원저
Lab Med Online
Vol. 9, No. 3: 126-132, July 2019
https://doi.org/10.3343/lmo.2019.9.3.126

진단혈액학 LMO

말초혈액에서 자동혈구분석기의 Large Unstained Cell, Blast Suspect, Delta Neutrophil Index II의 급성백혈병 재발예측에서의 유용성

Large Unstained Cell, Blast Suspect and Delta Neutrophil Index II Analyzed with Automated Hematology Analyzer as Parameters for the Prediction of Acute Leukemia Relapse

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Background: Here we investigated the clinical utilities of blast suspect, large unstained cell (LUC), delta neutrophil index II (DN II), and delta neutrophil index I (DN I), analyzed in peripheral blood samples with automated hematology analyzers to predict the relapse of acute leukemia.
Methods: We retrospectively reviewed the medical records of 112 patients, including 56 patients with acute leukemia relapse and 56 controls. Blast suspect, LUC, DN II, and DN I were compared between the control and leukemia relapse groups.
Results: Significant differences in blast suspect ($P<0.001$), LUC ($P<0.001$), DN II ($P<0.001$), and DN I ($P=0.002$) were observed between the leukemia relapse and control groups. The areas under the curve (AUC) value was 0.927 for blast suspect (95% confidence interval [CI]: 0.875-0.978, $P<0.001$), 0.868 for LUC (95% CI: 0.794–0.941, $P<0.001$), and 0.900 for DN II (95% CI: 0.841–0.960, $P<0.001$). Logistic regression analysis for the prediction of leukemia relapse revealed odds ratio values of 1.52 (95% CI: 1.26–1.96, $P=0.0002$) for blast suspect, 1.66 (95% CI: 1.27–2.42, $P=0.0019$) for LUC, 1.16 (95% CI: 1.08–1.29, $P=0.0014$) for DN II, and 1.05 (95% CI: 1.01–1.13, $P=0.0845$) for DN I.
Conclusions: Multiple parameters provided by automated blood cell analyzers may serve as powerful ancillary tools for the prediction and diagnosis of leukemia relapse.

Key Words: Acute leukemia; Automated hematology analyzer; Large unstained cell; Blast suspect; Delta neutrophil index

INTRODUCTION

The prognosis of acute leukemia has significantly improved since the inclusion of hematopoietic stem cell transplantation (HSCT) as an integral part of its treatment [1]. However, the relapse of acute leukemia after HSCT poses a great risk to the patients and is often associated with poor prognosis [2, 3]. Automated hematology analyzers evaluate multiple morphological and quantitative parameters such as nucleated red blood cells (NRBCs), large platelets, and left shift in addition to routine cytograms. ADVIA 2120i (Siemens Healthcare Diagnostics, Deerfield, IL., USA) provides multiple morphology flags such as large unstained cell (LUC), blast suspect, and immature granulocytes to assist in the identification of abnormal samples. LUC is defined as a large peroxidase-negative cell that may not be classified as a reactive lymphocyte, blast, or other cell type [4]. Blast suspect is a cell with dispersed chromatin as per the basophil/lobularity method of ADVIA 2120i (Siemens Healthcare Diagnostics). Delta neutrophil index (DN I) is a value obtained from the automatic calculation of immature

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Received: June 29, 2018
Revision received: November 10, 2018
Accepted: November 25, 2018

This article is available from http://www.labmedonline.org

granulocyte count, and is the difference in the count of white blood cells (WBCs) from the peroxidase channel and basophil/lobularity method [5]. DN II is the sum of DN I and LUC values. DN I and LUC are valuable tools that allow discrimination between acute promyelocytic leukemia (APL) and other types of leukemia [6]. In the present study, we investigated the clinical utilities of blast suspect, LUC, DN II, and DN I in peripheral blood samples to predict acute leukemia relapse.

MATERIALS AND METHODS

This retrospective observational study assessed the data obtained from 112 patients, including 56 patients with acute leukemia relapse, 29 with non-relapsed acute leukemia, and 27 with normal bone marrow between March 2012 and September 2017 at the Kyungpook National University Hospital. The patients with non-relapsed acute leukemia were either AML patients who achieved complete remission and underwent allogeneic HSCT or ALL patients in complete remission. Relapse of acute leukemia was reported in response to blast observed in more than 20% of all nucleated cells in the bone marrow aspirate smear except for those with AML with t(15;17)(q22;q21), t(8:21)(q22;q22.1), and inv(16)(p13.1q22).

Data were retrospectively collected from the medical records, and the following parameters were reviewed at relapse or follow-up: age, gender, complete blood cell count (CBC), WBC differential count, LUC, blast suspect, DN II, DN I, French-American-British (FAB) subtype, overall survival (OS) time, date of bone marrow aspiration, and date of allogeneic HSCT.

ADVIA 2120i (Siemens Healthcare Diagnostics) automatic blood analyzer was used for the calculation of CBC, LUC, blast suspect, DN II, and DN I. This analyzer is based on a flow cytometry method and employs two independent channels to analyze WBCs. One of these is a peroxidase channel containing 4-chloro-1-naphthol as the substrate for myeloperoxidase (MPO) from granulocytes that forms a black precipitate within cells. As the stained WBCs pass through the flow cell, a tungsten-halogen light source records and measures light scatter (size) and absorbance (staining intensity). The second channel is a basophil/lobularity channel that uses a 670 nm laser diode for the measurement of forward scatter (size) and side scatter (nuclear complexity). DN I was calculated as per the following formula: DN I (%) = (neutrophil subfraction and eosinophil subfraction assayed in peroxidase method)–(polymorphonuclear subfraction measured in basophil/lobularity method). LUC was defined by measuring the cells in the upper-left corner in the peroxidase method. DN II is the sum of DN I and LUC. Blast suspect was evaluated by measuring the cells in the blast area using basophil/lobularity method.

Statistical analysis was performed using R (http://www.r-project.org) and web-r (http://web-r.org). Continuous variables are presented as medians with interquartile range upon violation of the assumption of normality or as mean with standard deviation. Mann-Whitney *U* test or *t*-test was employed for comparison of continuous variables between the relapse and control groups. Logistic regression analysis was carried out to evaluate the relative prediction effect of LUC, blast suspect, DN II, and DN I for the detection of leukemia relapse in peripheral blood samples. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic performance of each parameter, while Kaplan–Meier OS analysis was carried out to estimate the distribution of OS. OS was measured from the date of diagnosis to the date of death or last follow-up. Statistical significance was set at $P<0.05$.

RESULTS

The clinical characteristics of the patients from leukemia relapse and control groups are summarized in Table 1. The data from a total of 112 cases including acute leukemia relapse, non-relapsed acute leukemia, and normal bone marrow were collected. Of the patients from leukemia relapse group, 40 and 14 were diagnosed with AML and ALL, respectively. In the control group, 14 and 12 patients were diagnosed with AML and ALL, respectively.

The most common FAB subtype among patients with AML relapse was AML M2 (32.2%).

The mean age was 53.1 ± 14.0 and 49.7 ± 15.6 years for the subjects from relapse and control groups, respectively. The relapse group comprised 25 women and 31 men, and the control group included 23 women and 33 men. The CBC results for the patients with relapse revealed the increase in the prevalence of anemia, leukocytosis, and thrombocytopenia as compared with the subjects from the control group (Table 1). We observed significant differences in blast suspect ($P<0.001$), LUC ($P<0.001$), DN II ($P<0.001$), and DN I ($P=0.002$) between the leukemia relapse and control groups and their median values (interquartile range)

Table 1. Clinical Characteristics of 112 patients

	Control (N=56)	Relapse (N=56)	*P*
Age (yr)	49.7 ± 15.6	53.1 ± 14.0	0.226
Sex			
F (%)	23 (41.1)	25 (44.6)	
M (%)	33 (58.9)	31 (55.4)	
BM blast (%) [IQR]	1.0 [0.6–1.4]	61.7 [44.0–85.8]	<0.001
PB blast (%) [IQR]	0.0 [0.0–0.0]	22.0 [6.5–60.0]	<0.001
Hb (g/dL)	12.3 ± 2.2	10.5 ± 2.1	<0.001
WBC (×10^9/L) [IQR]	5.8 [4.6–7.4]	6.3 [2.4–17.0]	0.509
ANC (×10^9/L) [IQR]	3.3 [2.3–4.6]	1.8 [0.6–4.6]	0.017
PLT (×10^9/L) [IQR]	183.0 [146.0–232.0]	45.0 [25.0–83.0]	<0.001
Subtype (%)			
N	27 (48.2)	0 (0.0)	
ALL	12 (21.4)	14 (25.0)	
ALL L1	3 (5.4)	1 (1.8)	
ALL L2	7 (12.5)	13 (23.2)	
ALL L3	2 (3.6)	0 (0.0)	
AML	14 (25.0)	40 (71.4)	
AML M0	0 (0.0)	2 (3.6)	
AML M1	1 (1.8)	0 (0.0)	
AML M2	3 (5.4)	18 (32.2)	
AML M3	1 (1.8)	2 (3.6)	
AML M4	6 (10.7)	4 (7.1)	
AML M4Eo	1 (1.8)	4 (7.1)	
AML M5a	0 (0.0)	2 (3.6)	
AML M5b	2 (3.6)	6 (10.7)	
AML M6	0 (0.0)	1 (1.8)	
AML M7	0 (0.0)	1 (1.8)	
MPAL	2 (3.6)	2 (3.6)	
T	1 (1.8)	0 (0.0)	

Data are presented as mean ± standard deviation or median [IQR].
Abbreviations: IQR, interquartile range; F, female; M, male; BM, bone marrow; PB, peripheral blood; DN I, delta neutrophil index I; DN II, delta neutrophil index II; LUC, large unstained cell; WBC, white blood cell; PLT, platelet; N, normal; T, T lymphoblastic leukemia; MPAL, mixed phenotype acute leukemia; Hb, hemoglobin.

Table 2. AUC of blast suspect, LUC, DN II, and DN I

Parameter	AUC (95% CI)	Cutoff (%)	Sensitivity (%)	Specificity (%)	*P*
Blast suspect	0.927 (0.875–0.978)	5.5	82.1	96.4	<0.001
LUC	0.868 (0.794–0.941)	3.9	83.9	89.3	<0.001
DN II	0.900 (0.841–0.960)	6.8	83.9	85.7	<0.001
DN I	0.670 (0.570–0.771)	2.2	57.1	76.8	0.639

Abbreviations: DN I, delta neutrophil index I; DN II, delta neutrophil index II; LUC, large unstained cell; AUC, area under the curve; CI, confidence interval.

Table 3. Results of univariate logistic regression analysis

Parameter	OR	95% CI	*P*
Blast suspect	1.52	1.26–1.96	0.0002
LUC	1.66	1.27–2.42	0.0019
DN II	1.16	1.08–1.29	0.0014
DN I	1.05	1.01–1.13	0.0845

Abbreviations: DN I, delta neutrophil index I; DN II, delta neutrophil index II; LUC, large unstained cell; CI, confidence interval; OR, odds ratio.

Table 4. Comparison of parameters between control and relapse groups with less than 5% blasts in the peripheral blood

Parameter	Control (N=9)	Relapse (N=9)	*P*
BM blast (%)	1.0 (0.6–1.3)	47.9 (45.0–60.2)	<0.001
PB blast (%)	0.0 (0.0–0.0)	1.0 (1.0–2.0)	0.002
Hb (g/dL)	11.5 ± 2.6	10.7 ± 1.5	0.440
WBC (×10^9/L)	5.5 (4.5–5.9)	2.1 (2.0–3.9)	0.038
ANC (×10^9/L)	3.6 (3.2–4.5)	1.3 (0.7–2.4)	0.050
PLT (×10^9/L)	172.6 ± 53.5	68.4 ± 71.0	0.003
Blast suspect (%)	0.5 (0.4–1.1)	2.0 (1.5–5.8)	0.019
LUC (%)	1.9 (0.9–3.5)	4.8 (2.6–5.6)	0.085
DN II (%)	0.9 (0.5–2.6)	11.1 (5.9–13.3)	0.024
DN I (%)	−0.9 (−1.6–0.3)	4.5 (−1.1–6.3)	0.102

Abbreviations: BM, bone marrow; PB, peripheral blood; WBC, white blood cell; ANC, absolute neutrophil count; PLT, platelet; LUC, large unstained cell; DN II, delta neutrophil index II; DN I, delta neutrophil index I; Hb, hemoglobin.

were 12.8% (7.0–36.8), 11.2% (4.7–52.5), 25.4% (9.6-61.0), and 2.8% (–1.0–6.0), respectively, in patients with leukemia relapse and 1.0% (0.4–2.1), 2.2% (1.4–3.2), 2.2% (–0.2–5.0), and –0.6% (–2.3–2.0) respectively, in those from the control group (Fig. 1).

In ROC curve analysis, all parameters except DN I showed good diagnostic performance. Blast suspect showed 82.1% sensitivity and 96.4% specificity at a cutoff value of 5.5%, while 83.9% sensitivity and 89.3% specificity was reported for LUC at a cutoff value of 3.9%. DN II showed 83.9% sensitivity and 85.7% specificity at a cutoff value of 6.8%, and the sensitivity and specificity of DN I at a cutoff value of 2.2% were 57.1% and 76.8%, respectively. The value of AUC was 0.927 for blast suspect (95% CI: 0.875–0.978, $P<0.001$), 0.868 for LUC (95% CI: 0.794–0.941, $P<0.001$), 0.900 for DN II (95% CI: 0.841–0.960, $P<0.001$), and 0.670 for DN I (95% CI: 0.570–0.771, $P=0.639$) (Table 2).

The median follow-up time for the patients with leukemia relapse was 16 months (range 2–86 months), and 31 patients (59.6%) died. In Kaplan–Meier survival analysis of blast suspect, LUC, DN II, and DN I, the patients with the values of blast suspect, LUC, and DN II higher than the third quartile showed shorter OS than those with the values in the first to third quartile. However, no statistical significance was observed for the survival analysis with DN I (Fig. 2).

To identify the values of blast suspect, LUC, DN II, and DN Iessential for the prediction of leukemia relapse, we determined the

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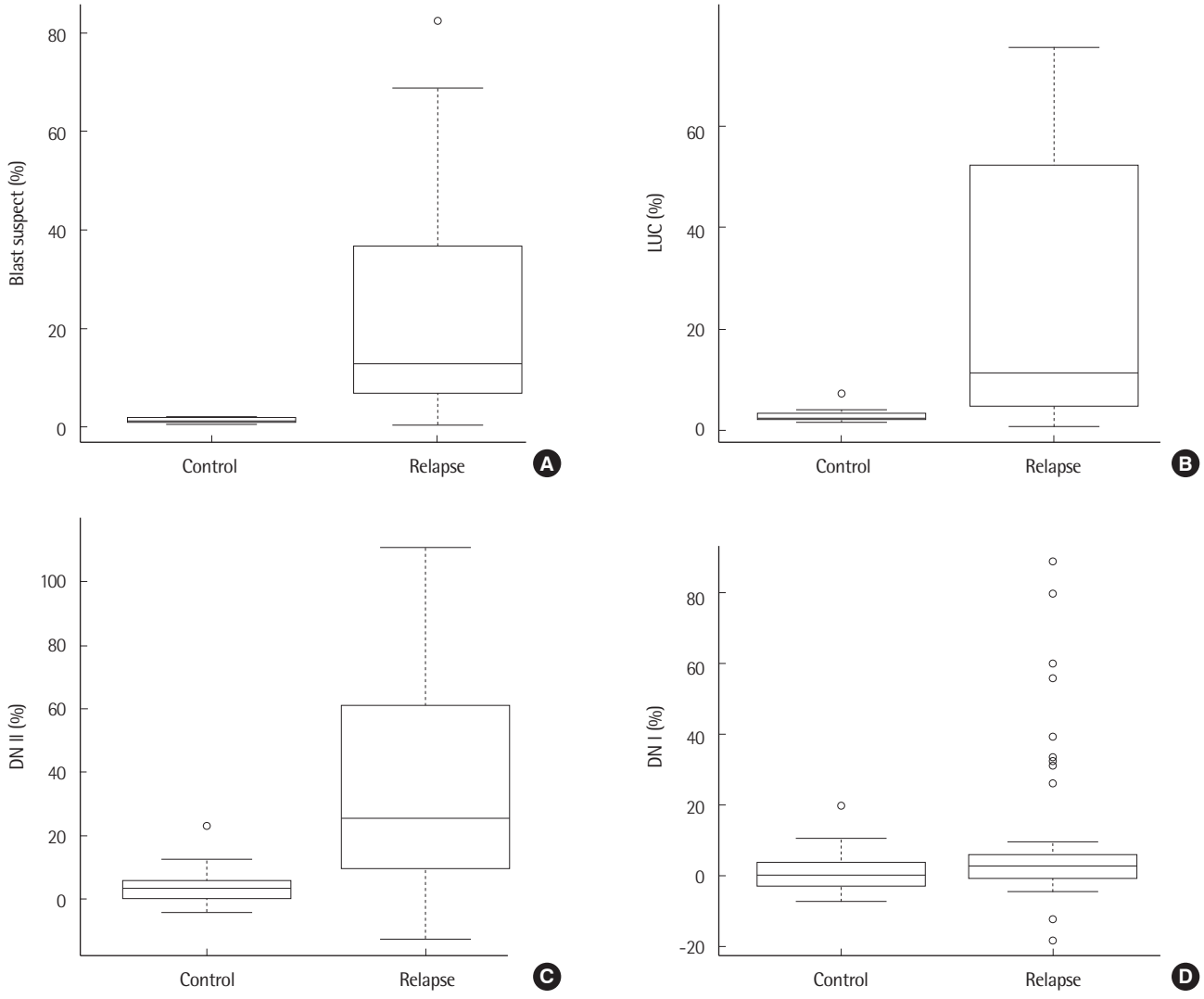

Fig. 1. Box plot of blast suspect (A), LUC (B), DN II (C), DN I (D) in control and leukemia relapse groups.

odds ratio (OR) by logistic regression analysis (Table 3). Univariate logistic regression analysis for the prediction of leukemia relapse revealed an OR value of 1.52 (95% CI: 1.26–1.96, $P=0.0002$) for blast suspect, 1.66 (95% CI: 1.27–2.42, $P=0.0019$) for LUC, 1.16 (95% CI: 1.08–1.29, $P=0.0014$) for DN II, and 1.05 (95% CI: 1.01–1.13, $P=0.0845$) for DN I.

Nine patients showed a blast percentage of less than five in the peripheral blood smear in response to leukemia relapse. In the control group, nine patients were randomly selected from those with normal bone marrow. To investigate whether blast suspect, LUC, DN II, and DN I serve as useful tools for the discrimination of leukemia relapse in cases when blast percentage was low in the peripheral blood, we compared leukemia relapse and control groups. Only blast suspect and DN II showed significantly different values with $P<0.05$ (Table 4).

DISCUSSION

Significant improvements have been reported in the treatment of acute leukemia over the last decade, as HSCT can provide cure and long-term survival in 35–40% of patients with adult leukemia [1]. However, leukemia relapse often leads to poor prognosis, and about 20–70% of patients die regardless of pre-transplant disease status, cytogenetic subtype, patient and donor age, and chemotherapy regimen [2, 3]. Although the detection of minimal residual disease with flow cytometry is a reliable method to predict leuke-

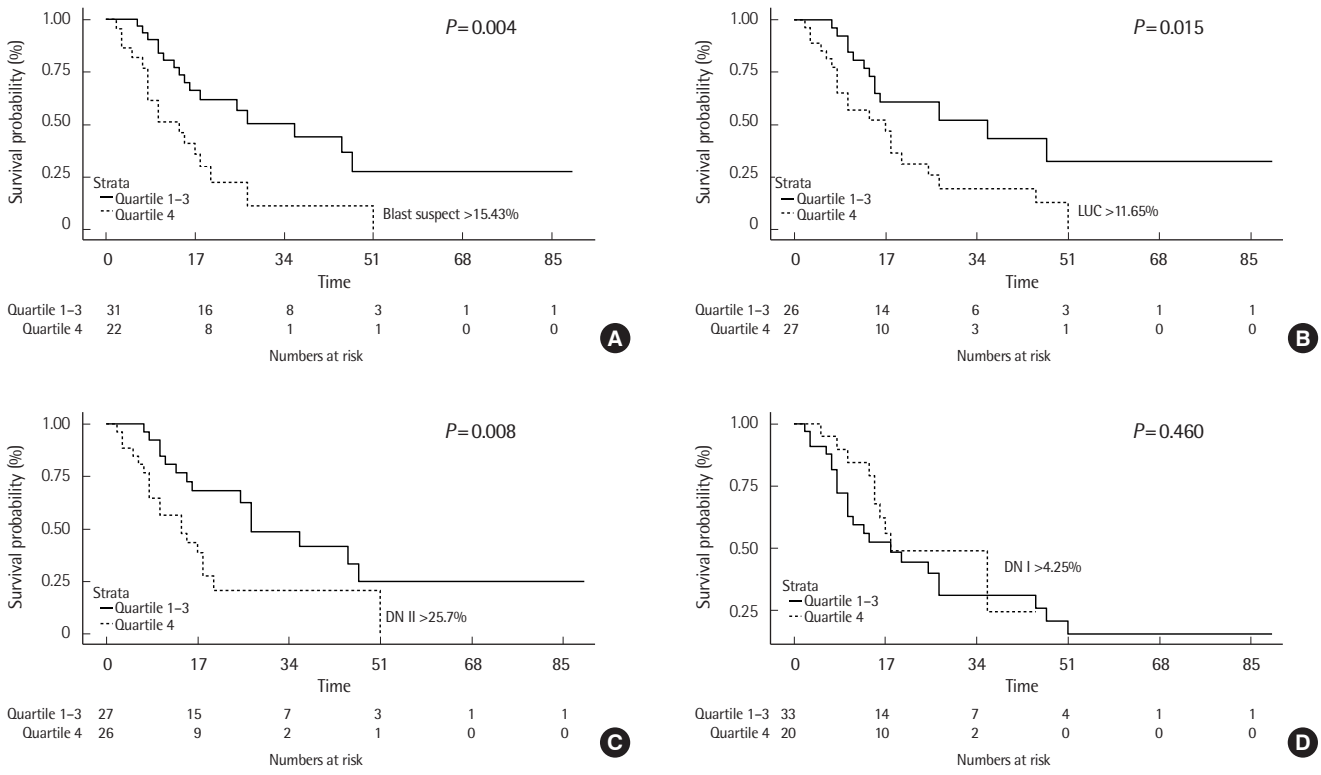

Fig. 2. Kaplan–Meier overall survival analysis of patients with leukemia relapse with blast suspect (A), LUC (B), DN II (C) and DN I (D). Dotted line represents patients with leukemia relapse in the third quartile (blast suspect >15.43%, LUC >11.65%, DN II >25.7%, DN I >4.25%) and solid line represents patients with leukemia relapse in the first to third quartile.

mia relapse, it necessitates highly skilled personnel and expertise and is time consuming [1]. Therefore, a simple and rapid method for the prediction of leukemia relapse may be helpful for both patients and clinicians.

The diagnosis of hematologic malignancies is based on bone marrow examination by experts, accompanied with complex and laborious cytochemical, molecular, and immunophenotyping tests. Although microscopic evaluation and manual counting of normal and abnormal cells have been deemed important for the diagnosis of hematologic malignancies, a simple, rapid, and cost-effective method for the differential diagnosis of hematologic malignancies is desirable [7, 8]. Since the introduction of automated blood cell analyzers in clinical laboratories, the technology has evolved to incorporate more refined laser-, optical-, and fluorescence-based detection systems [8]. Therefore, recent blood cell analyzers may provide more accurate and precise data with multiple parameters and morphology flags. Researchers have attempted to employ multiple parameters, indices, and cytograms provided by hematology analyzers for differentiation between pathologic and normal samples [9, 10]. Yang et al. [11] developed a model that can distinguish ALL and APL from AML using multiple parameters, including cell population data provided by DxH 800 Hematology Analyzer (Beckman coulter Inc, Brea, CA). Others have demonstrated very high specificity for AML diagnosis with a mean corpuscular volume ≥105 fL, mean corpuscular hemoglobin ≥36.5 pg, mean platelet volume ≥9.5 fL, monocytes ≥15.5%, and blasts ≥28.5% [10].

The role of LUC in the diagnosis and monitoring of acute leukemia has been interrogated in several studies [12-14]. Gibbs et al. suggested that LUC in ALL and AML may be differentiated with NRBC channel in ADVIA 2120i (Siemens Healthcare Diagnostics) [15]. LUC has been studied as a prognostic marker for B-cell CLL and may serve as a diagnostic and monitoring marker for viral infectious diseases [16, 17].

Jang et al. [6] evaluated the diagnostic role of LUC and DN I in the discrimination between APL and other leukemias in bone marrow samples. These authors showed that LUC serves as a better marker than DN I with higher AUC values in ROC curve analy-

https://doi.org/10.3343/lmo.2019.9.3.126

sis. Consistent with this observation, we found that LUC was a better marker than DN I for the prediction of leukemia relapse. DN II as the sum of DN I and LUC showed better diagnostic power for the prediction of leukemia relapse in ROC analysis. Of note, blast suspect showed the best discriminating power in ROC analysis. To our knowledge, this is the first study to evaluate the role of this marker. Analysis of patients with leukemia with less than 5% blast in the peripheral blood revealed the significant difference in blast suspect between patients from the control and leukemia relapse groups. Therefore, blast suspect in the peripheral blood may be a useful marker in the prediction of leukemia relapse.

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic cytokine often administered to patients suffering from myelosuppression after chemotherapy [18]. The administration of G-CSF causes neutrophilia and "left shift" toward more immature myeloid cells in normal subjects and may increase the number of peripheral blasts in patients with AML [19-21]. Neutrophilia usually resolves within 4 to 7 days after the discontinuation of G-CSF [19]. The use of G-CSF may impact other parameters, such as blast suspect, LUC, DN I, and DN II. In our cohort, most patients with AML had received HSCT and were free of G-CSF effects. Most patients with ALL were in complete remission after chemotherapy and were not administered with G-CSF within 2 weeks. Therefore, the changes in parameters such as blast suspects, LUC, and DN II could be solely associated with the pathologic state of peripheral blood samples.

A limitation of this study is that most patients with relapse showed blasts in peripheral blood smear. One could argue over the value of parameters such as blast suspect, LUC, and DN II required to predict leukemia relapse because peripheral blood smear is an easy, inexpensive, and established method to screen leukemia relapse. However, the interpretation of peripheral blood smear by experts is not always feasible, especially during night time and on weekends. Therefore, blast suspect, LUC, and DN II may serve as auxiliary parameters for the prediction of leukemia relapse especially when CBC results are abnormal. Further studies are warranted with cohorts of normal CBC and no blasts in the peripheral blood to completely evaluate the value of blast suspects, LUC, and DN II as independent parameters for leukemia relapse prediction.

In conclusion, although automated blood cell analyzer may not be a substitute for microscopic evaluation of the bone marrow in aspirated films, the use of multiple parameters provided by blood cell analyzers may serve as powerful ancillary tools for the prediction and diagnosis of leukemia relapse, especially in cases when CBC results are abnormal.

요 약

배경: 이 연구의 목적은 급성백혈병의 재발을 예측하는 데 있어서 말초혈액에서 자동혈구분석기가 제공하는 blast suspect, large unstained cells (LUC), delta neutrophil index II (DN II), delta neutrophil index (DN I) 변수의 임상적 유용성을 조사하는 데 있다.

방법: 저자들은 56명의 재발한 급성백혈병 환자군과 56명의 대조군의 의무기록을 후향적으로 검토하여 blast suspect, LUC, DN II, DN I을 비교하였다.

결과: 대조군과 급성백혈병 재발군에서 blast suspect ($P<0.001$), LUC ($P<0.001$), DN II ($P<0.001$), DN I ($P=0.002$)의 의미 있는 차이가 발견되었다. Blast suspect, LUC, DN II의 상대수행능 곡선분석에서 곡선 아래의 면적이 blast suspect는 0.927 (95% CI: 0.875–0.978, $P<0.001$), LUC는 0.868 (95% CI: 0.794–0.941, $P<0.001$), DN II는 0.900 (95% CI: 0.841–0.960, $P<0.001$)이었다. 각 변수의 생존분석에서 제3사분위값을 초과하는 값을 가지는 환자들의 전체 생존률이 제1-제3사분위값을 가지는 환자들보다 유의하게 단축되었다. 로지스틱 회귀분석에서 blast suspect, LUC, DN II, DN I의 오즈비는 1.52 (95% CI: 1.26–1.96, $P=0.0002$), 1.66 (95% CI: 1.27–2.42, $P=0.0019$), 1.16 (95% CI: 1.08–1.29, $P=0.0014$), 1.05 (95% CI: 1.01–1.13, $P=0.0845$)이었다.

결론: 자동혈구분석기가 제공하는 다양한 변수들을 백혈병 재발을 예측하는 데 보조적인 인자로 사용할 수 있다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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https://doi.org/10.3343/lmo.2019.9.3.126