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Diagnostic Importance of Flow Cytometry Multiparametric Immunophenotyping in Myelodysplastic Syndrome

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Abstract

Background: Myelodysplastic Syndromes (MDS) represent a heterogeneous group of clonal hematological disorders characterized by ineffective hematopoiesis, cytopenias, and a predilection to evolve into Acute Myeloid Leukemia (AML). While traditional diagnostic paradigms based on bone marrow morphology and cytogenetics are well-established, they can often be inconclusive, especially in low-grade cases. This study evaluates the diagnostic and prognostic utility of flow cytometry multiparametric immunophenotyping (FCMI) in MDS.

Methods: 50 patients with suspected MDS were subjected to leukocyte immunophenotyping between 2011 and 2013. Bone marrow samples were analyzed using a Beckman Coulter Epics XL flow cytometer, and multiple cell markers were studied. The control group comprised ten individuals with normal bone marrow.

Results: Flow cytometry revealed an immunophenotypic profile markedly more prevalent in suspected MDS patients than AML cases. This profile, characterized by the presence of CD34, CD117, HLA-DR, CD13, and CD33 cell markers, was found in 98% of MDS cases, aiding in their differentiation from AML cases. A significant increase in Side Scatter (SS) among myeloblastic cells further substantiated MDS diagnosis.

Conclusion: Our findings endorse the value of FCMI as a reliable complementary diagnostic

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tool in MDS, especially when traditional methods yield ambiguous results. Flow cytometry can improve disease staging and guide treatment strategies by differentiating MDS from AML. As diagnostic capabilities evolve, an integrated approach that combines traditional methods with flow cytometry could offer a more comprehensive diagnosis and nuanced understanding of MDS.

Keywords: Myelodysplastic Syndromes (MDS), Flow Cytometry Multiparametric Immunophenotyping (FCMI)

INTRODUCTION

Myelodysplastic Syndrome (MDS) is a biologically and clinically heterogeneous group of clonal diseases characterized by ineffective hematopoiesis and cytopenia due to increased apoptosis and a tendency to evolve into AML (1). Patients show dysplasia in one, two, or three cell lines in peripheral blood and cytopenia. Morphological examination of peripheral blood and bone marrow determines the dysplasia of cell lines, whereas cytogenetic analyses detect chromosomal changes (2). MDS is characterized by step-by-step genetic progression, possibly due to genetic predisposition and environmental factors (3). Using molecular biology methods to detect molecular changes beyond chromosomal abnormalities has enabled an understanding of molecular changes involved in MDS, making new therapies possible (4). Over 50% of patients with MDS have a normal karyotype (5). These patients are classified in the group with a good prognosis, although this can vary. In the International Prognostic Scoring System (IPSS), cytogenetic changes are taken as a basis, where more than 3, such changes have a poor prognosis. Identification of del (5q), del (20q) in patients with MDS suggests a more favourable prognosis (6, 7). Various processes essential for hematopoietic development, including transcription programs in differentiation, protein translation from ribosomes, proliferation, cellular signaling, epigenetic regulation, and stromal interaction, may not function normally, leading to an MDS phenotype (5). Treatment of patients with MDS is improving every day. However, due to various subtypes and different levels of prognosis for this pathology, the treatment and lifespan of patients with MDS can be difficult to understand (16). The number of blast cells in bone marrow is a crucial determinant of MDS staging (3).

MDS can be primary, also known as "de novo," or secondary due to previous medication or radiation therapy for another disease (4).

What tends to capture most people's attention is the incidence of a medical condition, which represents the number of new cases occurring within a specified timeframe. Taking into account the latest data from the SEER (Surveillance, Epidemiology, and End Results Program) Registry, the annual incidence of myelodysplastic syndrome (MDS) stands at 4.9 cases per 100,000 individuals. One intriguing aspect of MDS is the strong correlation between age and its incidence. For instance, within the 65 to 69 age group, the incidence notably rises to 13.9 cases per 100,000 people. When examining individuals aged 85 and older, this incidence further escalates to 64 cases per 100,000 people (14). This demonstrates a clear trend of increasing incidence with advancing age. It's crucial to differentiate between incidence and prevalence; incidence signifies new diagnoses, while prevalence encompasses both newly diagnosed cases and those previously diagnosed, with mens slightly more affected than women (14).

Most patients' MDS causes are unknown, but some risk factors have already been identified.

Radiation therapy and chemotherapy as treatment for a previous disease, exposure to certain chemicals, including smoking, pesticides, benzene, heavy metals like mercury or lead, and some congenital syndromes increase the risk for MDS (15).

Use of FCMI in MDS

Given that flow cytometry multiparametric immunophenotyping (FCMI) is a reliable, quick, and practical method for the quantitative and qualitative assessment of hematopoietic cells, this method has been considered a possible diagnostic tool for MDS. Simultaneous determination of several parameters of hematopoietic cells provides sufficient information to assist in diagnosing MDS. Determining the percentage of blast cells and the markers they express is essential in diagnosing MDS. A reduction in granularity in the myeloid series, which is revealed through Side Scatter, is a parameter that supports MDS (Fig. 1) (17). On the other hand, the loss of specific markers for the myeloid cell line or changes in their intensity compared to normal hematopoietic cells are also indicators of MDS. An increase in the ratio between the myeloid and lymphoid lines in favor of the latter is another indicator of MDS (18, 19, 20).

All these data contribute to diagnosing MDS, even in cases where cytogenetic or morphological changes have not been detected. Therefore, this methodology has diagnostic and prognostic value in MDS (21, 22).

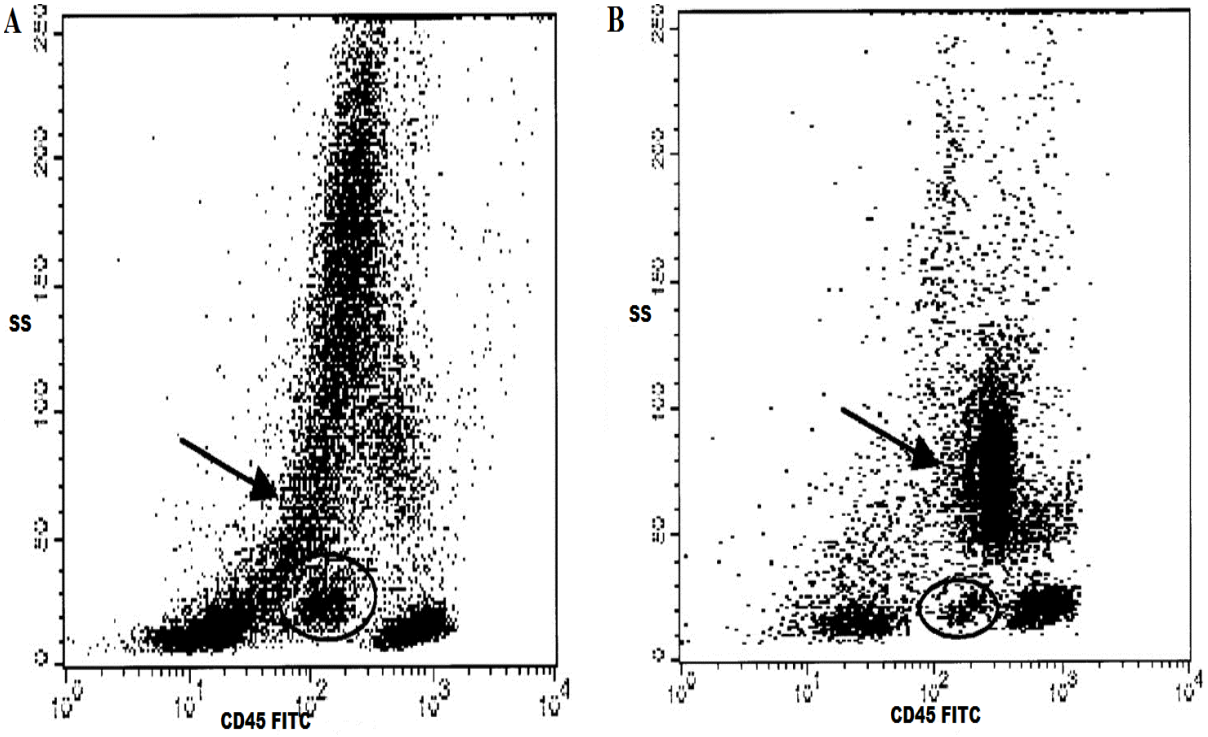

Figure 1. Distribution of cell populations of the bone marrow in two cases with MDS. The graphs show CD-45-SSC profiles of two patients. CD45 is depicted on the X-axes and on the Y-axes is SSC. In the both images the progenitor compartment is encircled and the arrows demonstrating the hypogranular neutrophils interfering with the progenitor compartment.

MATERIALS AND METHODS

Patients and studied samples

From 2011 to 2013, flow cytometry leukocyte immunophenotyping was performed on 50 samples taken from the bone marrow of patients with suspected MDS diagnosis, referred from the Haematology Service, University hospital Centre " Mother Teresa".

The biological materials for conducting FCMI were collected in tubes with K3EDTA anticoagulant.

For the study's control group comprising individuals with normal bone marrow, ten participants exhibited less than 5% myeloblasts in the granular series as determined by Side Scatter (SS) measurements. These subjects were not verified for monoclonality across any cell line.

On a cytomorphological basis, their white cell series remained unaltered, with the exception of anemia attributable to either iron or vitamin B12 deficiencies.

Immunophenotyping with Flow Cytometry of samples taken from the Bone marrow

Leukocyte immunophenotyping was conducted using a Beckman Coulter flow cytometer (COULTER EPICS XL-MCL). This device enables the simultaneous assessment of Forward Scatter (FS) and Side Scatter (SS), which analyze the cell's forward light scatter and right-angle light scatter respectively. These measures provide detailed information on the cell's size and granularity. Additionally, the cytometer can simultaneously detect up to four different fluorescently labeled cellular markers.

The flow cytometry employed a variety of monoclonal antibodies including CD3-PC5, CD4-PE, CD5-PE, CD7.PC5, CD8-ECD, **CD10-PC5**, CD11c-PE, **CD13-PE**, CD14-PC5, CD15-PC5, CD16-PE, CD19-ECD, CD33-PC5, CD34-PE, ECD, **CD45-FITC**, CD56-PE, CD64-PE, CD79a-PC5, **CD117-PC5**, **MPO-PE**, and **HLA-DR-ECD**. Four-color antibody panels, each conjugated with distinct fluorochromes and consistently incorporating CD45-FITC, were utilized to facilitate the delineation of normal and abnormal cell populations.

These specified markers are employed in each bone marrow analysis to delineate all cellular lines, with the markers presented in bold, being particularly indicative of the myeloid and myeloblastic cell lines.

RESULTS

General data of patients

A total of 50 patients suspected of having Myelodysplasia were included in the study, determined from cytological and clinical data. The material studied was bone marrow in all cases. The average age was 61.5±12.9 years, with a median age of 63, 95% CI 57.8-65.1 years. According to gender, 33 (66%) were males and 17 (34%) were females. The female-to-male ratio was 1:1.9. (Fig. 2).

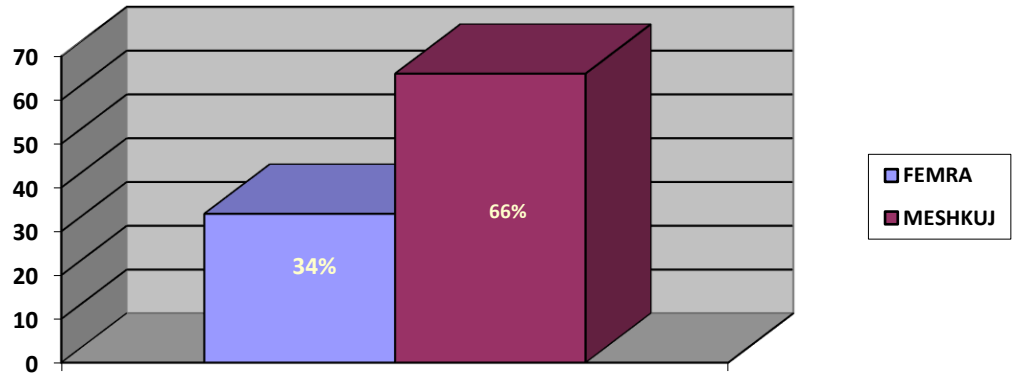

Figure 3. Graphical Representation of Patients According to Gender

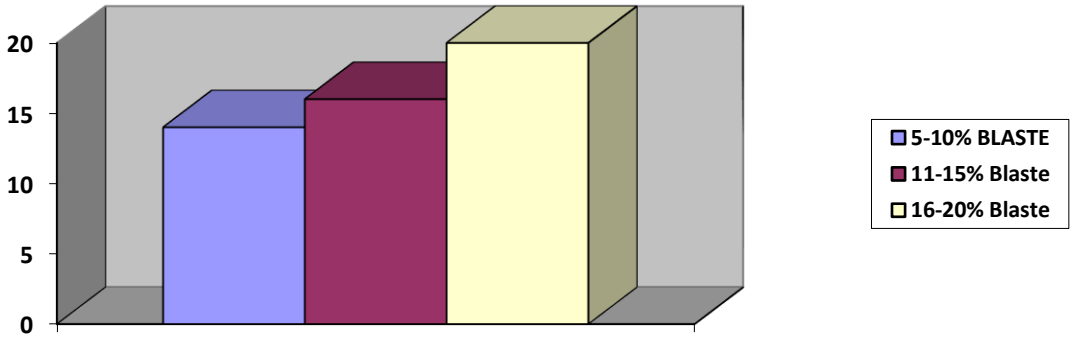

Figure 2. Frequency of Patients According to the Presence of Blast Cells in the Bone Marrow Identified with FCMI

Immunophenotypic Data of the Studied Samples

By studying markers for immature hematopoietic cells such as CD34, CD117, and HLA-DR, blast cells were present, ranging from 5-20% of all hematopoietic cells in all cases. (Fig.3).

Of these, 14 patients had 5-10% blast cells, specifically myeloblasts. Sixteen patients had 11-15% myeloblasts, and 20 patients had 16-20% myeloblasts. In all cases, an increase in Side Scatter (SS) was observed in the myeloblastic cells compared to the immature cells (maturing and above) in the normal marrow.

The markers CD34 and HLA-DR were present in the blast population in all cases. The marker CD117 was positive in 92% of the patients (Fig. 4).

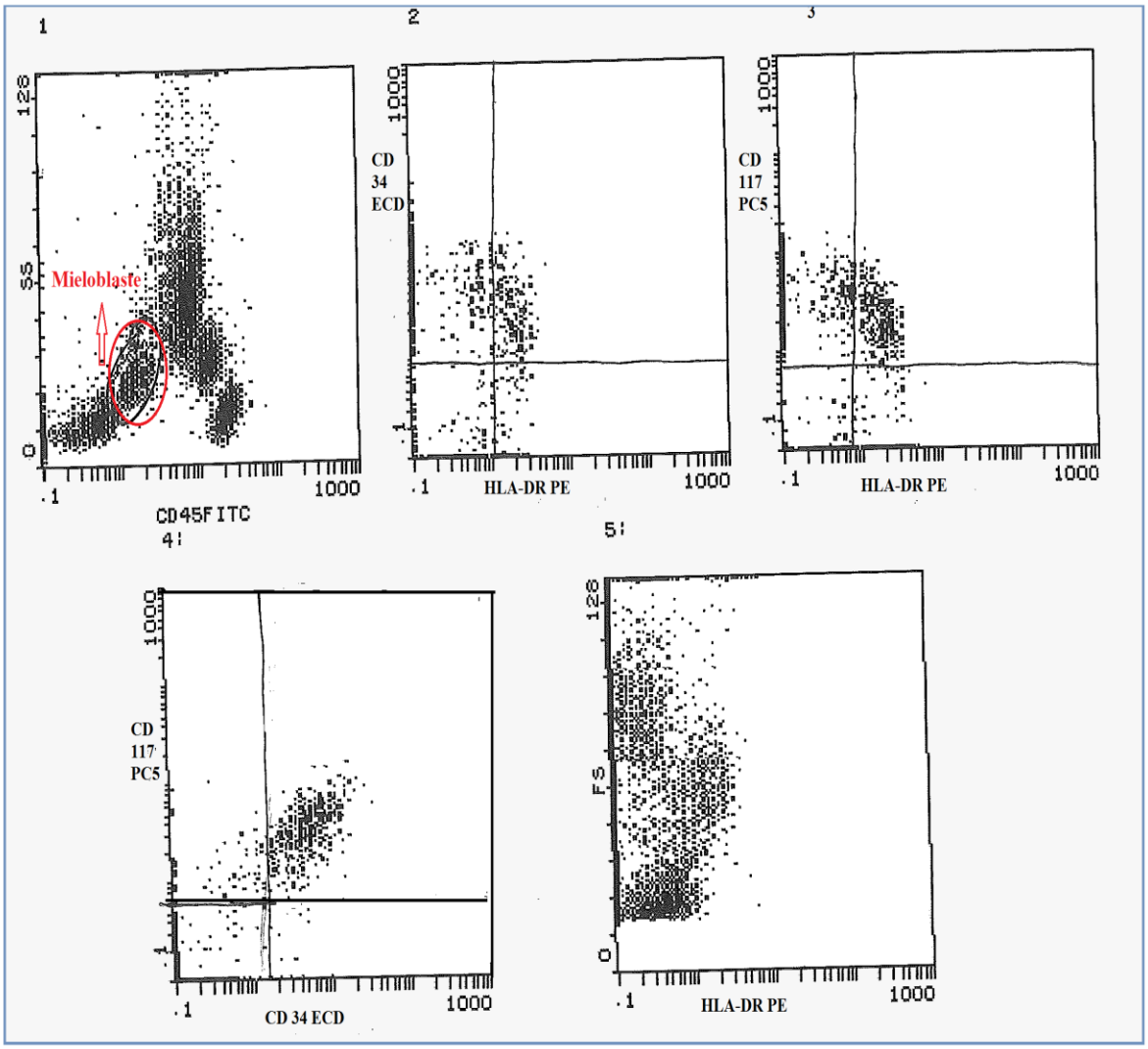

Figure 4. Presentation of a Case with MDS, Expression of Cellular Markers in the Myeloblastic Population

Table 1. Cellular Markers Expressed in the Blast Population in Suspected MDS Patients

Studied CD Markers (Number of Patients Studied)	Patients Positive for the Studied Marker N(%)	Patients Negative for the Studied Marker N(%)
CD45 (50)	50 (100%)	0 (0%)
CD34 (50)	50 (100%)	0 (0%)
HLA-DR (50)	50 (100%)	0 (0%)
MPO (50)	50 (100%)	0 (0%)
CD13 (50)	49 (98%)	1 (2%)
CD33 (50)	50 (100%)	0 (0%)
CD117 (50)	46 (92%)	4 (8%)

When comparing the expression of myeloblastic cell markers in AML with MDS, it was observed that CD34, CD117, and HLA-DR had a higher frequency in MDS than in AML (23).

DISCUSSION

Accurate diagnosis of MDS remains a significant clinical challenge. While traditional diagnostic approaches, such as bone marrow morphology and cytogenetics, are valuable, they often fail to offer a definitive diagnosis, especially in low-grade cases (24, 29). Our study reiterates the growing body of evidence that underscores the significance of flow cytometry in filling this diagnostic gap. We found a particular immunophenotypic profile (CD34+ CD117+ HLADR+ CD13+ CD33+) to be significantly more prevalent in MDS cases compared to AML, with a frequency of 98% in MDS versus 57% in AML ($p=0.0001$).

The high prevalence of this specific immunophenotypic profile in MDS correlates with Ogata et al.'s work, emphasizing flow cytometry's diagnostic utility for low-grade MDS (24, 26). Furthermore, the ability to differentiate between MDS and AML at an immunophenotypic level has profound implications for prognosis and treatment planning, especially given that MDS patients who transform into AML have a less favorable prognosis and are often non-responsive to chemotherapy (27).

In instances where cytological and cytogenetic changes are not sufficient for a conclusive MDS diagnosis, flow cytometry offers an additional layer of information (28). For example, our study found that the absence of CD10 was prevalent in 70% of MDS cases, which aligns with the results of Chang and Cleveland, who also found decreased CD10-positive mature granulocytes in MDS patients' bone marrow (25). This finding supports the hypothesis that the absence of CD10 could serve as a supplementary diagnostic feature, which is particularly helpful in cases where other diagnostic methods yield inconclusive results.

Rashidi et al. have previously outlined the utility of flow cytometry for differentiating between low-grade and high-grade MDS, suggesting that this technique could extend beyond mere diagnosis to include disease staging (27). Our findings could be an important extension of this aspect of flow cytometry, particularly in understanding the transformation from MDS to AML, which is critical for prognostic and treatment purposes.

Overall, our study further validates the role of flow cytometry as a robust tool for diagnosing and monitoring MDS, concurring with the existing literature (24, 26, 27, 29). It offers the ability to more precisely identify MDS at its various stages, differentiate it from AML, and facilitate more targeted treatment interventions (30). As we progress, a more integrated approach combining flow cytometry with traditional diagnostic methods could offer the most comprehensive means of diagnosing and managing MDS.

CONCLUSIONS

Our research underscores the pivotal role that flow cytometry can assume in the precise diagnosis and ongoing monitoring of

Myelodysplastic Syndromes (MDS). Through the identification of a distinct immunophenotypic profile (characterized by CD34+ CD117+ HLADR+ CD13+ CD33+), which is more frequently observed in MDS cases when compared to Acute Myeloid Leukemia (AML), we furnish additional substantiation for the diagnostic efficacy of flow cytometry, aligning with prior investigations. This holds particular significance because MDS patients transitioning into AML face a less favorable prognosis and often necessitate more aggressive treatment approaches such as stem cell transplantation.

Furthermore, the absence of CD10 in a notable proportion of MDS cases emerges as a noteworthy discovery. This particular feature, as highlighted in other global studies, could serve as an added diagnostic marker, particularly in situations where cytological and cytogenetic characteristics yield inconclusive results.

In summary, flow cytometry emerges as a valuable adjunctive diagnostic instrument that complements conventional methods, enabling a more precise diagnosis and the development of personalized treatment strategies. As MDS remains a diagnostic challenge, the integration of flow cytometry into routine diagnostic protocols has the potential to substantially enhance patient outcomes.

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Conflict of Interest Statement: The author declares that have no conflict of interest.

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