Original article

Diagnostic application of flow cytometry in myelodysplastic syndromes

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Abstract:

Background: *The diagnosis of myelodysplastic syndrome (MDS) is based on a combination of clinical history, morphological assessment, genetic studies and the exclusion of other diseases. Diagnosing MDS in uncertain morphology, especially in patients with normal karyotype or low myeloblast count remains a challenging issue.* ***Objective:*** *The primary objective was to evaluate the sensitivity and specificity of an in-house 12-parameter flow cytometry scoring method for diagnosing MDS.* ***Materials and Methods:*** *We collected 48 patients with cytopenia of unknown cause. The diagnosis of MDS was made using the WHO 2016 criteria. Flow cytometry criteria using 12 parameters included the following markers: %CD34, CD7/CD13 expression in progenitor cells, side scatter (SSC) of granulocyte/lymphocyte ratio, CD13CD16, CD16CD11b, CD13CD11b, CD15CD10 expression in maturing neutrophils, CD36CD14, %CD56 in monocyte, %CD34+CD10+ B-cells, CD71CD235a and CD36/CD235a in erythroid cells. The sensitivity and specificity of flow cytometry score for diagnosing MDS were analyzed. To validate the scoring method, we applied the panel to another set of cytopenic patients (n = 45) and evaluated its performance.* ***Result:*** *Of the 48 patients, 36 were diagnosed with MDS, while 12 did not meet the diagnostic criteria. A multiparameter flow cytometry score ≥ 3 showed 77.7% sensitivity, 100% specificity, 100% positive predictive value and 60% negative predictive value for MDS diagnosis. The parameters %CD34, SSC of granulocyte/lymphocyte ratio, CD13CD16, CD11bCD13, CD11bCD16 and CD71CD235a were strong indicators for MDS diagnosis. When applied to the validation set (n = 45), the results were comparable to those of the training set.* ***Conclusion:*** *Multiparameter flow cytometry is helpful in diagnosing MDS with inconclusive morphology. A score ≥ 3 appears to be an appropriate cut-off value for diagnosis.*

Keywords : ● *Myelodysplastic syndrome* ● *Flow cytometry*

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นิพนธ์ต้นฉบับ

การประยุกต์ใช้โฟลว์ไซโตเมทรีในการวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอส

จุฑาทิพย์ ประจวบจินดา[1] ภัทรศิตา กาลสุวรรณ[2] จันทิญา จันทร์สว่างภูวนะ[2,3] ศิโรรัตน์ ขอบบัวคลี่[2] และ จันทนา ผลประเสริฐ[2,3]
[1]กลุ่มงานอายุรกรรม โรงพยาบาลพุทธโสธร [2]ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ [3]ศูนย์เชี่ยวชาญเฉพาะทางด้านโลหิตวิทยาปริวรรต จุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อ

บทนำ *การวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอสอาศัยการซักประวัติทางคลินิก การตรวจร่างกาย การดูลักษณะความผิดปกติของเซลล์ในไขกระดูก รวมทั้งการตรวจความผิดปกติทางโครโมโซม ในบางกรณีการดูลักษณะความผิดปกติของเซลล์ในไขกระดูกอาจมีความไม่แน่นอนโดยเฉพาะอย่างยิ่งในผู้ป่วยที่มีโครโมโซมปกติหรือจำนวน myeloblast ต่ำ จึงทำให้การวินิจฉัยโรคไม่ชัดเจน* ***วัตถุประสงค์*** *เพื่อตรวจสอบความไวและความจำเพาะของการใช้โฟลว์ไซโตเมทรีในการนำมาวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอส* ***วิธีการดำเนินงานวิจัย*** *การวิจัยนี้ได้รวบรวมผู้ป่วยที่มีภาวะเม็ดเลือดต่ำโดยไม่ทราบสาเหตุที่สงสัยโรคไขกระดูกเสื่อมเอ็มดีเอสจำนวน 48 คน ได้ทำการซักประวัติ ตรวจร่างกาย การส่งตรวจทางไขกระดูกและการส่งตรวจโครโมโซมเพื่อวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอส โดยใช้เกณฑ์การวินิจฉัยตามองค์การอนามัยโลก ค.ศ. 2016 เลือดจากไขกระดูกของผู้ป่วยทุกรายได้รับการตรวจเพิ่มเติมโดยโฟลว์ไซโตเมทรีโดยใช้พารามิเตอร์ที่ศึกษาดังนี้ %CD34, CD7CD13 ของเซลล์ต้นกำเนิด, อัตราส่วนของการกระจายแสงด้านข้างของเม็ดเลือดขาวแกรนูโลไซต์/ลิมโฟไซต์, ความสัมพันธ์ระหว่าง CD13CD16, CD16CD11b, CD13CD11b, CD15CD10 ของเซลล์มัยอิลอยด์, ความสัมพันธ์ระหว่าง CD36CD14 และ %CD56 ของเซลล์โมโนไซต์, CD34CD10 ของเซลล์บีลิมโฟไซต์ในเซลล์ CD34 และ ความสัมพันธ์ของ CD36CD235a, CD71CD235a ของเซลล์เม็ดเลือดแดง หลังจากนั้นรวมผลคะแนนที่ได้ในผู้ป่วยแต่ละคน แล้วนำมาวิเคราะห์ความไวและ ความจำเพาะของการวินิจฉัยด้วยโฟลว์ไซโตเมทรี* ***ผลการวิจัย*** *จากผู้ป่วยที่มีภาวะเม็ดเลือดต่ำโดยไม่ทราบสาเหตุที่สงสัยโรคไขกระดูกเสื่อมเอ็มดีเอส จำนวน 48 คน พบว่ามีผู้ป่วย 36 รายที่ได้รับการวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอส ในขณะที่ผู้ป่วย 12 รายเป็นกลุ่มที่มีเม็ดเลือดต่ำแต่ไม่เข้าเกณฑ์การวินิจฉัยของโรคเอ็มดีเอส การใช้คะแนนโฟลว์ไซโตเมทรีตั้งแต่ 3 คะแนนขึ้นไปในการวินิจฉัยโรคไขกระดูกเสื่อมเอ็มดีเอส มีความไวร้อยละ 77.7 ความจำเพาะร้อยละ 100 ค่าการทำนายเมื่อผลเป็นบวกร้อยละ 100 และค่าการทำนายเมื่อผลเป็นลบร้อยละ 60 โดยพารามิเตอร์ที่มีความสำคัญได้แก่ %CD34, อัตราส่วนของการกระจายแสงด้านข้างของเม็ดเลือดขาวแกรนูโลไซต์/ลิมโฟไซต์, CD13CD16, CD11bCD13, CD11bCD16 และ CD71CD235a เมื่อทำการทดสอบในกลุ่มตรวจสอบอีก 45 รายพบว่าผลเป็นไปในทางเดียวกัน* ***สรุป*** *โฟลว์ไซโตเมทรีมีประโยชน์ในการช่วยวินิจฉัยโรคเอ็มดีเอสซึ่งผลรวมคะแนนตั้งแต่ 3 คะแนนขึ้นไป มีความเหมาะสมใช้เป็นเกณฑ์ในการช่วยวินิจฉัย*

คำสำคัญ : ● *โรคไขกระดูกเสื่อมเอ็มดีเอส* ● *โฟลวไซโตเมทรี*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2568;35:189-99.

Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell diseases characterized by cytopenia, dysplasia in one or more lineages, ineffective hematopoiesis, recurrent genetic abnormalities and increased risk of developing acute myeloid leukemia (AML).[1-3] The diagnosis of MDS is based on a combination of clinical history, morphological assessment and cytogenetic analysis according to the WHO 2016 criteria.[1-3] The thresholds defining dysplasia remain at 10% dysplastic cells in each lineage. Commonly observed dysplastic features include megaloblastoid erythroid maturation, erythroid precursor with nuclear abnormalities, ring sideroblasts, neutrophil hypolobulation or hypogranulation and small megakaryocytes.[2-4] It remains difficult but necessary to distinguish reactive causes of cytopenia and dysplasia from MDS prior to making a diagnosis of MDS, particularly when dysplasia is subtle and limited to a single lineage, or in patients with a normal karyotype or low myeloblast counts.[4] Due to these diagnostic challenges, additional assays can aid in the diagnosis of MDS, including flow cytometry, fluorescent in situ hybridization (FISH) and genomic sequencing techniques.[1-3] Previous studies have reported that multiparameter flow cytometry is a useful tool in supporting the diagnosis of MDS.[5-15] It can help identify abnormal phenotypic patterns and is particularly valuable in cases of minimal dysplasia, based on immunophenotypic abnormalities in myeloid progenitor, granulocyte, monocyte and erythroid lineages. A series of consensus guidelines has been published by the European Leukemia Net (ELN) MDS working group regarding the use of flow cytometry in the diagnostic work-up of patients with MDS.[10,13,14]

The primary objective of this study was to determine the sensitivity and specificity of an in-house 12-parameter flow cytometry scoring method for diagnosing MDS. The secondary objective was to correlate flow cytometry markers with the IPSS-R (Revised International Prognostic Scoring System) risk classification.[16]

Materials and methods

We conducted this retrospective and prospective cohort study at King Chulalongkorn Memorial Hospital. Patients were enrolled from January 2015 to January 2020.

Inclusion and exclusion criteria

Eligible patients were 20 years of age or older and presented with cytopenia in at least one lineage based on complete blood count (hemoglobin < 10 g/dL, platelet count < $100x10^9$/L, absolute neutrophil count < $1.8x10^9$/L). Other conditions that could potentially contribute to bone marrow dysplasia and/or cytopenia were excluded.

Study design

The study consisted of retrospective and prospective cohort investigations and included two cohorts of patients. The first cohort, used as a training set, included 48 patients. The second cohort, used for validation, included 45 patients. All patients underwent bone marrow studies, including bone marrow aspiration, cytogenetic analysis, flow cytometry and bone marrow biopsy. The diagnosis and classification of MDS were established using the WHO 2016 criteria.[1] Written informed consent was obtained from all patients in the prospective cohort. This study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Sample size calculation

$$n_0 = \frac{Z_\alpha^2\, p(1-p)}{d^2} = \frac{(1.96)^2(0.9)(0.1)}{(0.1)^2} = 35$$

n_0 = number of patients with MDS required for the flow cytometry test

p = estimated sensitivity of flow cytometry in diagnosing MDS = 0.6

Z_α = 1.96 (for a 95% confidence level)

d = allowable error = 0.1

Prevalence of MDS among cytopenic patients undergoing bone marrow aspiration = 0.46

To obtain 35 MDS cases, the total sample size required was calculated as: = 75

Flow cytometry studies on bone marrow cells

Bone marrow specimens were collected into ethylene diamine tetra acetic acid (EDTA) tubes and stained using

a whole-blood lysis technique and directly conjugated monoclonal antibodies. Immunophenotyping was performed using eight-color flow cytometry with various fluorochrome-conjugated antibodies. The scoring of our multiparameter flow cytometry was developed using a total of twelve parameters to evaluate bone marrow characteristics. Parameters were selected based on previous studies demonstrating high sensitivity and specificity and followed the recommendation of the IMDS Flow Group. These parameters included side scatter of neutrophils compared with lymphocyte, myeloid progenitor markers, granulocyte lineage markers, monocyte lineage markers, progenitor B lymphoid lineage markers and erythroid lineage markers (Table 1).[5-19]

A total flow cytometry score was calculated for each patient in both cohorts. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the multiparameter flow cytometry scoring system for diagnosing MDS were analyzed by comparing patients with MDS and those with non-clonal cytopenia (non-MDS).

Statistical analysis

Quantitative data were presented using medians and ranges. Categorial data were presented as percentages (%). Sensitivity and specificity of the flow cytometry score were calculated using the receiver operating characteristics (ROC) curve and 95% confidence intervals (CIs) for sensitivity and specificity and sensitivity were based on the binomial distribution. Correlations between flow cytometry markers and IPSS-R were analyzed using Spearman's correlation. Statistical analyses were performed using SPSS, Version 22.0.

Results

Baseline patient characteristics in the training and validation cohorts

A total of 48 and 45 patients with cytopenia of unknown cause were included in the training and validation cohorts, respectively (Table 2). Based on bone marrow and cytogenetic studies, 36 patients in the training cohort and 27 patients in the validation cohort were diagnosed with MDS, while the remaining patients did not meet the criteria for MDS. In the MDS group, low-risk MDS according to the R-IPSS score was the most frequent subtype, accounting for 32% and 41% of patients in the training and validation cohorts, respectively. The MDS subgroups classified according to the WHO 2016 criteria and cytogenetic findings are shown in Supplementary Table S1. Among patients without MDS in both cohorts, immune-mediated cytopenia (20%)

Table 1 Twelve parameters used in the analysis of dysplasia by inhouse flow cytometry (Each parameter counted as 1 score)

Bone marrow subset	Analyses	Aberrancy
Immature progenitor compartment	- % CD34 myeloblast cells in nucleated cell	≥ 2
	- Relationship of CD7+CD13+	Increase
Maturing neutrophils	- SCC of granulocyte ratio vs SSC of lymphocyte	≤ 6
	- Relationship of CD13 and CD16	Altered pattern
	- Relationship of CD16 and CD11b	Altered pattern
	- Relationship of CD13 and CD11b	Altered pattern
	- Relationship of CD15 and CD10	Altered pattern
Monocytes	- Relationship of CD36 and CD14	Altered pattern
	- expression CD56	> 20%
Progenitor B cell	% of CD34+ CD10+B cell in all CD 34+ cell	> 5
Erythroid compartment	Relationship CD71 and CD235a	Altered pattern
	Relationship CD36 and CD235a	Altered pattern

Table 2 Clinical and laboratory characteristics of the study population

	Training cohort				Validation cohort			
	MDS		NonMDS		MDS		NonMDS	
	N = 36	(%)	N = 12	(%)	N= 27	%	N = 18	%
Age								
20-39	3	8.33	2	16.66	0	0	0	0
40-59	8	22.22	3	25	5	18.52	5	27.78
≥ 60	25	69.44	7	58.33	22	81.48	13	72.22
Sex								
Male	25	69.44	3	25	12	44.44	6	33.33
Female	11	30.55	9	75	15	55.56	12	66.67
Hb								
< 8	11	30.55	2	16.66	12	44.44	1	5.56
8 to < 10	20	55.55	5	41.66	12	44.44	6	33.33
≥ 10	5	13.88	5	41.66	3	11.11	11	61.11
ANC								
≤ 800	9	25	1	8.33	6	22.22	0	0
> 800	27	75	11	91.66	21	77.78	18	100
Platelet								
< 50,000	9	25	3	25	8	29.63	5	27.78
50,000 to < 100,000	7	19.44	3	25	8	29.63	6	33.33
≥ 100,000	20	55.56	6	50	11	40.74	7	38.89

was the most common cause of cytopenia, followed by anemia in the elderly, cirrhosis, chronic kidney disease and drug-induced cytoepenia (each accounting for 10%) (Supplementary Figure S1).

Sensitivity and specificity of multiparameter flow cytometry in training and validation cohorts

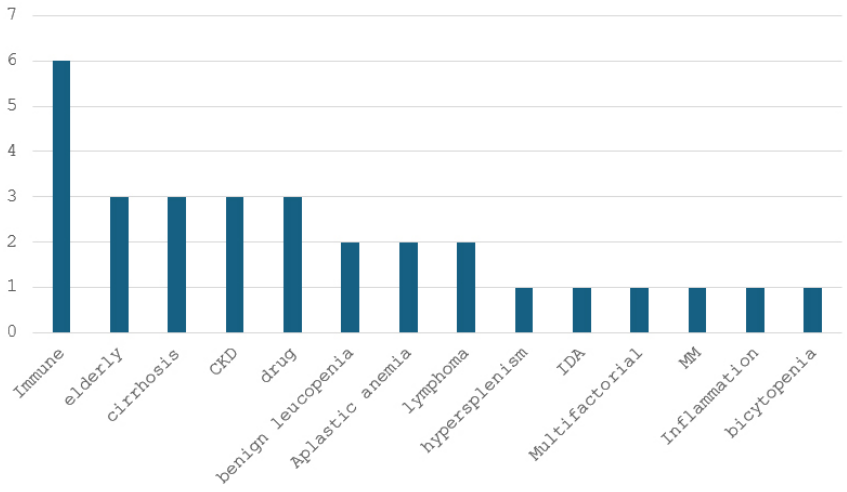
Multiparameter flow cytometry score was performed for all patients in both cohorts. In the training cohort, flow cytometry scores for the MDS and non-MDS groups are shown in Table 3. An optimal cutoff value of ≥ 3 was selected based on ROC curve analysis, with an emphasis on maximizing the specificity of the test. A multiparameter flow cytometry score ≥ 3 showed 80.56% sensitivity, 100% specificity, 100% PPV and 63.16% NPV for MDS diagnosis, with an area under the ROC curve (AU) of 0.948 (95%CI: 0.89-1.00) (Figure 1A). A multiparameter flow cytometry score of showed 100% sensitivity, 83% specificity, 78.2% PPV and 100% NPV for excluding MDS. In the validation cohort, flow cytometry scores are shown in Table 4. A score ≥ 3 yielded 51.85% sensitivity, 100% specificity, 100% PPV and 58.06% NPV for MDS diagnosis, with an AUC of 0.829 (95%CI: 0.709-0.095) (Figure 1B). A score of 0 in the validation set showed 88.89% sensitivity, 27.78% specificity, 64.86% PPV and 62.5% NPV for excluding MDS.

Correlation of individual flow cytometry parameters for diagnosing MDS

Flow cytometry markers that were strong indicators for MDS diagnosis included %CD34+ progenitor cells, granulocyte/lymphocyte SSC ratio, relationship of CD13CD16, CD11bCD13, CD11bCD16 of maturing neutrophils and relationship of CD71CD235a of erythroid compartment. In contrast, the monocyte markers CD36CD14 was a poor predictor for MDS (Supplementary Table S2 and Figure S2).

Supplementary Table S1 Diagnosis according to WHO 2016 classification, IPSS-R risk classification and cytogenetic result of MDS patients in Training cohort (N = 38) and validation cohort (N = 27).

	Training cohort		Validation cohort	
	N = 38	**%**	**N = 27**	**%**
WHO-classification				
• Del5q	2	5.12	0	0
• MDS SLD	2	5.12	6	22.22
• MDS MLD	13	33.33	9	33.33
• MDS EB I	4	10.25	3	11.11
• MDS EB II	6	15.38	1	3.70
• MDS-U	2	5.12	1	3.70
• AML-MRC	10	25.64	6	22.22
• MDS/MPN	0	0	1	3.70
IPSS-R				
• Very low risk	4	10.81	1	3.70
• Low risk	12	32.43	11	40.74
• Intermediate risk	5	13.51	5	18.51
• High risk	11	29.73	4	14.81
• Very high risk	5	13.51	6	22.22
Cytogenetic				
• Normal	25	67.56	15	55.55
• 45, X, -Y	3	8.11	0	0
• Del(5q)	2	5.41	0	0
• Complex	2	5.41	3	11.11
• Others [-7, +8, +21, der (18), del (20)]	5	13.51	7	25.92



CKD, chronic kidney disease; IDA, iron deficiency anemia; MM, multiple myeloma

Supplementary Figure S1 Causes of non-MDS cytopenia in both cohorts

Table 3 Flow cytometry score from training cohort

	Flow cytometer score														
	N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Score ≥ 3**
NonMDS	18	5	7	6	-	-	-	-	-	-	-	-	-	-	0 (0%)
All MDS	27	3	1	9	5	4	3	1	-	1	-	-	-	-	14 (51.85%)
Lower risk MDS	13	3	1	3	3	2	1	-	-	-	-	-	-	-	6 (46.15%)
Higher risk MDS	14	-	-	6	2	2	2	1	-	1	-	-	-	-	8 (57.14%)

Lower risk MDS = R-IPSS ≤ 3.5; Higher risk MDS = R-IPSS > 3.5

Table 4 Flow cytometry score from validation cohort

	Flow cytometer score														
	N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Score ≥ 3**
NonMDS	18	5	7	6	-	-	-	-	-	-	-	-	-	-	0 (0%)
All MDS	27	3	1	9	5	4	3	1	-	1	-	-	-	-	14 (51.85%)
Lower risk MDS	13	3	1	3	3	2	1	-	-	-	-	-	-	-	6 (46.15%)
Higher risk MDS	14	-	-	6	2	2	2	1	-	1	-	-	-	-	8 (57.14%)

Lower risk MDS = R-IPSS ≤ 3.5; Higher risk MDS = R-IPSS > 3.5

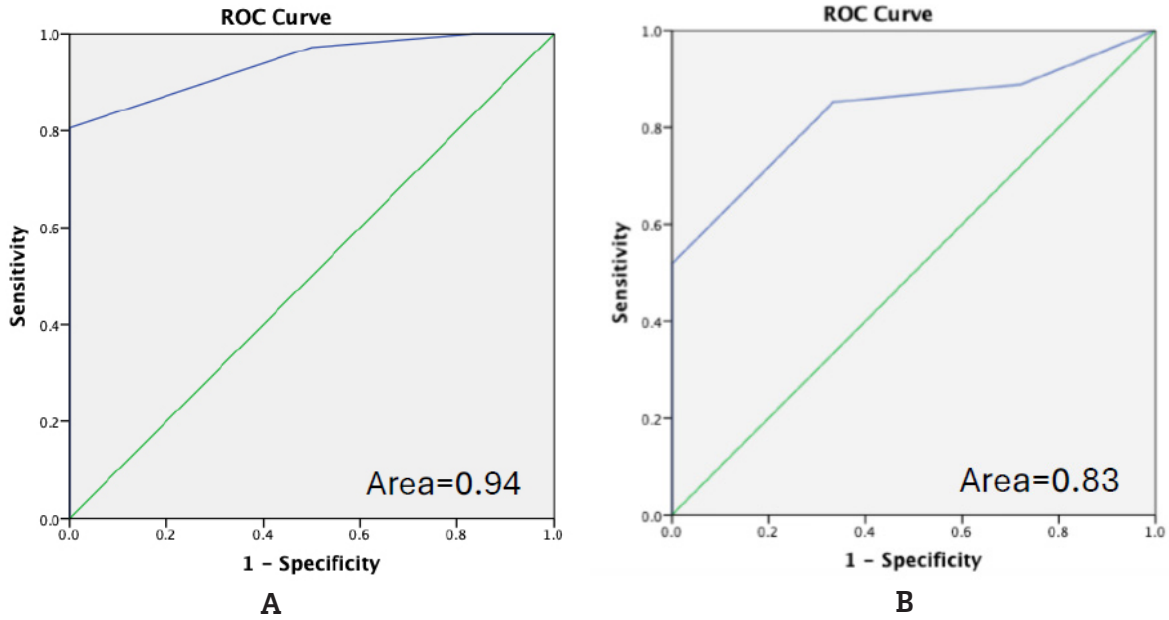


Figure 1 Receiver Operating Characteristic (ROC) curve of total flow cytometry score when predicting myelodysplastic syndrome. A) Training cohort, B) Validation cohort.

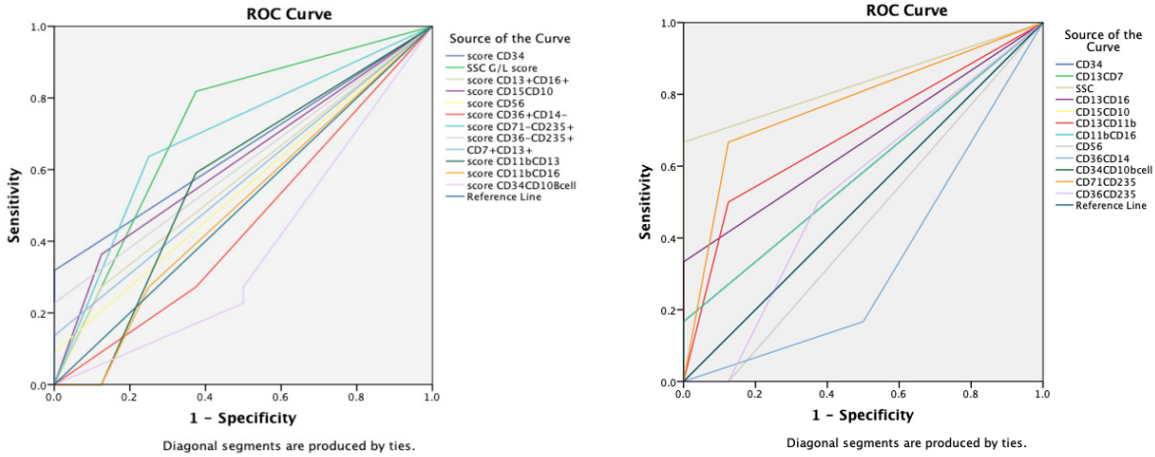
Correlation of flow cytometry markers with IPSS-R

A total of 63 MDS patients from both the training and validation cohorts were included to assess the correlation between IPSS-R scores and total flow cytometry scores using Spearman's correlation. The analysis showed a weak positive correlation between the IPSS-R score and total flow cytometry score (correlation coefficient = 0.365, p = 0.0284). However, there was no significant correlation between cytogenetic risk groups and flow cytometry scores (correlation coefficient = 0.15, p = 0.25).

Supplementary Table S2 Area under the curve (AUC) of 12 parameters when predicting myelodysplastic syndrome by ROC curve analysis.

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CD34	.583	.162	.606	.266	.901
CD13CD7	.583	.162	.606	.266	.901
SSC	.833	.127	.039	.585	1.000
CD13CD16	.667	.157	.302	.359	.975
CD15CD10	.583	.162	.606	.266	.901
CD13CD11b	.688	.153	.245	.388	.987
CD11bCD16	.583	.162	.606	.266	.901
CD56	.438	.158	.699	.128	.747
CD36CD14	.333	.150	.302	.040	.627
CD34CD10bcell	.500	.161	1.000	.184	.816
CD71CD235a	.771	.139	.093	.499	1.000
CD36CD235a	.531	.160	.846	.217	.845

[a]Under the nonparametric assumption; [b]Null hypothesis: true area = 0.5



A B

Supplementary Figure S2 Receiver Operating Characteristic (ROC) curve of each parameter when predicting myelodysplastic syndrome. A) Training cohort, B) Validation cohort.

Discussion

This retrospective and prospective cohort study confirmed that our in-house twelve-parameter flow cytometry panel can be used to support the diagnosis of MDS. Based on our results, we suggest a cut-off score ≥ 3 for confirming an MDS diagnosis, as it demonstrated high sensitivity and specificity. The presence of multiple aberrancies conferred a higher predictive value for MDS than single aberrancies. Performance in the validation cohort showed a slightly lower AUC compared to the training cohort, which was expected due to potential overfitting. However, the high AUC on the validation cohort suggests that the model generalizes well and maintains good predictive performance when applied to new data. Since there are no standard flow cytometry panels recommended by the ELNet working group, we selected parameters from previous studies that demonstrated high sensitivity and specificity.[5-19] To evaluate

dysplasia in the neutrophil compartment, we used the reference range for decreased SSC of neutrophils compared with lymphocytes from the Ogata score (≤ 6), which showed good predictive performance in our study (AUC 0.833), as indicated in ELN guidelines.[9,10] Dysplastic neutrophils were also identified by aberrant expression patterns between CD13 and CD16, CD13 and CD11b, CD11b and CD16, which differentiated MDS from non-clonal cytopenia and served as strong diagnostic markers (Figure 2).

Among these, the relationships between CD13/CD16 and CD13/CD11b were more predictive than the other two parameters (Supplementary Table S2). In evaluating the monocyte population, we assessed abnormal CD36 and CD14 expression, as well as CD56 expression. However, these markers proved to be weak predictors. This may have been due to missing data in some parameters, which could have led to falsely low scores in the MDS group. In the erythroid lineage, the lysis technique may affect the recovery of erythroid progenitors. Among the erythroid markers, the correlation between CD71 and CD235a was a more reliable predictor of dysplasia than CD36 and CD235a (Supplementary Table S2).

The IPSS-R is used to predict median survival and time to 25% AML transformation.[16] In our study, the IPSS-R demonstrated a weak positive correlation with

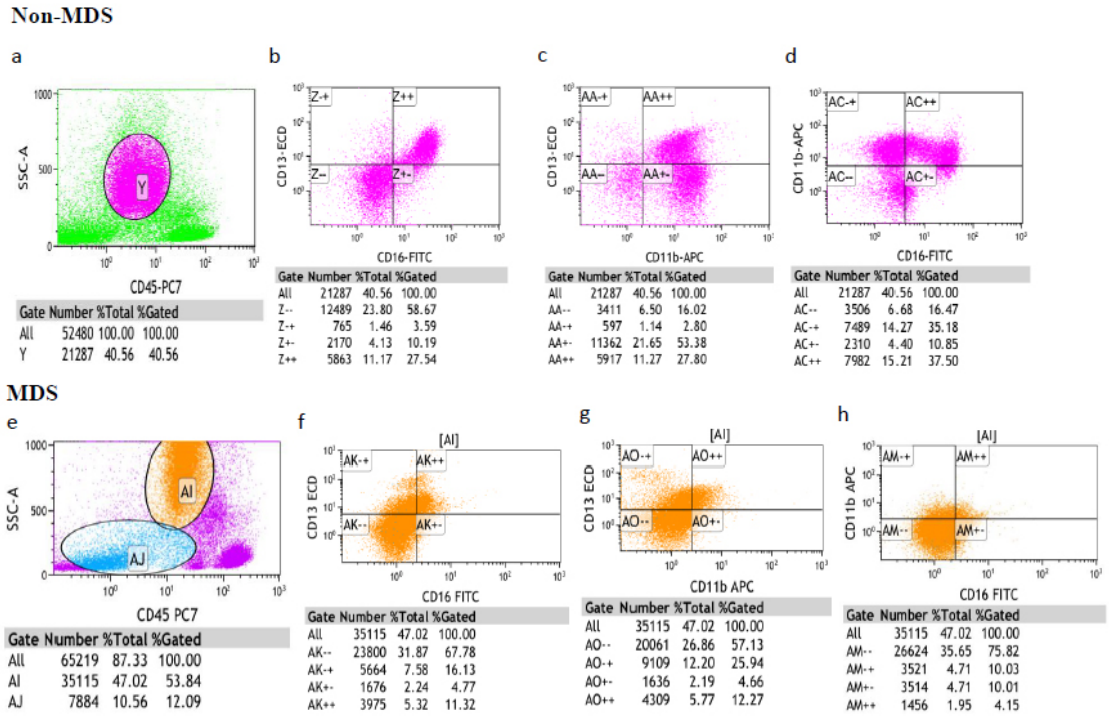


Figure 2 Immunophenotypic myeloid abnormalities in MDS compared to normal. Upper panel: analyzed gating is based on CD45 (x-axis) versus side light scatter (SSC, y axis) and granulocyte is demonstrated in the pink circle region in non-MDS patients (a). Normal pattern of correlation between CD13CD16, CD13CD11b and CD-11bCD16 of maturing neutrophils is shown in panel (b), (c) and (d). Lower panel: analyzed gating is based on CD45 (x-axis) vs. side light scatter (SSC, y axis) and granulocyte is demonstrated in the orange circle region in MDS patientsas shown in panel (e). Panel (f) shows decreased CD13+CD16+ in maturing myeloid in MDS when compared with that of nonMDS (b). Panel (g) shows decreased expression of CD11+CD13- in MDS when compared with that of non-MDS (c). The last panel (h) shows decreased expression of CD11b+CD16+ in MDS compared with nonMDS (d).

the flow cytometry score, whereas other studies have reported stronger correlations.[18,20-22] This discrepancy may be due to the small sample size in each subgroup. Although flow cytometry may help predict prognosis of MDS, this could not be demonstrated in our study due to the short follow-up period and loss to follow-up in some patients.

This study used the WHO 2016 criteria for diagnosis, which rely on morphology and cytogenetics. The WHO 2022 criteria include mutations to better define MDS subgroups, but morphology remains the central criterion in both versions. Thus, our flow cytometry platform remains applicable for diagnostic purposes under both the 2016 and 2022 WHO frameworks. The limitations encountered in this study included missing data for some parameters in the retrospective cohort and use of different fluorochromes in some patients. Although our target was 75 patients, only 48 were included in the training set and 45 in the validation set. A number of non-MDS control patients (n = 12 in the training set; n = 18 in the validation set) was also relatively small.

We analyzed the diagnostic utility of each flow cytometry parameter using ROC curve analysis. The SSC granulocyte/lymphocyte ratio had the highest AUC. Other strong parameters included the relationship of CD71CD235a in the erythroid compartment and the relationship of CD13CD16, CD13CD11b in the myeloid compartment. These parameters should be considered essential components of a diagnostic flow cytometry panel. However, reference ranges may vary slightly between institutions due to differences in fluorochromes and instrument settings.

Conclusion

Multiparameter flow cytometry is a valuable tool for supporting the diagnosis of MDS. A flow cytometry score ≥ 3 is an appropriate cut-off to support the diagnosis of MDS, whereas a score of 0 may help exclude MDS.

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Conflict of interest

The authors declare no conflict of interest.

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