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ORIGINAL ARTICLE

Evaluation of Neutrophil-Related Parameters by Hematology Analyzer for Detection of Myelodysplastic Syndromes

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ABSTRACT

Background: **Myelodysplastic syndromes (MDS) are characterized by dysplasia, which is crucial for diagnosis. Dysplastic features in the WHO classification include granulocyte size abnormalities. However, the relationship between granulocyte size and automated hematology analyzer parameters has not been investigated.**
In this study, we aimed to establish an objective marker for identifying dysplasia in MDS by evaluating the association between neutrophil-related parameters from an automated hematology analyzer and morphological dysplasia, including abnormalities in neutrophil size.
Methods: **Samples were collected from 75 MDS and 149 non-MDS cases and analyzed using XR-1000. The non-MDS cases were classified into six disease groups. Neutrophil-related parameters, including forward scatter (NE-FSC), side scatter (NE-SSC), and side fluorescence (NE-SFL), were analyzed. Morphological dysplasia was assessed by light microscopy using Wright-Giemsa-stained smears. The neutrophil area was quantified with ImageJ software.**
Results: **Compared with the other groups, the NE-FSC values were significantly lower in the MDS group than in the four disease groups ($p < 0.01$). The NE-SFL values were significantly lower in the MDS group than in the two disease groups ($p < 0.01$). No significant differences in NE-SSC were observed between the MDS group and any of the six disease groups. Among the MDS cases, 47 cases showed ≥ 10% neutrophil dysplasia (MDS with dysplasia) and 28 cases showed < 10% (MDS without dysplasia). The control group consisted of 50 non-MDS cases with normal blood counts and smears. When comparing the three groups, NE-FSC was highest in controls, intermediate in MDS without dysplasia, and lowest in MDS with dysplasia ($p < 0.01$). NE-SFL and NE-SSC were significantly lower in MDS with dysplasia compared with both controls and MDS without dysplasia ($p < 0.001$), with no difference between controls and MDS without dysplasia. Neutrophil area was significantly smaller in both MDS groups than in controls ($p < 0.001$) but did not differ between MDS with and without dysplasia. ROC analysis was performed to evaluate the diagnostic accuracy between the MDS and non-MDS groups, and the AUCs were 0.756 for NE-FSC, 0.632 for NE-SSC, and 0.694 for NE-SFL.**
Conclusions: **NE-FSC is a useful parameter for detecting neutrophil dysplasia and may improve diagnostic accuracy in MDS.**
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Supplementary Data

Table S1. Summary of demographic data and hematological data among seven groups.

Parameters		Group						
		Myelodys-plastic syndromes (n = 75)	Myelopro-liferative neoplasms (n = 10)	Aplastic anemia (n = 9)	Lymphoma (n = 43)	Solid tumor (n = 36)	Orthopedic disorder (n = 27)	Viral infection (n = 24)
Male:Female		52:23	7:3	5:4	25:18	14:22	11:16	9:15
Median (range)								
Age, years	(years)	74 (55 - 95)	70 (47 - 85)	49 (20 - 79)	73 (22 - 87)	65.5 (44 - 85)	67 (21 - 93)	41 (20 - 89)
White blood cells	($\times 10^9$/L)	3.2 (0.4 - 33.9)	14.0 (4.5 - 84.9)	7.6 (0.3 - 14.1)	5.8 (0.2 - 53.4)	6.3 (2.0 - 38.3)	5.4 (3.6 - 19.8)	6.2 (3.3 - 18.3)
Neutrophils	($\times 10^9$/L)	1.5 (0.1 - 31.7)	10.8 (1.8 - 71.8)	3.6 (0.1 - 12.5)	3.4 (0.1 - 40.9)	4.1 (1.0 - 25.0)	3.1 (1.7 - 17.2)	3.0 (1.4 - 16.9)
Red blood cells	($\times 10^{12}$/L)	2.73 (1.27 - 4.58)	4.12 (2.40 - 7.52)	4.48 (3.16 - 5.30)	3.30 (1.96 - 4.93)	4.26 (2.28 - 5.27)	4.48 (2.41 - 5.50)	4.51 (3.17 - 5.21)
Hemoglobin	(g/L)	88 (51 - 150)	135 (79 - 205)	136 (109 - 168)	103 (60 - 160)	127 (81 - 158)	139 (77 - 165)	136 (107 - 164)
Mean corpuscular volume	(fL)	97.2 (83.8 -128.0)	96.4 (69.7 - 117.8)	90.2 (77.7 - 102.5)	91.2 (79.3 - 99.7)	91.2 (81.7 - 108.0)	92.2 (82.8 - 98.7)	90.3 (82.7 - 102.8)
Platelets	($\times 10^9$/L)	123 (6 - 852)	439 (156 - 1,139)	263 (10 - 416)	152 (6 - 583)	224 (41 - 395)	204 (71 - 367)	224.5 (58 - 367)

Table S2. Comparison of XR-1000 neutrophil-related research parameters and cell area and nuclear area among myelodys-plastic syndromes subtypes.

Parameters	5q- (n = 1)	SLD (n = 5)	MLD (n = 27)	EB-1 (n = 11)	EB-2 (n = 28)	EB-F (n = 2)	Unclassifi-able (n = 1)	Kruskal Wallis test
	Median (range)							p-value
NE-SSC	140.1	145.7 (121.4 - 158)	137.5 (113 - 156.6)	138 (107.1 - 154.2)	143.3 (103.1 - 155.0)	151.55 (149.7 - 153.4)	109.6	0.24
NE-SFL	46.8	39.9 (28.9 - 64.5)	40.3 (26.1 - 63.4)	38.9 (32.5 - 54.2)	44.55 (30.2 - 61.9)	47 (46.3 - 47.7)	36	0.69
NE-FSC	102.8	89.8 (72.8 - 111.2)	86.4 (71.1 - 99.2)	83.5 (68.8 - 103.2)	93.3 (61.9 - 105.6)	101.65 (100.4 - 102.9)	66	0.11
Neutrophil cell area (μm^2)	176.899	170.845 (145.4 - 205.2)	191.5 (147.6 - 228.1)	172.1 (126.4 - 211.7)	191.1 (150.1 - 228.3)	170.6 (165.5 - 175.7)	156.139	0.31
Neutrophil nuclear area (μm^2)	77.34	71.6 (67.1 - 84.0)	76.1 (52.9 - 100.0)	66.7 (51.6 - 82.9)	75.7 (58.2 - 92.4)	60.8 (58.2 - 63.5)	74.1012	0.28

NE-SSC: side scatter calculated from neutrophil cell population, NE-SFL: side fluorescence intensity calculated from neutrophil cell popular-tion, NE-FSC: forward scatter calculated from neutrophil cell population, SLD: single lineage dysplasia, MLD: multi-LD, EB: excess blasts, EB-F: EB with fibrosis.

Table S3. Comparison of XR-1000 forward scatter (FSC) related research parameters and cell area and nuclear area among the three blood cells in the control group (n = 50).

Parameters	Group		
	Neutrophil mean (± SD)	Lymphocyte mean (± SD)	Monocyte mean (± SD)
XR-1000			
	NE-FSC	LY-Z	MO-Z
Forward scatter (FSC)	99.9 (± 2.9)	59.9 (± 1.8)	71.9 (± 2.7)
ImageJ			
Cell area (μm^2)	208.0 (± 13.4)	121.6 (± 15.3)	263.5 (± 15.3)
Nuclear area (μm^2)	76.9 (± 5.7)	74.3 (± 7.2)	120.4 (± 5.9)

NE-FSC: forward scatter calculated from neutrophil cell population, LY-Z: forward scatter calculated from lymphocyte cell population, MO-Z: forward scatter calculated from monocyte cell population.

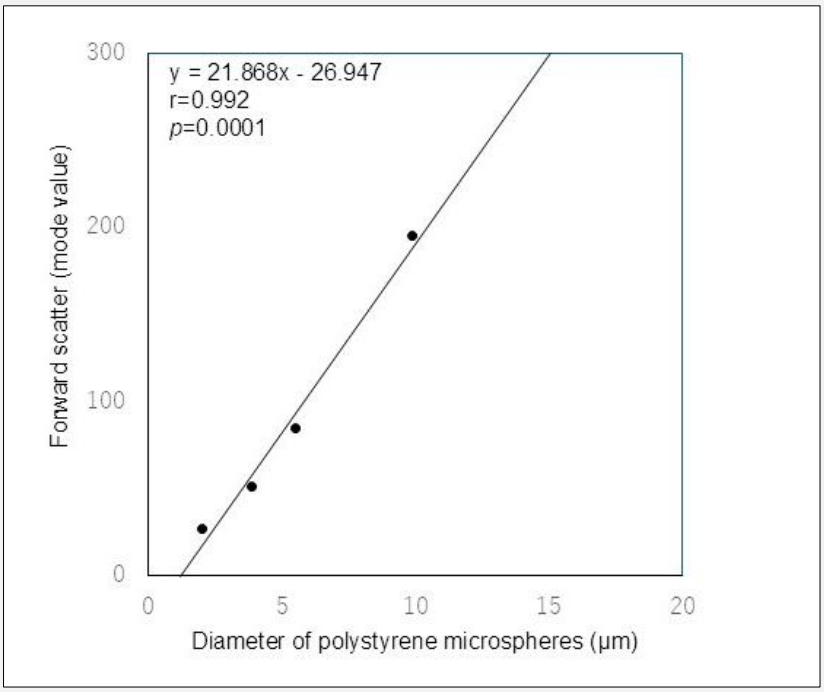

Figure S1. Results of measurements for Flow Cytometry Size Calibration Kit (Invitrogen) made of polystyrene beads.

Correlation between diameter of polystyrene microspheres and mode value of forward scatter (FSC) calculated from WDF scattergram.

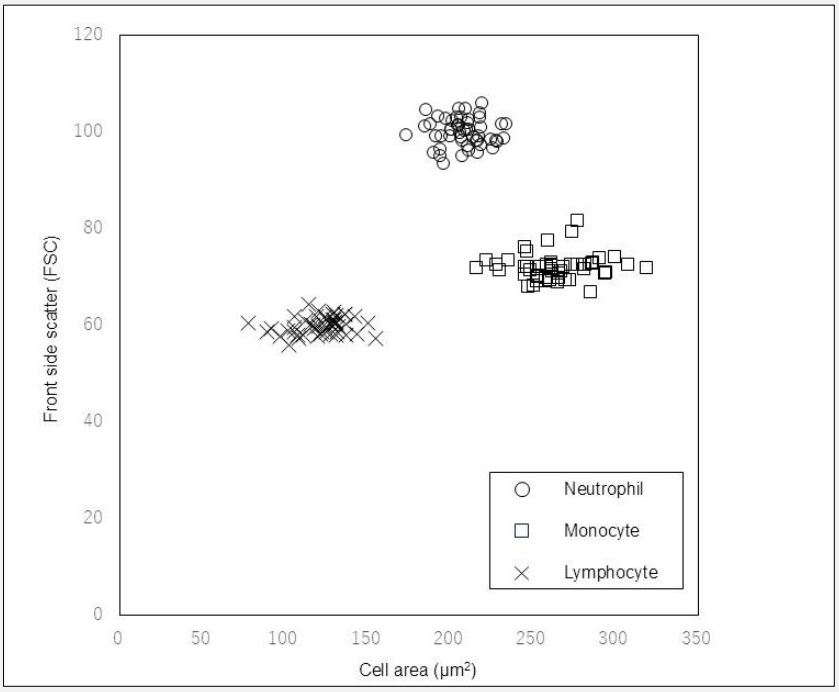

Figure S2. Correlation between cell area and forward scatter (FSC) of XR-1000 for three types of leukocytes in control group (n = 50). Different plot shapes were illustrated according to leukocyte types. No significant correlation was found between FSC and cell areas of three kinds of leukocytes, respectively. Three blood cells formed populations alone, not overlap with each other in scatter plot.