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Decreased CD10-positive granulocytes for the differential diagnosis of myelodysplastic syndrome

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Abstract: Myelodysplastic syndromes (MDS) are highly heterogeneous myeloid neoplasms, and a large number of patients are difficult to diagnose and classify by blood and bone marrow examination. As a surface marker of granulocyte, studies have shown CD10 can be used to define the degree of granulocyte maturation in MDS patients. However, whether it can be used for differential diagnosis of MDS and other hematological diseases remains inconclusive. To explore the value of CD10 for differential diagnosis of MDS, 60 newly diagnosed MDS, 20 aplastic anemia (AA) patients, and 35 iron-deficient anemia (IDA) patients were selected for this study. Bone marrow (BM) specimens were processed for surface marker analysis and labeled with pre-conjugated monoclonal antibodies. Stained cells were detected by flow cytometry. Our results indicated that CD10-positive granulocytes were significantly decreased in BM of MDS patients than AA and IDA patients, and the level of CD10-positive mature granulocytes was not associated with the clinical stages of malignancy. Receiver operating characteristic (ROC) areas under the curve (AUC) of CD10-positive granulocytes was 0.86 and 0.85, respectively, in MDS patients than the IDA group and AA group with good specificity and sensitivity. Further, CD10-positive granulocytes were increased after effective treatment. In conclusion, we found the decrease in CD10-positive granulocytes has a differential diagnostic value of MDS.

Introduction

Myelodysplastic syndromes (MDS) are heterogeneous myeloid neoplasms characterized by peripheral cytopenia, disordered differentiation of hematopoietic progenitors, and high risk of progression to acute myeloid leukemia (AML) (Nimer, 2008; Shastri *et al.*, 2017; Tefferi and Vardiman, 2009). Patients with MDS usually have hypercellular bone marrow (BM), showing dysplastic morphologic features in at least one hematopoietic lineage (Bennett and Orazi, 2009; Huang *et al.*, 2008). The diagnosis of MDS is clear if obvious morphological abnormalities are observed after standard or specific Perls' iron staining, or if specific cytogenetic abnormalities are present (Mathis *et al.*, 2013). If not, the diagnosis can be challenging and difficult, particularly when only subtle histomorphologic changes are present. Over the past 20 years, flow cytometry (FCM) has been a new approach in the diagnosis of patients with MDS under these circumstances (Alhan *et al.*, 2016; Craig and Foon, 2008; Mathis *et al.*, 2013). At present, although the value of several markers has been acknowledged, there is no single immunophenotypic marker that has proven to be able to discriminate accurately between MDS and other hematological diseases, such as aplastic anemia (AA) and iron-deficient anemia (IDA).

CD10, also called neutral endopeptidase, is a surface marker of granulocytes and lymphocytes and could be detected easily by FCM (Chang and Cleveland, 2000). As a maturation marker of granulocyte, some studies (Chang and Cleveland, 2000; Malcovati *et al.*, 2005; Moon *et al.*, 2010) have shown that CD10 expression in granulocytes can help define the degree of granulocyte maturation in MDS patients. However, whether CD10 can be used for differential diagnosis of MDS and other hematological diseases remains inconclusive. In this study, we selected AA and IDA as controls to explore CD10-positive granulocytes of MDS patients to explore the value of CD10 for differential diagnosis of MDS.

Materials and Methods

Patients

60 newly diagnosed MDS, 8 received effective therapy MDS patients, 20 newly diagnosed AA patients, and 35 newly

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diagnosed IDA patients were selected in our study from January 2016 to September 2018 in the Second Hospital of Anhui Medical University. The diagnosis and subtype identification of MDS were established according to the 2016 World Health Organization (WHO) criteria (Arber *et al.*, 2016), the stratification of prognosis was established on the basis of the international prognostic scoring system (IPSS) for MDS (Greenberg *et al.*, 1997). The clinical outcomes were defined according to the IWG 2006 criteria, Objective Response included complete response (CR), partial response (PR), and hematological improvement (HI). The criteria of effective treatment were according to Chinese guidelines for diagnosis and treatment of MDS (Chinese Society of Hematology, 2014). The diagnosis of AA was based on the International AA Study Group criteria. As BM aspiration is quite an invasive procedure, no health control was included in this study, and we chose 35 age- and sex-matched IDA patients as controls. The concurrence of autoimmune disease, human immunodeficiency virus (HIV), and syphilis were excluded for all enrolled individuals.

Flow cytometry

BM aspirate specimens were tested on a 2-laser FC-500 (Beckman Coulter, Miami, FL, USA). All monoclonal antibodies used in these studies were obtained from Beckman Coulter (Miami, FL, USA). These antibodies included CD34, CD10, CD19, CD33, and CD45. All samples were anticoagulated with heparin and processed for surface marker analysis within 4 h. About 100 μL of anti-coagulated BM sample was labeled with pre-conjugated monoclonal antibodies at 25°C for 20 min in the dark. After incubation, red blood cells were lysed and washed twice in phosphate-buffered saline (pH 7.4). Stained cells were quickly detected by FC-500 and analyzed using the CXP software (Beckman Coulter, USA). Cell debris was removed by FSC/SSC characteristic, and then lymphocytes, monocytes, and granulocytes were identified and classified according to their CD45/SSC. Granulocytes were identified by multiple gating of CD45/SSC and CD33/SSC.

Statistical analysis

Levels of CD10-positive granulocytes were compared among MDS patients, AA patients, and IDA controls using one-way ANOVA, followed LSD *t*-tests for pairwise comparisons. The level of CD10-positive granulocytes of MDS patients before and after effective therapy was compared by paired-samples *t*-test. Statistical analysis was performed using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). Diagnostic sensitivity and specificity were assessed by calculating the areas under the ROC curves (AUC). SPSS 17.0 was used to perform the ROC curve analysis. Two-sided *P*-values were calculated, and a difference was considered statistically significant if $p < 0.05$.

Results

Patient characteristics

A total of 60 newly diagnosed MDS patients, 20 AA patients, and 35 IDA patients were eligible for this analysis. The average age of MDS was 56.9 (24–86) years old, and 33 (55%) were male, 27 (45%) were female. The average age of AA patients was 53.8 (28–84) years old, and 9 (45.0%) were male, 11 (55.0%) were female. The average age of IDA was 61.7 (21–92) years old, and 16 (45.7%) were male, 19 (54.3%) were female. Tab. 1 showed the clinical characteristics of these patients in this study. There were no significant differences in gender and age among the three groups.

CD10-positive granulocytes were significantly decreased in BM of MDS patients.

Flow cytometry was used to analyze the CD10-positive granulocytes in BM. Our results indicated that the percentage of CD10-positive mature granulocytes was significantly decreased in MDS patients than AA group (29.27 *vs.* 51.01, $p = 0.000$) and IDA group (29.27 *vs.* 52.19, $p = 0.000$), there was no significant difference between AA and IDA groups (51.01 *vs.* 52.19, $p = 0.773$) (Fig. 1A). Typical histograms of CD10-positive granulocytic cells from a patient with IDA, a patient of MDS, and a patient with AA are shown in Figs. 1B–1D.

Receiver operating characteristic (ROC) curve for CD10-positive granulocytes

Sensitivity and specificity were determined by receiver operating characteristic (ROC) areas under the curve (AUC) to evaluate the differential diagnostic values of CD10. As shown in Fig. 2, the AUC of CD10-positive granulocytes was 0.493 (95% confidence interval [CI], 0.331–0.656; $p < 0.936$) in the IDA-AA group (Fig. 2A). The AUC of CD10-positive granulocytes was 0.86 (95% confidence interval [CI], 0.78–0.93; $p < 0.001$) in MDS-IDA group (Fig. 2B). The AUC of CD10-positive granulocytes was 0.85 (95% confidence interval [CI], 0.77–0.93; $p < 0.001$) in MDS-AA group (Fig. 2C). The results showed that the AUC value of IDA-AA group was less than 0.5, indicating that the sensitivity and specificity of CD10-Positive granulocytes to distinguish AA from IDA is extremely low, and this index has no differential diagnostic value of IDA and AA. But our results suggested that the CD10 was a valuable index for differential diagnosis

TABLE 1

Characteristics of IDA, AA and MDS patients

Groups	No. of patients	Average age (range)	Gender (M/F)
MDS	60	61.4 (24–86)	33/27
WHO classification			
MDS-MLD	26	58.4 (24–86)	14/12
RAEB1	11	66.6 (46–81)	7/4
RAEB2	23	62.2 (27–86)	12/11
IPSS stage			
inter-1	17	58.2 (36–86)	8/9
inter-2	17	60.6 (24–81)	11/6
high risk group	26	63.9 (29–86)	14/12
AA	20	53.8 (28–84)	9/11
IDA	35	56.8 (21–92)	16/19

IPSS: international prognostic scoring system; M: male; F: female; RAEB1: Refractory anemia with excess blasts-1; RAEB2: Refractory anemia with excess blasts-2; inter-1: inter-risk-1; inter-2: inter-risk-2

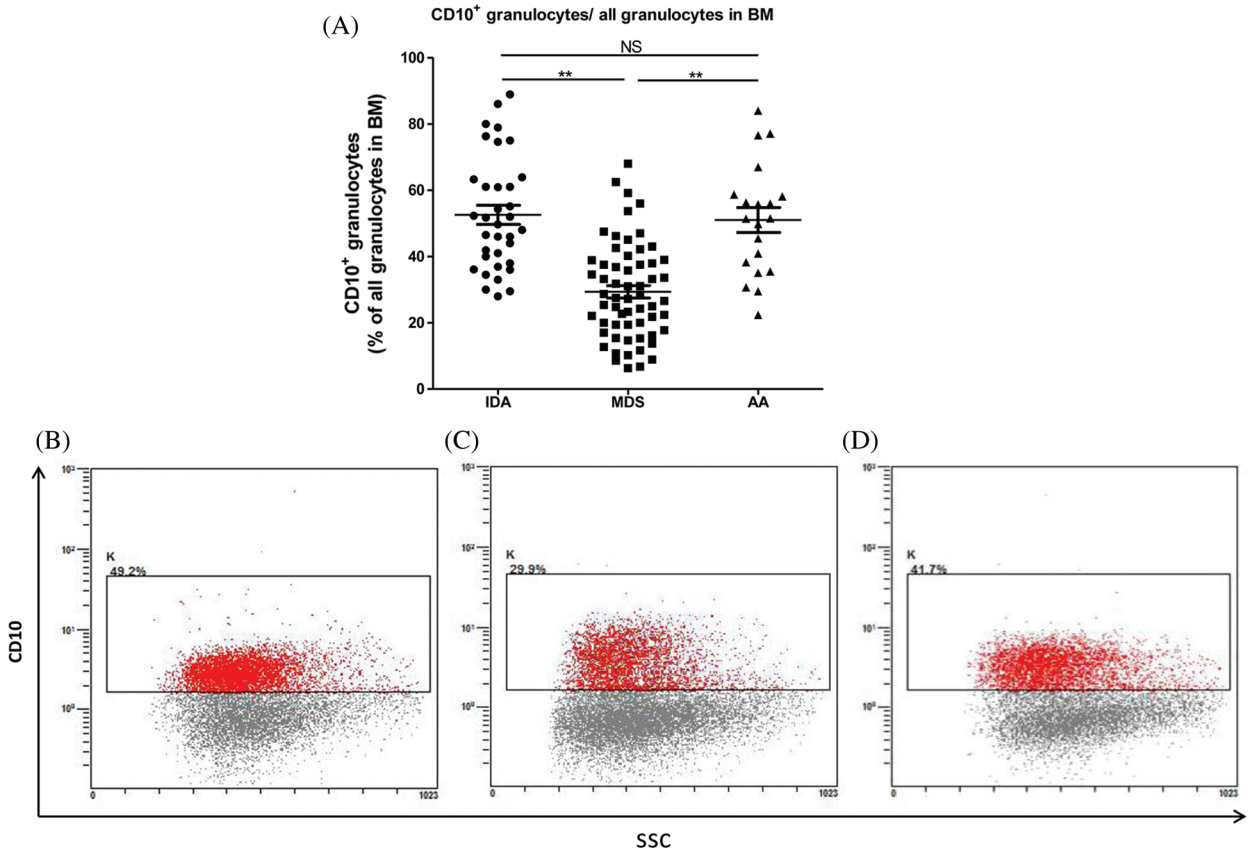

FIGURE 1. Comparison of CD10-positive granulocytes in newly diagnosed IDA patients, MDS patients, and AA patients.
(A) The level of CD10-positive granulocytes in newly diagnosed IDA patients, MDS patients, and AA patients. Each *dot* represents one individual. *Horizontal bars* indicate mean values. *$p < 0.05$; **$p < 0.01$. (B–D) Typical patterns of CD10-positive granulocytes in an IDA patient (B), an MDS patient (C), and an AA patient (D). The vertical coordinate represents staining with fluorescein isothiocyanate (FITC)-anti-CD10, and the horizontal coordinate represents side scatter (SSC) histograms. Gate K represents the region of granulocytic fraction in CD10-positive granulocytes.

of MDS and other hematological diseases, such as IDA and AA, both in terms of specified and sensitivity.

The levels of CD10-positive mature granulocytes were not associated with the clinical stages of malignancy

In order to analyze whether the percentage of CD10-positive granulocytes was correlated with the clinical stages of malignancy, we compared it according to the WHO classification and IPSS stage of MDS. As shown in Fig. 3, there were no significant differences among different subtypes (3A) and risk groups (3B) of MDS patients.

CD10-positive granulocytes were increased after effective treatment

In order to clarify whether the decreased CD10-positive granulocytes can recover after effective treatment, we examined the expression of CD10 in BM from 8 MDS patients who reached objective response after some treatment such as immunomodulatory therapy, demethylation therapy, and chemotherapy. Our result showed CD10-positive granulocytes were increased in BM of MDS patients after effective treatment ($p = 0.003$) (Fig. 4).

Discussion

MDS is a heterogeneous group of clonal hematologic disorders, and the diagnosis of it is established by correlating the clinical picture of refractory cytopenia with the morphologic abnormalities of the BM aspirate and biopsy, along with cytogenetic abnormalities (Platzbecker, 2019). However, the implementation of the WHO classification of MDS in clinical practice compels a refinement of the accuracy to detect marrow dysplasia (Arber *et al.*, 2016). FCM immunophenotyping has been proposed to be a valuable tool for marrow dysplasia evaluation in recent years (Barreau *et al.*, 2019; Craig and Foon, 2008; Westers *et al.*, 2017). In 2001, Stetler-Stevenson *et al.* (2001) performed BM FCM immunophenotyping of 44 patients with MDS and found that FCM was useful in difficult diagnosed cases in which morphology and cytogenetics were non-conclusive. These studies (Craig and Foon, 2008; Kern *et al.*, 2010; Malcovati *et al.*, 2005; Ogata *et al.*, 2002; Stetler-Stevenson *et al.*, 2001; Wells *et al.*, 2003) have shown the important role of FCM in the diagnosis of MDS. However, no single marker has been proven useful in diagnosing MDS, and there were no unified standards.

As a surface marker of granulocyte and lymphocyte, CD10 has been shown to appear late in granulocyte maturation. The previous studies (Chang and Cleveland, 2000; Malcovati *et al.*, 2005; Moon *et al.*, 2010) have already shown a significant decrease of CD10-positive granulocytes in the BM of patients with MDS by FCM. However, Chang and Cleveland (2000) only collected 7 patients in their study, and Malcovati *et al.* (2005) did not compare CD10

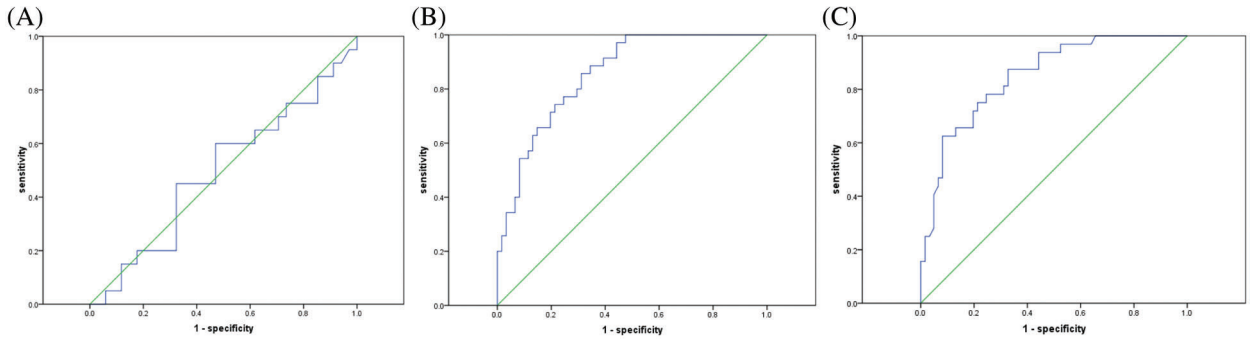

FIGURE 2. Receiver operating characteristic (ROC) curve for CD10-positive granulocytes.
(A) ROC curve of IDA-AA. The area under the ROC curve (AUC) of 0.493 (95% confidence interval [CI], 0.331–0.656; $p < 0.936$). (B) ROC curve of MDS-IDA. The area under the ROC curve (AUC) of 0.86 (95% CI 0.78–0.93; $p < 0.001$). (C) ROC curve of MDS-AA. The area under the ROC curve (AUC) of 0.85 (95% CI 0.77–0.93; $p < 0.001$).

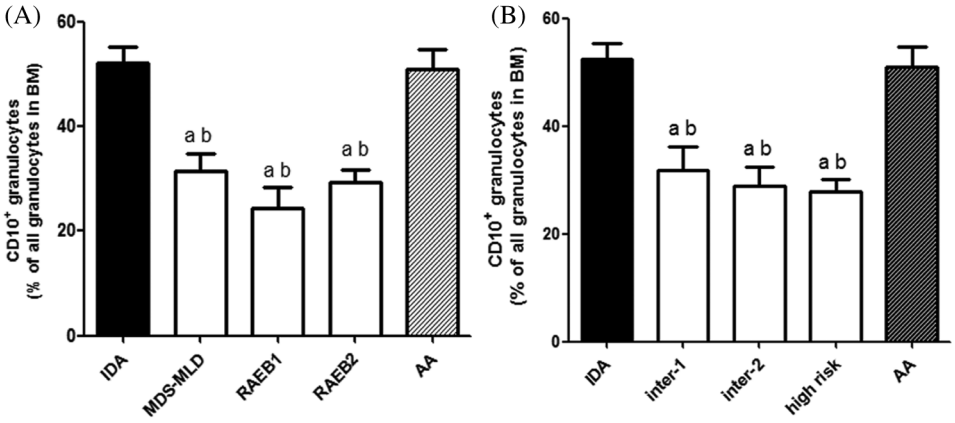

FIGURE 3. Clinical relevance of CD10-positive granulocytes in MDS patients.
(A) There were no significant differences in CD10-positive granulocytes levels of MDS patients by the WHO classification. (B) No significant differences in CD10-positive granulocytes level were seen in MDS according to the IPSS stage classification. a: $p < 0.01$, compared with IDA patients, b: $p < 0.01$, compared with AA patients.

expression among different subtypes and risk groups of MDS patients. Besides, these previous studies (Chang and Cleveland, 2000; Malcovati *et al.*, 2005; Moon *et al.*, 2010) have not made a comparison of CD10 expression with other cytopenic diseases and did not make a comparison before and after treatment.

In this study, we examined CD10-positive granulocytes in the BM of MDS, AA, and IDA patients by FCM. Our results clearly indicated a significant decrease of CD10-positive granulocytes in patients with MDS than AA and IDA patients, suggesting CD10 may be helpful in diagnosing MDS and in identifying MDS from other cytopenic diseases. Furthermore, the decreased CD10-positive mature granulocytes appeared to be present in different subtypes of MDS, and the decreased CD10-positive granulocytes were improved after effective treatment. These results indicate that CD10-positive granulocytes have clinical diagnostic and differential diagnostic value for MDS.

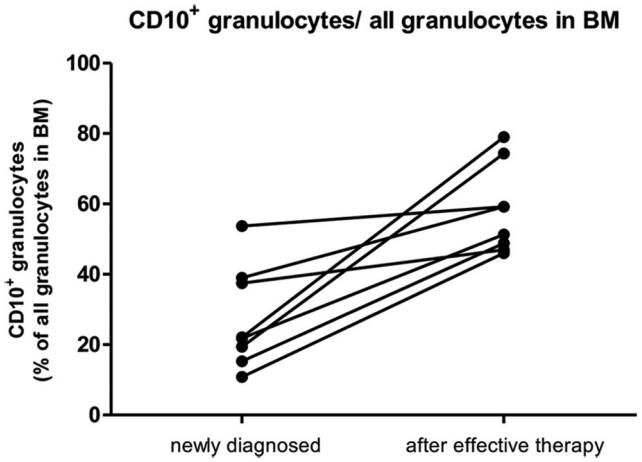

FIGURE 4. Comparison of CD10-positive granulocytes of 8 MDS patients when they were newly diagnosed and when they reached objective response after effective therapy. ($p = 0.003$).

There are several limitations in this analysis. First, as BM aspiration is quite invasive, we did not have healthy control in this study. Second, we did not analyze the relationship between the percentage of CD10-positive mature granulocytes and the follow-up of these patients, as many patients were lost to follow-up. Additionally, patients with other non-neoplastic disorders, such as hypersplenism, agranulocytosis, and reactive leukocytosis, should be studied to further determine the specificity and potential clinical applications of CD10-positive bone marrow granulocytes.

Our study demonstrated CD10-positive granulocytes were significantly decreased in BM of MDS patients than AA and IDA patients, indicating this index has a clinical and differential diagnostic value of MDS. Further studies are needed to determine whether it can be used in the flow cytometric scoring system.

Statement of Ethics: This studies was conducted ethically inaccordance with the World Medical Association Declarationof Helsinki, and approved by the Ethics Committee of the Second Hospital of Anhui Medical

University. All patients enrolled in the study have signed informed consent.

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's Contributions: Zhai Z designed this research; Wang J and Pan Y analyzed the data and wrote the manuscript; Wang H performed experiments; Tao Q collected clinical specimens.

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Conflicts of Interest: The authors declare that there is no conflict of interests with respect to the research, authorship, and/or publication of this article.

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