■ Original Article · Diagnostic Hematology ■

공격성NK세포백혈병 진단을 위한 CD7 항원 소실의 의의

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Frequent CD7 Antigen Loss in Aggressive Natural Killer-Cell Leukemia: A Useful Diagnostic Marker

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Background : Aggressive natural killer-cell leukemia (ANKL) is a rare neoplasm characterized by systemic proliferation of NK cells. However, the differential diagnosis of NK lymphoproliferative disorders is difficult because of the absence of a distinct diagnostic hallmark. Therefore, to identify diagnostic markers for ANKL, we analyzed the clinical data and laboratory findings obtained for 20 patients with ANKL.

Methods : From January 2000 to July 2007, 20 patients were diagnosed with ANKL on the basis of bone marrow studies. We retrospectively analyzed the clinical features and laboratory findings, including the complete blood count, Epstein-Barr virus status, immunophenotype, and the cytogenetic results.

Results : The subjects included 6 women and 14 men (median age, 44 yr; range, 2-70 yr). Cytogenetic studies were performed in 18 patients, and karyotypic abnormalities were observed in 9 patients (50%). None of the cytogenetic abnormalities were constantly observed in all the patients. However, 6q abnormalities were observed in 4 patients (4/18, 22%). The immunophenotype of the leukemic NK-cells was cytoplasmic CD3+, surface CD3-, CD16/56+, CD2+, and CD5-. Notably, the CD7 antigen was absent in 10 patients (50%). When the CD7 loss was combined with cytogenetic abnormalities, clonal markers could be identified in 75% of the ANKL cases.

Conclusions : The CD7 antigen loss was frequently observed in our series of ANKL patients. In conjunction with the cytogenetic findings, this characteristic immunophenotypic finding can serve as a reliable marker for the timely diagnosis of ANKL. Therefore, immunophenotypic analysis of CD7 expression should be included in the diagnosis of NK cell neoplasms. *(Korean J Lab Med 2009;29:491-6)*

Key Words : *Aggressive natural killer cell leukemia (ANKL), Clonal marker, Immunophenotype, CD7, Cytogenetics*

INTRODUCTION

Aggressive natural killer-cell leukemia (ANKL) is a rare neoplasm characterized by peripheral blood and bone marrow involvement of malignant NK cells. This neoplasm has a rapidly progressive clinical course and a fatal outcome

[1, 2]. The clinical manifestations of ANKL include fever, marked hepatosplenomegaly, lymphoadenopathy, coagulopathy, multiorgan failure, and a strong association with Epstein-Barr virus (EBV) [2-5]. ANKL is more prevalent in Asia than in Western countries [2, 3]; the prevalence of the disease is correlated with the distribution of EBV [6, 7]. In Korea, aggressive NK/T cell lymphoma accounts for 33% of all aggressive lymphomas [8].

Since ANKL has a fulminant course, a rapid and accurate diagnosis of the disease is extremely important. However, the available information on this disease is limited and the diagnosis is even more complicated in the cases with a low number of malignant cells. There are no validated clonal markers for diagnosing ANKL, except for cytogenetic abnormalities. The absence of a diagnostic hallmark has complicated the differential diagnosis from indolent NK cell lymphoproliferative disorders, including hemophagocytic lymphohistiocytosis. ANKL and benign NK cell disorders may show similar morphologic, immunophenotypic, and genetic characteristics (large granular lymphocytes, surface CD3$^-$/CD56$^{+/-}$, and germline T-cell receptor genes), and the diagnosis of ANKL is primarily dependent on the clinical features [9]. Therefore, to identify diagnostic markers for ANKL, we analyzed the clinical data and laboratory findings obtained from bone marrow studies in ANKL patients.

MATERIALS AND METHODS

1. Patients

From January 2000 to July 2007, 20 patients were diagnosed with ANKL on the basis of the morphologic and immunophenotypic (immunohistochemical and flow cytometric) findings from bone marrow studies conducted at Samsung Medical Center, Seoul, Korea. We retrospectively analyzed the clinical data and laboratory findings of these patients, including their complete blood count (CBC), Epstein-Barr virus (EBV) status, serum lactate dehydrogenase (LDH) levels, immunophenotypes, and the cytogenetic results from the medical records. EBV was detected using an in situ hybridization (ISH) technique and/or real-time quantitative PCR assays performed using the LightCycler EBV quantification kit (Roche Diagnostics; Mannheim, Germany).

2. Immunophenotypic analysis using flow cytometry and immunohistochemistry

The leukemic cells were typically large granular lymphocytes with pale or lightly basophilic cytoplasm that contained azurophilic granules. The surface antigens were analyzed by performing 3-color flow cytometric analysis on a FACSort flow cytometer (Becton Dickinson; San Jose, CA, USA) using monoclonal antibodies for cluster of differentiation (CD) 2, surface CD3, cytoplasmic CD3, CD5, CD7, CD10, CD19, CD20, CD23, CD25, FMC7, CD16/CD56, nuclear terminal deoxynucleotidyltransferase (TdT), and secretory immunoglobulin (sIg) (Becton Dickinson; San Jose, CA, USA). The cells that showed lymphoid light scattering and moderate CD45 expression were gated on the basis of the forward and side scatter characteristics (FSC/SSC) and CD45 antigen expression. ANKL was defined by the presence of monomorphic large cells with cytoplasmic CD3 and CD16/CD56 expressions. Immunohistochemical analysis of paraffin sections was performed using lineage-specific monoclonal and polyclonal antibodies, including CD3, CD20, CD56, CD4, and CD8; these analyses were performed in the Department of Pathology. To study normal CD7 expression, we performed flow cytometric analysis of the lymphocytes obtained from 10 healthy individuals (control group).

3. Cytogenetic analysis

A conventional culture method was used to perform cytogenetic analysis of the bone marrow cells obtained from 18 ANKL patients. Karyotype analyses were performed using the G-banding technique, and at least 20 metaphases were analyzed according to the recommendations of the international system for cytogenetic nomenclature (ISCN 2005).

4. Statistics

Statistical analysis was performed using MedCalc (Med-

Calc software; Mariakerke, Belgium). Chi-square test, Fisher's exact test, and ANOVA tests were performed to determine clinical or laboratory differences between the values for the CD7$^+$ group and the CD7$^-$ group. The overall survival was estimated using the Kaplan-Meier product-limit method and calculated from the date of diagnosis to the date of death or to the last follow-up. P values less than 0.05 were considered statistically significant.

RESULTS

The clinical features and laboratory characteristics of the 20 ANKL patients are summarized in Table 1. The subjects included 6 women (30%) and 14 men (70%) (median age, 44 yr; range, 2–70 yr). The most common presenting symptom was fever (85%). Hepatomegaly (14/20, 70%), splenomegaly (12/20, 60%), and lymphadenopathy (6/20, 30%) were also frequently observed. The peripheral blood counts showed variable findings; anemia (hemoglobin <10 g/dL) was predominant in 14 patients, and thrombocytopenia (platelet <100×10^9/L) was observed in 16 patients. The median hemoglobin concentration was 9.1 g/dL (range, 6.8–17 g/dL), and the median white blood cell count was 3,200/μL (range, 380–26,440/μL). The median large granular lymphocyte count in the bone marrow was 16% (range, 3–70%), and the median platelet count was 44×10^9/L (range, 11–250×10^9/L). Eighty-five percent of the patients showed LDH levels greater than the upper limit of the normal serum LDH levels, and the median serum LDH level was 3,369 IU/L (range, 279–43,200 IU/L). EBV was detected in 15 of the 18 patients (83.3%).

The immunophenotype of the leukemic NK cells, which was determined by flow cytometry and immunohistochemistry, was cytoplasmic CD3$^+$, surface CD3$^-$, CD16/56$^+$, CD2$^+$, and CD5$^-$. The NK cells in most of the patients were CD4$^-$ (13/16, 81%) and CD8$^-$ (11/14, 79%). The CD7 antigen was absent on the NK cells in 10 patients (10/20; 50%). CD7 antigens were present on the NK cells of all the subjects in the control group.

There were no statistically significant differences in the clinical or laboratory parameters such as age (P=0.554), gender (P=1.0), hepatomegaly (P=0.141), splenomegaly (P=1.0), lymphadenopathy (P=0.303), hemoglobin level (P=0.784), WBC count (P=0.249), platelet count (P=0.266), LDH level (P=0.608), or EBV positivity (P=1.0) between the CD7$^+$ and the CD7$^-$ ANKL patients. The median survival periods of CD7$^+$ and CD7$^-$ patients were 1 and 2 months, respectively, and the difference between these values was not statistically significant (P=0.17).

Complex chromosomal abnormalities were reported in 8 patients. None of the cytogenetic abnormalities were constantly observed in all the patients; however, 6q abnormalities were observed in 4 patients (4/18, 22%). 6q deletion was detected in 3 patients and attachment of additional genetic material to 6q23 was detected in 1 patient. All the 3 cases with 6q deletion showed absence of CD7 expression. Three patients presented with chromosome 7q abnormalities, including 2 with isochromosome 7. In this study, cytogenetic studies were performed in 18 patients and 9 of these patients showed karyotypic abnormalities. When CD7 loss was combined with cytogenetic abnormalities, clonal markers could be identified in 75% of the ANKL cases, compared to the 50% detection rate when we used only the cytogenetic results.

DISCUSSION

There was a considerable immunophenotypic difference among the series of ANKL patients in our study. The CD7 antigen was absent in 10 patients (10/20; 50%), and the proportion of CD7$^-$ANKL patients in our study was much higher than that reported in previous studies. In a review of the studies published from 1966 to 2003, Ruskova et al. [3] reported that CD7 expression was absent in 35.5% of 73 ANKL patients, while Suzuki et al. [10] reported that CD7 expression was absent in only 26% of 22 Japanese patients.

The immunophenotype for ANKL originated from mature NK cells is CD34$^-$, CD33$^-$, CD56$^+$; the cells also express T-cell-associated antigens such as CD2 and CD7 [11, 12]. The 40-kD glycoprotein CD7 is one of the earliest antigens appearing during the development of the T- and NK-cell lineage [13, 14], and this antigen is considered to play a role in cell adhesion and activation [15]. CD7 expression is the

Table 1. The clinical and laboratory characteristics of 20 patients with aggressive natural killer-cell leukemia

Case no	Sex	Age (yr)	Hb (g/dL)	WBC (/μL)	PLT (×10³/μL)	BM/PB LGL morphology (%)	Survival time (months)	sCD3	cCD3	CD4	CD7	CD8	CD16/56	EBV status	Cytogenetic study results
1	F	50	8.6	6,100	100	64.1/1	2	-	+	-	+	-	+	+	45,X,-X,**i(7)(q10)**,i(17)(q10), add(19)(p13)[5]/44,idem,-18[3]/ 45,idem,add(11)(p15)[2]/44, idem,add(3)(p26),-18[1]/44,idem, add(5)(p15.3),18[1]/ 46,XX[8]
2	F	14	6.8	5,700	36	8.4/1	1	-	+	-	+	-	+	NT	47,XX,+del(X)(q13),der(11) t(1;11)(q21;q25)[10]/46,XX[10]
3	M	37	10.3	3,200	79	3.9/0	4	-	+	+	-	NT	+	+	NT
4	F	62	9.1	900	44	12/0	1	-	+	-	+	-	+	+	79-85<4n>,XXXX,-X,**add(6)(q23), add(7)(q36)**,-8,del(8)(p21),-9,add(9) (p24),-10,der(10;13)(q10;q10),-12, add(12)(p13),+13, der(13;13) (q10;q10),der(13;17)(q10;q10), -18,der(18)t(1;18)(q21;p11.2), +20, -21,+22[cp8]/46,XX[12]
5	M	27	10.9	3,200	43	35/25	2	-	+	-	-	-	+	+	46,XY[13]
6	M	42	9.5	8,700	250	70/15	0.1	-	+	-	-	-	+	+	NT
7	F	20	9.6	2,620	91	3.8/1	96*	-	+	+	-	+	+	+	46,XX[20]
8	M	21	8.6	26,440	44	22.2/10	3	-	+	NT	+	NT	+	+	46,X,-Y,dup(1)(?q12q42),+8[15]/ 46,XY[5]
9	M	2	8.4	5,150	35	9.8/1	0.17	-	+	NT	+	NT	+	NT	92,XXYY[2]/46,XY[18]
10	M	21	11.3	790	11	23.6/0	12	-	-	+	-	NT	+	-	47,XY,**der(6)del(6)(p21.3)del(6) (q23q25)**,add(11)(p11.2), der(16;19)(q10;p10),der(22) t(16;22)(p11.2;q13),+2mar[1]/ 48,idem.+r[2]/46,XY[4]
11	M	48	9.7	1,260	112	2.6/1	F/U loss	-	+	NT	-	-	+	-	46,XY[20]
12	M	45	10.8	1,490	76	29.4/4	9	-	+	-	-	NT	+	+	46,XY[2]
13	F	48	7.7	15,950	18	37.5/7	1.6	-	+	-	-	-	+	+	88,XX,-X,-X,add(4)(q34), del(4)(q22),**del(6)(q21)x2, i(7)(q10)x2**,+8,+8,-11,del(11) (q12),der(13)t(5;13)(q12;p13), i(13)(q10),-14,-18,-18[cp16]/ 46,XX[4]
14	M	45	8.7	5,000	32	20.8/30	1	-	+	-	+	-	+	+	46,XY[8]
15	M	36	8.1	380	24	21.8/0	0.23	-	+	-	+	-	+	+	46,XY[20]
16	M	48	8.2	1,570	28	8/0	3	-	+	-	+	+	+	+	46,XY[20]
17	F	45	7.9	1,050	48	18.2/0	0.6	-	+	-	-	-	+	+	46,XX,add(3)(q26),+9,der (9;14)(p10;q10)[2]/46,XX[19]
18	M	70	9	700	37	13.2/0	1.6	-	+	-	-	+	+	+	47,XY,+X,+1,der(1;16)(q10;p10), add(3)(p23),**del(6)(q21)**,add(9) (p2?2),del(14)(q24),+16,-22[5]/ 46,XY[7]
19	M	16	10.5	5,500	104	8.6/7	11	-	+	-	+	-	+	-	46,XY[20]
20	M	60	17	18,800	47	12.4/0	0.27	-	+	NT	+	NT	+	+	46,XY[20]
	M:F 14:6	44 (2-70)	9.1 (6.8-17.0)	3,200 (380-26,440)	44 (11-250)	16/1 (3-70)/ (0-30)	1.3	0/20 (0%)	19/20 (95%)	3/16 (19%)	10/20 (50%)	3/14 (21%)	20/20 (100%)	15/18 (83.3%)	

*Alive, autologous peripheral stem cell transplantation was performed twice.
Abbreviations: WBC, white blood cells; PLT, platelets; BM, bone marrow; LGL, large granular lymphocytes; NT, not tested; M:F, male-to-female ratio.

first sign of differentiation of the hematopoietic stem cells committed to the NK lineage, and the expression of this antigen correlates with the various properties of the NK cells, including increased cloning efficiency, increased sensitivity to low-dose interleukin (IL-2), increased expression of IL2Rβ, and decreased requirement for stromal contact [14]. CD7 delivers a pro-apoptotic signal during the T-cell death induced by galectin-1; therefore, CD7-negative T cells may be resistant to apoptosis [16]. Although the absence of CD7 expression is considered to be a marker for the diagnosis of T cell leukemia/lymphomas such as Sezary cell leukemia and adult T cell leukemia associated with human T-cell leukemia virus-type 1 (HTLV-1) [17], it is not a very specific marker for malignant T cells because CD7 loss is rather frequently observed in normal T-cell development [18]. However, CD7 is associated with the differentiation of NK cells from uncommitted bone marrow (BM) progenitors, and this antigen is highly expressed in normal immature and mature NK cells [14]. Hence, the absence of CD7 expression in ANKL cases can have important implications on the diagnosis of ANKL. The CD7$^+$ group and the CD7$^-$ groups did not show any significant differences in the clinical findings, including the survival rates. Therefore, the impact of CD7 expression on clinical manifestation and prognosis may be insignificant.

The differential diagnosis of ANKL and other NK/T cell malignancies were also important. Surface CD3 and CD16/56 antibodies have been analyzed together to identify and determine the proportion of NK cells (surface CD3$^-$/CD16/56$^+$). The individual expressions of CD16 and CD56 could not be defined in our cases. However, specific T cell markers such as surface CD3 and TCR are not expressed in NK cell malignancies. Furthermore, aggressive clinical features and disseminated disease are distinctive characteristics of ANKL. ANKL can develop from both CD56^{bright}/CD16$^{dim/negative}$ and CD56^{dim}/CD16^{bright} NK cells [19].

After the first report on the pathologic role of EBV in ANKL in 1989 [20], EBV detection analysis has been performed to determine its influence on the clonality of NK cell proliferation. Although EBV has been detected in the tumor tissue in approximately 85% of ANKL patients [3],

EBV infection is also associated with nasopharyngeal carcinoma, gastric cancer, and various other lymphoproliferative diseases, including Hodgkin lymphoma, extranodal NK/T cell lymphoma, Burkitt lymphoma, and post-transplant lymphoproliferative disorders [21-23]. Therefore, the diagnostic value of determining the EBV status in these diseases is still debatable.

In the absence of a definite clonal marker, cytogenetic analysis has been the most important factor for the laboratory-based diagnosis of ANKL. However, no constant cytogenetic abnormalities have been identified in ANKL cases. Aberrations involving the chromosome 6q appear to be the most frequent cytogenetic abnormality [24]. None of the cytogenetic abnormalities were constantly observed in all the patients. However, there were 6q abnormalities in 4 cases and 7q abnormalities in 3 cases, including 2 cases with isochromosome 7, which is the most frequent abnormality for hepatosplenic T-cell lymphoma [25]. These abnormalities are not unique to ANKL, and half of the ANKL cases showed a normal karyotype; therefore, the diagnosis based solely on cytogenetic analysis is not sufficient to prove clonality in NK cell proliferation. CD7 expression was absent in all 3 cases of 6q deletion and all these cases showed an aggressive clinical outcome. However, a large-scale study of the correlation between 6q deletion and CD7 loss in ANKL is required to obtain any definitive conclusions.

In the present study, we report that the characteristic immunophenotypic findings, absence of CD7 antigen can be considered as reliable diagnostic markers for the timely diagnosis of ANKL. When the CD7 loss was combined with cytogenetic abnormalities, clonal markers could be identified in 75% of ANKL cases, compared to 50% being detected only by the cytogenetic results. Therefore, immunophenotypic analysis of CD7 expression should be included in the diagnosis of ANKL. This approach would be helpful for rapid and accurate diagnosis, thereby facilitating appropriate management of ANKL patients.

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