Journal of Hematology & Thromboembolic Diseases
ISSN: 2329-8790

Brahimi et al., J Hematol Thromb Dis 2013, 1:4
DOI: 10.4172/2329-8790.1000e104

Editorial Open Access

The Role of Flow Cytometry in the Diagnosis of Myelodysplasic Syndromes

Mohamed Brahimi[1,2*], Mohammed Nazim Bennaoum[1], Hassiba Lazreg[1], Affaf Adda[1], Fadela Attaf[2], Abdessamad Arabi[2], Soufi Osmani[2], Nabil Yafour[2] and Mohamed Amine Bekadja[2]

[1]*Department of Hemobiology, Institution University Hospital, Oran, Algeria*
[2]*Department of Hematology and Cell Therapy, Institution University Hospital, Oran, Algeria*

Myelodysplastic Syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by dysplastic features of erythroid and/or myeloid and/or megakaryocytic lineages, a varying percentage of blast cells, progressive bone marrow failure and enhanced risk to evolve towards acute myeloid leukemia [1].

The diagnosis of MDS is straightforward if an increase in blasts and/or ringed sideroblasts and/or presence of chromosomal aberration as evidence of clonal myelopoiesis, are detected. In other words, a diagnostic challenge exists in low-grade MDS without ringed sideroblasts with normal karyotype. For this reason many laboratory scientists have investigated the use of Flow Cytometry (FCM) to increase the sensitivity and specificity of diagnosis in such cases [2].

Most authors examined bone marrow cells using multiparameter FCM, applying a pattern recognition approach [3]. As maturation and differentiation of hematopoietic cells is abnormal in MDS, FCM identifies dysplasia by detecting deviations from the normal pattern of antigen expression [3,4]. Data interpretation is not based solely upon whether an antigen is 'positive' or 'negative'. Instead, interpretation is based on the examination of staining patterns demonstrated by a series of antibody combinations. This approach requires an extensive knowledge of the normal patterns of antigen expression during differentiation [3].

The flow cytometric abnormalities typically detected in MDS include: abnormal co-expression of antigens that are normally present at different stages of differentiation, abnormal intensity of antigen expression, presence of aberrant lymphoid lineage antigens expressed on myeloid precursors, and abnormally decreased side scatter properties due to hypo granularity of granulocytes [3].

In general, the antigens expressed on normal myeloid precursors (such as CD34, CD117, and HLA-DR) and those on immature granulocytes (such as CD13 and CD33) are increased in MDS. In contrast, antigens that are expressed on mature granulocytes (such as CD10, CD11b, CD11c, CD16, and CD64) are decreased in MDS [5].

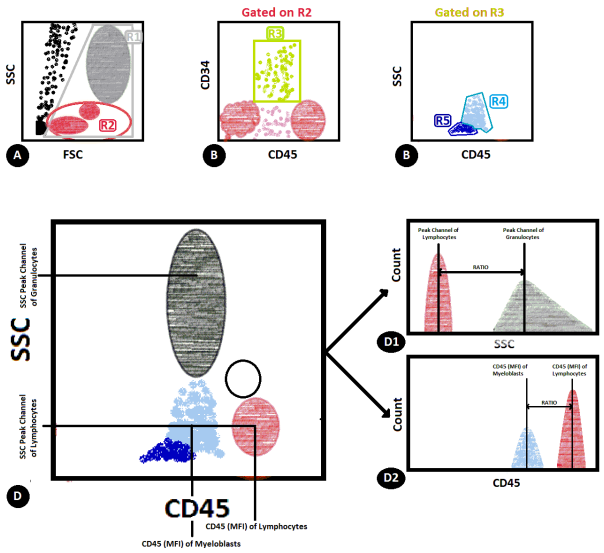
Among all the quantitative changes, CD34 abnormality is most thoroughly studied. The percentage of CD34 cells is usually proportional to the number of blasts. Therefore, its percentage increases progressively from RA and RARS to RAEB and leukemic transformation [6].

Qualitative changes are mainly manifested as aberrant expression of nonmyeloid markers, namely, T-cell, B-cell, and natural killer cell markers. CD7 and CD19 can be detected on maturing myeloid cells or monocytes [7]. CD56 may be found on myeloblasts and maturing myeloid cells and monocytes [7]. Abnormal patterns of CD11b versus CD16 expression or CD13 versus CD16 expression have also been identified in MDS cases [3,7].

Finally, the low side-scatter property in the nonblastic myeloid cells in MDS cases represents the presence of hypogranular granulocytes [8].

This approach requires sufficient experience and knowledge of the normal (control) patterns of haematopoietic cells in FCM, which is the basis on which examiners identify abnormal MDS flow patterns. In other words, this approach is an FCM version of cytomorphology [2].

Obviously, there are many cases in which FCM patterns are intermediate between typical control patterns and typical MDS



(A): All nucleated cells (R1) and cells with relatively low SSC (R2) are gated.
(B): Cells in R2 in panel (A) are displayed on a CD45/CD34 plot, and CD34+ cells with intermediate CD45 expression are gated (R3).
(C): Cells in the R3 gate are plotted on a CD45/SSC display. In the CD45/SSC dot plot, we can identify a cell population consisting predominantly of CD34+ B-cell progenitor cells which formed an easily recognizable cluster (R5) that had the lowest SSC and relatively low CD45 expression. Other CD34+ cells (R4) showing more SSC and a wider distribution of CD45 expression consisted predominantly of myeloblasts.
(D) All nucleated cells are plotted on a CD45/SSC display.
Histogram (D1) SSC of lymphocytes (in red) and granulocytes (in gray). SSC peak channel values (SSC channel number where the maximum number of cells occurs) of both fractions were computed.
Histogram (D2) shows CD45 expression of lymphocytes (in red) and CD34+ myeloblasts (in cyan). Mean fluorescence intensity (MFI) of CD45 of both populations are computed.

Figure 1: Ogata's gating strategy cells stained with CD34 and CD45 antibodies [8-11].

***Corresponding author:** Mohamed Brahimi, Department of Hemobiology, Institution University Hospital, 269 Hai, Ennakhla Canastel 31132 Oran, Algeria, Tel: 00213 771 48 57 39; Fax: 00213 41 42 16 36; E-mail: bmw73dz@yahoo.fr

Received July 21, 2013; **Accepted** October 17, 2013; **Published** October 19, 2013

Citation: Brahimi M, Bennaoum MN, Lazreg H, Adda A, Attaf F, et al. (2013) The Role of Flow Cytometry in the Diagnosis of Myelodysplasic Syndromes. J Hematol Thromb Dis 1: e104 doi: 10.4172/2329-8790.1000e104

Parameters	Cut-off values	Variable weighted score
CD34+ Myeloblast cluster size (%)*	≥ 2	1
CD34+ B-progenitor cluster size (%)**	≤ 5	1
Lympocytes to Myeloblasts CD45 ratio	≤ 4 or ≥ 7.5	1
Granulocytes to Lymphocytes SSC ratio	≤ 6	1

* in all nucleated cells; **in all CD34+ cells.

Table 1: Ogata Scoring System for the diagnosis of MDS: a diagnosis of MDS is formulated in presence of a FCM-score value ≥ 2 [8-11].

patterns. Furthermore, a large panel of antibodies is usually required, and many of those antibodies are not commonly used in routine FCM [2].

Thus in order to become universally applicable for the diagnosis of MDS: First, the parameters used in MDS FCM should have sufficient specificity and sensitivity, secondly, the data of these parameters should be reproducible even if different flow operators, and hopefully even if different laboratories, analyze them and finally, these parameters should be easily understood by the majority of clinicians who use this diagnostic tool.

Ogata and co-workers developed a diagnostic scoring system, based on four objective flow cytometric parameters: 1) CD34+ myeloblast-related cluster size 2) CD34+ B-progenitor-related cluster size, 3) myeloblast CD45 expression and 4) granulocyte side scatter value. Figure 1 and Table 1 describes the gating strategy and the calculations of the MDS diagnosis score [2,9,10].

This scoring system has several advantages: first this later uses only a single antibody combination containing CD45 and CD34 (CD10/CD19 or CD10/CD13-CD33 are optional), secondly Ogata score is not based on pattern recognition approach but on four calculated parameters and finally, since this score is based on sizes of clustered cells and ratios of internal populations there is no need process normal bone marrow controls [2,9,10].

This diagnosis scoring system has been validated by European Leukemia NET group in a multicenter study on a large cohort of patients: "learning cohort" (n=538) to define the score and a "validation cohort" (n=259). The sensitivity and specificity of the Ogata score in this study were 69% and 92%, respectively and the likelihood ratio of the flow cytometric score was 10 [11].

However, some difficulties can be noticed when using this score such as: 1) nucleated cells are gated on FSC/SSC scattergram without any nuclear stain; 2) the separation of the CD34+ subpopulations is unclear due to hypogranulation of these clustered cells, 3) CD34 negative myeloblasts are not gated using this strategy 4) peripheral blood contamination of the bone marrow sample can lead to an underestimation of the myéloblasts and B-progenitors.

Since the late 1980's, many laboratories have been working to develop MDS FCM and are still struggling to determine suitable flow parameters. Ogata score seems to be an interesting tool, but its sensitivity must yet to be improved, maybe by adding more flow cytometric parameters. However, it must be kept in mind that the diagnosis of the MDS is first of all based on cytomorphology features, presence of ringed sideroblasts and chromosomal aberrations. FCM should be considered as an auxiliary technique which brings supplementary details.

References

1. van de Loosdrecht AA, Alhan C, Béné MC, Della Porta MG, Dräger AM, et al. (2009) Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica 94: 1124-1134.
2. Ogata K (2008) Diagnostic flow cytometry for low-grade myelodysplastic syndromes. Hematol. Oncol 26: 193-198.
3. Stetler-Stevenson M, Yuan CM (2009) Myelodysplastic syndromes: the role of flow cytometry in diagnosis and prognosis. Int J Lab Hematol 31: 479-483.
4. Kussick SJ (2007) Multiparametric flow cytometry in the diagnosis of myelodysplastic syndromes and related disorders. LabMedicine 38: 305-313.
5. Sun T (2008) Flow Cytometry and Immunohistochemistry for Hematologic Neoplasms. Wolters Kluwer Health.
6. Orfao A, Ortuno F, de Santiago M, Lopez A, San Miguel J. (2004) Immunophenotyping of acute leukemias and myelodysplastic syndromes. Cytometry Part A 58: 62-71.
7. Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, et al. (2003) Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood 102: 394-403.
8. Della Porta M G, Lanza F, Del Vecchio L (2011) Flow cytometry immunophenotyping for the evaluation of bone marrow dysplasia. Cytometry B Clin Cytom 80: 201-211.
9. Ogata K, Kishikawa Y, Satoh C, Tamura H, Dan K, et al. (2006) Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes. Blood 108: 1037-1044.
10. Ogata K, Della Porta M G, Malcovati L, Picone C, Yokose N, et al. (2009) Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. Haematologica 94: 1066-1074.
11. Della Porta M G, Picone C, Pascutto C, Malcovati L, Tamura H, et al. (2012) Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study. Haematologica 97: 1209-1217.