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소아 급성백혈병 환자에서 조기 골수성 감별 인자로서의 Delta Neutrophil Index

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Delta Neutrophil Index as an Early Marker for Distinguishing Myeloid from Childhood Acute Leukemia

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Background: The accurate and early diagnosis of acute myeloid leukemia (AML) is important to choose proper treatment option depending on the risk stratification. The delta neutrophil index (DNI) is a relatively new blood marker that indicates the proportion of immature granulocytes in peripheral blood circulation. This study aimed to evaluate the diagnostic value of the DNI for detecting AML in the early phase of acute leukemia.

Methods: We retrospectively analyzed laboratory tests and bone marrow study results of 163 pediatric patients with acute leukemia admitted to the emergency department, who were diagnosed with acute leukemia. An automatic analyzer (ADVIA 2120 Hematology System; Siemens Healthcare Diagnostics, Forchheim, Germany) was used to measure the DNI in the peripheral blood of each patient.

Results: The mean DNI was significantly different between the AML (N=39) and non-AML (N=124) groups ($P<0.05$), and the DNI was the only significant marker for predicting AML in patients with acute leukemia (odds ratio, 1.328; $P<0.05$). The DNI more than 4.4% has the highest predictability for distinguishing the patients with AML from the patients with acute leukemia. The mean DNI of the acute promyelocytic leukemia (APL, N=8) group was statistically higher than that of the non-APL group (N=31, $P=0.019$), but the DNI was not significant in the univariate logistic regression analysis.

Conclusion: The DNI might be a promising peripheral blood marker for predicting AML in the early work-up of patients with acute leukemia.

Key Words: Leukemia, Child, Acute myeloid leukemia

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Introduction

Acute leukemia is the most common malignant neoplasm occurring in childhood [1], and acute lymphoblastic leukemia (ALL) is responsible for the majority (approximately 80%) of childhood acute leukemia, whereas acute myeloid leukemia (AML) comprises the most of the rest [2]. Prior to the 1960's, childhood ALL and AML had very low 5-year survival rates of <10% [3]. However, recent data from National Cancer Institute's Surveillance, Epidemiology, and End Results Program showed that the 5-year event-free survival rate for children with ALL was up to 90% and for children with AML was up to 66% [4,5]. Although diagnostic tools for leukemia have greatly improved in recent times, there are still several difficulties in accurately diagnosing acute leukemia, especially in children [6].

The delta neutrophil index (DNI) indicates the ratio of circulating immature granulocytes to total neutrophil count. The DNI is calculated using an automated blood cell analyzer [7] with two separate channels-the myeloperoxidase (MPO) channels and nuclear lobularity channels [8]. By subtracting the fraction of mature polymorphonuclear leukocytes from the sum of the MPO-reactive cells, we can estimate the fraction of immature granulocytes such as metamyelocytes, myeloctyes and promyelocytes [9]. After the appearance of this new biological marker, many studies have aimed at identifying clinical relationships between the DNI and several diseases [9-15]. The DNI seems to have diagnostic and prognostic value for infectious diseases, including sepsis and severe systemic inflammation. Furthermore, studies have reported associations between the DNI and rheumatologic diseases such as vasculitis [12-13]. However, thus far, only a few studies have focused on the importance of the DNI as marker for the myeoloid leukemia [14].

In this study, we aimed to determine the clinical significance of the DNI as a new blood marker to distinguish AML from acute leukemia in children with acute leukemia.

Materials and Methods

1) Study design and patients

We retrospectively analyzed the medical records of 179 pediatric patients (age 0-18 yr at the time of diagnosis) diagnosed with acute leukemia from January 2011 to October 2016 at a single tertiary academic hospital. Of them, 16 patients were excluded from the study due to the lack of definite bone marrow confirmation results (blast count for ≥ 20% from bone marrow aspiration or bone marrow pathological confirmation).

The results for complete blood count, DNI, routine chemistry laboratory results, C-reactive protein levels, lactate dehydrogenase levels, aspartate transaminase (AST) levels, alanine aminotransferase (ALT) levels, bone marrow biopsy with aspiration, and cytogenetic analysis were assessed for all eligible patients. According to the bone marrow study and cytogenetic analysis, we assorted all eligible patients into two groups: AML and non-AML. ALL and mixed-phenotype acute leukemia (MPAL) were classified as non-AML. We categorized ALL into three subgroups only according to cell linage and presence of philadelphia chromosome to first screen whether further classification of ALL would be necessary: B linage ALL, T linage ALL, and ALL with the Philadelphia chromosome. The AML group was categorized as each subgroups according to the World Health Organization classification of AML [16].

2) The DNI and other variants

We collected laboratory data of all eligible patients from the peripheral blood specimens collected at the time of the visit to the pediatric emergency department. The DNI was calculated using an automatic cell analyzer (ADVIA 2120 Hematology System, Siemens Healthcare Diagnostics, Forchheim, Germany) as per the following formula: DNI=[sum of the neutrophil sub-fraction and the eosinophil sub-fraction measured in the myeloperoxidase (MPO) channel]−(the polymorphic neutrophil sub-fraction measured in the nuclear lobularity channel).

3) Statistical analysis

Continuous variables were expressed as mean±standard deviation, and categorical variables were done as number and the percentage. Two-sample t-test or the Mann-Whitney U-test was used to compare the means of continuous variables between two groups. The univariate logistic regression analysis was used to examine the odds ratio (OR) of each independent variable predicting AML in patients with acute leukemia. Sensitivity and specificity along with predictability of the DNI for distinguishing the patients with AML from the patients with acute leukemia was calculated using receiver operating characteristic (ROC) curve. The optimal cut-off value of the DNI was selected when Youden's index (defined as sensitivity+specificity-1) was maximized. For all statistical analyses, SPSS software version 23 for windows (IBM Corp., Armonk, NY, USA) was used and *P*-values <0.05 were considered statistically significant.

Results

A total of 163 patients were eligible for this study and diagnosed with acute leukemia by bone-marrow study. Of these, 39 patients (24%) were diagnosed with AML, and the remaining patients were diagnosed with ALL (N=122, 75%) or MPAL (N=2, 1%) (Table 1). The percentage of male (N=21, 54%) did not differ much from that of female (N=18, 46%) in AML patient group, while the percentage of male

Table 1. Clinical characteristics and types of leukemia of total patient

	Non AML (N=124)			AML (N=39)	
Gender	Male		78 (63%)	Male	21 (54%)
	Female		46 (37%)	Female	18 (46%)
Types	ALL (N=122)	B linage ALL	90 (73%)	AML with t(8;21)(q22;q22) ; RUNX1/RUNX1T1	6 (15%)
				AML with inv(16)(p13q22);CBFB-MYH11	2 (5%)
				Acute promyelocytic leukemia t(15;17); PML/RARA	8 (21%)
		T linage ALL	22 (18%)	AML with mutated CEBPA	2 (5%)
				AML with myelodysplasia-related changes	1 (3%)
				Therapy-related myeloid neoplasm	1 (3%)
		ALL with the Philadelphia chromosome	10 (7%)	AML without maturation	4 (10%)
				AML with maturation	7 (18%)
				Acute myelomonocytic leukemia	5 (12%)
	MPAL (N=2)		2 (2%)	Acute monocytic leukemia	1 (3%)
				Myeloid leukemia associated with Down syndrome	2 (5%)

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MPAL, mixed-phenotype acute leukemia.

Table 2. Comparison of clinical characteristics between the AML and non-AML patients

Parameters	Total (N=163)	Non-AML (N=124)	AML (N=39)	*P*-value
Age (yr)	7.94 (0.3-18)	7.61 (0.3-18)	8.99 (0.7-17.9)	0.18
WBC count ($\times10^3/\mu L$)	49219.8±97309.4	48750±95919.1	50713.3±102884.9	0.91
Hb (g/dL)	8.7±2.5	8.6±2.7	8.7±2.0	0.77
Plt count ($\times10^3/\mu L$)	106.4±102.32	114.4±109.5	80.9±70.3	0.027[a)]
LDH (IU/L)	1232.2±2184.9	1312.0±2383.5	978.5±1369.7	0.41
AST (IU/L)	52.9±101.7	60.6±115.3	28.4±17.3	0.003[a)]
ALT (IU/L)	43.5±119.2	51.0±135.7	19.6±15.7	0.013[a)]
CRP (mg/L)	27.4±42.1	28.1±39.8	25.3±49.2	0.72
DNI (%)	5.8±15.8	0.8±2.5	21.8±26.5	<0.001[a)]

[a)] $P<0.05$. AML, acute myeloid leukemia; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine aminotransferase; CRP, C-reactive protein; DNI, delta neutrophil index.

(N=78, 63%) was relatively bigger than that of female's (N=46, 37%) in non AML patients (Table 1). The age of total patients enrolled in the study varied from 0.25 to 18 years old. The mean age of total patient group was 7.94 and the mean age of AML (8.99) and non AML patient group (7.61) each did not show significant differences (P=0.18) (Table 2). Patients with ALL (N=122) were assorted into three subgroups according to the lymphoid cell lineage and presence of the Philadelphia chromosome. Ninety (73%) of the 124 non-AML patients had B linage ALL and 22 (18%) had T linage ALL. The remaining 12 patients (9%) consisted of 10 Philadelphia ALL patients (7%) and 2 mixed phenotype acute leukemia patients (2%) (Table 1). Comparison of variables between the AML and non-AML groups revealed that platelet count, AST, and ALT levels and mean DNI of the AML group were significantly different from those of the non-AML group. However, WBC count, hemoglobin, LDH, and CRP level did not show significant differences between two patient groups (Table 2). Of the four variables that showed significant differences, the mean DNI between the AML group (21.63±25.53) and non-AML group (0.80±2.49) was especially significant (P <0.001, Table 2).

We evaluated each variable using the logistic regression model to determine their odds ratios for predicting AML in patients with acute leukemia. Among them, the DNI was the only statistically significant predictor of AML (OR, 1.328; 95% CI; 1.155-1.527; P<0.001), not the platelet count (P=0.178) or AST (P=0.215) and ALT (P=0.779) levels (Table 3). Subsequently, we used a ROC curve to examine the diagnostic accuracy of using the DNI to distinguish AML from acute leukemia; the area under the ROC curve showed a value of 0.815 (P<0.01) (Fig. 1). According to our data regarding the ROC curve, the highest sensitivity for distinguishing AML from acute leukemia patient group was 71.8% at the DNI value of 0.85% (Specificity, 83.1%). The optimal cut-off value of DNI using Youden's index in this ROC curve was 4.4%, whereas the sensitivity was 69.2% and the specificity was 94.4%. Using the cut-off value of 4.4% in our study, only 7 non-AML patients had peripheral blood DNI values over this cut-off value, whereas a majority of AML patients had DNI values above the cut-off value (Fig. 2). We listed all 7 non-AML patients and their clinical characteristics who had the DNI value above 4.4%, the cut-off value suggested according to Youden's index in Table 4. There were 3 B linage ALL patients (43%), 3 T linage ALL patients (43%), and 1 ALL patient with Philadelphia chromosome (14%). All 7 patients showed negative result for both blood and urine cultures meaning there were no proven bacterial infections. The immunologic marker of anti-myeloperoxidase (MPO) was also negative in all 7 patients. However, the CRP levels were 'posi-

Table 3. Univariate logistic regression analysis (AML vs non-AML)

	Odds ratio (95% CI)	P-value
Age (yr)	0.963 (0.866-1.072)	0.49
WBC count ($\times 10^3/\mu L$)	1.000 (1-1)	0.395
Hb (g/dL)	1.020 (0.817-0.273)	0.861
Plt count ($\times 10^3/\mu L$)	0.995 (0.989-1.002)	0.178
LDH (IU/L)	1.000 (0.999-1.000)	0.372
AST (IU/L)	0.973 (0.931-1.016)	0.215
ALT (IU/L)	0.779 (0.975-1.035)	0.779
CRP (mg/L)	0.992 (0.974-1.010)	0.386
DNI (%)	1.328 (1.155-1.527)	<0.001[a)]

[a)]P<0.05. AML, acute myeloid leukemia; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine aminotransferase; CRP, C-reactive protein; DNI, delta neutrophil index.

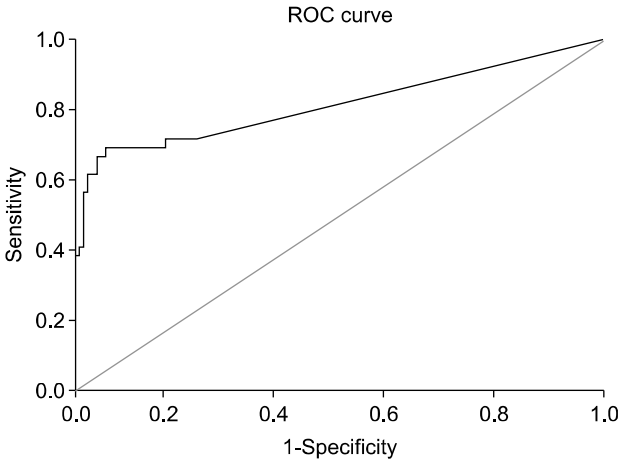

Fig. 1. Receiver operating characteristic (ROC) curves of delta neutrophil index (DNI) for predicting AML. The area under the ROC curve for distinguishing AML from acute leukemia is 0.815 (P<0.01). The optimal cut-off of DNI using Youden's index is 4.4% (Sensitivity, 69.2%, specificity 94.4%).

tive' in 5 of 7 patients (71%, CRP level above 8 mg/L was counted meaningfully elevated) (Table 4).

We divided the AML group into 11 subgroups according the World Health Organization classification of AML (Table 1). Among the 39 patients with AML, 8 patients (21%) were diagnosed with acute promyelocytic leukemia (APL). The rest of each subgroup for AML had too small number of patients to analyze respectively. The mean age of APL and non APL group did not show any statistically meaningful differences (P=0.905). The mean DNI and lactate dehydrogenase level showed significant differences between the APL and non-APL groups among patients with AML (P=0.019 and P=0.015, respectively), while WBC count (P=0.152), platelet count (P=0.067), AST (P=0.851), ALT (P=0.772), and CRP level (P=0.328) did not (Table 5). However, according to the univariate logistic regression, none of the variables including LDH level (P=0.270) and the DNI level (P=0.085) had significant odds ratio for predicting APL from AML patients (Table 6).

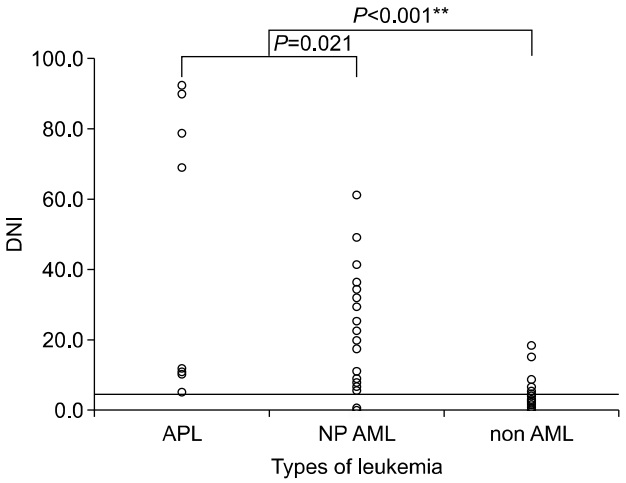

Fig. 2. The delta neutrophil index (DNI) according to the subgroup of acute leukemia. The marked line parallel to the "Types of leukemia" on the graph indicates a DNI of 4.4%, a cut-off value suggested in our study. Twenty nine of the 39 patients with AML (74%) had a DNI >4.4% whereas only 7 patients with ALL (5.6%) and no patients with MPAL had the DNI >4.4%. APL, acute promyelocytic leukemia; nP AML, non promyelocytic acute myeloid leukemia; AML, acute myeloid leukemia.

Discussion

The DNI is a new blood marker that represents the proportion of immature granulocytes in the peripheral circulation and is unfamiliar to clinicians worldwide [7]. To date, several studies have reported the DNI as an effective diagnostic and prognostic marker of sepsis and severe systemic inflammation [9-12]. Since the DNI is calculated by subtracting the number of multi-lobulated blood cells from the sum of MPO-positive blood cells, all disease conditions that increase the number of MPO-positive cells in blood circulation could show high peripheral blood DNIs. The understanding MPO and MPO-rich cells is critical to interpreting the clinical meaning of the DNI and its relationship with such diseases.

The MPO is a peroxidase enzyme most abundantly expressed in polymorphonuclear neutrophils that produce hypochlorous acid, a potent oxidant with antimicrobial activity in the human body [17]. Many studies have thus far highlighted the clinical significance of the MPO in various

Table 4. Clinical characteristics of non AML patients with high DNI

Patient No.	Types of leukemia	Gender	Age (Yr)	WBC ($\times 10^3/\mu L$)	DNI (%)	Blood culture	Urine culture	Anti-MPO	CRP (mg/L)
1	B linage ALL	M	3.3	26,120	4.8	Negative	Negative	Negative	11.7
2	B linage ALL	M	13	56,400	4.7	Negative	Negative	Negative	7.5
3	B linage ALL	M	12.3	36,340	18.3	Negative	Negative	Negative	8.0
4	T linage ALL	F	14.3	11,460	15.0	Negative	Negative	Negative	17.8
5	T linage ALL	F	3.3	493,410	5.8	Negative	Negative	Negative	0.6
6	T linage ALL	F	15.5	4,410	4.8	Negative	Negative	Negative	66.4
7	ALL with Ph	F	6.8	5,080	8.4	Negative	Negative	Negative	72.2

ALL, acute lymphoblastic leukemia; WBC, white blood cell; CRP, C-reactive protein; DNI, delta neutrophil index; MPO, myeloperoxidase; Ph, Philadelphia chromosome.

Table 5. Comparison of clinical characteristics between the APL and non-APL patients

	APL (N=8)	Non-APL (N=31)	Total (N=39)	*P*-value
Age (yr)	9.0 (1.25-17.1)	9.0 (0.7-17.92)	9.0 (0.7-17.92)	0.905
WBC count ($\times10^3/\mu L$)	15518.8±17079.3	59795.8±113685.8	50713.3±102884.9	0.152
Hb (g/dL)	8.1±1.7	8.9±2.1	8.7±2.0	0.597
Plt count ($\times10^3/\mu L$)	46±41.8	89.9±73.8	80.9±70.3	0.067
LDH (IU/L)	459.1±299.6	1112.6±1504.9	978.5±1369.7	0.015[a)]
AST (IU/L)	26.3±8.8	28.9±18.9	28.4±17.3	0.851
ALT (IU/L)	20.5±16.7	19.4±15.7	19.6±15.7	0.772
CRP (mg/L)	39.3±86.1	21.7±35.6	25.3±49.2	0.328
DNI (%)	46.1±39.7	15.5±17.9	21.8±26.5	0.021[a)]

[a)] $P<0.05$. APL, acute promyelocytic leukemia; AML, acute myeloid leukemia; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine aminotransferase; CRP, C-reactive protein; DNI, delta neutrophil index.

Table 6. Univariate logistic regression analysis (APL and non-APL groups)

	Odds ratio (95% confidence interval)	*P*-value
Age (yr)	0.846 (0.586-1.221)	0.371
WBC count ($\times10^3/\mu L$)	1.000 (0.999-1)	0.352
Hb (g/dL)	1.372 (0.456-4.123)	0.573
Plt count ($\times10^3/\mu L$)	1.004 (0.980-1.028)	0.751
LDH (IU/L)	0.992 (0.979-1.006)	0.270
AST (IU/L)	0.923 (0.684-1.246)	0.601
ALT (IU/L)	1.085 (0.919-1.280)	0.336
CRP (mg/L)	1.033 (0.980-1.089)	0.227
DNI (%)	1.113 (0.985-1.258)	0.085

APL, acute promyelocytic leukemia; AML, acute myeloid leukemia; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine aminotransferase; CRP, C-reactive protein; DNI, delta neutrophil index.

diseases including non-infectious diseases [18,19]. In addition, as the specific expression of the MPO is restricted only to myeloid precursors and leukemic cells, the diagnosis of hematologic malignancies of myeloid cell origin, such as AML, involves the use of MPO staining and MPO messenger RNA expression as an immunochemistry marker [20]. Thus, since the lymphoblasts and lymphoid lineage cells usually do not express MPO, this enzyme could be a good biological marker for distinguishing AML among patients with acute leukemia. Peripheral blood DNI, which indirectly reflects the percentage of MPO-expressing cells in peripheral circulation, is expected to be higher in patients with AML than in those with ALL. This might explain the results from our study, which showed significantly higher peripheral blood DNIs in the AML group compared to the non-AML group; and an OR of 1.328 for peripheral blood DNI as a predictor of AML in patients with acute leukemia ($P<0.001$).

In our study, 28 of the 122 patients with ALL (24%) showed elevation of the DNI (>0%). Of these, 7 patients with ALL (6%) had a peripheral blood DNI above the cut-off DNI of 4.4% for distinguishing AML from acute leukemia. Surprisingly, we observed a relatively high DNI in patients with ALL, although lymphoid lineage cells usually do not express MPO and are therefore expected to have a DNI of approximately 0%. Such a high peripheral blood DNI in patients with ALL may be explained by the following reasons. First, patients with ALL concurrently having infectious diseases at the early phase can yield relatively high peripheral blood DNIs. Although patients with ALL do not show MPO-positive cells, the presence of infectious diseases could lead to expression of MPO-abundant neutrophils, providing a high peripheral blood DNI in the laboratory findings. Another explanation is MPO-expressing B-cell ALL. Thus far, several studies worldwide have reported patients with ALL expressing the MPO [21,22]. Oberley *et al.* reported a study on isolated MPO expression in pediatric patients with B-cell ALL and its clinical significance [23]. They concluded that patients with B-cell ALL who expressed MPO had an increased rate of relapse and

a worse event-free survival than patients with B-cell ALL who did not express MPO. In our study, 7 patients with ALL who showed relatively high peripheral blood DNIs, 3 patients had B linage ALL, 3 patients had T linage ALL, and 1 patient had ALL with the Philadelphia chromosome. None of the 7 patients showed anti-MPO expression in the immuno-chemistry examination while 5 of 7 patients (71%) had 'positive' CRP levels assuming presence of infectious conditions. Thus, we concluded that there were no isolated MPO-expressing B-cell ALL patients in our patient pool and, that the patients with ALL showing relatively high peripheral blood DNIs are likely due to the presence of inflammatory conditions.

Thus far, two studies attempted to determine the diagnostic significance of the DNI in the context of acute leukemia. Jang *et al.* did not show significant differences in the DNI of bone marrow between the APL and non-APL groups [24]. In contrast, Ko *et al.* showed that the peripheral blood DNIs of the APL group were significantly higher than those of non-APL group and suggested that the peripheral blood DNI in early-stage APL could be an independent predictor of APL [14]. Contrary to the findings of Ko *et al.*, our study showed that the peripheral blood DNI values can be a useful marker for predicting AML among children with acute leukemia in the early phase of disease. To our knowledge this is the first study not only to examine the efficacy of the DNI as diagnostic marker for hematologic malignancies in children, but also to evaluate and document the possible usefulness of peripheral blood DNI for distinguishing AML from pediatric acute leukemia patients. In addition, comparison of the peripheral blood DNI between the APL and non-APL groups did not reveal any significant difference. One of the possible reasons for this might be the small number of total APL (N=8) and non-APL (N=31) patients enrolled in the study, which failed to yield meaningful statistical output. To date, bone-marrow studies with cytogenetic analyses are the gold standard for diagnosing acute leukemia. However, some difficulties and pitfalls persist in bone-marrow study alone for such early distinction and accurate diagnosis of acute leukemia [6,15], especially in children who have to undergo general anesthesia for bone-marrow studies. Although peripheral blood DNI cannot be a confirmatory test, it can be a faster and easier screening test to provide an early estimation of AML from acute leukemia patients even in the emergency department. Consequently, with the help of peripheral blood DNI as an early screening ancillary test of bone marrow, timely risk-stratification and accurate diagnosis are possible, which could lead to early treatment initiation of acute leukemia in children and a better outcome.

Despite our important findings, this study has several limitations. First, it is a retrospective study with a small number of patients. Thus, selection bias may exist in our results. Second, we could not sufficiently examine the inflammatory signs and laboratory tests to exclude infectious diseases in each patient. Although the blood cultures, urine cultures, and blood C-reactive protein levels were considered, other parameters were not examined enough to properly determine the isolated effects of infection or systemic inflammation on patients' DNIs. Third, as mentioned earlier, the number of patients in the MPAL and APL groups was too small to yield a statistically significant output. In patients with MPAL, in particular, we did not find any statistically significant results, as there were only 2 patients who had the DNI value of 0% and 4.2%, respectively. Theoretically, patients with MPAL are expected to have higher peripheral blood DNIs than patients with ALL, since the former express the MPO. Therefore, although the incidence of MPAL is low, MPAL should be considered a possible diagnosis in patients with acute leukemia who have relatively high peripheral blood DNIs. Finally, since the number of total AML patients was small, the sensitivity of DNI as an early marker for distinguishing AML from acute leukemia patients was relatively low. Actually, our study produced up to 71.8% of sensitivity which is relatively low to state the superiority of DNI as a good screening marker. However, we carefully believe that with more number of AML patients in the study, the DNI might yield a better results.

In conclusion, although this study was conducted with small number of patients and came out with relatively low sensitivity of DNI cut-off result, DNI may be a blood marker for AML, and its estimation in the early phase of a diagnostic work-up could be helpful for distinguishing AML

from children with acute leukemia. The DNI can be a subsidiary tool for rapid recognition of AML, resulting in timely risk-stratification and treatment initiation. With further prospective, multicenter studies on DNI in other subgroups of patients with AML, DNI can be a promising diagnostic and differentiation marker for predicting AML in acute leukemia patients and its clinical outcome.

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