Transient Deformation of Neutrophils in Kawasaki Disease

Yumi Koike, MD[1], Ryu Yanagisawa, MD, PhD[2,3], Yoshifumi Ogiso, MD, PhD[4], Yoshiaki Cho, MD[1], Kisei Minami, MD[1], Kouichi Takeuchi, MD, PhD[1], Kazuo Sakashita, MD, PhD[2,3], and Tsukasa Higuchi, MD, PhD[1]

In the treatment of Kawasaki disease, resistance to high-dose immunoglobulin intravenous (IGIV) can occur. The neutrophil morphology analyses in 17 patients revealed that transient pseudo-Pelger-Huët anomaly was more frequently detected in the IGIV-resistant group. This finding may aid the prediction of IGIV resistance. *(J Pediatr 2016;173:238-41).*

High-dose immunoglobulin intravenous (IGIV) treatment in the acute stage of Kawasaki disease (KD) has been shown to be an effective treatment; however, 10%-20% of patients are resistant to initial treatment.[1,2] These patients may require additional dose(s) of IGIV or other therapies.[2] Because these patients may have the greatest risk of developing a coronary artery lesion (CAL),[1-3] several prediction-scoring systems for assessing resistance to IGIV in patients with KD have been proposed that base the prediction on patient background characteristics and laboratory data. Although these scoring methods can predict resistance to IGIV in a convenient and versatile way, the predictive probability remains only 70%-80%.[1,4,5] In addition, these methods may not always be suitable or adaptable for all ethnicities.[6,7] Therefore, further enhancement of precision is desirable.

Although a variety of cytokines are produced in patients with KD, granulocyte colony-stimulating factor (G-CSF) is one cytokine known to increase neutrophil counts in KD.[8-11] Higher neutrophil counts and immature forms are reported to be related to the risk for resistance to IGIV treatment and the development of CAL.[7,11-14] The number of neutrophils is one factor used in predictive scoring for IGIV resistance.[4] However, there have been few reports of neutrophil morphologic analysis in KD. We conducted a retrospective analysis of morphologic abnormalities in KD and made a study of the potential utility of neutrophil morphologic features as an additional risk-scoring factor for predicting IGIV resistance.

Methods

This retrospective study included pediatric patients (aged ≤6 years) who were diagnosed with KD and who received IGIV (2 g/kg) treatment at Nagano Children's Hospital from June 2011 to May 2015. The diagnosis of KD was made based on the diagnostic guidelines.[15] We collected a peripheral blood smear at diagnosis and reevaluated the morphologic analysis of neutrophils. The morphology of neutrophils was analyzed by a pediatric hematologist and a pediatric pathologist. We also compared the clinical features and clinical data between IGIV-responsive and -resistant groups. Furthermore, we compared the score using previous risk-scoring systems for IGIV resistance.[1,4,5] Resistance to IGIV treatment was defined as fever persisting beyond 24 hours after conclusion of IGIV administration or recrudescent fever associated with KD symptoms after an afebrile period.[4] Statistical analysis was performed using Fisher exact test for the comparison of sex in the 2 groups. Mann-Whitney U tests were applied for all other statistical analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[16] Statistical significance was defined as $P < .05$. The Nagano Children's Hospital Institutional Review Board approved this study.

Results

A total of 17 patients were eligible for participation in this study. Although 10 patients were responsive to IGIV monotherapy, 7 patients were resistant. The summarized result of the comparison of the 2 groups is shown in the **Table**. A comparison between patients who responded to IGIV monotherapy and those who did not respond showed significantly elevated aspartate aminotransferase and alanine aminotransferase levels in nonresponders. Although our patients were limited in number, one of the risk-scoring systems for IGIV resistance predicted IGIV resistance (Sano Score).[5]

In the analysis of neutrophil morphologies, all patients showed some transient abnormalities. Based on the observation of deformed neutrophils, we categorized abnormalities into 3 types: (1) the pseudo-Pelger-Huët anomaly

CAL	Coronary artery lesion
G-CSF	Granulocyte colony-stimulating factor
IGIV	Immunoglobulin intravenous
KD	Kawasaki disease
Pseudo-PHA	Pseudo-Pelger-Huët anomaly

From the Departments of [1]General Pediatrics and [2]Hematology/Oncology, Nagano Children's Hospital, Azumino, Japan; [3]Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan; and [4]Department of Clinical Pathology, Nagano Children's Hospital, Azumino, Japan

Supported by the Japan Society for the Promotion of Science (JSPS KAKENHI; 26860790 [to R.Y.]). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2016.02.065

Table. Comparison of clinical variables between IGIV responsive and resistant group

Characteristics	Response to treatment of IGIV		*P* value
	Responsive (N = 10)	Resistant (N = 7)	
Sex (male:female)	5:5	6:1	.304
Age (y)	4.0 ± 2.2	3.0 ± 1.7	.729
The days of illness at diagnosis of KD (d)	5.0 ± 1.3	5.0 ± 0.9	.622
Number of symptoms of KD	4.0 ± 1.1	5.0 ± 0.8	.281
Risk-scoring systems for IGIV resistance			
Egami score[1]	2.0 ± 0.6	2.0 ± 1.1	.484
Kobayashi score[4]	1.0 ± 1.9	4.0 ± 2.0	.062
Sano score[5]	1.0 ± 0.6	2.0 ± 1.1	.031
Laboratory data at diagnosis of KD			
White blood cell/mm^3	11 000 ± 4700	12 700 ± 4700	.813
Hemoglobin (g/dL)	12.2 ± 0.8	11.6 ± 0.5	.261
Hematocrit (%)	35.6 ± 2.4	34.2 ± 1.8	.204
Platelets/mm^3	277 000 ± 111 000	263 000 ± 208 000	.962
Albumin (g/L)	3.8 ± 0.5	2.8 ± 1.1	.128
AST (IU/L)	31.0 ± 7.1	146.0 ± 641.1	.011
ALT (IU/L)	20.5 ± 42.9	40.5 ± 372.5	.016
Total bilirubin (mg/L)	5.0 ± 2.4	8.0 ± 8.9	.105
Sodium (mEq/L)	136.5 ± 2.6	134.5 ± 3.4	.382
C-reactive protein (mg/L)	79.1 ± 95.7	150.3 ± 72.4	.475
Absolute neutrophil count/mm^3	8500 ± 5000	8900 ± 5200	.887
Percentage of deformation in total neutrophils			
Pseudo-PHA (%, range)	5.5 ± 5.7 (1.0-22.0)	10.0 ± 3.1 (5.0-13.0)	.005
3- to 4-segmentation anomaly (%, range)	4.0 ± 5.1 (1.0-19.0)	3.0 ± 4.2 (2.0-15.0)	.536
Hypersegmentation (%, range)	1.0 ± 2.1 (0.0-7.0)	2.0 ± 3.5 (0.0-10.0)	.921

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase.
Statistical analysis was performed with Fisher exact test in comparison of sex in the 2 groups. Mann-Whitney U tests were applied for all of the other statistical analyses. Score is shown as mean ± SD.

(pseudo-PHA); (2) 3- to 4-segmentation anomaly; and (3) hypersegmentation (Figure 1). Although the percentage of deformed neutrophils in each patient was quite different, all patients had pseudo-PHA (1%-22%; median, 6% of the total neutrophils in peripheral blood) and 3- to 4-segmentation anomaly (1%-19%; median, 3% of the total neutrophils in peripheral blood). In addition, 11 patients (64.7%) showed hypersegmentation (0%-10%; median,

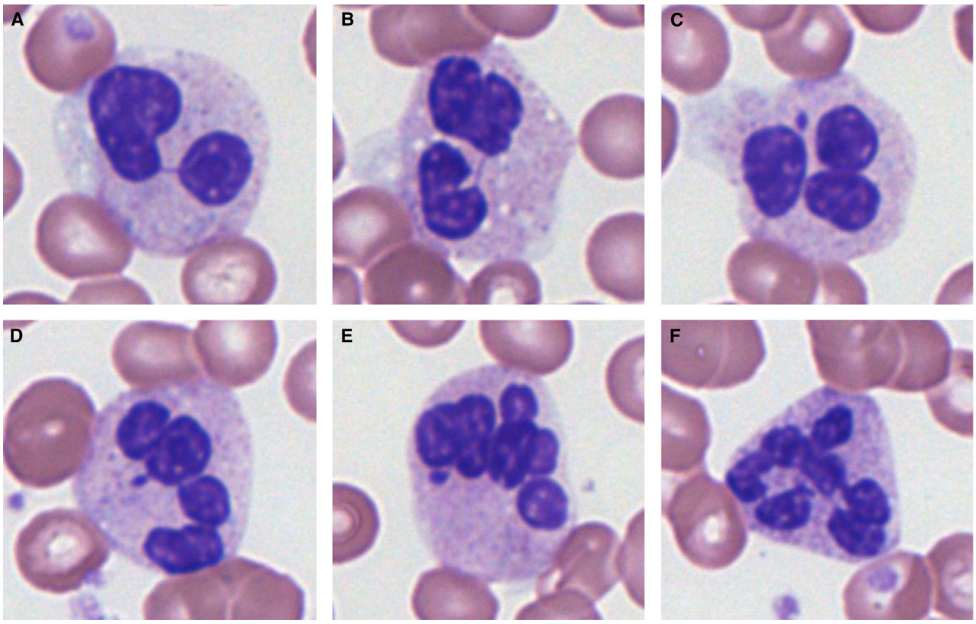

Figure 1. Deformation of neutrophils on the peripheral blood smear. **A-B,** Pseudo-PHA. **C-D,** 3- to 4-segmentation anomaly. **E-F,** Hypersegmentation.