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ORIGINAL ARTICLE

A Multiparametric Flow Cytometry Immunophenotypic Scoring System for the Diagnosis and Prognosis of Myelodysplastic Syndromes

JING HUANG [1,3*], PEILONG LAI [1,2*], MAOHUA ZHOU [1], JIANYU WENG [1,2], ZESHENG LU [1], XIN DU [1,2]

** both authors contribute equally to this publication and are co-first authors*
[1] Department of Haematology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, 510080, P.R. China
[2] Southern Medical University, Guangzhou, 510515, P.R. China
[3] Department of Haematology, Central Hospital of Longgang, Shenzhen, 518116, P.R. China

SUMMARY

Background: **Myelodysplastic syndromes (MDS) are a group of clonal stem/progenitor cell diseases. The diagnosis of MDS, especially in the early stages, represents a particular challenge. Therefore, this study aimed to develop a multiparametric flow cytometry (FCM) method to analyze the immunophenotypic characteristics of MDS patients and establish a FCM scoring system to evaluate its potential for the diagnosis, classification, and prognosis of MDS.**
Methods: **41 bone marrow samples from MDS patients and 30 bone marrow samples from non-MDS patients were subjected to multiparametric flow cytometry. ROC curve and Spearman's correlation analysis were performed to combine the results of hematology, morphology, and cytogenetic analyses of these samples.**
Results: **Based on the proportion of blasts and the expression of antigens on cell populations, we established a BM FCM scoring system. The system has a good correlation rate (p = 0.006) for MDS diagnosis; the 95% confidence interval was 0.921 - 0.997 and the sensitivity and specificity were 90.2% and 86.7%, respectively. The BM FCM scores of the MDS group was significantly higher than the non-MDS group (p = 0.008). Moreover, there were positive correlations between FCM scores and MDS classification, karyotype, and IPSS and WPSS scores.**
Conclusions: **We have established a BM FCM scoring system that may provide a useful adjunct to existing tests for accurate diagnosis and prognosis of MDS.**
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KEY WORDS

Myelodysplastic syndrome, Flow cytometry, Immunophenotype, Scoring System

INTRODUCTION

Myelodysplasia syndromes (MDS) are a group of clonal stem cell disorders characterized by ineffective hematopoiesis which leads to quantitative and qualitative cell abnormalities with one or more peripheral blood (PB) cytopenias, recurrent cytogenetic abnormalities, and an increased risk of progression to acute myeloid leukemia (AML) [1,2]. Traditionally, the gold standard for the diagnosis of MDS has relied on the integration of information obtained from clinical evaluation of the patients, peripheral blood counts, morphologic review of peripheral blood smears and bone marrow aspirates and biopsies, and cytogenetics [3]. Due to the variation of clinical presentations of MDS patients, not all clinically suspected cases demonstrate definitive morphologic dysplasia[4,5]. In addition, the cytogenetic abnormalities are only present in roughly 40% of cases and non-MDS cytopenias may mimic MDS morphologically [6]. Therefore, the diagnosis of MDS especially at early stages represents a particular challenge. Ancillary studies that could aid in the diagnosis of MDS would be very informative, especially for morphologically intermediate and/or cytogenetically normal cases.

The maturation of hematopoietic cells from the primitive progenitor cell is a tightly controlled process, which

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is manifested immunophenotypically by the sequential expression of a variety of antigens [7-9]. Immunophenotyping using flow cytometry (FCM) is a potentially objective and reliable method to identify antigenic abnormalities of differentiating neoplastic cells and has been extensively employed in the diagnosis of many haematological malignancies [10]. However, the utility of immunophenotyping in the diagnosis of MDS has not been well established. In early studies of myelodysplasia, a wide variety of individual immunophenotypic abnormalities have been nicely summarized [11]. Nevertheless, these studies largely represent a catalogue of abnormalities without directly addressing their utility in a diagnostic and prognostic setting [12]. More recently, many immunophenotypic abnormalities in different BM cell compartments have been reported and an increasing number of "dysplastic features" are being identified in MDS patients [11,13-15].

Consequently, there is great interest in the analysis of clinically significant immunophenotypic data in order to assist in the diagnosis and prognosis of MDS [16-18]. While FCM has been applied only to a relatively small number of surface antigens in MDS in the past 2 decades [19-22], several recent studies have investigated the application of FCM in MDS diagnosis, with most of them focused on myelomonocytic cells [12-13,17,23-25]. Applying a larger panel of antibodies to study the abnormal maturation patterns of myelomonocytic cells has shown diagnostic value in MDS [12-13,17,24-25]. However, the practical usefulness of such an FCM approach in the diagnosis of MDS has not yet been verified by multiple groups and, therefore, has not yet reached general application [2]. Scoring systems have proven to be useful tools for diagnosis and treatment strategies in hematological malignancies. Considering the marked heterogeneity of MDS, it would be particularly helpful to develop a scoring system for the diagnosis and prognosis of MDS patients. The aim of this study was to use multiparametric FCM to analyze the abnormalities in blasts, granulocytes, monocytes, lymphocytes, and nucleated erythrocytes in MDS and non-MDS patients. Using the immunophenotypic characteristics, we established the FCM scoring system, examined the correlation between the FCM scores and disease risk using the validated International Prognostic Scoring System (IPSS) and WHO classification-based Prognostic Scoring System (WPSS) scores [26] and explore the clinical significance of this scoring system for the diagnosis, classification, and prognosis of MDS.

MATERIALS AND METHODS

Patients

Between July 2009 and March 2010, 41 bone marrow samples were collected from newly diagnosed MDS patients, and 30 bone marrow samples were obtained from non-MDS patients. All samples were analyzed by flow cytometry and the results were retrospectively correlated with morphologic, cytogenetic, and hematologic parameters. The median age of the MDS group was 62 years (range 25 - 87 years) including 29 males and 12 females. Using the WHO (2008) criteria (Supplementary Table 1), the patients were classified as follows: 10 refractory cytopenia with multilineage dysplasia (RCMD), 16 refractory anemia with excess of blasts-I (RAEB-I), 15 refractory anemia with excess of blasts-II (RAEB-II) (Table 1). The median age of non-MDS patients was 58 years (range 26 - 78 years) including 14 males and 16 females. They were classified as follows: 15 Aplastic Anemia (AA), 5 Iron Deficiency Anemia (IDA), 4 Idiopathic cytopenia of uncertain significance (ICUS), 4 Anemia of Chronic Disease (ACD), and 2 Idiopathic Thrombocytopenic Purpura (ITP) (Table 1). This study was approved by the Ethics Committee of Guangdong General Hospital and informed consent was obtained from all patients.

Morphological diagnosis

The morphological evaluation was performed independently by at least 2 hematopathologists experienced in MDS diagnosis without the knowledge of the FCM findings. For each individual case, routine hematoxylin and eosin (H&E) histologic sections and well-prepared Wright-Giemsa-stained smears were evaluated. The Perls reaction for iron was performed on BM aspirates and silver impregnation stain for reticulin was performed on biopsy samples, if necessary. To strictly define morphologic dysplasia, the features of dyserythropoiesis (DysE), dysgranulopoiesis (DysG), and dysmegakaryopoiesis (DysM) had to be present in at least 10% of cells of the respective lineage. Dysplasia was defined as uni-lineage if it was limited to a single lineage, while multi-lineage dysplasia was considered to be present when two or more lineages showed those features.

Cytogenetic analysis

Fixed preparations obtained from cultured (24 and 48 hour) bone marrow samples were G-banded, and a minimum of 20 metaphase spreads were examined. The identification of abnormal clones was based on the criteria defined by the International System for Human Cytogenetic Nomenclature [27].

Four-color flow cytometry analysis

All samples were collected in tubes that contained heparin sodium and processed within 24 hours of collection. For all specimens, four-color flow cytometry was performed on a FACS Calibur flow cytometer equipped with a 15 mW argon laser excited at 488 mm and 635 mm (Becton-Dickinson Biosciences, BDB, San Jose, CA, USA) using commercially available reagents. Immunofluorescence staining was performed using a standardized direct stain-lyse-wash technique. Briefly, 5×10^5 to 1×10^6 cells were incubated with appropriate amounts of titrated antibodies for 20 minutes at room temperature in the dark; erythroid cells were then bulk

Table 1. The basic data of MDS and non-MDS groups.

Group	N	Gender		Median Age (years)	Types	N
		Male	Female			
MDS	41	29	12	62 (25-87)	RCMD	10
					RAEB-I	16
					RAEB-II	15
non-MDS	30	14	16	58 (26-78)	AA	15
					IDA	5
					ICUS	4
					ACD	4
					ITP	2

Abbreviations: RCMD: refractory cytopenia with multilineage dysplasia; RAEB-I: refractory anemia with excess of blasts-I; RAEB-II: refractory anemia with excess of blasts-II; AA: Aplastic Anemia; IDA: Iron Deficiency Anemia; ICUS: Idiopathic cytopenia of uncertain significance; ACD: Anemia of Chronic Disease; ITP: Idiopathic Thrombocytopenic Purpura.

Table 2. The combinations of antibodies used for MDS flow cytometry immunophenotypic analysis.

Tube	FITC	PE	PERCP	APC
1	IgG	IgG	CD45	IgG
2	CD64	CD10	CD45	CD14
3	CD15	CD33	CD45	HLA-DR
4	CD34	CD5	CD45	CD117
5	CD16	CD11b	CD45	CD13
6	CD61	CD66d	CD45	CD56
7	CD71	GPA	CD45	CD105
8	CD8	CD25	CD45	CD4
9	CD7	CD133	CD45	CD19
10	CD38	CD123	CD45	CD34

Note: CD133 was purchased from eBioscience company, CD105 was purchased from BioLeged company, CD13 and CD66d were purchased from BD Pharmingen company, other antibodies were purchased from Becton Dickinson company.

Table 3. The comparison of FCM scores between MDS and non-MDS groups.

Group	N	FCM scores ($\bar{X} \pm S$)
MDS	41	32.78 ± 5.45*
non-MDS	30	18.03 ± 5.92
t value	-	10.865
p value	-	0.008

Note: Independent-Samples T test * vs. non-MDS group $p = 0.008$.

Table 4. The comparison of FCM scores among MDS subtypes (RCMD, RAEB-I, and RAEB-II).

Group	N	FCM scores ($\bar{X} \pm S$)
RCMD	10	29.00 ± 5.45*
RAEB-I	16	30.38 ± 3.12#
RAEB-II	15	37.87 ± 2.92
F value	-	21.03
P value	-	0.025

Note: * RCMD vs. RAEB-I $p = 0.875$, RCMD vs. RAEB-II $p = 0.002$, # RAEB-I vs. RAEB-II $p = 0.004$.

Table 5. The relationship between FCM scores and MDS subtypes.

Classification	FCM scores					
	20-	25-	30-	35-	40-	Total
RCMD	3	1	4	2	0	10
RAEB-I	0	6	9	1	0	16
RAEB-II	0	0	2	7	6	15
Total	3	7	15	10	6	41

Note: Spearman rank order correlation analysis: $r = 0.656$, $p = 0.021$.

Table 6. The relationship between FCM scores and karyotypes.

Karyotypes	FCM scores					
	20-	25-	30-	35-	40-	Total
Good	3	7	14	6	4	34
Intermediate	0	0	1	1	0	2
Poor	0	0	0	3	2	5
Total	5	7	15	10	6	41

Note: Spearman rank order correlation analysis: $r = 0.410$, $p = 0.008$.

Table 7. The relationship between FCM scores and IPSS scores.

IPSS scores	FCM scores					
	20-	25-	30-	35-	40-	Total
Low (0)	3	0	0	0	0	3
Intermediate-I (0.5-1)	0	7	13	3	0	23
Intermediate-II (1.5-2.0)	0	0	2	3	4	9
High (≥ 2.5)	0	0	0	4	2	6
Total	3	7	15	10	6	41

Note: Spearman rank order correlation analysis: $r = 0.791$, $p = 0.016$.

Table 8. The relationship between FCM scores and WPSS scores.

WPSS scores	FCM scores					
	20-	25-	30-	35-	40-	Total
Very Low (0)	0	0	0	0	0	0
Low (1)	0	0	1	1	0	2
Intermediate (2)	3	3	7	2	0	15
High (3-4)	0	4	6	3	4	17
Very High (5-6)	0	0	1	4	2	7
Total	3	7	15	10	6	41

Note: Spearman rank order correlation analysis: $r = 0.425$, $p = 0.006$.

Table 9. The relationship between karyotypes and IPSS scores.

IPSS scores	karyotypes			
	Good	Intermediate	Poor	Total
Low (0)	3	0	0	3
Intermediate-I (0.5-1)	23	0	0	23
Intermediate-II (1.5-2.0)	8	1	0	9
High (≥ 2.5)	0	1	5	6
Total	34	2	5	41

Note: Spearman rank order correlation analysis: $r = 0.703$, $p = 0.009$.

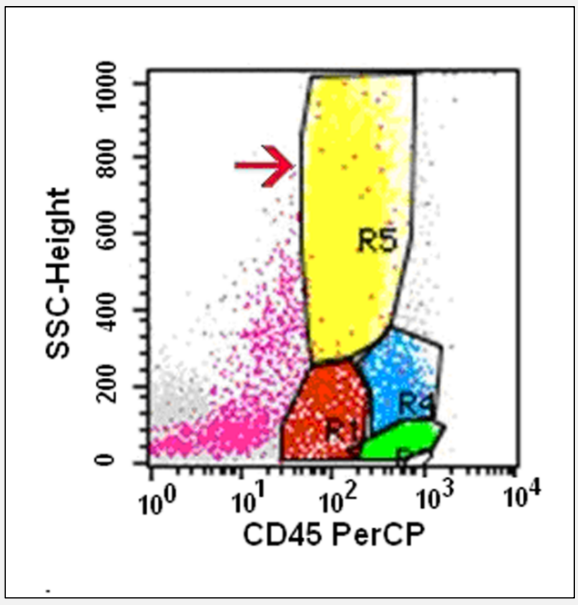

Figure 1. CD45 vs. SSC of non-MDS patients.

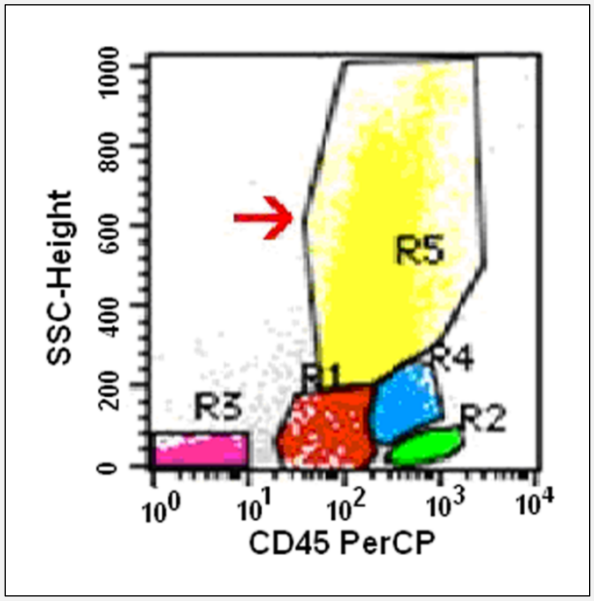

Figure 2. CD45 vs. SSC of MDS patients.

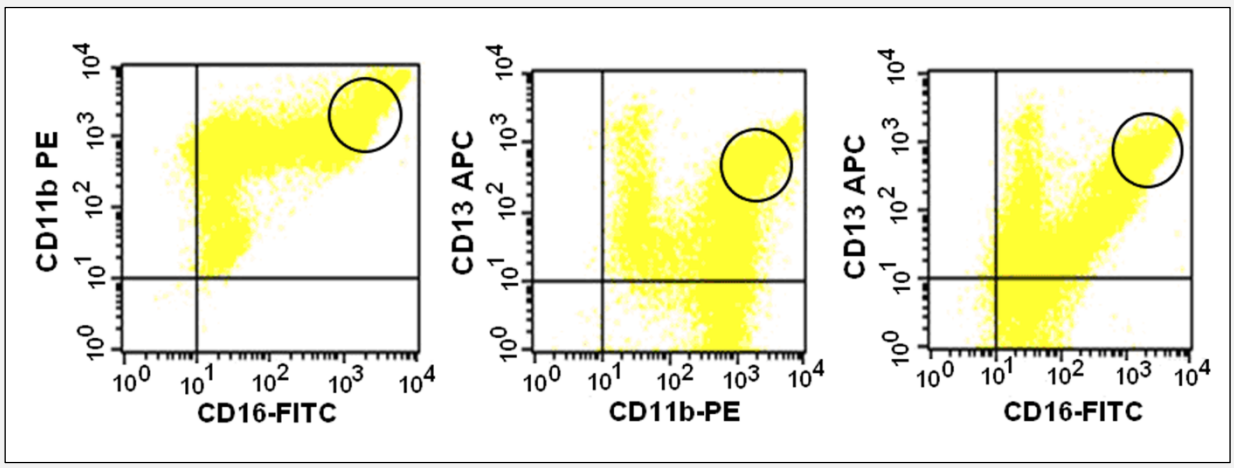

Figure 3. CD16 vs. CD11b, CD11b vs. CD13, and CD16 vs. CD13 of non-MDS patients.

lysed with buffered ammonium chloride, and the remaining cells were washed once with phosphate-buffered saline-bovine serum albumin (PBS-BSA)-azide (pH 7.4) and resuspended in 0.1% paraformaldehyde [25]. After staining, data from at least 100,000 cells were acquired. For data analysis, the CellQuest software program was used. The following panel of antibodies was used in all cases (Table 2). The bone marrow cell populations including blast cells, maturing granulocytes, maturing monocytes, maturing lymphocytes, and

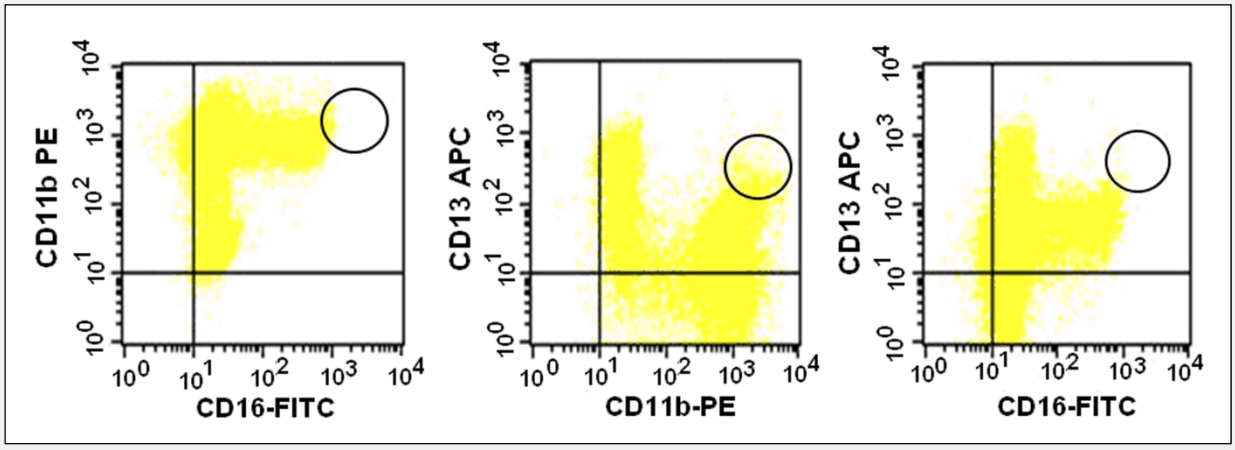

Figure 4. CD16 vs. CD11b, CD11b vs. CD13, and CD16 vs. CD13 of MDS patients.

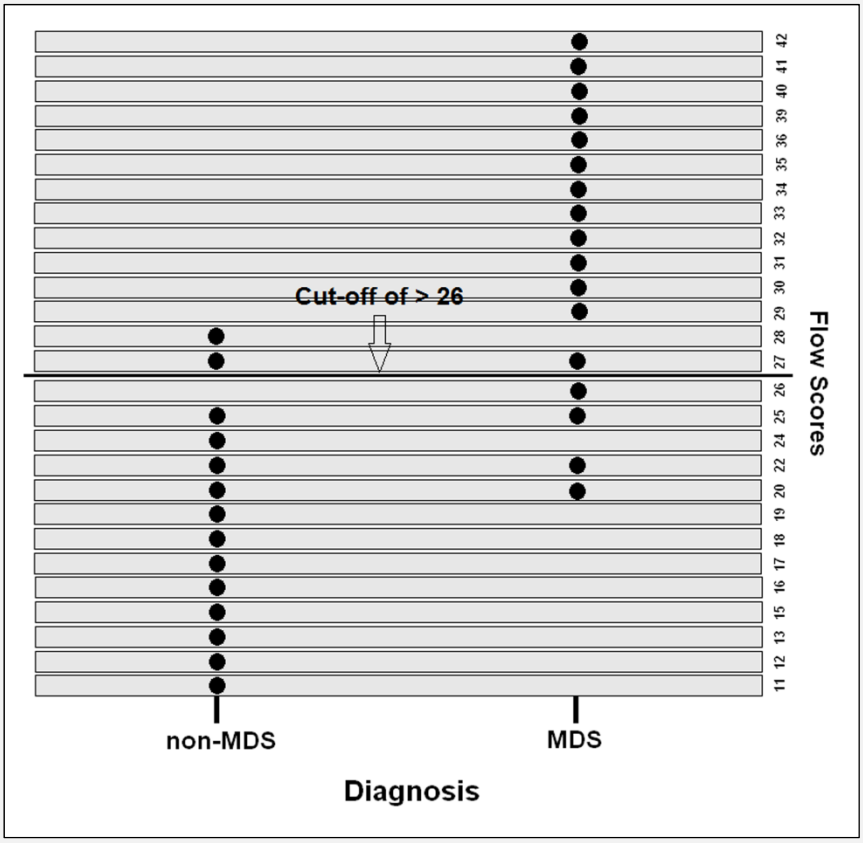

Figure 5. Establishment of the BM MDS Score. The BM MDS Score was calculated by combining the expressions of antigens of bone marrow cells and the quantity of blast in patients with MDS as described. As illustrated, the BM MDS Score was significantly higher in patients with MDS than non-MDS (p = 0.006).

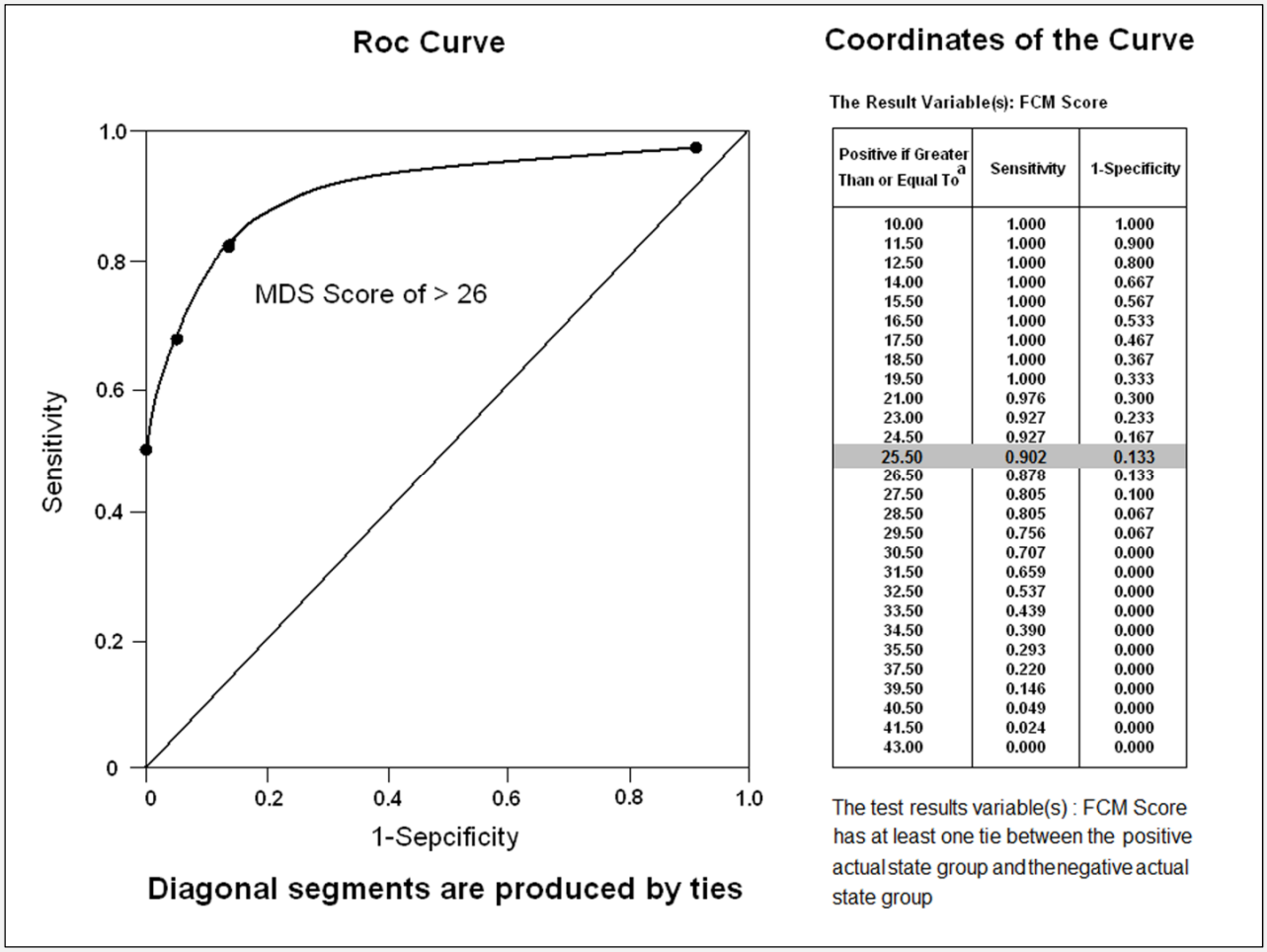

Figure 6. The ROC Curve of the FCM Scoring System. Receiver operating curve (ROC) analysis demonstrates that using a cutoff higher than 26 to identify MDS patients minimizes false-positive and false-negative results.

nucleated erythrocytes were analyzed using CD45 staining and side scatter (SSC) defined as follows: blast cells: $CD45^{dim}/SSC^{low-int}$; mature granulocytes: $CD45^{int}/SSC^{int-bright}$; mature monocytes: $CD45^{int-bright}/SSC^{int}$; mature lyphocytes: $CD45^{bright}/SSC^{low}$; nucleated erythrocytes: $CD45^{negativ}/SSC^{low}$. The gating strategies were shown in figures 1 - 4.

Definition of FCM scoring system

Base on the proportion of blast and the expressions of antigens on cell populations, we made the following definitions [17,28-31]: For blast cell populations: 0: < 20%; 1: 20-40%; 2: ≥ 40%. For the expression of antigens of different cell types: X indicates the expression of antigens, $\bar{X}$ indicates the average percentage of each antigen of all samples, d indicates $X-\bar{X}$, SD indicates standard deviation. According to the percentage of each antigen, the scores were defined as follows: 0: $|d| < |SD|$, 1: $|SD| < |d| < 2|SD|$, 2: $|d| \geq 2|SD|$.

The detection of the antigens in different cell types is listed as below: CD34, CD38, CD123, CD33, CD133, HLA-DR, CD7, CD117, and CD13 antigen staining of blast cells; CD11b+CD16+, CD10, CD11b, CD16, CD13, CD33, CD15, HLA-DR, CD56, CD66d, and CD117 antigen staining of mature granulocytes; CD7, CD15, HLA-DR, CD33, CD14, CD13, CD61, CD64, and CD56 antigen staining of mature monocytes; CD25+CD4+, CD25+CD8+, CD4, CD5, CD7, CD8, CD19, CD25, and CD56 antigen staining of mature lymphocytes; GPA+CD105+, GPA+CD71+, CD71+ CD105+, CD71, CD105, and GPA antigen staining of nucleated erythrocytes.

Supplementary Table 1. WHO classification and criteria for the myelodysplastic syndromes (2008).

Disease	Blood findings	Bone marrow findings
Refractory anemia (RA)	Anemia No or rare blasts	Erythroid dysplasia only < 5% blasts < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only ≥ 15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1×10^9/L monocytes	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines < 5% blasts in marrow No Auer rods < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias < 5% blasts No Auer rods < 1×10^9/L monocytes	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines ≥ 15% ringed sideroblasts < 5% blasts No Auer rods
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenias < 5% blasts No Auer rods < 1×10^9/L monocytes	Unilineage or multilineage dysplasia 5% - 9% blasts No Auer rods
Refractory anemia with excess blasts-1 (RAEB-2)	Cytopenias < 5% blasts No Auer rods < 1×10^9/L monocytes	Unilineage or multilineage dysplasia 10% to 19% blasts Auer rods ±
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Unilineage dysplasia in granulocytes or megakaryocytes < 5% blasts No Auer rods
MDS associated with isolated del (5q)	Anemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts No Auer rods Isolated del (5q)

Supplementary Table 2. Expressions of antigens on BM Blasts of MDS and non-MDS groups.

Group	N		Blasts								
			CD34	CD38	CD123	CD33	HLA-DR	CD133	CD7	CD117	CD13
MDS	41	$\bar{X}$	45.82*	80.98	64.35*	75.98	67.24	54.40*	41.55	34.48	55.05
		S	23.51	19.06	15.57	20.24	16.26	20.90	15.03	20.11	19.60
non-MDS	30	$\bar{X}$	24.98	75.30	51.31	80.55	63.00	34.03	43.61	38.90	51.85
		S	15.97	19.46	19.76	11.91	18.25	14.70	15.59	20.54	23.57
T value	-	-	4.445	1.229	3.109	-1.106	1.031	4.821	-0.560	-0.905	0.622
P value	-	-	0.000	0.223	0.003	0.273	0.306	0.000	0.577	0.368	0.536

Note: Independent-Sample T Test, * vs. non-MDS group $p < 0.05$

Supplementary Table 3. Expressions of antigens on BM Granulocytes of MDS and non-MDS groups.

Group	N		Granulocytes									
			CD10	CD11b	CD13	CD15	CD16	CD33	HLA-DR	CD56	CD66d	CD117
MDS	41	$\bar{X}$	85.12*	96.64*	73.64	97.44*	94.96*	96.25	33.83	10.33	96.44*	7.98
		S	8.73	4.34	16.62	3.76	3.76	3.99	17.24	9.14	3.87	6.05
non-MDS	30	$\bar{X}$	79.30	93.39	77.37	94.31	87.48	93.80	35.82	15.50	93.46	11.44
		S	10.24	6.81	15.22	6.03	13.19	5.99	19.26	14.15	6.14	10.32
T value	-	-	2.580	2.292	-0.966	2.511	2.983	1.945	-0.457	-1.686	2.341	-1.773
P value	-	-	0.012	0.027	0.337	0.016	0.005	0.058	0.649	0.096	0.024	0.081

Note: Independent-Sample T Test, * vs. non-MDS group $p < 0.05$

Supplementary Table 4. Expressions of antigens on BM Monocytes of MDS and non-MDS groups.

Group	N		Monocytes								
			CD7	CD15	HLA-DR	CD33	CD64	CD14	CD13	CD61	CD56
MDS	41	$\bar{X}$	83.07	92.29*	88.83	94.05*	92.80*	69.38	85.36	86.41	22.28
		S	11.46	5.32	7.91	4.42	5.71	17.15	13.49	10.71	18.81
non-MDS	30	$\bar{X}$	77.86	85.08	84.44	89.54	86.46	65.75	80.97	82.58	18.18
		S	12.66	9.77	10.84	7.78	7.90	19.40	11.42	10.39	14.47
T value	-	-	1.810	3.994	1.879	2.855	3.740	0.833	1.443	1.505	0.998
P value	-	-	0.075	0.001	0.066	0.007	0.000	0.408	0.153	0.137	0.322

Note: Independent-Sample T Test, * vs. non-MDS group $p < 0.05$

Supplementary Table 5. Expressions of antigens on BM Lymphocytes of MDS and non-MDS groups.

Group	N		Lymphocytes								
			CD25+CD4+	CD25+CD8+	CD4	CD5	CD7	CD8	CD19	CD25	CD56
MDS	41	$\bar{X}$	16.84	4.91*	37.48	62.64*	80.21*	31.12*	15.37*	23.29	25.06
		S	5.23	2.42	12.09	19.80	7.75	8.69	10.37	13.38	11.52
non-MDS	30	$\bar{X}$	15.96	3.27	39.57	71.51	73.87	25.60	25.45	23.48	24.99
		S	6.27	1.28	14.30	7.13	8.62	6.27	8.95	5.39	9.56
T value	-	-	0.640	3.685	-0.666	-2.644	3.244	2.958	-4.182	-0.073	0.027
P value	-	-	0.524	0.000	0.508	0.011	0.002	0.004	0.000	0.942	0.978

Note: Independent-Sample T Test, * vs. non-MDS group $p < 0.05$

Supplementary Table 6. Expressions of antigens on BM Nucleated Red Cells of MDS and non-MDS groups.

Group	N		Nucleated Red Cells					
			GPA+CD105+	GPA+CD71+	CD71+CD105+	CD71	CD105	GPA
MDS	41	$\bar{X}$	11.97	20.29*	13.00	40.14*	17.96*	35.99*
		S	9.09	12.83	8.37	28.21	9.85	21.64
non-MDS	30	$\bar{X}$	8.81	14.54	12.87	28.17	13.83	26.92
		S	3.69	8.43	5.84	13.27	6.10	10.76
T value	-	-	2.008	2.276	0.078	2.380	2.172	2.318
P value	-	-	0.050	0.026	0.938	0.020	0.033	0.024

Note: Independent-Sample T Test, * vs. non-MDS group $p < 0.05$

Statistical analysis

ROC Curve and Spearman rank order correlation analyses were performed. A p-value of 0.05 or less was considered statistically significant.

RESULTS

The diagnostic efficacy of the FCM scoring system

Based on the proportion of blast cells and the expression of antigens on cell populations (Supplementary Table 2-6), we established the BM FCM scoring system. This scoring system has a good correlation rate for MDS diagnosis ($p = 0.006$), 95% confidence interval is 0.921 - 0.997, and the sensitivity and specificity are 90.2% and 86.7%, respectively (Figure 5 and 6). The BM FCM scores of the MDS group are significantly higher than those of the non-MDS group ($p = 0.008$), and the score is increased with the progression of the disease ($p = 0.025$). Meanwhile, there are positive correlations between FCM scores and MDS classification, karyotype, IPSS scores, and WPSS scores ($r = 0.656$, $r = 0.410$, $r = 0.791$, and 0.425, $p = 0.021$, $p = 0.008$, $p = 0.016$ and 0.006, respectively, Table 3 - 8).

Cytogenetic analysis

7 out of 41 MDS patients had a complex chromosome karyotype and all of them were RAEB-II, accounting for 17% of MDS patients and 47% of RAEB-II patients. There was a positive correlation between abnormal chromosome karyotype and IPSS scores ($r = 0.703$, $p = 0.009$) (Table 9).

DISCUSSION

Flow cytometry has been widely used for the diagnosis, characterization, and classification of various hematologic malignancies [32-34]. However, the utility of FCM for MDS has not been well defined although a variety of immunophenotypic abnormalities have been detected in MDS patients [33]. Flow cytometry for the diagnosis and prognosis of MDS is based upon the knowledge that the maturation of hematopoietic lineages is tightly controlled, leading to a predictable normal pattern of antigen expression at different stages of differentiation. The abnormal granulocytic, monocytic, lymphocytic, and erythroid differentiation in MDS would result in the deviations from the normal pattern of antigen expression and help distinguish MDS [13-14,17,25].

Subsequently, multiparametric flow cytometric studies examining the full spectrum of granulocytic, monocytic, lymphocytic, and erythroid immunophenotypic abnormalities have confirmed that numerous multi-lineage abnormalities are characteristic of MDS, and flow cytometric testing increases the sensitivity and specificity of diagnosis [2,13-15,17,25,30,34-35]. Additionally, these immunophenotypic abnormalities correlate with cytogenetic and diagnostic subclassification systems for MDS [16,32]. To aid in the interpretation of FCM results, a numeric system for scoring the immunophenotypic abnormalities into normal/mild, moderate or severe categories was developed, which demonstrated 100% specificity for MDS and even predicted relapse after transplant and overall survival in a manner independent of IPSS [17]. Subsequent studies confirmed the prognostic value of flow cytometry testing in MDS [14,17,22,36-38]. Unfortunately, there is a lack of standardization for scoring immunophenotypic abnormalities in MDS [17,37].

Three- or four-color flow cytometry has been utilized for the characterization of expression patterns in different lineages. For example, three-color FCM identified aberrant antigen expression in 2 or more lineages in 88% of MDS cases with 100% correlation between FCM and cytogenetic abnormalities [13]. Three-color FCM was also employed for the analysis of the immunophenotypes of blast-enriched specimens from MDS patients [16]. Wells et al. [17] introduced the concept of a flow cytometric scoring system to quantitate the extent of the abnormalities. This scoring system was recently validated in an independent four-color FCM stu-

dy [39] focusing on myelomonocytic precursors in low and intermediate-risk MDS groups. Although there was a wide variability in flow scores with respect to the 3 IPSS cytogenetic risk categories, flow scores did correlate with WPSS [25,40]. Notably, we observed positive correlations between FCM scores and IPSS scores or WPSS scores ($r = 0.791$ and $r = 0.425$, $p = 0.016$ and 0.006, respectively), which indicates the potential value of our system for the prognosis of MDS.

Compared to the previous studies [13,15-18,28,36], our approach has advantages in that it maintains the ability to integrate the heterogeneity of phenotypic changes observed in MDS and examines the full spectrum of granulocytic, monocytic, lymphocytic, and erythroid immunophenotypic abnormalities. In the present study, we focused on BM cells and combined data on immunophenotypic variables to create the BM FCM scoring system, a multivariate predictor. This system is relatively comprehensive compared to previous systems because it is performed on the full spectrum of BM cells. Using the BM FCM score, we were able to separate MDS patients from non-MDS controls with a high level of accuracy ($p = 0.006$). When we chose 26 as a cut-off point, we got the best sensitivity and specificity (90.2% and 86.7%, respectively). Only 4 cases of MDS were not diagnosed and 4 cases of non-MDS were mis-diagnosed. Unfortunately, due to the limitation of the case numbers, this scoring system is not validated in patients with cytopenias and/or hematopoietic dysplasia to determine the potential application of our system to differentiate MDS from other hematologic conditions. This is our next plan. The BM FCM scores of the MDS group were significantly higher than the non-MDS group ($p = 0.008$). Importantly, there were positive correlations between FCM scores and MDS classification (RCMD, RAEB-1, and RAEB-2), as well as karyotype ($r = 0.656$ and $r = 0.410$, $p = 0.021$ and $p = 0.008$, respectively). These results reveal that the higher the BM FCM score the higher the probability of a diagnosis of high-grade MDS and detecting a complex chromosome karyotype. While no data associating the BM FCM score with the corresponding lifetime are currently available, due to short follow-up, we will accumulate these data for the evaluation of its predictive ability.

In summary, we have established a BM FCM scoring system based on FCM analysis of BM cells that may provide a useful adjunct to existing tests for accurate diagnosis and prognosis of MDS. The BM FCM score was derived from a relatively comprehensive immunophenotypic approach that performed well in patients with clinical characteristics of MDS. Further multicenter studies are needed to confirm these preliminary data and to assess correlations between the BM FCM score and clinical parameters relevant in the evaluation of MDS patients. Although this method is laborious and expensive, it increases the sensitivity and specificity of the diagnosis of MDS. When combined with other clinical and laboratory parameters, this method provides a new paradigm for the diagnosis and prognosis of MDS.

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Declaration of Interest:

The authors declare no competing financial interests.

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Correspondence:
Xin Du, M.D., Ph.D.
Department of Haematology
Guangdong General Hospital
Guangdong Academy of Medical Sciences
Guangzhou, 510080, P.R. China
Tel.: +86-20-83827812-62122
Fax: +86-20-83889772
Email: miyadu@hotmail.com