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Delta-neutrophil index: a potential predictor of coronary artery involvement in Kawasaki disease by retrospective analysis

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Abstract

DNI is the immature granulocyte fraction provided by a blood cell analyzer, which is determined by subtracting the fraction of mature polymorphonuclear leukocytes from the sum of myeloperoxidase-reactive cells. We aimed to evaluate the role of Delta-neutrophil index (DNI) in cardiac prognosis prediction in children with Kawasaki disease (KD). Medical records of 193 patients were retrospectively reviewed. The values of DNI, white blood cells, erythrocyte sedimentation rate, the percent of polymorphonuclear leucocytes, C-reactive protein, aspartate transaminase, alanine aminotransferase, total bilirubin data of children with KD were analyzed. Also, sex and age of children were compared. The value of DNI was higher in children with cardiac complications [median 0.8 (0–0.26) vs 5.3 (3.55–8.95); $P < 0.001$]. The ROC curves showed that DNI was a better predictor of cardiac complications than other parameters. The best cutoff value for DNI to predict cardiac complications was 5.55% with sensitivity of 80% and specificity of 82% (AUC 0.883, 95% confidence interval [CI] 0.807–0.959, $P < 0.05$). DNI could serve as a facile and useful marker to predict cardiac complications in children with KD, as it is included in a routine complete blood count.

Keywords Delta-neutrophil index · Kawasaki disease · Prognosis · Coronary aneurysms

Introduction

Kawasaki disease (KD) has been known to be a systemic inflammatory response by an infection in those who are genetically predisposed [1]. Appropriate treatment with intravenous immunoglobulin (IVIG) with aspirin can reduce acute inflammatory responses, but coronary artery lesions (CAL) developed in approximately 5–10% of patients [2, 3]. It is important to identify these patients who may need more aggressive initial therapy [4–6]. Recent evidence suggests that immature granulocyte (IG) percentage could be better indicator of severe inflammatory response than traditional acute-phase reactants. The delta-neutrophil index (DNI), which reflects a fraction of circulating IG has been reported to be associated with increased mortality in the condition of severe infection or inflammation such as hypersensitivity reactions to iodinated contrast media, and in patients with myocardial infarction. However, the value of DNI in patients with KD has never been evaluated. We evaluated the potential of delta-neutrophil index as a biomarker predicting the occurrence of coronary artery lesions (CAL) in Kawasaki disease (KD).

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Patients and methods

We reviewed the medical records of all consecutive patients with KD and the medical records of children who were hospitalized for KD at Konyang University Hospital between June 2002 and July 2007 while DNI was determined in our center. The Institutional Review Board (IRB) of Konyang University Hospital approved (2019-01-009). Informed consent was exempted by the IRB.

Patients

Two-hundred and twenty-four children were hospitalized for KD during the period. Only those who fulfilled the diagnostic criteria for KD were included in this analysis. The thirty-one patients were excluded because their clinical presentations did not meet the diagnostic criteria of Kawasaki disease. One hundred ninety-three patients with KD were enrolled in our study. None of the patients had a missing value of DNI because DNI was measured simultaneously by performing complete blood cell counts (CBC).

Methods

We analyzed the clinical and the initial laboratory data including complete blood count (CBC), the percentage of polymorphonuclear cell counts (PMN), erythrocyte sedimentation rate (ESR), serum levels of C-reactive protein (CRP), electrolyte, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, and DNI at the time of admission before initial treatment. Blood sampling was conducted at the time of first admission that is before treatment and 48 h after completion of IVIG infusion. If fever persists, additional blood tests were done depending on the condition of the patients.

Diagnosis and treatment of Kawasaki disease

The diagnosis of KD was established based on American Heart Association guideline [7] which included fever accompanied by the presence of at least four of the following five findings: bilateral conjunctival injection, changes in the lips and oral cavity, non-purulent cervical lymphadenopathy, polymorphous exanthema, and changes in the extremities. We have treated the KD patients as follows; IVIG (2 g/kg/day) infusion was done over 10–12 h with 50 mg/kg of aspirin. A patient was considered afebrile when body temperature remained below 37.5 °C for more than 24 h. Laboratory tests were performed at the acute phase and 36–48 h after IVIG treatment.

IVIG responsiveness and echocardiographic examinations

IVIG resistance was defined as persistent or recrudescent fever (body temperature > 38.0 °C) after more than 36 h following completion of IVIG infusion (2 g/kg). Additional dose of IVIG was given for IVIG non-responders. The echocardiography was accomplished by a pediatric cardiologist to identify cardiac complication in acute phase, 4 weeks and 8 weeks later. Following the guidelines of Japanese Ministry of Health guidelines, we defined CAL as an increment of the internal diameter of 3 mm (≤ 5 years old) or 4 mm (> 5 years old), or 1.5 × larger of internal diameter than the adjacent segment [8].

Delta-neutrophil index

The difference between the leukocyte differentials assayed in the MPO channel and those measured in the nuclear lobularity channel was designated as delta-neutrophil index (DNI) [9], which corresponds to the fraction of immature granulocytes (IG) in circulating blood. The automatic cell counter (ADVIA 120, Siemens, Inc.) measured DNI simultaneously by performing CBC without additional order or cost and reported it with the results of CBC. The same autoanalyzer was used for all the tests to detect the DNI during that period.

Statistical analysis

All of the data were analyzed using IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA). We checked the distribution of all parameters by Kolmogorov–Smirnov test. All data except the value of ESR and PMN were not normally distributed. So, the clinical characteristics and laboratory findings were statistically analyzed via a Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Multivariable logistic regression analysis was performed using the factors that had been selected by univariable analysis. As our data were not normally distributed, quantitative variables were presented as the median (25th–75th percentiles). A P value < 0.05 was considered significant.

Results

Demographic and clinical characteristics

Male outnumbered female by 120:73. The range of age of patients was 1.5 month–10 year 3 months. The duration of

fever before admission was 4 days (2–15 days). 45 patients (23.3%) did not respond to the first IVIG therapy and CAL was developed in 25 (13.0%) patients. 45 patients (23.3%) did not respond to the first IVIG therapy of whom 36 children were administered additional IVIG. Nine patients of them eventually responded to methylprednisolone pulse therapy, 15 mg/kg for 3 consecutive days.

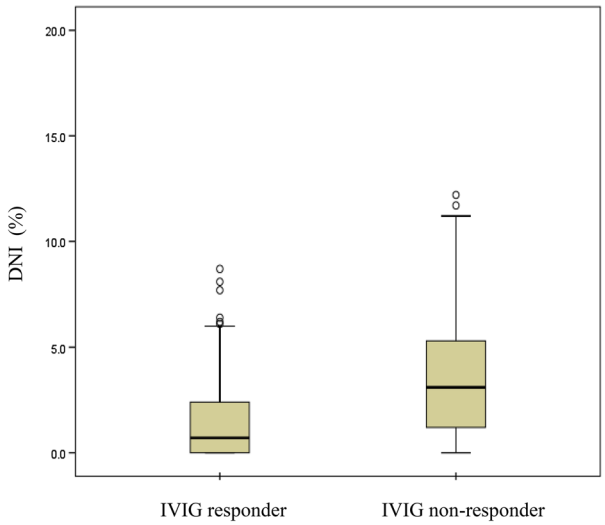

Fig. 1 DNI distribution in patients with KD according to IVIG responsiveness. *DNI* delta-neutrophil index, *KD* Kawasaki disease, *IVIG* intravenous immunoglobulin, *CAL* coronary artery lesions

IVIG responsiveness and delta-neutrophil index

The serum level of acute phase reactants such as WBC, PMN, ESR, CRP and total bilirubin was higher in IVIG-resistant group than IVIG-responsive group. Also, the value of DNI was higher in IVIG-resistant patients (Fig. 1). However, only DNI, total bilirubin and CRP were statistically significant in multivariate analysis (Table 1). 19 (42%) of 45 patients developed CAL in the IVIG-resistant group, while 6 (4%) of 148 patients were confirmed to have CAL in the IVIG-responsive group ($P < 0.05$).

Coronary artery lesions (CAL) and delta-neutrophil index (DNI)

The value of DNI was higher in children with CAL, their median values were 0.7 (0–8.1) and 3.1 (0–59.0), respectively ($P < 0.001$) (Fig. 2). The patients with CAL also had higher value of CRP, total bilirubin, and longer fever duration before treatment which were significant in multivariate analysis ($P < 0.05$) (Table 2).

Receiver-operating characteristic curve analysis was performed to determine the best DNI cutoff value for predicting CAL in patients with KD. The area under the curve (AUC) of DNI was 0.883 [95% confidence interval (CI) 0.807–0.959, $P < 0.05$]. As for CRP and total bilirubin, AUC were 0.786 (95% CI 0.691–0.881, $P < 0.05$) and 0.695 (95% CI 0.579–0.811, $P < 0.05$) (Table 3). The best DNI cutoff value was 5.55% which predicted CAL with sensitivity and specificity, of 89% and 75%, respectively ($P < 0.05$) (Fig. 3).

The KD patients were divided into two groups according to the best cutoff DNI value, 5.55%. There were 153

Table 1 Comparison of demographic and clinical characteristics in patients with Kawasaki disease according IVIG responsiveness

	IVIG responsive	IVIG resistant	*P* value	
			Univariate	Multivariate
Age (months)	23.2 (11.6–41.3)	29.1 (17.1–48.9)	0.275	
Gender				
Male	54	19	0.341	
Female	94	26		
Fever days	4 (3.0–5.0)	4 (3.0–5.0)	0.619	
WBC (/mm^3)	13,085 (10,425–17,637)	17,560 (12,380–20,345)	0.042	0.834
PMN (%)	65.35 (53.9–75.6)	77.4 (64.35–85.20)	0.017	0.464
ESR (mm/h)	48.5 (32.0–63.3)	68 (50.0–80.0)	0.023	0.059
AST (U/L)	40 (28.0–78.3)	39 (26.0–116.0)	0.882	
ALT (U/L)	33.5 (16.0–120.0)	85 (19.5–187.5)	0.127	0.793
TBIL (mg/dL)	0.44 (0.31–0.59)	0.57 (0.42–1.00)	0.001	0.006
DNI (%)	0.7 (0.0–2.40)	5.3 (3.10–11.9)	< 0.001	< 0.001
CRP (mg/dL)	4.88 (3.17–9.17)	13.91 (13.25–19.0)	< 0.001	< 0.001

Values are the median (25th–75th percentiles) unless otherwise indicated

CAL coronary artery lesions, *ESR* erythrocyte sedimentation rate, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *TBIL* total bilirubin, *DNI* delta-neutrophil index, *CRP* C-reactive protein

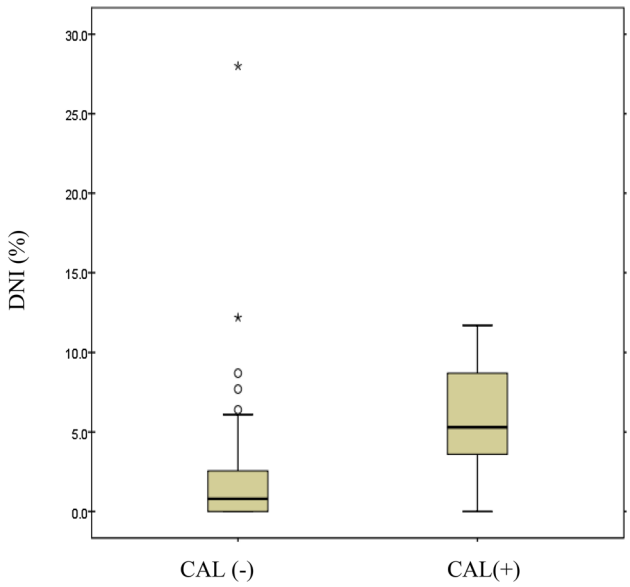

Fig. 2 DNI distribution in KD patients with CAL and without CAL. *DNI* delta-neutrophil index, *KD* Kawasaki disease, *CAL* coronary artery lesions

patients (79%) in group I (DNI < 5.55%) and 40 (21%) in group II (DNI > 5.55%). The CAL was developed in 7 (4.5%) in group I and 22 patients (55%) in group II. The patients showing IVIG unresponsiveness were 25 patients (16.3%) and 20 (50%) in group I and II, respectively. Group II had a significantly higher odds of CAL occurrence (OR 25.4, 95% CI 9.803–45.45, $P = 0.001$). The odds of IVIG unresponsiveness were five times higher in group I than group II (OR 5.12, 95% CI 3.333–7.874, $P = 0.005$) (Table 4). Actual positive and negative predictive values were 55% and 95%, respectively.

Discussion

We identified that the DNI values higher than 5.55% strongly associated with the development of CAL and IVIG unresponsiveness. The patients with increased DNI showed a higher probability of CAL occurrence (OR 25.4, 95% CI 9.803–45.45, $P < 0.001$) and tend to be resistant to IVIG unresponsiveness (OR 5.12, 95% CI 3.333–7.874, $P = 0.005$). The sensitivity, specificity, positive and negative predictive values were 89%, 75%, 55%, and 95%, respectively. The positive predictability tends to be generally low in disease with lower prevalence rates, so we thought that higher negative predictability was meaningful.

Recently, as an important biomarker of KD that has been widely evaluated was NT-proBNP. Song et al. [10] reported that serum NT-pro BNP levels and PMN percentage might be useful predicting markers for IVIG resistance and CAL in patients with KD. We also identified that age-adjusted NT-pro BNP could be an important biomarker in KD [11]. The cutoff values of 629–1300 pg/mL predicted IVIG unresponsiveness and CAL with a sensitivity and specificity of 70–79% and 58–77%, respectively. DNIT showed higher sensitivity than NT-pro BNP. Another interesting biomarker is neutrophil-to-lymphocyte ratio (NLR) [12]. An NLR 2 days after IVIG that exceeded 1.0 was predictive of coronary aneurysm development and IVIG resistance. This cutoff value predicted aneurysm with sensitivity, specificity, positive predictive value, and negative predictive value of

Table 2 Comparison of demographic and clinical characteristics in patients with Kawasaki Disease according to development of CAL

Characteristics	CAL (−) ($N = 168$)	CAL (+) ($N = 25$)	*P* value	
			Univariate	Multivariate
Age (months)	24.15 (11.80–42.39)	29.16 (15.37–43.55)	0.011	0.781
Gender				
Male	65	8	0.605	
Female	103	17		
Fever days (days)	4 (3.0–5.5)	4 (3.0–5.5)	0.027	0.034
WBC (/mm^3)	13,655 (10,547–17,577)	18,330 (12,077–23,070)	0.001	0.051
PMN (%)	66 (55.0–78.0)	74 (64.9–86.7)	0.001	0.467
ESR (mm/h)	50 (32.75–68.25)	60 (49.5–79.5)	0.001	0.759
AST (U/L)	39 (28.0–75.0)	57 (30.5–120.5)	0.655	
ALT (U/L)	35 (15.75–129.5)	105 (28.0–214)	0.263	
TBIL (mg/dL)	0.44 (0.33–0.59)	0.68 (0.43–2.14)	0.034	0.049
DNI (%)	0.8 (0.00–2.52)	5.3 (3.55–8.95)	< 0.001	< 0.001
CRP (mg/dL)	5.72 (3.49–12.12)	14.8 (11.45–18.80)	< 0.001	0.012

Values are the median (25th–75th percentiles) unless otherwise indicated

CAL coronary artery lesions, *ESR* erythrocyte sedimentation rate, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *TBIL* total bilirubin, *DNI* delta-neutrophil index, *CRP* C-reactive protein

Table 3 Area under curve (AUC) in ROC curve

	AUC	95% CI		*P* value
DNI	0.883	0.807	0.959	0.000
Fever days	0.530	0.411	0.649	0.630
CRP	0.786	0.691	0.881	0.000
TBIL	0.695	0.579	0.811	0.002

AUC area under the curve, *ROC* receiver operation characteristic, *CI* confidence interval, *DNI* delta-neutrophil index, *fever days* duration of fever before initial treatment, *CRP* C-reactive protein, *TBIL* total bilirubin

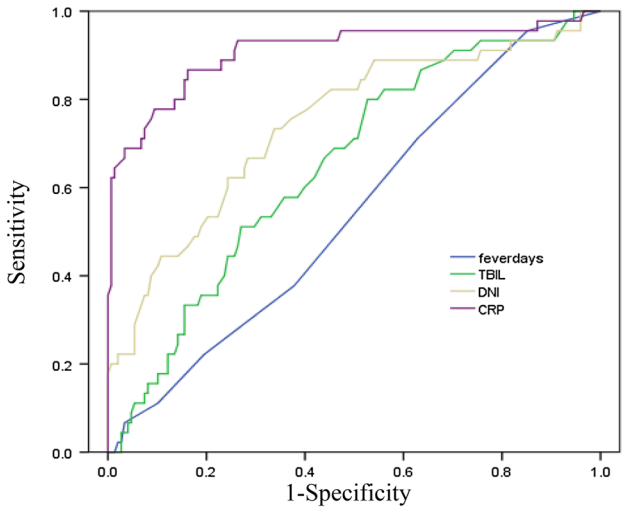

Fig. 3 Receiver-operating characteristic curves for the development of coronary artery lesions in patients with Kawasaki disease. *DNI* delta-neutrophil index, *feverdays* the duration of fever before admission, *CRP* C-reactive protein, *TBIL* total bilirubin

87%, 63%, 6%, and 99%, respectively which showed comparable to DNI. This seems to be a good index, but it has to be verified by other researchers. There have been numerous studies to reveal the prognostic factors that associated with IVIG responsiveness and CAL development in KD [13–16].

Based on recent series of studies, the fraction of immature granulocytes could be a better indicator of sepsis of severe inflammation [17]. The index of left shift such as WBC, ANC, or band counts has been considered to assess the severity and prognosis of Kawasaki disease in which there is an explosive increase of cytokines. Cytokines accelerate the release of cells from the bone-marrow reserve pool, leading to an increased number of immature cells. Influx of immature leucocyte is not limited to the neutrophil but the number of immature granulocytes including eosinophils increase in the peripheral circulation. However, measurement of immature granulocytes is not easy because manual counting is cumbersome, and is not accurate even by a trained clinic-pathologist [18, 19]. Recently, DNI was developed as an indicator of immature granulocytes; it is calculated by an automated blood cell analyzer [20]. The complete blood count is routinely checked in admitted patients, so DNI can be easily obtained without additional examination or cost. The value of DNI has been known to be correlated with overt DIC, bacterial isolation rate, and mortality in patients with suspected sepsis [19, 21]. Recently, DNI was also implicated in sterile inflammation, such as AMI or post-resuscitation after cardiac arrest [22]. The hemodynamic instability or severe inflammation could affect critical regulatory mechanisms for neutrophil release from the bone marrow.

As far as we know, this is the first study about DNI in KD. However, our study was a limitation which is retrospective and relatively small sized. We hope that large-scaled prospective studies will perform to validate whether DNI is an effective biomarker of KD. Also, KD is sometimes difficult to distinguish from a severe viral or bacterial infection, but increased fraction of immature granulocytes is not specific for KD. More studies are also warranted to evaluate the benefit of combining DNI with other biomarkers, such as NT-pro BNP which has been a widely used biomarker in KD.

Also, DNI is an index which a specific auto-analyzer could provide, so there may be a limit DNI being widely used. Actually, the type of auto-analyzer has been changed and DNI is not available in our hospital. That is why our study period is from 2002 to 2007. If the fraction of immature granulocyte will be proven to be useful not only in sepsis or shock but also in KD, various types of blood

Table 4 Coronary artery lesions and IVIG responsiveness in Kawasaki disease patients with high or low value of DNI

	DNI (< 5.55%)	DNI (> 5.55%)	OR	95% CI		*P* value
CAL			25.4	9.803	45.45	< 0.001
(+)	7	22				
(−)	146	18				
Responsiveness to IVIG			5.12	3.333	7.874	< 0.001
(+)	25	20				
(−)	128	20				

ROC receiver operation characteristic, *IVIG* intravenous immunoglobulin, *CAL* coronary artery lesions, *OR* odds ratio, *CI* confidence interval, *DNI* delta-neutrophil index

auto-analyzer could provide the information of immature granulocyte.

Conclusions

Our study demonstrated that DNI, which reflects the proportion of immature granulocytes in circulating blood, may be a new predictive biomarker in patients with KD. The high value of DNI could help to identify patients with a risk of developing CAL. Thus, incorporating the immature granulocyte assay into the initial laboratory workup could improve the early detection of high risk of patients with KD.

Author contributions Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; all authors. Drafting the work or revising it critically for important intellectual content; all authors. Final approval of the version to be published; all authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; all authors. All authors take full responsibility for the integrity of the study and the final manuscript. No part of the manuscript has been copied from elsewhere or previously published in whole or in part.

Compliance with ethical standards

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval The Institutional Review Board (IRB) of Konyang University Hospital approved. Approval number: 2019-01-009. Date of approval; 2019. 01.16.

Informed consent Informed consent was exempted by the IRB.

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