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Original Article

Simplified flow cytometry scoring for diagnosis and prognosis of myelodysplastic symptom

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Abstract: A flow cytometric score (FCM-score) to diagnose myelodysplastic syndromes (MDS) was proposed in 2012 that used four parameters to distinguish low-grade MDS from non-clonal cytopenias. This study was carried out to further simplify the method for better clinical application. Combinations of antibodies CD34, CD19, CD33 and CD45 were analyzed for the four parameters. Compared with the published method that used low side scatter (SSC) and CD45 expression to separate B lymphocyte progenitor cells and myeloblasts, our method (MFCM-Score) used CD19 and CD33 to separate B lymphocyte progenitor cells and myeloblasts within the $CD34^{+}CD45^{dimm}$ population. Subjects were analyzed and compared using the two schemes. In addition, the relationships between the MFCM-Score and the Revised International Prognostic Scoring System (IPSS-R) for MDS were analyzed. There was no significant difference between the MFCM-score and FCM-score in the diagnosis of MDS ($P > 0.05$); MFCM-score had a positive correlation with the IPSS-R prognosis classification for MDS (Spearman r = 0.848, $P < 0.001$). All parameters in the MFCM-score were positively correlated to the IPSS-R grades in MDS ($P < 0.01$). Our work demonstrates that the FCM score using four parameters is simple and practical for screening MDS patients and the MFCM-score could be used to evaluate the risk of MDS patients.

Keywords: Myelodyplastic syndromes, flow cytometry, prognostic scoring system, immunophenotype

Introduction

Myelodysplastic syndromes (MDS) are a group of heterogeneous diseases characterized by ineffective hematopoiesis, peripheral blood cytopenias, dysplasia in one or more myeloid lineage cells in bone marrow, and are classified by the WHO as myeloid neoplasms [1]. Ineffective hematopoiesis often leads to a high risk of progression to leukemia [2, 3]. The incidence rate is about 5 cases per 100000 persons per year in the general population, but increases to 20 to 50 cases per 100000 persons per year after the age of 60 years [4, 5]. According to WHO criteria, the classification of myelodysplastic syndromes is based on peripheral cytopenias and morphological evaluation of bone marrow dysplasia, as well as on other evidences, such as clonal cytogenetic abnormalities and/or ringsideroblasts. However, in clinical practice, when patients are negative for these diagnostic markers, MDS diagnosis is not always straightforward [6-8]. Therefore, auxiliary diagnosis for MDS is needed. Qualitative and quantitative flow cytometry (FCM) are well developed and play a very important role in the diagnosis of blood system diseases [9]. At present, MDS-specific set of antigens or antibodies has not been identified for flow cytometry-based diagnosis. However, since flow cytometry is capable to diagnose reactive and clonal proliferations of bone marrow hematopoietic cells, it has been explored as the auxiliary tool for MDS diagnosis [10, 11]. FCM-based MDS diagnosis is accurate, practical and widely used in clinics. However, the methods are still complicated, need to use many antibodies and are highly expensive, not suitable for use in developing countries [12-15]. To resolve these issues, a flow cytometric score (FCM-score) was proposed for MDS patients capable of distinguishing low-grade MDS from non-clonal cyto-

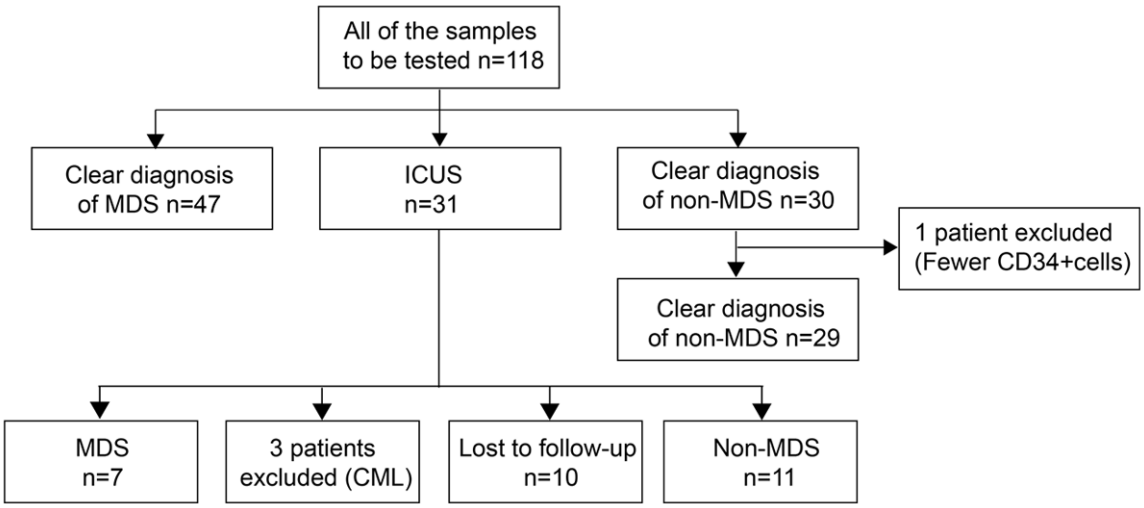

Figure 1. Flow chart for patient selection, grouping and follow-up. A total of 118 patients suspected of MDS was enrolled, consisting of 47 and 30 unequivocal MDS and non-MDS patients and 31 Idiopathic cytopenia of undetermined significance (ICUS) patients. After 6-month follow-up, 7 of the ICUS patients were classified as MDS and 12 as non-MDS. 10 patients were lost and 3 patients were excluded because the final diagnosis was CMALL. In 30 a definitive diagnosis of MDS patients, 1 patient was excluded because of the lack of $CD34^+$ cells, leaving 54 MDS and 40 non-MDS for analyses.

penias, and the method is simple and has high sensitivity and specificity [16]. To further improve this method, we investigated the gating strategy and cluster of differentiation (CD) markers in FCM for MDS diagnosis and our findings demonstrate that the improved FCM-score has better accuracy, objectiveness and clinical application.

Materials and methods

Patients

A total of 118 patients suspected of MDS because of peripheral blood cytopenia from the Second Affiliated Hospital, Anhui Medical University, China, were enrolled in this study. The selection process is described in **Figure 1**. Patients were diagnosed and classified in accordance with the minimum diagnostic criteria established by the Conference on MDS (Vienna, 2006) and the 2008 WHO criteria respectively [6, 17]. All patients were subjected to morphological evaluation, cytogenetic analysis and hemosiderin staining. Biopsy was carried out in case the morphology was difficult to diagnose. If there was no evidence of clonality by genetic studies, the patient was classified as having idiopathic cytopenia of undetermined significance (ICUS) and needed to be observed clinically for 6 months before MDS was diagnosed [7]. At the same time, patients were assessed for prognostic risk according to the Revised International Prognostic Scoring System (IPSS-R) for MDS [18]. The differential diagnosis of MDS and non-clonal cytopenias diseases were based on medical history, clinical characteristics, morphological changes and response to treatment. Patients were grouped into MDS (n = 45) and non-clonal cytopenias (n = 41) groups and their clinical and demographic data were retrieved and are shown in **Table 1**. This study was approved by the ethics committee of Anhui Medical University and strictly abided by the Declaration of Helsinki revised in 2000.

Sample preparation

Bone marrow samples with heparin as anticoagulant were collected and the nucleated cell counts were adjusted to $5\text{-}8\times10^5$ per tube and incubated in the dark with fluorescent-labeled monoclonal antibodies (CD34-FITC, CD19-PE, CD33-APC, CD45-PC7, Beckman Coulter, Miami, FL, USA) and treated with NH_4Cl for 10 min before FACS analysis on a flow cytometer (FC500 MPL, Beckman Coulter). EXPO 32 MultiComp software (Beckman Coulter) was used for data acquisition and analysis. At least 10^5 nucleated cells and 500 $CD34^+$ cells were acquired for most samples. Isotype-matched antibodies were used as controls.

Table 1. The clinical and biological characteristics of the study population

Clinical and biological characteristics	Non-MDS (n = 40)	MDS (n = 54)
Median age, year (range)	64 (22-81)	66 (20-86)
Gender (female/male)	15/25	21/33
B12/folate deficiency	3	0
Anemia associated with iron deficiency	2	0
Chronic liver disease	6	0
Aplastic anemia	6	0
Anemia associated with renal failure	2	0
Anemia of chronic disorders	1	0
Drug-induced cytopenias	1	0
Cytopenia associated with marrow infiltration	1	0
Autoimmune cytopenia	3	0
Idiopathic thrombocytopenic purpura	8	0
Infective cytopenia	3	0
Paroxysmal nocturnal hemoglobinuria	1	0
Solid tumors related cytopenia	2	0
Hemophagocytic syndrome (HPS)	1	0
RCUD	0	3
RARS	0	6
RCMD/RS	0	25
MDS-U	0	1
RAEB-1	0	12
RAEB-2	0	7
Karyotype		
Good plus very good, intermediate, Poor plus very poor	0	39, 14, 1
Marrow blasts (%)		
≤ 2, 2-5, 5-10, > 10	0	31, 4, 12, 7
Hb (g/L)		
≥ 100, 80-100, < 80	0	8, 10, 33
PLT		
≥ 100, 50-100, < 50	0	12, 18, 24
ANC		
≥ 0.8, < 0.8	0	34, 20
IPSS-R		
Very-low, low, intermediate, high, very-high	0	2, 11, 19, 18, 4

Scoring

Four parameters (cutoff) reported previous were used for scoring, i.e. myeloblast-related cluster size (myeloblast-related cells/all nucleated cell), B-progenitor-related cluster size (B-progenitor-related cells/all $CD34^+$ cells), CD45 mean fluorescence intensity (MFI) ratio (lymphocytes/myeloid blast cells) and SSC peak channel ratio (granulocyte/lymphocyte) [16]. A value of 1 was assigned to each parameter and MDS was diagnosed if a FCM-score was ≥ 2.

Gating strategy

First, on the forward scatter (FSC)-vs-side scatter (SSC) dot plot (**Figure 2A**), all nucleated cells were gated (P1) after removing adhesion cells and impurities, and cells with relatively low SSC were gated (P2) and then plotted on a CD45 vs CD34 display (**Figure 2B**). Next, $CD34^+$ cells with intermediate CD45 expression ($CD34^+$ $CD45^{dimm}$) were gated (P3) within all nucleated cells (P1) with low SSC characteristics (P2) (**Figure 2C**). Using the gating strategy as described [16], $CD34^+CD45^{dimm}$ (P3) were plot-

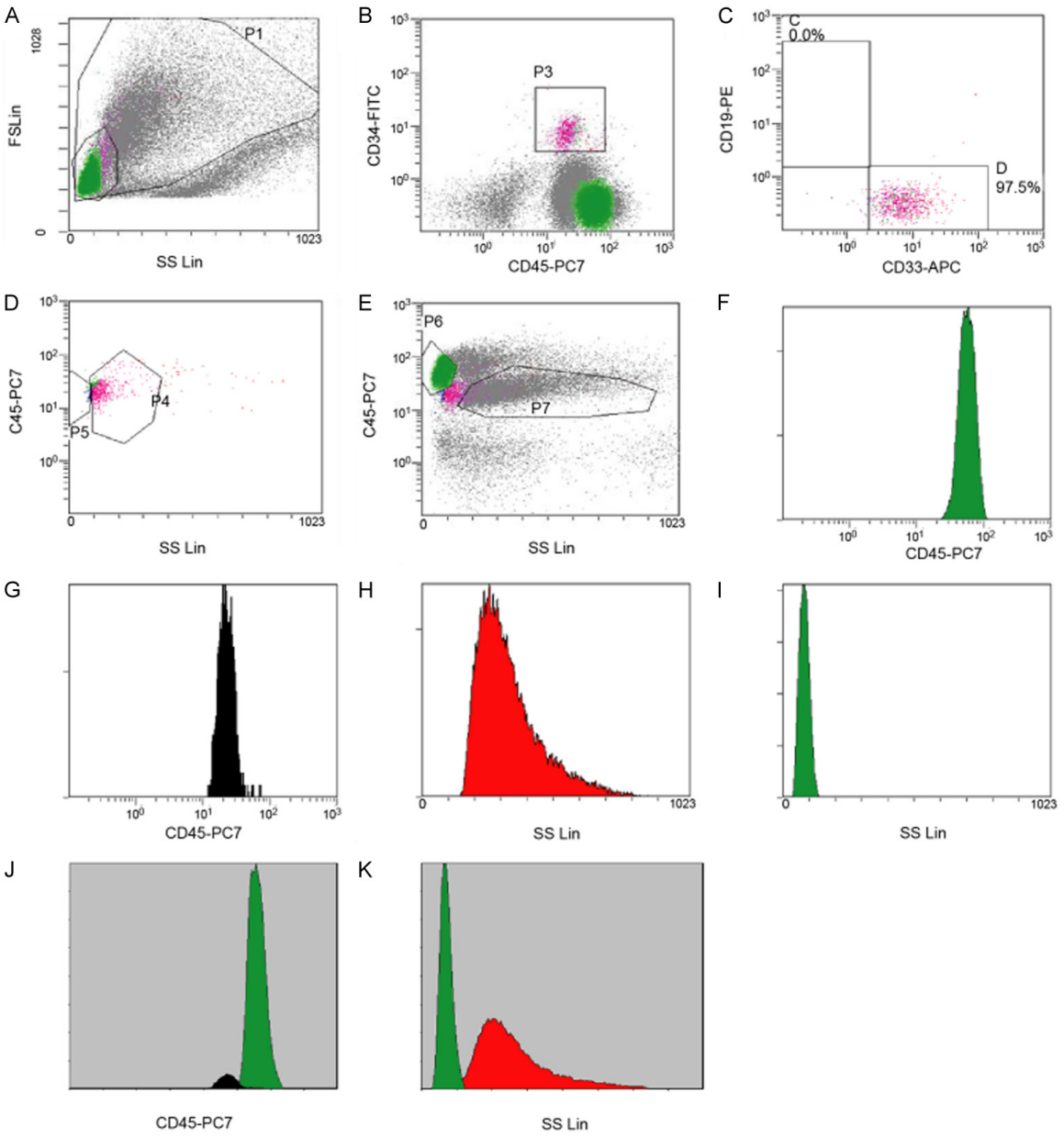

Figure 2. Flow cytometry analysis of scoring parameters from samples of cells stained with antibodies against CD34, CD19, CD33 and CD45. A. All nucleated cells were gated P1 with cell debris removed, cells with relatively low SSC were gated P2. B. P2 was plotted on a CD45 versus CD34 display, $CD34^{+}CD45^{dimm}$ cells were gated P3. C. P3 were plotted on a CD19/CD33 display, B-progenitor-related cluster C and the myeloblast-related cluster D. D. Cells in the P3 gate were plotted on a CD45-versus-SSC display, P5 denotes B-progenitor-related cluster, P4 denotes the myeloblast-related cluster. E. All nucleated cells were plotted on a CD45-versus-SSC display, P6 denotes lymphocytes, P7 denotes granulocytic cells. F, G. Mean fluorescence intensity (MFI) of CD45 expression on lymphocytes P6 and myeloblasts D. H, I. Peak channel ratio of SCC of granulocytic cells P7 and lymphocytes P6. J. 6 and 7 were computed using the software. K. 8 and 9 were computed using the software.

ted on a CD45/SSC display (**Figure 2D**), B-progenitor cells were deemed to have the lowest SSC and relatively low CD45 expression (P5) and other $CD34^{+}$ cells (P4) showing more SSC and CD45 expression were identified as myeloblasts and were gated, respectively. If B-progenitor blasts were separated from myeloblasts by SSC and CD45 expression using

Table 2. MFCM-score in patients with and without MDS

Variables	MDS	Non-MDS	*P*
Number of patients (n)	54	40	
Median age, year (range)	66 (20-86)	64 (22-81)	0.123
Median myeloblast-related cluster size 1, % (range)	2 (0.04-16.75)	0.43 (0.01-2.3)	< 0.001
Median B-progenitor-related cluster size, % (range)	0.7 (0-10)	5.51 (0-44)	0.001
Median mean fluorescence intensity, (range)	4.1 (1.8-16.1)	4.8 (2.3-10)	0.341
Median SSC peak channel ratio 4, (range)	5.7 (3.3-9.6)	6.7 (4.9-7.9)	< 0.001
Median MFCM-score	3 (1-4)	1 (0-3)	< 0.001

reported algorithm [16], it might be influenced by operator experience. Therefore, a slight change was made on the gating strategy to distinguish B-progenitor blasts from myeloblasts: $CD34^{+}CD45^{dimm}$ (P3) were plotted on a CD19/CD33 display (**Figure 2C**). The B-progenitor cells (C) and the myeloblasts (D) were then identified by positive expression of CD10 and CD33, respectively. In addition, it is also feasible to identify the B-progenitor cells by CD19 [13]. At the same time, all cells could be plotted on a CD45/SSC display (**Figure 2E**) and lymphocytes (P6) and granulocytic cells (P7) were gated [16]. CD45 MFI on lymphocytes (P6 gate) and myeloblasts (D gate) were plotted (**Figure 2F** and **2G**), and the both fractions were computed and plotted (**Figure 2H**). Similarly, SSC peak channel ratios of granulocytic cells (P7 gate) and lymphocytes (P6 gate) were plotted (**Figure 2I** and **2J**), and the both fractions were computed and plotted (**Figure 2K**). Using this simplified gating strategy, four CD antibodies were used to analyze the four parameters for a FCM-score.

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). For normally distributed variables, Student's t test was used to compare means between groups. The Mann-Whitney U test was used to compare the differences with non-Gaussian distribution. Chi square test of paired four grid data was used to compare the detection rates by MFCM- and FCM-score. Pearson correlation analysis was used for numeric type tests enabled to investigate data distribution. Spearman correlation analysis was used for ranking correlation tests. A value of $P < 0.05$ was considered significant.

Results

Baseline characteristics and FCM-score

There was no significant difference between MDS and non-MDS groups in age (median 66, range 20-86 vs 64, range 22-81, $P = 0.123$). The myeloblast-related cluster size was significantly greater in MDS than in non-MDS patients (median 2, range 0.04-16.75, vs median 0.43, range 0.01-2.3, $P < 0.001$), B-progenitor-related cluster size was smaller in MDS than in non-MDS patients (median 0.7, range 0-10 vs 5.51, range 0-44, $P = 0.001$), CD45 MFI ratios were similar in the two groups ($P = 0.341$). It was noted that the MDS group did not conform to the Gaussian distribution, while the non-MDS group was in line with the Gaussian distribution. SSC peak channel ratio was less in MDS than in non-MDS patients (median 5.7, range 3.3-9.6 vs 6.7, range 4.9-7.9, $P < 0.001$). The total score was significantly higher for MDS than for non-MDS patients ($P < 0.001$, **Table 2**).

MFCM-score was better than FCM-score

The two schemes were used to analyze the study populations at the cut-off of 2, and evaluated for their diagnostic performance. Using the FCM-score, 49 of 54 MDS cases were diagnosed (sensitivity 90.7%); 7 false-positive (misdiagnosed) was found in non-MDS (specificity 82.5%); the agreement between clinical and FCM-score diagnosis was 87.2%, the PPV and NPV were 87.5% and 86.8%, respectively (**Table 3**). Using the MFCM-score, 52 of 54 MDS cases were correctly diagnosed (sensitivity 96.3%) and there were 5 false-positive in non-MDS patients (specificity 87.5%) (**Table 4**), the agreement between clinical and FCM-score diagnosis was 92.6%, the PPV and NPV were 91.2%

Table 3. Comparison of MDS diagnosis by FCM-score and clinical examination

FCM-score diagnosis	Clinical diagnosis		
	MDS	Non-MDS	Total
MDS	49 (a')	7 (b')	56 (a'+b')
Non-MDS	5 (c')	33 (d')	38 (c'+d')
Total	54 (a'+c')	40 (b'+d')	94 (a'+b'+c'+d')

Sensitivity = [a'/(a'+c')] ×100% = 90.7%. Specificity = [d'/(b'+d')] ×100% = 82.5%. Positive predictive value = [a'/(a'+b')] ×100% = 87.5%. Negative predictive value = [d'/(c'+d')] ×100% = 86.8%. Diagnosis agreement rate = [(a'+d')/(a'+b'+c'+d')] ×100% = 87.2%. Positive likelihood ratio, +LR = Sensitivity/(1- Specificity) = 5.2. Negative likelihood ratio, -LR = (1- Sensitivity)/Specificity = 0.1.

Table 4. Comparison of MDS diagnosis between MFCM-score and clinical examinations

MFCM-score diagnosis	Clinical diagnosis		
	MDS	Non-MDS	Total
MDS	52 (a)	5 (b)	57 (a+b)
Non-MDS	2 (c)	35 (d)	37 (c+d)
Total	54 (a+c)	40 (b+d)	94 (a+b+c+d)

Sensitivity = [a/(a+c)] ×100% = 96.3%. Specificity = [d/(b+d)] ×100% = 87.5%. Positive predictive value (PPV) = [a/(a+b)] ×100% = 91.2%. Negative predictive value (NPV) = [d/(c+d)] ×100% = 94.6%. Diagnosis agreement rate = [(a+d)/(a+b+c+d)] ×100% = 92.6%. Positive likelihood ratio, +LR = Sensitivity/(1- Specificity) = 7.7. Negative likelihood ratio, -LR = (1- Sensitivity)/Specificity = 0.04.

and 94.6%, respectively (**Table 3**). The false-positive five patients included two patients with idiopathic thrombocytopenic purpura, one patient with the anemia of chronic disorders, one with anemia associated with renal failure who had a long history of hemodialysis, one with cytopenia associated with marrow infiltration due to the metastasis of lung cancer. Chi square tests showed that the diagnostic rates by MFCM-score and FCM-score were similar ($P > 0.05$).

The 94 patients with MDS were scored by two schemes respectively. The diagnosis cut-off score was ≥ 2. The scores were different in 11 patients due to FCM scheme. 5 out of the 11 were correctly diagnosed due to use of MFCM-score, two of them were in MDS group and the remaining three were in non-MDS group. Using the simplified gating strategy, 10 out of the 11 patients had the score change due to changes in B-progenitor-related cluster size and one due to changes in myeloblast-related cluster size (**Table 5**).

MFCM-score and IPSS-R for MDS were positively related

IPSS-R for MDS is predominantly based on a new comprehensive cytogenetic scoring system (MDS cytogenetic scoring system) and marrow blasts. It defines five risk groups with different clinical outcome [18-20]. The relationships of MFCM-score in non-MDS group and IPSS-R are shown in **Table 6**. Due to limited number of samples in the Very low and Very high groups of IPSS-R (2 and 4, respectively), the two groups were combined with adjacent groups to reduce error. In the non-MDS group, patients with MFCM-score of 0–1 and over 1 (or 2-4) accounted for 87.5% (35/40) and 12.5% (5/40) of the group, respectively. In all patients with MDS, the percentages for MFCM-score of 0-1 and over 1 was 7.7% (2/54) and 92.3% (52/54), respectively. When non-MDS patients were compared with Very low and low patients, Very low and low groups had more patients with the score over 1 (≥ 2, which was the cut-off for MDS) and a higher median MFCM-score (12.5% median 1 vs 84.6% median 2). The Int group of MDS patients and Very high and high patients showed a similar percentage of MFCM-score over 1 (all 100%), however, the Very high and high patients showed a higher median MFCM-score (median 3 vs median 3.5). As seen in **Figure 3** and **Table 6**, from non-MDS to MDS, MFCM-score had a positive correlation with non-MDS and IPSS-R (Spearman r = 0.848, $P < 0.001$).

As shown in **Table 7**, the patients with high-grade MDS showed higher myeloblast-related cluster size, from non-MDS to MDS, the size was on the rise, suggesting that the size is a positively correlated with IPSS-R (Spearman's rank correlation: r = 0.732, $P = 0.01$). Increased number and aberrant antigen expression of $CD34^+$ cells in BM precursors have been shown to be independent risk factors for survival [21, 22]. There was a positive correlation between B-progenitor-related cluster size and IPSS-R risk (Spearman's rank correlation: r = 0.549, $P < 0.01$, **Table 7**), indicating that the patients with low-grade MDS have lower B-progenitor-

Table 5. Comparison of MDS diagnosis based on MFCM-score, FCM-score and clinical examinations

Sample	Myeloblast-related cluster size		B-progenitor-related cluster size		CD45 mean fluorescence intensity ratio		SSC peak channel ratio		Score		Diagnosis based on		
	MFCM	FCM	MFCM	FCM	MFCM	FCM	MFCM	FCM	MFCM-score	FCM-score	MFCM-score	FCM-score	Clinical examination
1	0	0	0	1	0	0	0	0	0	1	Non-MDS	Non-MDS	Non-MDS
2	0	0	0	1	1	1	0	0	1	2	Non-MDS	MDS	Non-MDS
3	0	0	0	1	0	0	1	1	1	2	Non-MDS	MDS	Non-MDS
4	0	0	1	0	0	0	0	0	1	0	Non-MDS	NM	Non-MDS
5	1	1	1	0	0	0	0	0	2	1	MDS	NM	MDS
6	0	0	1	0	1	1	0	0	2	1	MDS	NM	MDS
7	0	0	1	0	0	0	1	1	2	1	MDS	NM	MDS
8	0	0	0	1	1	1	1	1	2	3	MDS	MDS	MDS
9	1	1	1	0	1	1	0	0	3	2	MDS	MDS	MDS
10	1	0	1	1	1	1	1	1	4	3	MDS	MDS	MDS
11	1	1	1	0	1	1	1	1	4	3	MDS	MDS	MDS

Table 6. The relationship of MFCM-scores with the non-MDS group and the IPSS-R prognosis classification for MDS

MFCM-score	No. MDS (*n* = 40)	MDS group (*n* = 54)			
		Very-low and Low (n = 13)	Int (n = 19)	High and Very high (n = 22)	Total (n = 54)
0	12 (30%)	0	0	0	0
1	23 (57.5%)	2 (15.4%)	0	0	2 (3.7%)
2	3 (7.5%)	10 (76.9%)	3 (15.8%)	3 (13.6%)	16 (29.6%)
3	2 (5%)	1 (7.7%)	14 (73.7%)	8 (36.4%)	23 (42.6%)
4	0	0	2 (10.5%)	11 (50%)	13 (24.1%)
≥ 2	5 (12.5%)	11 (84.6%)	19 (100%)	22 (100%)	52 (92.3%)
Median	1	2	3	3.5	3

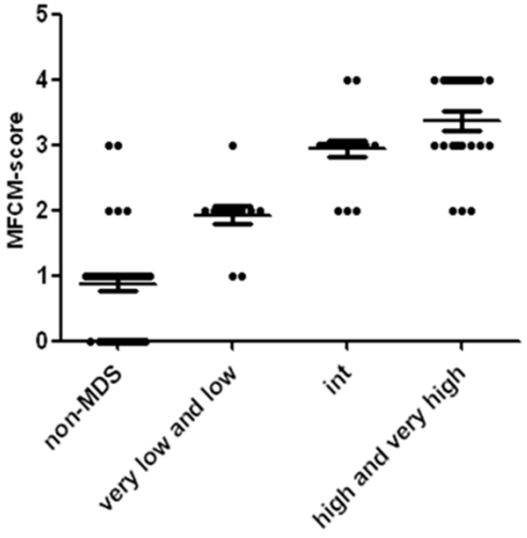

Figure 3. Correlation of MFCM-score with non-MDS and IPSS-R in patients with MDS. MFCM-score had a positive correlation with IPSS-R prognosis classification (Spearman r = 0.848, *P* < 0.01).

related cluster size. Interestingly, the MFI ratios were similar between MDS and non-MDS groups, but they had a positive correlation with IPSS-R risk (Spearman's rank correlation: r = 0.434, P < 0.01, **Table 7**). Finally, SSC peak channel ratio also showed a positive correlation with IPSS-R risk (Spearman's rank correlation: r = 0.543, *P* < 0.01, **Table 7**).

Discussion

In most MDS patients, one or more types of blood cells are low in number. For precise diagnosis of MDS, it is necessary to rule out other causes that lead to the reduction of peripheral blood cells, such as B12/folate deficiency, chronic liver disease, anemia of chronic, aplastic anemia disorders, anemia associated with renal failure, anemia associated with iron deficiency, drug-induced cytopenias, cytopenia associated with marrow infiltration, autoim-

Table 7. The relationship between MFCM-score parameters and IPSS-R for MDS and non-MDS groups

Parameter	NO MDS (*n* = 40)	MDS group (*n* = 54)			Spearman *r*	*P*-value
		Very low and Low (*n* = 13)	Int (*n* = 19)	High and Very high (*n* = 22)		
Myeloblast-related cluster size	1 (2.5%)	1 (7.8%)	6 (31.6%)	21 (95.5%)	0.735	< 0.01
B-progenitor-related cluster size	18 (45%)	12 (92.3%)	18 (94.7%)	22 (100%)	0.549	< 0.01
CD45 mean fluorescence intensity ratio	10 (25%)	7 (53.8%)	12 (63.2%)	17 (77.3%)	0.434	< 0.01
SSC peak channel ratio	6 (15%)	4 (30.8%)	13 (68.4%)	17(77.3%)	0.543	< 0.01

mune cytopenia, and other hematopoietic stem cell disorders [16, 23]. The diagnosis and classification of MDS are based on morphological evaluation of bone marrow dysplasia and cytogenetic abnormalities and/or ringsideroblasts as well as other specific markers. However, some MDS patients may not have specific markers [8, 24]. FCM analysis approved by European LeukemiaNet is an important auxiliary tool for the diagnosis of MDS and has been intensively reported [12-15]. With multi-parameter FCM analysis, abnormal phenotypes of MDS patients can be classified despite the inadequacy of morphology and cytogenetic [25]. However, those analysis methods are often complicated, need many antibodies and are costly. In addition, some methods are not very reproducible. A reproducible FCM-score with 69% of sensitivity and 92% of specificity was proposed in 2012 [16], which used four parameters to distinguish low-grade MDS from non-clonal cytopenias. The method is relatively simple, economical and suitable for clinical use.

In this study, we further modified the antibody combinations and gating strategy to enhance its clinical practicability. Different from the published FCM -score that uses low SSC and CD45 to separate progenitor B-cell blasts and myeloblasts, our MFCM-score uses CD19 and CD33 to separate progenitor B-cell blasts and myeloblasts from myeloid blasts within the $CD34^{+}CD45^{dimm}$ population with better specificity and effectiveness. It also reduces the influence of the operators. Chi square test showed that there is no statistical difference in MDS diagnosis between the two methods, while the sensitivity of MFCM-score and the specificity are slightly better than those of FCM-score. The positive and negative likelihood ratios of MFCM-score are also better than the FCM-score too. As such, our method may identify more patients with > 5% of bone marrow blasts. Taken together, our method is simple and convenient to operate with low cost. It only needs a set of antibody combination and 4-color flow cytometry instrument to analyze the four parameters. Since the four parameters are calculated as ratio, the influence of the peripheral blood dilution is minimized and there is no need to make tedious correction of $CD34^{+}$ cells [8, 16, 25].

Our analysis also showed that the MFCM-score is positively correlated with IPSS-R. As a consequence, from non-MDS to MDS, the MFCM-scores and percentage of MFCM-score above 1 increase with patient's MDS grade. In all patients with MDS, only two false negative patients were confirmed and they were in low and very low risk group. Rank correlation analysis indicated that MFCM-score is an independent prognostic factor for survival in MDS patients, suggesting the score may serve as a prognostic indicator. In addition, the relationship of each parameter of the MFCM-score and the IPSS prognosis was also analyzed. The percentage of every MFCM-score parameter is found increasing with MDS grade. Correlation analysis showed that these parameters are positively associated with the IPSS-R. Therefore, we can follow up the treatment and survival of MDS patients to validate the clinical significance of the MFCM-score in the treatment and prognosis of patients. The MFCM-score covers not only the low-grade MDS, but also all MDS subtypes, and it also has prognostic value for survival and can be used for treatment tracking.

There are limitations in the study. It was a single-center study with relatively smaller sample size and diversity of patient population was limited. The patients were followed up for relatively short period. It is therefore necessary to validate our method in large, multi-center and prospective studies.

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Disclosure of conflict of interest

None.

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