급성백혈병에서 이상항원 공동발현의 임상적 의의

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Clinical Significance of Co-expression of Aberrant Antigens in Acute Leukemia

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Background: Acute leukemias co-expressing myeloid and lymphoid antigens but does not meet the criteria for biphenotypic acute leukemia (BAL) is common, however its clinical significance is not fully defined.

Methods: In this study, clinical features of 68 co-expressing (myeloid and lymphoid) acute leukemias diagnosed between January 2000 and December 2006 were studied and compared with those of a control group of patients (pure AML or ALL).

Results: Age, gender, initial Lactate dehydrogenase (LDH) level and cytogenetics were not different between the co-expressing group and the control group. But, the initial bone marrow blast percent was significantly higher in the co-expressing group (70% vs. 54.5%, *P*=0.003). Fifty five percent (16/29) of ALL and 30% (52/172) of AML patients showed myeloid and lymphoid markers concomitantly. The lymphoid antigen positive AML (Ly+ AML) patients showed significantly shorter survival rates than pure AML patients (4 year survival rate, 17.6% vs. 45.6%, *P*=0.002). However hematopoietic stem cell transplantation (HST) abrogated the difference (4 year survival rate, 54.7% vs. 50.6%, *P*=0.894). In ALL patients, survival rate was not affected by myeloid antigen co-expression (4 year survival rate 26.1% vs. 20%, *P*=0.954).

Conclusion: Co-expression of lymphoid markers in AML should be regarded as a poor prognostic factor and more aggressive treatment such as HST should be considered. ***(Korean J Hematol 2009;44:67-73.)***

Key Words: Acute leukemia, Immunophenotyping, Co-expression, Prognosis

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INTRODUCTION

Acute myeloid or lymphoid leukemias are classified according to their morphology and immunological markers. A proper immunophenotyping strategy allows for the diagnosis and classification of acute leukemia in over 99% of cases, but the immunological markers of myeloid and lymphoid leukemia are not totally exclusive. The co-expression of differentiation antigens associated with two or more different lineages on blast cells is a frequent finding in acute leukemia (20~50%).[1-3] Some of them are classified as biphenotypic acute leukemia (BAL) by a scoring system of the European group for immunological classification of leukemia (EGIL), which uses number and degree of specificity of the markers.[4] BAL has been established as a distinct clinico-biological entity with poor prognosis.[5,6] However, much is still to be discovered about the clinical characteristics and significance of co-expression of two or more lineage leukemia markers (lymphoid antigen positive AML, Ly+ AML; myeloid antigen positive ALL, My+ ALL) not classified as BAL. There were several reports about the clinical significance of the co-expression of leukemia markers, but most of them were not comprehensive and did not show clinical outcomes related to hematopoietic stem cell transplantation (HST).[7-9] We retrospectively analyzed the clinical characteristics and treatment outcomes of Ly+ AML and My+ ALL patients, and compared them with a historical control group who were diagnosed as single lineage acute leukemia at the same time at the same institute.

MATERIALS AND METHODS

1. Patients

A total of 209 patients who were newly diagnosed as acute leukemia between January 2000 and December 2006 in Ajou University Hospital were included in this study. Sixty eight out of 209 patients were Ly+ AML or My+ ALL at diagnosis. Clinical information including age, sex, initial lactate dehydrogenase (LDH) level, bone marrow blast percent, chromosomal abnormality, treatment and outcome was obtained from the medical records.

Table 1. Panel of antibodies used

B-lymphoid	T-lymphoid	Myeloid	Non-lineage Ag
CD10	CD2	CD13	CD34
CD19	CD3	CD14	HLA-DR
CD20	CD5	CD33	
CD22	CD7	anti-MPO*	
CD79a	Tdt†		

Abbreviations: CD, cluster of differentiation; HLA, human leukocyte antigen; anti-MPO, anti-myeloperoxidase; Tdt, terminal deoxytransferase
*anti-MPO was tested only when the leukemic cell looked like myeloid lineage, †Tdt was tested only when the leukemic cell looked like lymphoid lineage.

2. Immunophenotyping

Flow cytometry using a panel of monoclonal antibodies against lymphoid and myeloid antigens was used to determine the phenotype of leukemic blasts. The antigen panel used is given in Table 1. Peripheral blood and/or bone marrow mononuclear cells were used in assay. A marker was considered positive if expressed in $>20\%$ of blasts by flow cytometry, and myeloperoxidase was considered positive if expression was $>3\%$.

3. Cytogenetic analysis

Cytogenetic analysis was performed on heparinized unstimulated bone marrow specimens cultured for 24 to 48 hours in RPMI-1640 medium with 15% fetal calf serum using blocking with excess thymidine, arresting with colcemid, banding with 2×SSC and trypsin, and staining with Giemsa.

4. Treatment

The ALL remission induction regimen was hyper-CVAD (Odd cycles; cyclophosphamide 300 mg/m^2 bid for D1-D3, vincristine 2mg/day on D4

and D11, daunorubicin 50mg/m^2 or idarubicin 12 mg/m^2 on D4, dexamethasone 40mg for D1-D4 and D11-D14, methotrexate 1g/m^2 on D1, cytosine arabinoside 3g/m^2 every 12 hours on D2 and D3, Even cycles; methotrexate 200mg/m^2, leucovorin 15mg every 6 hours for 8 doses, cytosine arabinoside 3g/m^2 every 12 hours for 4 doses, methylprednisolone 50mg for D1-D3, CNS prophylaxis; methotrexate 12mg intrathecally on D2).[10] In AML, the standard 3/7 regimen (idarubicin 12mg/m^2 for 3 days and cytosine arabinoside 200mg/m^2 for 7 days) was used for remission induction except M3 subtype.[11] After achieving complete remission (CR), AML patients received post-remission therapy including HST or several cycles of consolidation chemotherapy stratified by chromosomal abnormality; ALL patients received intensified serial cycles of chemotherapy with cranial irradiation or intrathecal chemotherapy. In case of failure to achieve CR, re-remission induction therapy was attempted with another regimen if the patient could tolerate this.

5. Statistical analysis

Patients' age, gender, initial LDH level, bone marrow blast percent, cytogenetic data, treatment and outcomes were compared with those of the historical control group. Clinical data were analyzed using a two-tailed Student's *t*-test and χ^2 test. Survival was compared using Kaplan-Meier survival curves. A log rank test was used to examine statistical significance

RESULTS

1. Demographic and clinical characteristics

Of the 209 patients, 172 were AML, 29 were ALL and 8 were BAL with EGIL criteria [4].

Table 2. Clinical characteristics of the patients

	Historical control group	Co-expression group	*P*-value
Number	133 (120 AML, 13 ALL)	68 (52 Ly+ AML, 16 My+ ALL)	
Median Age	44 (16～83)	43 (20～80)	.895
Sex	1 : 0.77 (75/58)	1 : 0.7 (40/28)	.528
LDH	516IU/ml	619IU/mL	.380
Chromosomal abnormality	52/133 (39%)	27/68 (40%)	.897
Good	12 (9%)	10 (15%)	
Intermediate	81 (61%)	39 (57%)	
Poor	40 (30%)	17 (25%)	
Blast % in BM	54.5%	70%	.003

Abbreviations: LDH, lactate dehydrogenase; BM, bone marrow.

Table 3. Co-expression profiles of acute leukemia

AML		ALL	
Incidence of markers in AML	Ly+ AML in each FAB subtype	Incidence of markers in ALL	My+ ALL in each FAB subtype
CD19 19 (11%)	AML M0 4/9 (44%)	CD13 12 (41%)	ALL L1 5/10 (50%)
CD7 17 (10%)	AML M1 10/26 (38%)	CD33 10 (34%)	ALL L2 10/16 (63%)
CD22 14 (8%)	AML M2 21/45 (47%)	CD14 1 (3%)	ALL L3 1/3 (33%)
CD5 14 (8%)	AML M3 3/35 (9%)		
CD10 2 (1%)	AML M4 10/37 (27%)		
	AML M5 3/11 (27%)		
	AML M6 1/7 (14%)		
	AML M7 0/2 (0%)		

Abbreviations: Ly+ AML, lymphoid antigen positive AML; My+ ALL, myeloid antigen positive ALL; CD, cluster of differentiation.

BAL was excluded from this study. 68 patients (52 Ly+ AML, 16 My+ ALL) showed aberrant expression of counter part lineage surface markers. Median follow-up was 52 months (ranges 2~79 months). There was no statistically significant difference between the study group and the control group except mean bone marrow blast percent (70% vs. 54.5%, P=0.003). The clinical characteristics are listed in Table 2.

2. Immunophenotype

CD19 was the most frequent lymphoid marker in AML (11%). CD7 (10%), CD22 (8%), and CD5 (8%) were also common, whereas CD10 was expressed in only 2 patients (1%). Twenty-seven AML patients expressed B-lineage antigens and 25 patients expressed T-lineage antigens. One patient expressed both B- and T-lineage antignes. The most common FAB subtype in Ly+ AML was M2 (40%), followed by M4 (19%), M1 (19%), M0 (8%), M3 (6%), M5 (6%), and M6 (2%). The lymphoid antigen expression rate was relatively high in AML M0, M1, and M2 (44%, 38%, and 47%, respectively), relatively low in M4 and M5 (27% each), and very low in M3 and M6 (9% and 14%, respectively) (Table 3). CD34 and HLA-DR expression was significantly higher in Ly+ AML patients than the control group. (CD34: 65% vs. 41%, P=0.033, HLA-DR: 86% vs. 59%, P=0.024). In ALL, CD13 was aberrantly expressed in 41% and CD33 was expressed in 34%; 1 patient expressed CD14 (3%). The co-expression profile is given in Table 3.

3. Treatment and clinical outcome

Complete remission (CR) was achieved in 82% of Ly+ AML patients with remission induction therapy; this was not different from the control group (80%) (Table 4). Four year overall survival of Ly+ AML was shorter than the control group but not statistically significant (31.3% vs. 46.4%, P=0.059) (Fig. 1A). Treatment outcomes were not different between B-lineage antigen expressing AML and T-lineage antigen expressing AML (Data not shown). Seventeen out of 52 Ly+ AML patients received autologous or allogeneic HST. Of the seventeen patients, twelve patients had HST for poor prognostic chromosomal abnormalities at diagnosis and 5 patients for relapsed AML.

Table 4. Details of treatment in AML

	Ly− AML	Ly+ AML	P-value
Remission rate	96/120 (80%)	42/52 (81%)	.908
HST	46/120 (38%)	17/52 (33%)	.484
Auto-PBSCT	2 (4%)	2 (12%)	
Allo-BMT	26 (57%)	9 (53%)	
Allo-PBSCT	18 (39%)	6 (35%)	
Cause of death	58	31	
Disease progression	37 (64%)	19 (61%)	
Infection	17 (30%)	9 (29%)	
TRM	4 (7%)	3 (10%)	

Abbreviations: Ly− AML, lymphoid antigen positive AML; Ly+ AML, lymphoid antigen negative AML; HST, hematopoietic stem cell transplantation; Auto-PBSCT, autologous peripheral blood stem cell transplantation; Allo-BMT, allogeneic bone marrow transplantation; Allo-PBSCT, allogeneic peripheral blood stem cell transplantation; TRM, treatment related mortality.

Without HST, 4 year survival rate of Ly+ AML patients was significantly lower than that of pure AML group (17.6% vs. 45.6%, P=0.002) (Fig. 1B). Allogeneic or autologous HSCT significantly improved probability of 4year survival rate of Ly+ AML up to 54.7% (Fig. 1C); this was similar to the control group (50.6%). Four year survival rate of My+ ALL patients was 26%. The clinical features and treatment outcomes of My+ ALL were not different from those of pure ALL, regardless of HST (Fig. 1D).

DISCUSSION

The immunophenotyes of leukemic cells have been integrated into treatment outcomes of patients with acute leukemia. Acute leukemias with both lineage characteristics are classified as BAL by EGIL classification, and several studies suggested a poor clinical outcome for BAL[5,6] compared with single lineage acute leukemias. Expre-

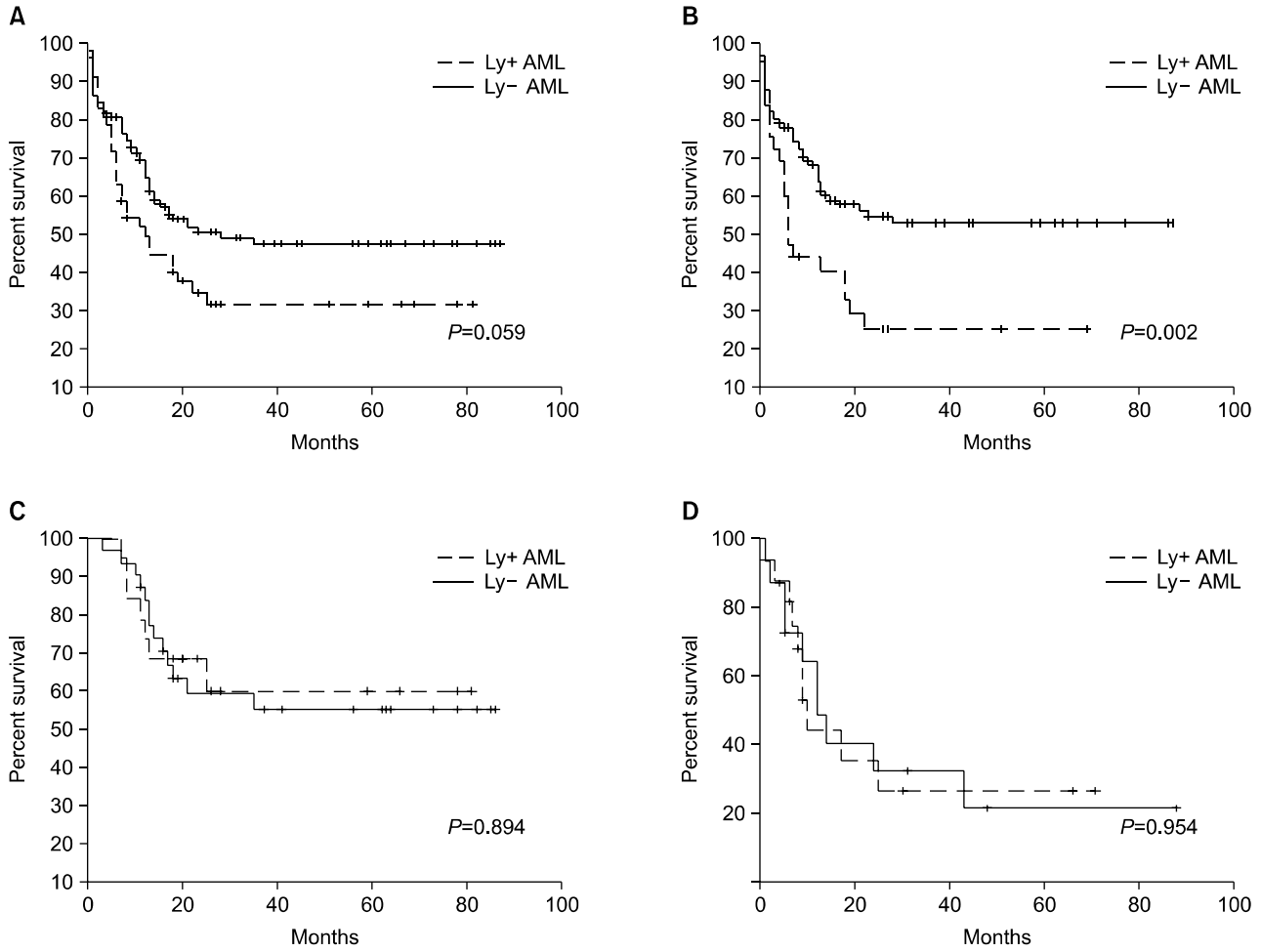

Fig. 1. Survival of patients with co-expression against matched control. (A) Overall survival of AML patients, (B) Survival of AML patients without HST. (C) Survival of AML patients with HST. (D) Overall survival of ALL patients.

ssion of CD7 on AML, not only for BAL, was also reported as a poor prognostic marker.[7] On the other hand, in one study done in the 1990s, CD2 or CD19-positive AML showed higher complete remission rates, longer time before failure and longer overall survival.[9] In this study, Ly+ AML patients (not BAL) showed a tendency toward shorter survival than the historical control group. Furthermore, subgroup analysis showed that non-transplanted Ly+ AML exhibited significantly shorter survival than the historical control group. However, in transplanted patients this survival difference was abrogated. Based on this finding, we can carefully conclude that lymphoid antigen co-expression could be considered as a poor prognostic factor in AML and more aggressive treatment such as transplantation should be considered.

Co-expression of leukemia markers may represent the prematurity of leukemic cells. In our study, lymphoid antigens were expressed more in AML M0, M1, and M2 than in M3, M4, or M5. CD34 and HLA-DR were more frequently detected in the group of patients in our study; this finding was not different from previous studies.[6,12]

Concomitant expression of counterpart antigens (lymphoid or myeloid) is common in acute leukemia and showed great variation between studies (20～50%).[1-3] Fifty five percent of all ALL patients expressed myeloid antigens and 30 percent of all AML patients co-expressed lymphoid antigens in our study. Because our antigen panel did not include leukemia markers such as

CD24, CD64, and CD117 in the routine work-up, the incidence of co-expression of leukemia markers could be underestimated; however, our result was comparable to previous studies.

Initial blast percent was significantly higher in Ly+ AML patients than in pure AML patients. Higher blast count could be translated into a low rate of complete remission (CR) achievement, more complications due to heavy treatment and the easy appearance of resistant strains. This feature may contribute to the poor clinical outcomes of Ly+ AML. However our study documented a similar rate of CR achievement with remission induction therapy.

Previous studies revealed diverse aberrant expression of leukemia markers. In ALL, CD33, CD13, and CD14 were frequently co-expressed and CD2, CD3, CD5, CD7, CD19, CD20, and CD22 were common in AML.[1,2,13,14] CD19 was the most common marker in Ly+ AML followed by CD7, CD5, CD 22, and CD10 in our study. In My+ ALL, CD13 was most common, followed by CD33 and CD14.

Cytogenetic analysis was performed on all patients. Chromosomal abnormalities were noted in 40% of co-expressing acute leukemias, and this was not different from the historical control group. There have been several reports about the frequent association of t(4;11) translocation and myeloid antigen co-expression in ALL,[15,16] and significant correlation between myeloid antigen expression and TEL-AML1 fusion (resulting from the t(12;21) (p13;q22) translocation) in childhood ALL.[17,18] In our study, no specific chromosomal abnormality was observed in co-expressing acute leukemia.

The optimal chemotherapeutic regimen for co-expressing acute leukemia is another issue. The study group received 3/7 regimen or hyper-CVAD according to the main lineage of leukemic cells without any modification; the remission rate was similar to that of the historical control group. Theoretically, chemotherapy for BAL should target both AML and ALL, but combining both lineage regimens showed high toxicities and complications.[6] A recent report showed that treatment regimens designed for ALL provide a better response rate than AML regimens in BAL[19]; however, there have been no studies on co-expressing leukemia. Because Ly+ AML showed poor outcome with an AML regimen, the addition of vincristine or corticosteroid should be investigated as part of remission induction therapy or hematopoietic stem cell transplantation should be considered in first remission.

There are limitations in concluding as to the clinical significance of the aberrant expression of counterpart lineage markers with single center data, but our study has demonstrated the possibility that acute myeloid leukemia co-expressing lymphoid markers has a different tumor cell biology, and that treatment of these patients should contain more aggressive initial therapy such as hematopoietic stem cell transplantation regardless of chromosomal study.

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요 약

배경: 급성백혈병에서 양형성급성백혈병 (Biphenotypic acute leukemia)의 진단 기준에 맞지 않는 골수성 및 림프구성 항원의 공동발현은 흔히 관찰되지만 임상적인 의미는 충분히 확립되어 있지 않다.

방법: 본 연구에서는 2000년 1월부터 2006년 12월 사이에 아주대학교병원에서 급성백혈병을 진단받은 환자들 중 골수성 및 림프구성 항원을 공동발현하는 68명의 환자들을 대상으로 대조군과 비교하여 임상적인 특성 및 치료결과를 후향적으로 분석하였다.

결과: 나이, 성별, 젖산탈수소화효소, 염색체 이상은 공동발현군과 대조군에서 차이가 없었으나 진단 당시의 골수 모세포의 비율은 공동발현군에

서 유의하게 높았다(70% vs. 54.5%, P=0.003). 급성림프구성백혈병의 55% (16/29명), 급성골수성백혈병의 30% (52/172명)에서 골수성 항원과 림프구성 항원의 공동발현을 나타냈다. 림프구성 항원을 공동발현한 급성골수성백혈병의 생존율은 골수성 항원만 발현한 급성골수성백혈병보다 낮았으나(4년 생존율, 17.6% vs. 45.6%, P=0.002) 조혈모세포 이식을 시행한 경우에는 생존율의 차이가 없었다(4년 생존율, 54.7% vs. 50.6%, P=0.894). 급성림프구성백혈병에서는 골수성 항원의 발현여부에 따라 생존률의 차이가 없었다 (4년 생존율, 26.1% vs. 20%, P=0.954).

결론: 급성골수성백혈병에서 림프구성 항원의 공동발현은 불량한 예후인자로 간주되어야 하며 이런 환자들에서는 조혈모세포이식술 등의 보다 적극적인 치료가 고려되어야 한다.

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