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Peripheral Blood Lymphokine-Activated Killer Cell Activity Can Predict Response to Conventional Chemotherapy in Aggressive Non-Hodgkin's Lymphomas

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말초혈액내의 림포카인 활성 세포(LAK)의 활성도 측정은 진행성 비호지킨 림프종에 대한 고식적 치료 반응의 예측인자임

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Abstract

Background / objective Combination chemotherapy has transformed aggressive non-Hodgkin's lymphoma from a fatal disease into one that is often curable. However, many patients still die of their disease. Patients at high risk who are not effectively treated with current regimens may benefit from new experimental approaches. To determine whether in vitro measurement of lymphokine-activated killer (LAK) activity can predict results after conventional chemotherapy, we analyzed the correlation between in vitro LAK activity and tumor response. ***Methods*** Forty-two newly diagnosed intermediate or high-grade non-Hodgkin's lymphoma patients were enrolled. LAK cells were made by incubating mononuclear cells from peripheral blood with IL-2 for 72 hours, and measured its activity against RAJI cell line by counting released 51Cr during 4 hours. In those patients who got chemotherapy, value of LAK activity as a predictor of response to conventional treatment was assessed. ***Results*** 1. The mean LAK activities of peripheral blood from patients and controls were 69.7% and 75.1%, respectively (P = 0.079) 2. An analysis of the LAK activity in patients with respect to various clinical and laboratory variables including age, sex, LDH, performance status, extranodal sites, stage, B symptom, bone marrow involvement, CD3-positive cell fraction, and CD56-positive cell fraction showed that B symptom (P = 0.036) was correlated with LAK activity. 3. Twenty-six patients were eligible to response assessment. The objective response was achieved in 23 cases (88.5%). A complete response was achieved in 15 cases (57.7%), and partial response in 8 cases (30.8%). Three cases did not respond to chemotherapy (11.5%). 4. LAK activity, B symptom, age, LDH, and IPI were predictive of complete response (P = 0.005, 0.010, 0.011, 0.011, and 0.024, respectively). ***Conclusion*** LAK activity was the best predictor of complete response to conventional chemotherapy in intermediate or high-grade non-Hodgkin's lymphomas. It means it can also be an important predictor of long-term survival. More aggressive chemotherapy including high dose chemotherapy followed by stem cell rescue is suggested for those with low LAK activities.

Key Words: Non-Hodgkin's lymphoma, Prognosis, LAK

Introduction

Non-Hodgkin's lymphoma is a highly complex and heterogenous group of malignant disease of the lymphoreticular system. Combination chemotherapy has transformed aggressive non-Hodgkin's lymphoma from a fatal disease into one that is often curable. However, many patients still die of their disease. The identification of different risk groups would also aid in the design and interpretation of therapeutic trials. Patients with a good chance at cure after standard therapy can be excluded from participating in unproved, potentially toxic trea-

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tment.[1]

In previous analyses of relatively small numbers of patients with non-Hodgkin's lymphomas, a variety of clinical characteristics were consistently associated with outcome: the age at diagnosis, the presence or absence of systemic (B) symptoms, performance status, the serum lactate dehydrogenase (LDH) concentration, the number of nodal and extranodal sites of disease, tumor size, and stages. Recently, International Prognostic Index (IPI) was developed by multicenter cooperation, and was found to be powerful prognostic predictor.[2] Unfortunately, the IPI system is also limited by several factors. In the absence of measurable biologic properties that correlate with tumor aggressiveness and response to therapy, investigators have focused on clinical, pathologic, and serologic variables that offer an indirect but reproducible manner of assessing risk and outcome. In addition to clinical factors, there have been several other factors including serum beta2-microglobulin[3] and tumor growth fraction.[4] High tumor growth fraction measured by flow cytometry or tritiated thymidine labeling is associated with a poor prognosis, but in general, these methods have been too cumbersome to be performed routinely.

Incubation of murine or human lymphocytes in the interleukin 2 for 3-4 days leads to the development of cytotoxic lymphocytes capable of killing a wide range of fresh tumor targets, but not normal cells in a 4 hour chromium release assay. These cells are called lymphokine activated killer (LAK) cells.[5] LAK cells can lyse natural killer (NK)-sensitive as well as NK-resistant tumor cells in a non-MHC-restricted manner.[6] In previous reports using RAJI cell line, a natural killer cell-resistant cell line, as a target, the difference of LAK activities between non-Hodgkin's lymphomas and normal controls was not significant.[7] There was no difference in LAK activities between stage I/II and stage III/IV non-Hodgkin's lymphomas, between stage I/II non-Hodgkin's lymphomas and normal controls, and between stage III/IV non-Hodgkin's lymphomas and normal controls.[7,8] In Hodgkin's lymphomas, LAK activities were significantly lower than controls.[9] Overall, there have been a few studies on the peripheral blood LAK activities of lymphoma patients over normal control, and the relation of LAK activity and important clinical parameters, but, to our knowledge, there is still no study on the LAK activity as a prognostic indicator in lymphomas.

The purpose of this study is to determine the LAK activity of lymphoma patients as compared with normal controls. Secondarily, we tried to determine the clinical factors affecting LAK activity. Finally, to determine whether in vitro measurement of LAK activity can predict results after conventional chemotherapy, we analyzed the correlation between in vitro LAK activity and tumor response.

For that purpose, we made LAK cells by incubation of mononuclear cells from lymphoma patients with IL-2, and measured its activity against lymphoma cell line.

Materials and Methods

1. Patients and controls

Patients were eligible if they had biopsy-confirmed non-Hodgkin's lymphoma; stage I to IV disease; and histologic features representing any intermediate-grade or high-grade disorder other than lymphoblastic lymphoma. Patients were excluded if they had any of the following: previous treatment with chemotherapy or radiotherapy; lymphoma associated with the acquired immunodeficiency syndrome; a history of neoplasm. Controls consisted of healthy donors without any immediate past history of major illness.

2. Evaluation of the patients according to clinical and laboratory finding.

Pretreatment evaluation included a history and physical examination; complete blood and differential leukocyte counts, including a search for abnormal lymphoid cells; renal- and liver-function tests; measurement of serum lactate dehydrogenase, beta2-microglobulin, calcium, uric acid and fasting blood glucose; measurement of

CD3-positive and CD3-negative/CD56-positive fraction; measurement of peripheral blood LAK activity; urinalysis; chest radiography; and abdominal and pelvic computed tomography. Bilateral bone marrow aspirates and biopsy specimens from the iliac crest were required. Cerebrospinal fluid was obtained for analysis if clinically indicated or if the bone marrow contained lymphoma cells.

3. Preparation of effector cells and target cells

Heparinized peripheral blood samples were collected from patients and controls. Mononuclear cells were separated on Ficoll-Hypaque gradient (Pharmacia, Sweden). Adherent cells were eliminated by plastic adherence for 90 minutes at 37'C in humidified atmosphere of 5% CO_2. The non-adherent peripheral blood lymphocytes were obtained by gentle washing with warm saline. The non-adherent lymphocytes (2×10^6 cells/mL) were incubated with culture media in the presence of recombinant IL-2 (50 U/mL, human IL-2, Genzyme, Cambridge, MA). After 72 hours, the effectors were washed with RPMI 1640 medium, and cytotoxicity was assessed against ^{51}Cr-labeled targets after adjustment of the cell count to 4×10^6 cells/mL.

RAJI, a human Burkitt lymphoma cell line was used as a target cell. One million cells were labeled with $Na_2{}^{51}CrO_4$ for 60 minutes at 37'C in 96-well, U-bottom tissue culture plates (Costar, Cambridge, Massachusetts). Unbound ^{51}Cr was removed by three washes with medium and cells were suspended in FCS-containing medium.

4. Cytotoxicity assay

A short-term 4 hour ^{51}Cr release assay was carried out.[10] The effector cells were added to 10^5/mL labeled targets at effector-to-target cell ratios of 40:1, and assayed for ^{51}Cr release as previously described. Triplicate cultures were set up in round-bottomed microtiter plates (Nunc, Denmark) and incubated for 4 hours at 37'C in 5% CO_2. At the end of the incubation period, 100 uL supernatant was harvested and cytotoxicity was calculated by using gamma counter (Packard Instrument Inc, IL). The results have been expressed as percent cytotoxicity.

$$\frac{\text{test cpm - spontaneous cpm}}{\text{total cpm - spontaneous cpm}} \times 100$$

Spontaneous release was determined by incubating the targets in culture medium of 10% FCS-RPMI 1640, and total release was determined by incubating targets in 1N HCl.

5. Eligibility to treatment

Patients were eligible to treatment if they had measurable, biopsy-confirmed non-Hodgkin's lymphoma; stage I to IV disease; and histologic features representing any intermediate-grade or high-grade disorder other than lymphoblastic lymphoma (i.e., patients in working formulation groups D through H and group J). There were no age restrictions. Patients were excluded if they had overt central nervous system disease, marked impairment of cardiac function, indicated by an abnormal result on multiple-gated acquisition scanning in patients with a history of such impairment; a carbon monoxide-diffusing capacity below 50 percent; or a serum creatinine concentration of 1.7 mg per deciliter (150 mol per liter) or more and a calculated serum creatinine clearance of 60 ml per minute or less.

6. Treatment modality

All chemotherapy was administered exactly as described in the original reports of the regimens. CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)[11] or CHOP-like was given in six to eight consecutive 21-day courses unless progressive disease developed. Central nervous system prophylaxis was carried out in the ProMACE-CytaBOM (etoposide, cyclophosphamide, doxorubicin, vincristine, bleomycin, methotrexate, cytosine arabinoside, prednisone),[12] as was initially recommended, but not in the CHOP group. Vincristine doses did not exceed 2.0 mg. Modification of

dosages because of hematologic or other toxicity was based on precise guidelines in the initial reports.

7. Assessment of response

Only patients treated with chemotherapy regimen with proven efficacy with intention to cure were enrolled for response analysis. If patients want to stop infusing chemotherapy, it was allowed, and if it is before analysis of response, they are excluded when analyzing the response to treatment.

All patients underwent repeat staging every 3 courses and after therapy ended. Complete remission has traditionally been defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks; remission is verified by repeating all radiographic tests previously yielding positive findings. With the advent of modern radiographic techniques such as computed tomography and magnetic resonance imaging, residual abnormalities of various sizes have frequently been detectable after treatment, making an accurate assessment of complete responses very difficult. Therefore, in this study the rate of complete response was estimated conservatively: no peripheral disease could be present, and any abnormalities detected on abdominal or chest radiography had to be less than 2.5 cm in diameter with negative Gallium scanning.[13] A partial remission was indicated by a decrease of more than 50 percent in the sum of the products of the maximal perpendicular diameters of the measured lesions, lasting at least four weeks. Disease progression was indicated by the appearance of new lesions or by a 25 percent increase in the size of preexisting lesions.

8. Statistical analysis

All 42 patients were included to analyze the factors associated with LAK activity. Only patients who got qualified chemotherapy for more than 3 months with intention to achieve cure were included in determining factors for treatment responses. The LAK activities of patients and controls were compared by student's t-test. The correlation between patients' characteristics and LAK activities and between patients' characteristics and responses were done by linear regression test. Statistical analysis was performed with SPSS version 8.0.

Results

1. Characteristics of the patients

The presenting characteristics of 42 patients with intermediate or high-grade non-Hodgkin's lymphomas were included in Table 1. Mean value of CD3-positive cell was 56.9% (SD, 12.1%) and CD3-negative/CD56-positive cell 19.0% (SD, 9.6%).

2. LAK cell activity

The mean LAK activities of peripheral blood lymphocytes from patients and controls were 69.7% (SD, 12.4%; range 48.8 to 91.2%) and 75.1% (SD, 13.7%; range 40.6 to 95.2%), respectively. The difference of LAK activity between two groups was not significant (P = 0.079) (Figure 1). An analysis of the LAK activities in patients with respect to various clinical and laboratory

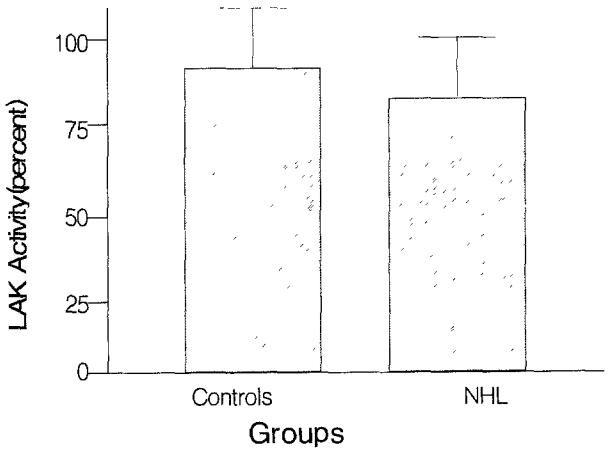

Fig. 1. Comparison of LAK activities between controls and intermediate or high-grade non-Hodgkin's lymphomas

variables including age, sex, LDH, performance status, extranodal sites, stage, B symptom, bone marrow involvement, CD3-positive cell fraction, and CD3-negative/CD56-positive cell fraction showed that B symptom (P = 0.036) was correlated with LAK activity in non- Hodgkin's lymphoma patients (Table 2).

3. Response to treatment

Fifteen out of 42 patents were not inadequate to assess response. Seven patients were under multicenter trial to assess the efficacy of new combination chemotherapy. Six patients did not finish more than 3 courses of chemotherapy required to assess the response pending patient's desire in four cases, and their medical condition (cerebrovascular accident and hepatic insufficiency, respectively) in two cases. Two patients who got salvage chemotherapy or salvage radiation therapy were excluded. One patient diagnosed as lymphoblastic lymphoma was also excluded.

Twenty-six patients were eligible to response assessment. The objective response was achieved in 23 cases (88.5%). A complete response was achieved in 15 cases (57.7%), and partial response in 8 cases (30.8%). Three cases did not respond to chemotherapy (11.5%) (Table 3).

Linear regression method revealed that LAK activity, B symptom, age, LDH, and IPI were predictive of complete response (P = 0.005, 0.010, 0.011, 0.011, and 0.024, respectively) (Table 4).

Table 1. Clinical characteristics of 42 cases of intermediate or high-grade non-Hodgkin's lymphomas

Characteristics	Number	%
Sex		
Male : Female	27 : 15	64.3 : 35.2
Age (years)		
≤ 60	27	64.3
> 60	15	35.7
Pathologic subtypes (working formulation)		
E (Diffuse small cleaved cell)	5	11.9
F (Diffuse mixed small and large cell)	10	23.8
G (Diffuse large cell)	20	47.6
H (Large cell immunoblastic)	5	11.9
I (Lymphoblastic)	1	2.4
J (Small non-cleaved cell)	1	2.4
LDH (IU/L)		
Normal	19	45.2
Abnormal (> 450)	23	54.8
Performance status (ECOG*)		
0, 1	25	59.5
2, 3, 4	17	40.5
Stage (Ann-Arbor)		
I, II	12	28.6
III, IV	30	71.4
B symptom		
Absent	18	42.9
Present	24	57.1
Bone marrow invasion		
Absent	33	78.6
Present	9	21.4
Extranodal Site(s)		
0, 1	33	78.6
> 1	9	21.4
IPI† (No. of risk factors)		
Low risk (0, 1, 2)	25	59.5
High risk (3, 4, 5)	17	40.5
Serum beta2-microglobulin‡		
Normal	19	54.3
Abnormal (> 1.9)	16	45.7

* : eastern cooperative oncology group
† : international prognostic index
‡ : data are not available in 7 cases.

Table 2. Multivariate analysis of factors affecting LAK activities of peripheral blood in intermediate or high-grade non-Hodgkin's lymphomas

Factors	B	beta	t	P value
B symptom	10.010	0.450	2.248	0.036

R^2 = 0.249, F = 3.31 (P = 0.057).
Factors analyzed were age, LDH, performance state, extranodal site, stage, B symptom, BM involvement, serum beta2-microglobulin, CD3-positive cell fraction, CD3-negative/CD56-positive cell fraction, and IPI.

Discussion

Non-Hodgkin's lymphoma is a highly complex and heterogenous group of malignant disease of the lymphoreticular system. Combination chemotherapy has transformed aggressive non-Hodgkin's lymphoma from a fatal disease into one that is often curable. However, many patients still die of their disease, underscoring the need for more accurate methods of prospectively identifying patients with different long-term prognoses. The identification of those at "high" or "low" risk could have important therapeutic implications. Patients at high risk who are not effectively treated with current regimens may benefit from new experimental approa-

ches, whereas those at low risk may do well with standard therapy but sustain severe toxic reactions without additional benefit if they are treated with experimental regimens. The identification of different risk groups would also aid in the design and interpretation of therapeutic trials.

The tumor stage of patients with aggressive non-Hodgkin's lymphoma is currently determined with the Ann Arbor classification, which was originally developed for Hodgkin's disease. This classification emphasizes the distribution of nodal disease sites because Hodgkin's disease commonly spreads through contiguous groups of lymph nodes. Since the patterns of disease spread in Hodgkin's disease and non-Hodgkin's lymphoma are different, it is not surprising that the Ann Arbor classification system is less accurate in identifying prognostic subgroups of patients with aggressive non-Hodgkin's lymphoma.[2)]

In previous analyses of relatively small numbers of patients with this disease, a variety of clinical characteristics were consistently associated with outcome: the age at diagnosis, the presence or absence of B symptoms, performance status, the serum LDH concentration, the number of nodal and extranodal sites of disease, tumor size, and the distinction between localized disease (Ann Arbor stage I or II) and advanced disease (stage III or IV). These features were thought to reflect the tumor's growth and invasive potential (LDH level, tumor stage, tumor size, number of nodal and extranodal sites of disease, and the presence or absence of bone marrow involvement), the patient's response to the tumor (performance status and status for B symptoms), and the patient's ability to tolerate intensive therapy (performance status, bone marrow involvement, and age). Many investigators identified a subgroup of clinical features that remained independently significant in multivariate analyses of their patients and used this subgroup to develop prognostic models that would predict a given patient's risk of death. Although the specific clinical features in these models differed, all models included the measurements of disease volume and extent of tumor involvement at presentation. To develop a better prognostic-factor model for aggressive non-Hodgkin's lymphoma, 16 institutions and cooperative groups in the United States, Europe, and Canada participated in the project, and developed IPI.[2)] IPI is calculated by counting the risk factors, age, LDH, performance state, extranodal site, and stage.

Unfortunately, the IPI system is limited by several factors. First, the authors treat the relative risks of the five independent variables as equal. Second, in the model the cutoff point for LDH is set at the upper limit of normal regardless of most predictive level (20 percent above normal). Third, inaccurate analysis of extranodal site lymphomas. Fourth, the authors chose 60 years of age as a cutoff point because "this dichotomy was most commonly used in previous analyses. Finally, there was no concern about immunohistochemical staining. After development of IPI scoring system, there are many reports concerning prognostic indicators.

In this study, we analyzed the value of peripheral blood LAK activity as a prognostic parameter after conventional chemotherapy. LAK cells are developed by incubation of murine or human lymphocytes in the interleukin-2 (IL-2) for 3-4 days. LAK cytotoxicity prior to IL-2 culture is low in peripheral blood, but is significantly increased following IL-2 co-culture.[14)] LAK cells have the ability to kill a wide range of cultured or fresh tumor targets, but not normal cells in a 4-hour chromium release assay.[5)] LAK cells can lyse NK-sensitive as well as NK-resistant tumor cells in a non-MHC-restricted manner. Recombinant IL-2 manifest its antitumor effects through stimulation of T cell dependent and mostly NK cell-dependent immune reactivity.[15)] IL-2 acts as a pleiotropic mediator within the immune system, having a variety of effects via specific cell surface receptors. The interaction of IL-2 with the IL-2 receptor induces proliferation and differentiation of a number of T cell subsets, and stimulates a cytokine cascade that includes various interleukins, interferons and tumor necrosis factors. Antitumor effects of IL-2 appears to be mediated by its effects on natural killer, lymphokine activated killer and other cytotoxic cells.[16)] NK cells in human peripheral

blood express Fc gamma receptor type III and initiate antibody-dependent cell-mediated cytotoxicity (ADCC) on binding to the antibody. The ADCC response requires simultaneous binding of the antigen-combining site of an antibody with the antigen and the Fc region to Fc gamma receptors on the effector cell.[6]

There have been several reports concerning LAK activities in malignant tumors from 1980's. According to the relatively recent previous studies, the overall peripheral blood LAK activity against RAJI cells was significantly lower in acute leukemia, chronic myelogenous leukemia, bladder cancer, and Hodgkin's lymphomas compared with normal controls.[9,10,17,18,19] On the contrary, difference of LAK activities between non-Hodgkin's lymphomas and normal controls was not significant,[7] and there was no difference in LAK activities according to stages in non-Hodgkin's lymphomas.[7,8]

Although there have been some reports on the LAK activities of lymphoma patients over normal control and their relation with important clinical parameters like stage, there has been no study on the value of peripheral blood LAK activities as a possible prognostic factor in lymphomas. So, it seems interesting to determine the association of clinical factors with LAK activities, and to analyze the correlation between in vitro LAK activity and tumor response to conventional chemotherapy in intermediate or high-grade non-Hodgkin's lymphoma patients.

In this study, the mean LAK activities of peripheral blood from lymphoma patients showed only a trend to be lower than normal controls. The result is similar to that of previous reports.[7,8](Fig. 1)

We analyzed the association of LAK activity in patients with various clinical and laboratory variables including age, sex, LDH, performance status, extranodal sites, stage, B symptom, and bone marrow involvement. Because most of the precursors of LAK cells are NK cells and T cells,[3,7] it is also needed to evaluate the correlation of the concentration of these precursors and LAK activities. For that purpose, we used the CD3-positive cell fraction as T cell marker, and CD3-negative/CD56-positive cell fraction as NK cell marker as CD3-negative/CD56-positive cells are mostly NK cells.[20,21] In this study, peripheral blood LAK activities were correlation with B symptom ($P = 0.036$), and there was no correlation with stages, and the latter is the same as previous reports.[7,8](Table 2)

It is critically important to select patients for intensive or experimental therapy only if they have a low chance for cure with conventional treatment. Thus some consideration will be given to pretreatment prognostic factors. To evaluate the value of peripheral blood LAK activities as a prognostic index, it was needed to restrict the patients to those who got CHOP, CHOP-like, ProMACE- CytaBOM or equivalent. According to the important phase III studies on efficacy of chemotherapeutic regimens in intermediate or high-grade non-Hodgkin's lymphomas, 2nd or 3rd generation chemotherapeutic regimens like ProMACE-CytaBOM, m-BACOD, or MACOP-B was comparable to CHOP, a 1st generation chemotherapeutic regimens.[22,23] We excluded lymphoblastic lymphoma in this study because the therapeutic plan is usually different, and it may need regimens applicable to acute lymphoblastic leukemias. They behave similarly to T-cell acute lymphoblastic leukemia. One patient with CVP (cyclophosphamide, vincristine, prednisone) was also excluded, because CVP is known to be inferior to CHOP in its efficacy in previous report. Another seven patients were also excluded because they were enrolled to multicenter clinical trial to evaluate the efficacy of THP-COP (cyclophosphamide, pirarubicin, vincristine, prednisone) regimen, and the efficacy of the regimen is unknown.

Among the twenty-six patients eligible to response assessment, complete response rate was 57.7%, and it is comparable with our recent study on efficacy of ProMACE-CytaBOM regimen in intermediate or high-grade lymphomas.[24]

We evaluated the value of LAK activity on complete response to conventional chemotherapies. We did not analyze the value of LAK activity on overall survival of non-Hodgkin's lymphoma, because it is around 55% in 5 years after treatment,[24] and it takes long time to evaluate. An important point of previous studies with

Table 3. Clinical characteristics of 26 cases of intermediate or high-grade non-Hodgkin's lymphomas enrolled to assessment of response to conventional chemotherapy

Characteristics	Number	%
Sex		
Male : Female	15 : 11	57.7 : 42.3
Age (years)		
≤ 60	19	73.1
> 60	7	26.9
Pathologic subtypes (working formulation)		
E (Diffuse small cleaved cell)	5	19.2
F (Diffuse mixed small and large cell)	3	11.5
G (Diffuse large cell)	14	53.8
H (Large cell immunoblastic)	4	15.3
LDH (IU/L)		
Normal	12	46.2
Abnormal (> 450)	14	53.8
Performance status (ECOG)		
0, 1	18	69.2
2, 3, 4	8	30.8
Stage (Ann-Arbor)		
I, II	9	34.6
III, IV	17	65.4
B symptom		
Absent	11	42.3
Present	15	57.7
Bone marrow invasion		
Absent	20	76.9
Present	6	23.1
Extranodal Site(s)		
0, 1	22	84.6
> 1	4	15.4
IPI (No. of risk factors)		
Low risk (0, 1, 2)	18	69.2
High risk (3, 4, 5)	8	30.8
Serum beta2-microglobulin*		
Normal	4	20.0
Abnormal (> 1.9)	16	80.0
Treatment regimen		
ProMACE-CytaBOM	13	50.0
CHOP	10	38.5
CHOP-like	3	11.5

ECOG, eastern cooperative oncology group; IPI, international prognostic index
*data are not available in 6 cases

doxorubicin-containing combination chemotherapy is that the same factors that predict a poor outcome also predict a low chance of achieving a complete response. So, achieving complete response is prerequisite to long term survival, and without getting complete response to 1st line chemotherapy, the prognosis is miserable.[1),2)]

According to linear regression method, LAK activity, B symptom, age, LDH, and IPI were predictive of complete response (P = 0.005, 0.010, 0.011, 0.011, and 0.024, respectively) to conventional chemotherapy for intermediate or high-grade lymphomas (Table 4). The collinearity was not significant pending collinearity test. Among them, age, B symptom, and LDH are well-known predictors of prognosis, and also they are three of five components of IPI scoring system. The most interesting one is that LAK activity was most important out of those factors in this study, and we can conclude that peripheral blood LAK activity is an independent prognostic factor in intermediate or high-grade non-Hodgkin's lymphomas.

We do not know what is the reason for the difference in effector activity of LAK cells in two response groups, complete responder and non-complete responder. In this study, there was no evidence that LAK activities are dependent upon the concentration of their precursors, NK cells and T cells. So, there may be some unknown factor affecting both responsiveness of IL-2 receptor of effector cells to IL-2, and of host lymphoma cells to conventional chemotherapies.

Our results do not have any value as a predictor of adoptive immunotherapy with IL-2[25)] or high dose chemotherapy followed by stem cell transplantation. We have a plan to evaluate whether peripheral blood LAK activity can be a predictor of survival after high dose chemotherapy in addition to conventional chemotherapy, because there is no consensus about that.

Table 4. Multivariate analysis of factors affecting complete response in intermediate or high-grade non-Hodgkin's lymphomas

Factors	B	beta	t	P value
LAK activity	-0.023	-0.588	-3.373	0.005
B symptom	0.625	0.631	2.959	0.010
Age	0.018	0.571	2.910	0.011
LDH	0.722	0.729	2.931	0.011
IPI	-0.439	-0.984	-2.522	0.024

R^2 = 0.648, F = 4.48 (P = 0.011).
Factors analyzed were age, LDH, performance state, extranodal site, stage, B symptom, BM involvement, serum beta2-microglobulin, LAK activity, and IPI. The results of collinearity statistics (VIF) were less than 10 in all factors.

Conclusion

There was no significant difference in peripheral blood LAK activities between intermediate or high-grade non-Hodgkin's lymphomas and controls. LAK activities of patients were correlated with presence of B symptoms, but not with the fraction of their suggested precursors, NK cells and T cells.

LAK activity was the best predictor of complete response to conventional chemotherapy. It means it can also be an important predictor of long-term survival given the facts that long-term survival is attained only after getting complete response, and without complete response to 1st line chemotherapy, the prognosis is miserable. As a result, in those with low LAK activity, more aggressive chemotherapy including high dose chemotherapy followed by stem cell rescue is suggested.

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국문 초록

목적 : 복합화학요법은 과거 치명적이던 진행성 비호지킨림프종을 치료가 가능하게 하였지만 아직도 많은 환자들이 이 병으로 사망한다. 만일 치료전에 고식적인 화학요법으로는 치료가 어려운 고위험군으로 판단된다면 새로운 치료방법을 고려해 볼 수 있을 것이다. 이에 따라 저자는 말초혈액의 림포카인으로 활성된 세포 (LAK)가 화학요법의 결과를 예측할 수 있는 지표로서의 가치가 있는가를 분석하였다.

방법 : 치료경력이 없는 중등도 이상의 비호지킨 림프종 환자 42명을 대상으로 하였다. LAK 활성은 말초혈액의 단핵세포를 분리하여 IL-2와 72 시간 배양한 뒤, 표적세포인 RAJI 세포주와 4 시간 동안 반응시켜 유리된 ^{51}Cr 양을 측정하였다. 복합화학요법을 받은 환자를 대상으로 LAK 활성을 포함한 여러 인자와 치료 반응과의 관계를 분석하였다.

결과 :

1. 환자와 대조군의 말초혈액 LAK 활성의 평균은 각각 69.7%와 75.1%로서 유의한 차이가 없었다 (P = 0.079).
2. 환자의LAK 활성과 임상 및 검사상의 지표들, 즉 연령, 성별, LDH, 운동능력, 림프절외 장소의 병변, 병기, B 증상, 골수 침범, CD3 양성 세포비, CD3음성/CD56양성 세포비의 상관관계를 분석한 결과, LAK 활성은 B 증상과 유의한 상관관계가 있었다 (P = 0.037).
3. 26명에서 치료에 대한 반응을 분석한 결과, 전체 관해율이 88.5% (완전관해율 57.7%, 부분관해율 30.8%)였다. 3예 (11.5%)는 치료에 반응이 없었다.
4. LAK 활성, B 증상, LDH, IPI등이 완전관해를 예측할 수 있는 지표였다 (각각 P = 0.005, 0.010, 0.011, 0.011, 0.024).

결론 : LAK 활성은 중등도 이상의 비호지킨 림프종에 대한 고식적 화학요법의 효과를 가장 잘 예측하는 지표였는데, 이에 따라 LAK 활성은 장기 생존율을 예측케 하는 가치가 있다. LAK 활성이 낮은 군에서는 고용량 화학요법 등 강력한 치료가 필요하리라 생각된다.