single report regarding these patients, although the number of cases was limited in that study. **Methods** : We analyzed the clinicopathological features of ALK+ ALCL and ALK- ALCL in 30 Korean patients diagnosed with primary systemic ALCL. **Results** : ALK expression was detected in 60% of all ALCL patients (18/30), and there was no statistical significance to ALK expression in overall survival. Patients with ALK+ ALCL were younger in age and had negative bcl-2 expression; these differences were statistically significant. Tumors positive for ALK protein and granzyme B expression, and negative for bcl-2 expression with a null-cell phenotype tended to have better survival outcomes, although this trend failed to reach statistical significance ($p < 0.2$), probably due to the limited number of cases in this study. **Conclusion** : ALK protein expression and the absence of bcl-2 in tumor cells tend to result in better survival despite the failure of this trend to achieve statistical significance. Further studies that examine potential pathologic prognostic factors combined with the expression of ALK and apoptotic factors such as bcl-2 are needed. Additional larger-scale studies are also needed to conclude that ALK expression has no prognostic significance among Koreans.

Key Words : Lymphoma, large-cell; Ki-1; Anaplastic lymphoma kinase; bcl-2, proto-oncogene proteins

Anaplastic large cell lymphoma (ALCL) was first described by Stein *et al.* as a large-cell non-Hodgkin lymphoma characterized by a bizarre morphology that often exhibits intrasinusoidal and paracortical infiltration of lymph nodes.¹ ALCL, as defined in the recently published WHO classification, is a distinct non-Hodgkin lymphoma with T/null-cell lineage that has anaplastic cytological features and expresses CD30.² Among non-Hodgkin lymphoma, ALCL is among those with the most favorable prognosis if chemotherapy is given. Based on genetic and immunophenotypic features combined with clinical parameters, systemic nodal anaplastic large cell lymphoma can be divided into two major subgroups. The first is anaplastic lymphoma kinase (ALK)-positive. The t(2;5)(p23;q35) is the most common translocation in ALCL, which leads to the formation of a chimeric protein, p80^{NPM-ALK}.³ The polyclonal antibody against p80^{NPM-ALK} that recognizes ALK as well as the monoclonal antibody ALK1 and anti-ALK (cytoplasmic portion) monoclonal antibody (ALKc) have made it possible to further categorize ALCL as a separate entity from Hodgkin lymphoma.⁴ Thirty to sixty percent of systemic ALCL are reported to express ALK, and ALK expression is strongly related to younger age groups and lower

international prognostic index (IPI) risk groups.⁵⁻⁷ The second group, ALK-negative ALCL, is more heterogeneous in immunophenotype and clinical behavior, and prognostic parameters are needed to determine treatment strategies for individual patients.⁸ Several studies have reported that ALK+ ALCL patients have better overall survival than patients with ALK- ALCL.^{6,7} However, no prognostic significance was attributed to ALK expression in Koreans in the only Korean report about these two groups, although the number of cases was limited.⁹ Therefore, the first aim of this study is to evaluate the prognostic significance of ALK expression in Korean patients with primary systemic ALCL.

Recent studies suggest that apoptosis may play a significant role in the better prognosis reported for patients with ALK+ ALCL.¹⁰ Conversely, there have been some reports that bcl-2 positive cases of ALCL display a statistical trend toward worse survival, in conjunction with ALK expression.¹⁰

In this study, we investigated whether ALK expression is related to clinical outcome in primary systemic ALCL with T/null phenotype among Koreans. As it relates to apoptosis, differences in expression of bcl-2 and granzyme B were also investigated and

related to ALK expression. Here, we report the clinico-pathological analysis of 30 Korean patients with primary systemic ALCL in a single institution.

MATERIAL AND METHODS

Clinical parameters

The thirty patients included in this study were diagnosed with systemic ALCL of T-cell or null-cell lineage at the Korea Cancer Center Hospital between 1991 and 2005. No cases of primary cutaneous ALCL or secondary ALCL were included. Overall, there were 20 males and 10 females, including four pediatric patients, with a median age of 40 years (range 7-70 years). No patients had received prior lymphoma therapy, and all cases were reclassified using the WHO classification. Detailed information regarding the clinical characteristics and treatment was available in 25 of 30 cases. All the patients were treated with chemotherapy, primarily using the CHOP polychemotherapeutic regimen. From the medical records of these 25 patients, the patients' information and clinical data were investigated; the pertinent data included sex, age, Ann Arbor tumor stage at presentation, B symptoms, performance state, number of extranodal sites, serum levels of lactate dehydrogenase (LDH), therapy, and the follow-up record in each patient. Patients were stratified according to the IPI. IPI was applied for five risk factors: age (<60 vs >60), tumor stage (I-II vs III-IV), performance status (0-1 vs ≥ 2), LDH level (normal vs. high), and number of extranodal involvement (0-1 vs ≥ 2). Based on these criteria, patients were divided into two risk groups: low/low-intermediate (IPI 0 to 2) and high/high-intermediate (IPI >2). Survival time was measured from the time of initial diagnosis until last follow-up or death due to disease in 29 patients (survival time: 1 month-10.5 year).

Histology and Immunohistophenotyping

Tissues from all cases were routinely formalin-fixed and paraffin-embedded. All were stained with hematoxylin and eosin (H&E), CD20 (L26; 1:100, Zymed), BSAP (pax 5; 1:50, Dako), CD3 (monoclonal; 1:50-1:100, Dako), CD45RO (UCHL-1; 1:50, Dako), CD30 (BerH2; 1:30, Dako), granzyme B (1:30, Dako), ALK-1 (anaplastic lymphoma kinase 1; 1:50, Dako), CD56 (1:50, Novo), and bcl-2 (1:50, Dako). EMA (1:50, Dako), CD15 (LeuM1; 1:20, Dako), cytokeratin (1:100, Zymed), HMB45 (1:50, Dako), vimentin (1:200, Dako), LCA (1:100, Dako), and

CD4 (1:50, Dainona) as needed. Cases were classified as T-cell lineage if they were positive for one or more of the T-cell antigens CD45RO, CD43 and CD3 and lacked reactivity for the B-cell-associated antigens CD20 and BSAP. A null-cell phenotype was assigned to cases that did not express either T- or B-cell-associated markers. All ALCL cases uniformly expressed CD30 and were negative for CD20 and CD15.

Statistical analysis

Survival curves were constructed by the Kaplan-Meier method. All p-values are based on two-tailed statistical analysis with p-value <0.05 considered significant. Correlation between the two groups was examined with the χ^2 test or Fisher's exact test. All analyses were performed using the SPSS statistical software (version 10.0).

RESULTS

Histologic findings

Twenty-seven patients were classified as common type ALCL and three belonged to the lymphohistiocytic variant. The affected lymph nodes were partially or near-totally involved in a sinusoidal pattern and/or a cohesive growth pattern of neoplastic cells. All cases contained a variable proportion of cells with eccentric, horse-

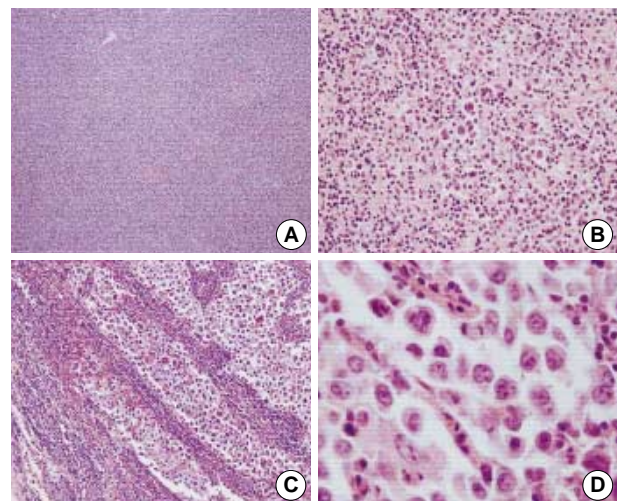


Fig. 1. Histology of common type of systemic ALCL (H&E). (A) Tumor cells replace the normal architecture of underlying lymph node. (B) The tumor cells are composed of cells with eccentric or kidney-shaped nuclei and multinucleated cells. (C) ALCL showing sinusoidal pattern. (D) Note the irregular morphology of tumor cells of ALCL.

shoe or kidney-shaped nuclei, and multinucleated cells (Fig. 1).

ALK expression and immunohistochemical findings

The immunohistochemical results of ALCL are summarized in Table 1 and Fig. 2; these results were categorized as ALK+ ALCL and ALK-ALCL. All cases were positive for CD30 and negative for CD20. Eighteen of the 30 patients (60%) were ALK+ ALCL cases according to ALK-1 antibody staining. Thirteen of 30 cases (43%) expressed the T-cell immunophenotype and the others were from the null-cell lineage. T-cell expression was more frequent in ALK- ALCL (7/12, 58%) than in ALK+ ALCL (6/18, 33%). All tested cases were negative for CD15 and CD56. ALK expression negatively correlated with bcl-2 positivity (p=

Table 1. Immunophenotypic expression according to ALK expression

	ALK-positive ALCL	ALK-negative ALCL
CD30 (n=30)*	18/18 (100%)	12/12 (100%)
CD15 (n=14)*	0/9 (0%)	0/5 (0%)
CD56 (n=30)*	0/18 (0%)	0/12 (0%)
CD20 (n=30)*	0/18 (0%)	0/12 (0%)
T cell marker (n=30)* (CD45 and/or CD3)	6/18 (33%)	7/12 (58%)
bcl-2 (n=22)*	1/11 (9%)	6/11 (55%)
granzyme B (n=19)*	8/9 (89%)	4/10 (40%)

*n, total number.

0.03); one of 11 ALK+ ALCLs (9%) expressed bcl-2 while bcl-2 was detected in 6 of 11 ALK- ALCLs (55%). On the other hand, expression of ALK+ positively correlated with granzyme B pos-

Table 2. Clinical features according to ALK expression

	ALK-positive ALCL	ALK-negative ALCL	p-value
Mean age (range)	32.8 yrs (7-55)	50.7yrs (31-70)	0.018
Male/Female (n=30)*	11/7	9/3	0.505
Stage (n=24)*			
I&II	4/15 (27%) & 3/15 (20%)	2/9 (22%) & 1/9 (11%)	0.611
III&IV	5/15 (33%) & 3/15 (20%)	1/9 (11%) & 5/9 (56%)	
IPI (n=24)*			
Low	9/15 (60%)	4/9 (44%)	0.418
Low-intermediate	0/15 (0%)	2/9 (22%)	
High-intermediate	5/15 (33%)	3/9 (33%)	
High	1/15 (7%)	0/9 (0%)	
Performance score (n=24)*			
0-2	10/15 (67%)	8/9 (89%)	0.611
3-4	5/15 (33%)	1/9 (11%)	
Number of E.I. [†] (n=24)*			
0-1	10/15 (67%)	8/9 (89%)	0.171
>1	5/15 (33%)	1/9 (11%)	
LDH (n=24)*			
Normal	5/15 (33%)	5/9 (56%)	0.132
High	10/15 (67%)	4/9 (44%)	
Death (n=29)*	4/17 (24%)	6/12 (50%)	0.237

*n, total number; [†], Number of extranodal involvement.

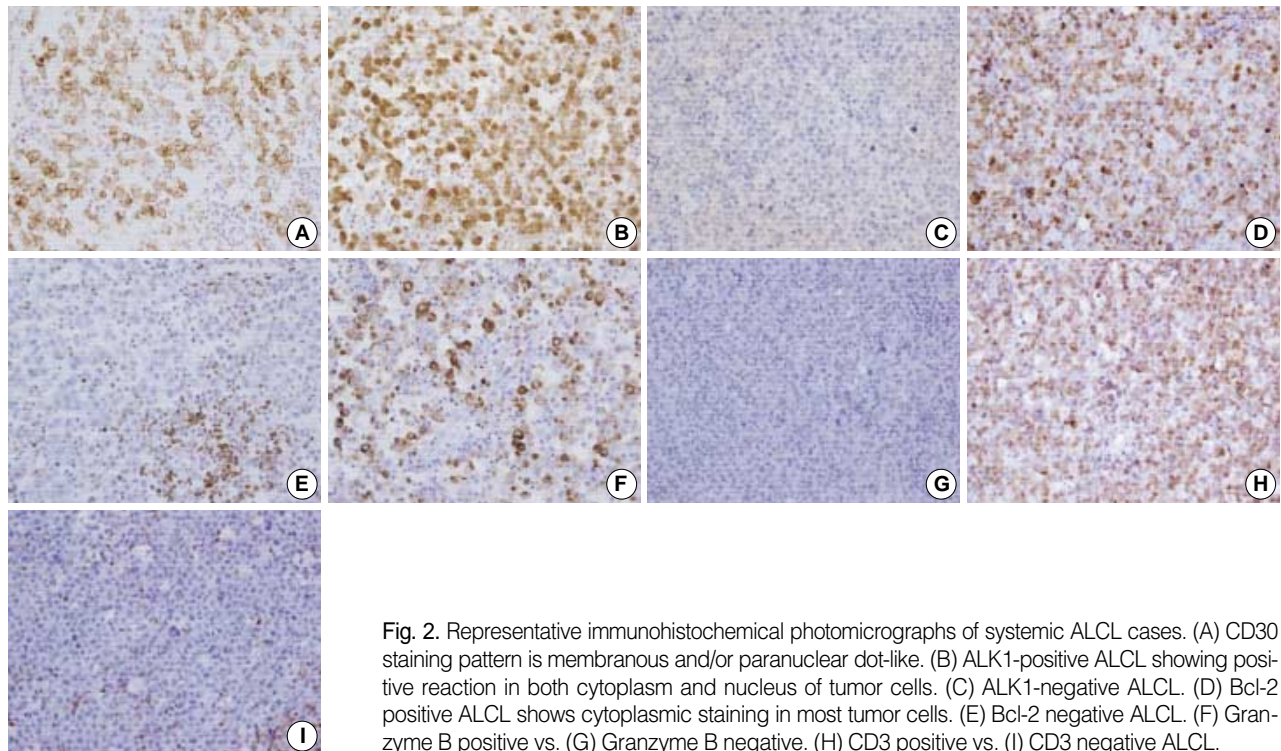


Fig. 2. Representative immunohistochemical photomicrographs of systemic ALCL cases. (A) CD30 staining pattern is membranous and/or paranuclear dot-like. (B) ALK1-positive ALCL showing positive reaction in both cytoplasm and nucleus of tumor cells. (C) ALK1-negative ALCL. (D) Bcl-2 positive ALCL shows cytoplasmic staining in most tumor cells. (E) Bcl-2 negative ALCL. (F) Granzyme B positive vs. (G) Granzyme B negative. (H) CD3 positive vs. (I) CD3 negative ALCL.

itivity. Granzyme B expression was detected more frequently in ALK+ ALCLs (8/9 cases, 89%) than in ALK- ALCLs (4/9 cases, 44%). Thus the expression of the cytotoxic molecule granzyme B was found more frequently ($p=0.204$), but *bcl-2* expression was found significantly less often in the ALK+ group compared with the ALK- group ($p=0.03$).

Clinical features and survival in ALK+ vs ALK- ALCL

A comparison of the clinical characteristics of ALK+ and ALK- cases is summarized in Table 2. Patients presenting with ALK+ systemic ALCL were significantly younger than those with ALK- systemic ALCL: the mean age of ALK+ patients was 32.8 years vs 50.7 years for ALK- patients ($p=0.02$). ALK+ ALCL frequently occurred in the first four decades of life, in contrast to ALK-

ALCL. We conducted a survival analysis including of 29 ALCL patients with clinical follow-up data, and comparing ALK+ with ALK- ALCL (Fig. 3A, B). Overall survival was 77.8% (ALK+ ALCL) vs 54.6% (ALK- ALCL). Nine of 29 ALCL died of the disease, including 4 deaths out of 17 ALK+ ALCL patients and 5 deaths out of 11 ALK- ALCL patients (Table 3). Although the ALK- group tended toward a shorter overall survival than the ALK+ group, this trend did not reach a statistical significance (Fig. 3B). There were no notable differences between the ALK+ and ALK- groups in other clinical variables (Table 2).

Other clinicopathological variables related to survival in ALCL

Apart from ALK expression, we also conducted a survival anal-

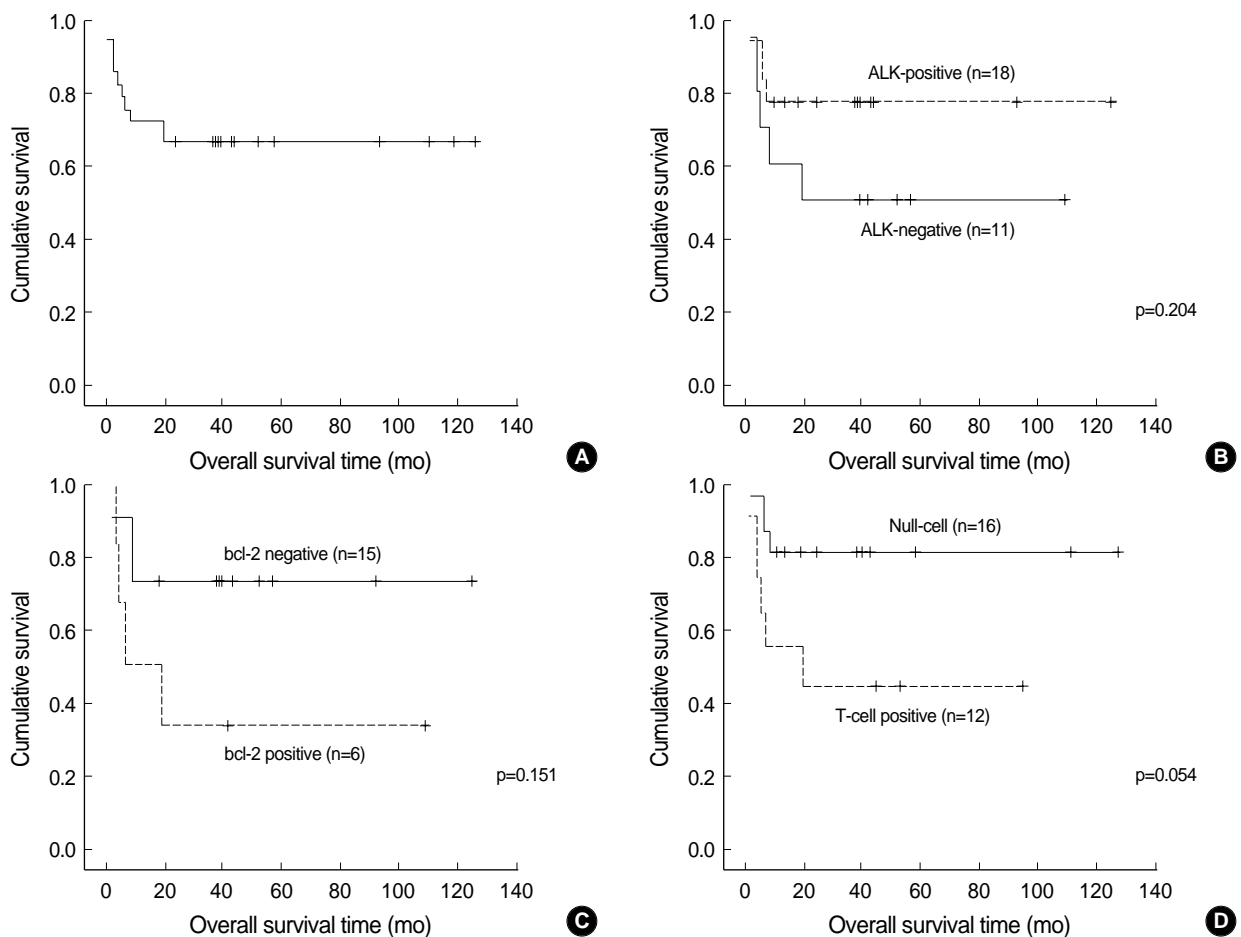


Fig. 3. Overall survival according to immunophenotype in patients with ALCL. (A) Overall survival curve of all 29 patients with systemic ALCL. (B) Comparison of overall survival according to ALK expression, including 18 ALK-positive and 11 ALK-negative cases. The ALK-positive group tends to show better survival, which has no statistical significance ($p=0.204$). (C) Comparison of overall survival according to *bcl-2* expression. Negative expression of *bcl-2* tends to show better survival, which has no statistical significance ($p=0.151$). (D) Comparison of overall survival according to T-cell (CD45RO and/or CD3) or null-cell phenotype. Null-cell phenotype tends to show better survival, which do not reach a statistical significance ($p=0.054$).

ysis utilizing other pathologic and clinical variables. Although not statistically significant probably due to the limited number of cases in this study, bcl-2 negativity, null-cell phenotype and granzyme B expression each showed a trend toward favorable outcome (Fig. 3C, D). Among clinical variables, the IPI score and the number of extranodal involvement demonstrated prognostic significance in this series of ALCL patients. Overall survival curves based on IPI score (Table 3, Fig. 4A) reveal significantly better survival for ALCL with low IPI scores (L & LI) compared with those with high IPI scores (H & HI) ($p=0.022$). ALCL with one or no extranodal involvement exhibited significantly better overall survival than those with two or more extranodal

involvement (0-1 vs ≥ 2 , $p=0.039$) (Fig. 4B). No other clinical parameters or immunohistochemical markers showed prognostic significance in this study.

DISCUSSION

In this study, we investigated whether ALK expression correlates with favorable prognosis in Korean patients with ALCL. Our series included biopsy specimens obtained from 30 untreated patients with systemic ALCL of T- or null-cell lineage. Eighteen tumors were ALK+ ALCL (60%) and 12 tumors were ALK- ALCL (40%), a prevalence that is comparable to those reported in other studies.^{6,7,11} Several studies have reported that patients with ALK+ ALCL patients have better overall survival than patients with ALK- ALCL, patients,^{5,7} whereas this study failed to show a significant difference in survival outcome between these two groups. We did find, however, that ALK+ ALCL patients in this study showed a trend toward favorable prognosis, although this trend did not reach statistical significance ($p=0.2$). This might be partially attributed to the limited number of cases examined.

Clear clinicopathologic differences were found between the ALK+ ALCL and ALK- ALCL groups in this series. Patients with ALK+ ALCL are younger, and their tumors are negative for bcl-2 expression, as has been suggested by others.^{5-7,10,12} The expressions of ALK and bcl-2 were inversely correlated in this study ($p=0.03$). Bcl-2 expression was not detected in ALK+ tumors, with the exception of one case, whereas ALK- ALCL expressed bcl-2 in 6 of 11 cases tested. Other reports have also indicated that bcl-2 expression levels are strongly related to ALK

Table 3. Prognostic significance in ALCL

	Total	Alive	Death	
ALK (n=29)*				
Positive	18	14	4	.205
Negative	11	6	5	
Bcl-2 (n=21)*				
Positive	6	4	2	.151
Negative	15	11	4	
Granzyme B (n=17)*				
Positive	11	9	2	.294
Negative	6	3	3	
Null cell type (n=28)*				
Null cell type	16	13	3	.054
T cell type	12	6	6	
International Prognostic Index (n=23)*				
Low*	15	13	2	.022
High†	8	4	4	
Number of extranodal involvement (n=23)*				
0-1	17	15	2	.039
≥ 2	6	2	4	

*, Low & Low-intermediated; †, High & High-intermediated.

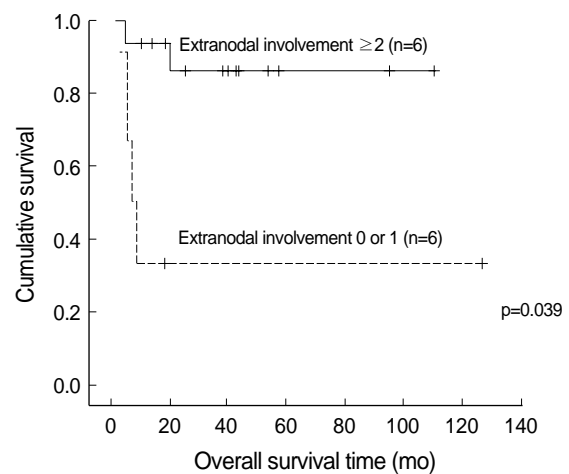
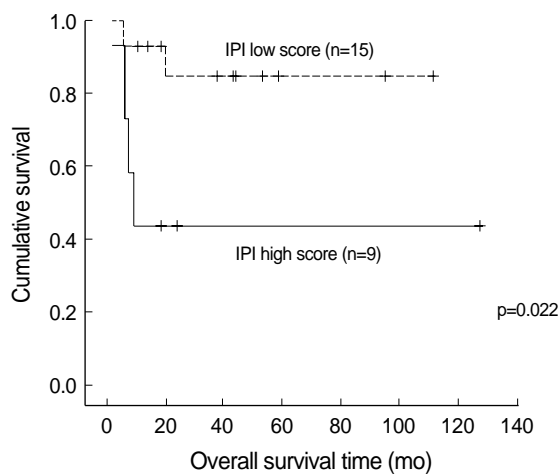


Fig. 4. Prognostic significance according to IPI score and number of extranodal involvement. (A) The IPI low score group (low & low-intermediate) shows better survival than IPI high score group (high & high-intermediate) in patients with systemic ALCL ($p=0.022$). (B) ALCL with no or one extranodal involvement show a better survival than those with two or more extranodal involvement ($p=0.039$).

status,¹² comparable to our results. Other investigators explain that the difference in survival between ALK+ and ALK- ALCL is related to differences in the levels of apoptosis inhibition.^{10,12} The absence or low expression of the anti-apoptotic protein bcl-2 has been suggested to play a probable role in the good chemotherapeutic response observed in ALK+ ALCL patients.¹² In addition to bcl-2 expression, other apoptotic mechanisms including caspases and granzyme B have also been associated with ALK expression in ALCL.¹²⁻¹⁴ The immunohistochemical study by ten Berge *et al.*¹² demonstrated significantly higher levels of active caspase 3 in the ALK+ ALCL than in ALK- ALCL. Low expression of caspase 3-positive tumor cells based on immunohistochemistry has also been reported that it is related to poor prognosis, both in Hodgkin lymphoma and ALCL.¹⁵ Granzyme B and caspases share the unique ability to cleave their respective targets on the carboxyl side of aspartate residues. This allows granzyme B to activate the stress-induced pathway via Bid or to activate caspase 3 directly.^{13,14} Such observations may explain the our results of positive relationship we observed between granzyme B and ALK expression and the negative correlation between bcl-2 positivity and ALK expression. No other clinical parameters including IPI were significantly different when comparing ALK+ ALCL and ALK- ALCL patients in this study.

Apart from ALK expression, we also analyzed other clinicopathological variables with regard to clinical outcome. In our series, IPI score and the number of extranodal involvement had a statistical significance in terms of overall survival. ALCL patients with a low IPI scores (low & low-intermediate group) had significantly better prognoses than those with IPI high scores (high & high-intermediate group) ($p=0.022$). Patients with one or no extranodal involvement are also associated with favorable prognosis ($p=0.039$). While other studies have suggested that normal serum LDH at diagnosis, an IPI score of 3 or less, and expression of ALK protein are associated with a favorable clinical outcome,⁷ we found no relationship between serum LDH level and clinical outcome in our study. In this study, no other clinical variables related to the clinical outcome of patients with systemic ALCL, except IPI score and number of extranodal involvement.

As for the relationship between pathological variables and overall survival, the null-cell phenotype and granzyme B expression, in addition to ALK expression and bcl-2 negativity, each exhibited a trend toward favorable outcome, but did not achieve statistical significance. High bcl-2 expression is reportedly related to poor clinical outcome in various type of lymphoma, including systemic ALCL, other studies have suggested that the percentage of bcl-2+ tumor cells is a strong prognostic marker, inde-

pendent of other clinical parameters,^{10,12} however, our data did not detect a statistical prognostic significance for bcl-2 expression ($p=0.151$). Inhibition of the apoptosis cascade may be responsible for resistance to chemotherapy-induced apoptosis.¹⁶⁻²⁰ Although apoptotic mechanisms have important in prognostic significance in patients with ALCL, no definite prognostic relation of granzyme B is suggested.²¹ In this study, granzyme B expression showed a tendency toward better survival, but this trend did not have statistical significance. No other distinctions have been found in cases with a T-cell versus a null-cell phenotype as defined in the recently published WHO classification.² But, patients with T-cell phenotype showed poorer prognosis compared to null-cell type, we also found a high proportion of T-cell phenotype associated with ALK- ALCL in this series.

In summary, we have failed to show statistical prognostic significance of ALK expression, in this series of 30 Korean patients with systemic ALCL. On the other hand, we have shown that a low IPI score and one or no extranodal involvement are prognostic factors that both predict a favorable clinical outcome, and that ALK protein expression and the absence of bcl-2 expression in the tumor cells tend to indicate better survival despite the failure to achieve statistical significance. Larger studies that examine potential pathologic prognostic factors in combination with the expression of ALK and apoptotic factors such as bcl-2 are needed for further analysis of these relationships. More studies with larger series of patients are also needed to conclude that there is no prognostic significance in ALK expression among Koreans.

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