Study on the Diagnostic and Prognostic Value of Integrated Flow Cytometry Scoring in Myelodysplastic Syndromes (Postprint)

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Date: 2022-09-30T00:00:00+00:00

Abstract

Background The gold standard for diagnosing myelodysplastic syndromes (MDS) is based on bone marrow morphology, progenitor cell enumeration, and cytogenetics. However, dysplasia is not specific to MDS, and diagnosis is often challenging when bone marrow smear quality is suboptimal, dysplasia is inconspicuous, or blasts are not significantly increased, particularly in cases with normal karyotype. Multiparameter flow cytometry (MFC) has become an important co-criterion for MDS diagnosis. Some studies have suggested that the integrated flow cytometric score (iFS) is the optimal scoring system for MFC-based MDS diagnosis, but research in Chinese populations has not been reported.

Objective To explore the diagnostic and prognostic value of iFS in MDS using MFC, and to identify an appropriate MFC scoring system for clinical disease diagnosis and prognostic evaluation.

Methods We retrospectively analyzed the immunophenotypes of 83 MDS cases and 77 non-MDS cases diagnosed and treated in the Department of Hematology, Affiliated People' s Hospital of Ningbo University from January 2019 to April 2022. Phenotypic abnormalities were interpreted according to iFS, and chi-square test was used to compare the probability of phenotypic abnormalities in each lineage between the two groups. Diagnostic scoring was performed according to iFS, and receiver operating characteristic (ROC) curve was plotted to analyze the sensitivity, specificity, and area under the curve (AUC) of iFS. Spearman rank correlation analysis was used to evaluate the correlation between iFS assessment grade and Revised International Prognostic Scoring System (IPSS-R) in MDS patients. Kaplan-Meier method and Log-rank test were applied to compare event-free survival between patients assessed as MDS by iFS and other MDS patients.

Results The probabilities of phenotypic abnormalities in myeloid progenitor cells, granulocytic and/or monocytic lineage, and erythrocytes in the MDS group were 71.1%, 73.5%, and 60.2%, respectively, which were significantly higher than those in the non-MDS group (1.3%, 18.8%, and 14.2%) (P<0.05). The specificity and sensitivity of iFS for diagnosing MDS were 93.5% and 81.9%, respectively, with an AUC of 0.921 (95% CI: 0.876–0.967). The sensitivity for diagnosing lower-grade MDS and lower-grade MDS with normal karyotype was 66.7% and 65.0%, respectively. iFS assessment grade was positively correlated with IPSS-R (r=0.411, P<0.05). Event-free survival was significantly shorter in patients assessed as MDS by iFS compared with other MDS patients (36.4 months vs. not reached, 2=5.71, P<0.05).

Conclusion iFS can compensate for the limitations of morphology and cytogenetics, providing diagnostic and prognostic information for MDS.

Full Text

Study on the Diagnostic and Prognostic Value of Integrated Flow-Score in Myelodysplastic Syndrome

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Funding: National Natural Science Foundation of China (Grant No. 81872433); Ningbo Medical Science and Technology Program (Grant No. 2020Y45)

Abstract

Background: The gold standard for diagnosing myelodysplastic syndrome (MDS) is based on bone marrow morphology, progenitor cell count, and cytogenetics. However, dysplasia is not specific to MDS, and diagnosis is often challenging when bone marrow smear quality is poor, dysplasia is not obvious, or blasts are not significantly increased, especially in cases with normal karyotype. Multiparameter flow cytometry (MFC) has become an important co-criterion for MDS diagnosis. Some studies suggest that integrated flow-score (iFS) is the optimal scoring system for MFC-based MDS diagnosis, but no studies have been reported in Chinese populations.

Objective: To explore the diagnostic and prognostic value of iFS by MFC in MDS, and to identify an appropriate MFC scoring system for clinical diagnosis and prognosis evaluation.

Methods: We retrospectively analyzed the immunophenotypes of 83 MDS cases and 77 non-MDS cases treated at the Department of Hematology, Affiliated People' s Hospital of Ningbo University from January 2019 to April 2022. Phenotypic abnormalities were interpreted according to iFS. Chi-square test was used to compare the probability of phenotypic abnormalities in each cell lineage between the two groups. Diagnostic scores were performed according to iFS, and receiver operating characteristic (ROC) curves were plotted to analyze sensitivity, specificity, and area under the curve (AUC). Spearman-rank correlation analysis was used to evaluate the relationship between iFS evaluation grade and Revised International Prognostic Scoring System (IPSS-R). Kaplan-Meier method and Log-rank test were used to compare event-free survival between MDS patients evaluated as MDS by iFS and other MDS patients.

Results: The probabilities of phenotypic abnormalities in myeloid progenitors, granulocytic and/or monocytic lineages, and erythroid lineage in the MDS group were 71.1%, 73.5%, and 60.2%, respectively, significantly higher than those in the non-MDS group (1.3%, 18.8%, and 14.2%) ($P<0.05$). The specificity of iFS for MDS diagnosis was 93.5%, sensitivity was 81.9%, and AUC was 0.921 (95%CI: 0.876–0.967). The sensitivities for low-grade MDS and low-grade MDS with normal karyotype were 66.7% and 65.0%, respectively. iFS evaluation grade was positively correlated with IPSS-R ($r=0.411$, $P<0.05$). Patients evaluated as MDS by iFS had significantly shorter event-free survival than other MDS patients (36.4 months vs not reached, $^2=5.71$, $P<0.05$).

Conclusion: iFS can compensate for the limitations of morphology and cytogenetics, providing valuable diagnostic and prognostic information for MDS.

Key words: Myelodysplastic syndrome; Flow cytometry; Diagnosis; Prognosis; Immunophenotype

Chinese Library Classification:

Introduction

Myelodysplastic syndrome (MDS) is a group of heterogeneous, clonal myeloid hematopoietic stem cell malignancies characterized by cytopenias, dysplasia in one or more lineages, genetic instability, and a high risk of transformation to leukemia [1]. Despite rapid developments in new diagnostic methods, the gold standard for MDS diagnosis remains based on bone marrow morphology, progenitor cell count, and cytogenetics [2]. Dysplasia is not specific to MDS, and diagnosis is often challenging when bone marrow smear quality is poor, dysplasia is not obvious, or blasts are not significantly increased, especially in cases with normal karyotype or without MDS-related genetic abnormalities.

Multiparameter flow cytometry (MFC) evaluation of abnormal immunophenotypes in bone marrow hematopoietic cells has become an important co-criterion for MDS diagnosis [2,3]. The widely used Ogata score [4] demonstrates good

specificity (>90%) but low sensitivity (30–70%) for MDS diagnosis [4-7]. In 2017, Cremers et al. [6] developed the integrated flow-score (iFS) using 44 flow cytometric parameters, incorporating the Ogata score along with analysis of dysplastic features in immature and mature granulocytic and monocytic lineages and erythroid lineage, providing comprehensive evaluation of all cell lineages. Oelschlaegel et al. [7] considered iFS to be the optimal scoring system for MDS diagnosis. Nevertheless, no studies on this scoring system have been reported in Chinese populations, particularly in low-grade MDS without increased blasts, and it has not been widely applied.

Therefore, this study retrospectively analyzed the immunophenotypes of 83 MDS cases and 77 non-MDS cases, applied iFS for diagnostic scoring, and explored its potential in MDS diagnosis and prognosis to identify an appropriate MFC scoring system for clinical disease diagnosis and prognosis evaluation.

Methods

Study Population and Clinical Characteristics Patients who visited the Department of Hematology at the Affiliated People' s Hospital of Ningbo University due to one or more lineages of peripheral blood cytopenias from January 2019 to April 2022 were enrolled. Based on bone marrow cytomorphology, immunophenotyping, chromosome analysis, bone marrow biopsy, gene sequencing, and following the minimal diagnostic criteria for MDS [2], 83 patients were diagnosed with myelodysplastic syndrome after 0–12 months of follow-up. This group included 53 males (64%) and 30 females (36%), aged 25–84 years with a median age of 63 years. Among patients suspected of MDS, 77 were diagnosed with non-clonal cytopenias and enrolled in the non-MDS group, including 40 males (52%) and 37 females (48%), aged 25–89 years with a median age of 68 years. There were no statistically significant differences in gender and age between the MDS and non-MDS groups (^{2}=2.327, P=0.127; Z=-0.442, P=0.658, respectively). Patient clinical characteristics are shown in Table 1 . All study subjects provided informed consent, and the study was approved by the Ethics Committee of the Affiliated People' s Hospital of Ningbo University (2022-(Research)-031).

Specimen Processing and MFC Data Acquisition Fresh bone marrow (1–2 mL) was collected in EDTA anticoagulant tubes. Sample processing followed the standardized operating procedures for MDS from the International/European LeukemiaNet (ELN) [8]. The antibody panels used were: CD34-FITC/CD33-PE/CD45-PERCP/CD13-PECY7/CD117-APC/HLA-DR-APC-H7, CD16-FITC/CD56-PE/CD45-PERCP/CD13-PECY7/CD11b-APC, CD15-FITC/CD64-PE/CD45-PERCP/CD34-PECY7/CD11c-APC/CD14-APC-H7, CD38-FITC/CD34-PE/CD45-PERCP/CD19-PECY7/CD10-APC/CD20-APC-CY7, CD3-FITC/CD7-PE/CD45-PERCP/CD4-PECY7/CD5-APC/CD8-APC-H7, and CD2-FITC/CD36-PE/CD45-PERCP/CD123-

PECY7/CD71-APC. Monoclonal antibodies were purchased from BD Biosciences (USA). (5–10)$×10^{5}$ bone marrow cells were incubated with antibodies for 15 minutes, followed by red blood cell lysis using BD FACS Lysing Solution. After washing, samples were analyzed on a BD FACS Canto II multiparameter flow cytometer, with at least 100,000 nucleated cells and 250 progenitor cells acquired per tube. Patient MFC data were analyzed retrospectively and blindly using FACSDiva Version 6.1.3 software.

MFC Gating Strategy and Diagnostic Scoring The hierarchical gating strategy followed ELN guidelines [8,9]. Fluorescence parameter/Time two-dimensional dot plots were used to select stable fluidic regions. Forward Scatter (FSc)/Side Scatter (SSc) two-dimensional dot plots were used to remove debris. FSc-A/FSc-H two-dimensional dot plots were used to exclude doublets. Bone marrow nucleated cells were displayed on CD45/SSc two-dimensional dot plots, with gates set for myeloid progenitors ($CD34^+$ and/or $CD117^+$CD45dim SScint), B-progenitor cells ($CD19^+CD34^+$CD45dimSSclow), lymphocytes (CD45hiSSclow), granulocytes (CD45dimSScint/hi), monocytes (CD45hiSScint), and nucleated red blood cells (CD45neg/dim). The four parameters of the Ogata score are shown in Table 2 . SSc peak values and CD45 mean fluorescence intensity values were calculated by software. When granulocytes overlapped with progenitors and/or monocytes, they were identified using appropriate markers (e.g., CD34, CD117, CD15, CD64, etc.) [8]. Antigen expression within each gate was analyzed separately. iFS [6] interpretation is shown in Table 2.

Cytogenetic Analysis and IPSS-R Chromosome analysis was performed in 83 MDS patients, though 2 cases had no dividing cells. IPSS-R [10] was used for cytogenetic risk classification and prognostic stratification in 81 MDS patients.

MDS Treatment and Patient Follow-up MDS treatment regimens followed the Chinese Guidelines for Diagnosis and Treatment of Myelodysplastic Syndromes (2019 edition) [11]. Follow-up data were obtained by reviewing inpatient and outpatient medical records and through telephone follow-up until April 30, 2022. Event-free survival was defined as the time from diagnosis to transformation to leukemia or death. Patients without these events were censored at the last follow-up date or the date of allogeneic hematopoietic stem cell transplantation.

Statistical Analysis Categorical variables in patient characteristics were described using frequency percentages, while continuous variables were described using median and range. SPSS 26 software was used for statistical analysis. Chi-square test or Fisher's exact test was used for categorical variables, and rank-sum test was used for non-normally distributed continuous variables when comparing groups. Using WHO 2016 diagnostic criteria as the gold standard, ROC curves

were plotted to evaluate the diagnostic value of Ogata and iFS. Spearman-rank analysis was used for variable correlation. Kaplan-Meier method and Log-rank test were used for survival analysis. P<0.05 was considered statistically significant.

Results

Phenotypic Abnormalities in MDS and Non-MDS Groups Based on iFS interpretation (Table 2), phenotypic abnormalities in each lineage were assessed. The proportions of myeloid progenitor abnormalities in the MDS and non-MDS groups were 71.1% (59/83) and 1.3% (1/77), respectively, with a statistically significant difference (^{2}=82.998, P<0.001). Granulocytic and/or monocytic lineage phenotypic abnormalities were observed in 73.5% (61/83) of the MDS group, significantly higher than 18.8% (14/77) in the non-MDS group (^{2}=49.074, P<0.001). Additionally, 60.2% (50/83) of MDS cases showed $ 2*erythroid phenotypic abnormalities compared to* 14.2^{2}$=35.76, P<0.001).

Diagnostic Performance of iFS for MDS MDS cases with bone marrow blasts <5% were classified as low-grade MDS subgroups, including MDS-SLD, MDS-MLD, MDS-RS, MDS-U, and MDS with isolated 5q-. The Ogata score threshold was $ $2 points. Based on Ogata score and lineage phenotypic abnormalities, iFS evaluation categorized patients as "A" (no evidence of MDS by MFC), "B" (suggestive of limited MDS evidence by MFC), or "C" (consistent with MDS by MFC).

The results showed that 81.9% (68/83) of MDS patients were evaluated as C by iFS. All 38 patients with MDS-EB1 and MDS-EB2 (100%) were evaluated as C. Among 45 low-grade MDS patients, 42.4% (19/45) had Ogata scores $ 2 *and were all evaluated as C by iFS. Of the remaining 26 patients with Ogata scores* < 2, 11 *were classified as MDS by iFS, while 15 cases (7 MDS − SLD, 8 MDS − MLD) were not assigned to MDS : 3 were evaluated as A and 12 as B. Different iFS evaluation results showed statis*
P<0.001). In the non-MDS group, 5 cases (6.5%) were incorrectly evaluated as C. The numbers and percentages of patients evaluated as A, B, and C in each group are shown in Figure 1 [Figure 1: see original paper].

The specificity of iFS for MDS diagnosis was 93.5%, sensitivity was 81.9%, and area under the curve (AUC) was 0.921 (95%CI: 0.876–0.967), with a Youden index of 0.754. In low-grade MDS and low-grade MDS with normal karyotype, the sensitivities were 66.7% and 65.0%, respectively. These results are shown in Figure 2 [Figure 2: see original paper] and Table 3 .

Correlation Between iFS and MDS Prognosis iFS evaluation grades (A, B, C) in MDS patients were not correlated with cytogenetic risk classification (P>0.05). Due to the limited number of very low-risk samples in IPSS-R (only 1 case), very low-risk and low-risk groups were combined to reduce error. There

was a statistically significant positive correlation between iFS evaluation grades and IPSS-R ($P<0.05$), as shown in Table 4 .

Follow-up data were available for 83 MDS patients with a median follow-up time of 12 (0.2–41) months. Patients evaluated as C had significantly shorter median event-free survival time than those evaluated as A or B (36.4 months vs not reached, $^{2}=5.71$, $P<0.05$), as shown in Figure 3 [Figure 3: see original paper].

Discussion

The WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2016) lists MFC-detectable immunophenotypic abnormalities in MDS, including abnormal maturation patterns of CD11b/CD13 or CD13/CD16 in myeloid lineages; cross-lineage expression of CD56 and/or CD7 on progenitors, granulocytes, or monocytes; reduced granulocyte SSc; and increased coefficient of variation or reduced expression intensity of CD71 or CD36 on erythroblasts. Over the years, multiple MDS flow cytometric scoring systems have been developed, describing different antigen expressions and scoring strategies for MDS diagnosis. The Ogata score [4], proposed through multicenter large-sample studies on the ELN platform, analyzes progenitor and mature granulocyte abnormalities with high specificity but low sensitivity. Mathis et al. [12] evaluated increased coefficient of variation of erythroid CD36 and CD71 combined with hemoglobin levels to assist MDS diagnosis, achieving 88% sensitivity and 89% specificity, but subsequent studies did not confirm these results well [7]. iFS combines the ELN MDS Flow Working Group evaluation system [13] with the erythroid score [14], integrating the Ogata score with abnormalities across all lineages, representing the most comprehensive MDS diagnostic scoring system to date [15].

Our results demonstrate that based on iFS interpretation, the detection rates of phenotypic abnormalities in myeloid progenitors, granulocytic and/or monocytic lineages, and erythroid lineage were all significantly different between MDS and non-MDS groups, all significantly associated with MDS diagnosis. Cremers et al. [6] reported that iFS specificity (95%) was superior to Ogata score (86.9%), while Oelschlaegel et al. [7] noted that iFS specificity (86%) was inferior to Ogata score (94%). Our study found iFS specificity (93.5%) was essentially comparable to Ogata score (94.8%), possibly related to different antibody panels or ethnic differences. Multiple research groups have shown [16-19] that bone marrow progenitor cell quantity and immunophenotypic abnormalities are very useful for distinguishing MDS from non-clonal cytopenias, likely because MDS is essentially a clonal abnormality of stem cells with high probability of phenotypic abnormalities in progenitor cells, which are less affected by infection or inflammation compared to mature cells [15]. In our non-MDS group, only 1 case (1.3%) showed myeloid progenitor phenotypic abnormalities, while granulocytic and/or monocytic and erythroid abnormalities were observed in 14 (18.8%)

and 11 (14.2%) cases, respectively, suggesting that progenitor cell phenotypic abnormalities are more specific for MDS diagnosis. Ogata score $ $2 has high specificity, and iFS analyzes myeloid progenitor phenotypic abnormalities in addition to Ogata score, which may explain why iFS evaluation C also has high specificity, even though combining granulocytic/monocytic with erythroid abnormalities may increase iFS evaluation grade.

Our study found iFS sensitivity was 81.9%, superior to Ogata score (68.7%). iFS also outperformed Ogata score in AUC, Youden index, and positive and negative likelihood ratios, particularly in low-grade MDS and low-grade MDS with normal karyotype, where iFS improved Ogata sensitivity from 42.2% and 40.0% to 66.7% and 65.1%, respectively, with positive likelihood ratios of 10.3 and 10.0. These results suggest iFS is an effective method for MDS differentiation, consistent with previous studies [6,7]. Among 45 low-grade MDS patients, 26 had Ogata scores <2, of which 11 were classified as MDS by iFS. Increasing evidence shows that MDS has no single specific immunophenotypic marker, and that multiple phenotypic abnormalities in stem/progenitor cells [17-19], mature granulocytic/monocytic lineages [19,20], and erythroid lineage [12,14,21] can suggest underlying MDS clonal abnormalities. In MDS patients with reduced or absent CD34 expression on myeloid progenitors, CD34 <2% but with progenitor phenotypic abnormalities, or dysplasia only in mature granulocytic/monocytic or erythroid lineages, Ogata score rarely reaches 2 points, whereas iFS can identify some MDS patients with Ogata <2, demonstrating superior sensitivity.

Porta et al. [22] proposed that Ogata score may provide additional survival information for low-grade MDS. Dysplasia of progenitor cells, granulocytes, and monocytes in MDS correlates with IPSS, IPSS-R, and overall survival [15,20,23], but studies on the correlation between iFS and prognostic stratification have not been reported. Our results show that iFS evaluation grade is positively correlated with IPSS-R, with the proportion of patients evaluated as C increasing as MDS prognostic score rises. All 15 patients evaluated as A or B belonged to low-risk or intermediate-risk IPSS-R categories. However, iFS was not correlated with cytogenetic risk classification, possibly due to small patient numbers in cytogenetic subgroups requiring larger cohorts for analysis. Additionally, we followed up treatment and survival of 83 MDS patients, finding that patients evaluated as C had significantly shorter leukemia-free or survival time than those evaluated as A or B, indicating that iFS can provide prognostic information for MDS, particularly when prognostic scoring is unavailable due to bone marrow fibrosis, poor smear quality, or absence of dividing cells in chromosome analysis. This is a single-center study with small sample size, short follow-up time, and few cases of death due to loss to follow-up, requiring continued follow-up and larger samples for multivariate survival analysis.

The greatest challenge in diagnosing MDS is distinguishing it from reactive causes of cytopenias and dysplasia. Multiparameter flow cytometry provides a more objective and reliable diagnostic tool, and iFS comprehensively analyzes

phenotypic abnormalities across all lineages, facilitating MDS diagnosis, particularly in normal karyotype MDS without increased blasts or ring sideroblasts. Furthermore, this scoring system provides prognostic information, offering important prognostic basis for rational clinical treatment.

Author Contributions

CHEN Ying contributed to study conception and design, feasibility analysis, case collection, data analysis, and manuscript writing. LI Ji-peng provided research guidance, manuscript revision, funding support, and overall responsibility and supervision. YE Pei-pei contributed to case collection and clinical guidance.

Conflict of Interest

The authors declare no conflict of interest.

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Note: Figure translations are in progress. See original paper for figures.