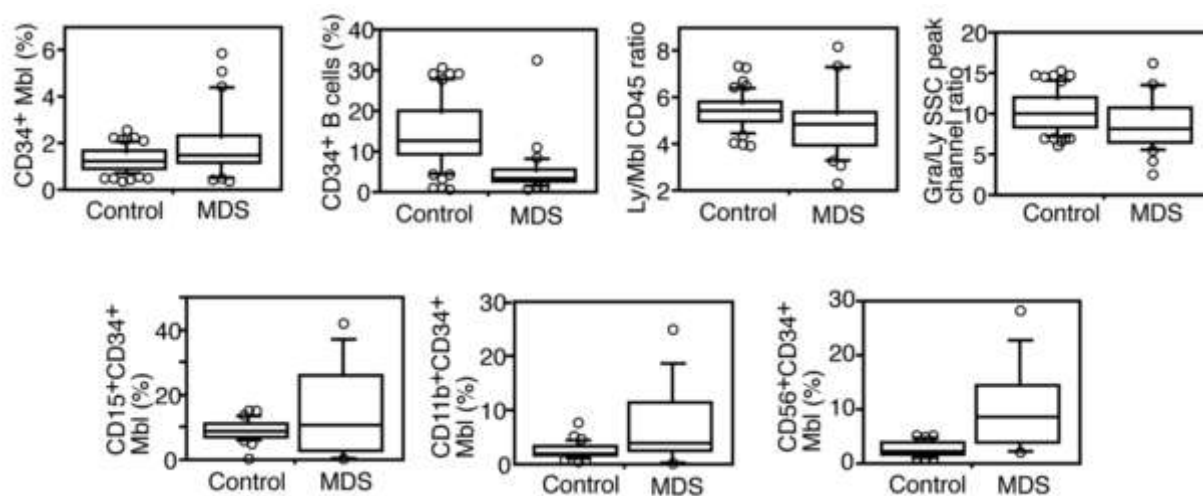


## Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study

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**Online Supplementary Figure S1.** Analysis of seven FCM parameters in the first (upper four panels) and second cohorts (lower three panels). The horizontal lines in each boxplot represent the 90th, 75th, 50th, 25th, and 10th percentiles. Circles are outliers. Controls are nonclonal cytopenic patients, and MDS indicates low-grade MDS patients lacking RS.

Online Supplementary Table S1. Interlaboratory variability of FCM results.

Parameter	CV (%)	
	Mean	Range
CD34 <sup>+</sup> myeloblasts (%)	12.9	2.6–24.3
CD34 <sup>+</sup> B cells (%)	23.4 (16.8) <sup>1</sup>	4.7–69 (4.7–35.5) <sup>1</sup>
Ly/Mbl CD45 ratio	9.9	2.8–18.2
Gra/Ly SSC peak channel ratio	14.5	8.2–20.9
CD34 <sup>+</sup> myeloblasts expressing CD11b (%)	40	6.2–83.7
CD34 <sup>+</sup> myeloblasts expressing CD15 (%)	46.1	19.3–64.0
CD34 <sup>+</sup> myeloblasts expressing CD56 (%)	50.9 (39.1) <sup>2</sup>	17.4–173.2 (17.4–97.9) <sup>2</sup>
In each of 10 cell samples, CV values were determined from data analyzed by three laboratories. Then, mean CV values (ranges) were calculated with the results of all cell samples as shown.		
<sup>1</sup> Data from two samples were around 1% in all institutions, and thus only a slight difference between institutions resulted in large CV values in these samples. Data in parentheses are data when these two samples were excluded.		
<sup>2</sup> Data from three laboratories for one sample were 0%, 0%, and 3.3%, which resulted in a large CV value. Data in parentheses are data when this sample was excluded.		

Online Supplementary Table S2. Flow scores of patients in the second cohort.

	Scoring using 4 parameters <sup>1</sup>						Scoring using 7 parameters <sup>2</sup>					
	0	1	2	3	4	2 or more	0	1	2	3	4	2 or more
<b>Nonclonal cytopenia</b>	26	4	0	0	0	0/30 (0%)	26	4	0	0	0	0/30 (0%)
<b>Low-risk MDS</b>	6	1	5	1	0	6/13 (46.2%)	1	3	5	3	1	9/13 (69.2%)
Data are number of patients or percentages in parentheses.												
<sup>1</sup> Parameters used were CD34 <sup>+</sup> myeloblasts, CD34 <sup>+</sup> B cells, Ly/Mbl CD45 ratio, and Gra/Ly SSC peak ratio.												
<sup>2</sup> All seven parameters were used.												

Online Supplementary Table S3. Flow scores of MDS patients in the prospective cohorts as a function of characteristics.

Characteristic	Flow score using 4 parameters			Flow score using 7 parameters		
	0 or 1	2 or more	<i>p</i> value	0 or 1	2 or more	<i>p</i> value
<b>Japanese MDS</b>						
<b>Dysplasia<sup>1</sup></b>						
Multilineage	11	9	0.86	6	14	0.80
Erythroid alone	11	8		5	14	
<b>Karyotype<sup>2</sup></b>						
Good	19	11	0.31	11	19	0.17
Intermediate	4	4		1	7	
Poor	1	3		0	4	
<b>Transfusion<sup>3</sup></b>						
Dependent	10	8	0.81	6	12	0.59
Independent	16	11		7	20	
<b>IPSS</b>						
Low	9	1	0.036	6	4	0.028
Intermediate-1	14	14		6	22	
Intermediate-2	1	3		0	4	
<b>WPSS</b>						
Very low	3	3	0.056 (high vs. others)	1	5	0.12 (high vs. others)
Low	14	7		7	14	
Intermediate	2	0		2	0	
High	1	4		0	5	
<b>Italian MDS</b>						
<b>Dysplasia<sup>1</sup></b>						
Multilineage	8	37	0.0066	2	34	0.026
Erythroid alone	18	22		7	21	
<b>Karyotype<sup>2</sup></b>						
Good	18	36	0.28	5	41	0.22
Intermediate	3	5		2	4	
Poor	0	5		0	4	
<b>Transfusion<sup>3</sup></b>						
Dependent	7	19	0.81	1	21	0.17
Independent	18	43		7	36	
<b>IPSS</b>						
Low	17	32	0.33	4	31	0.72
Intermediate-1	8	24		4	22	
Intermediate-2	0	3		0	3	
<b>WPSS</b>						
Very low	10	17	0.007 (very low & low vs. others)	3	15	0.035 (very low & low vs. others)
Low	13	20		5	20	
Intermediate	1	16		0	16	
High	1	6		0	5	

<sup>1</sup> "Multilineage" includes RCMD and RCMD-RS. "Erythroid alone" includes RA and RARS.

<sup>2</sup> Cytogenetic categories used in IPSS and WPSS.

<sup>3</sup> Definition used in WPSS. Data on 1 patient from each cohort were unavailable.