

The Role of Flow Cytometry in the Diagnosis of Myelodysplastic Syndromes

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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].

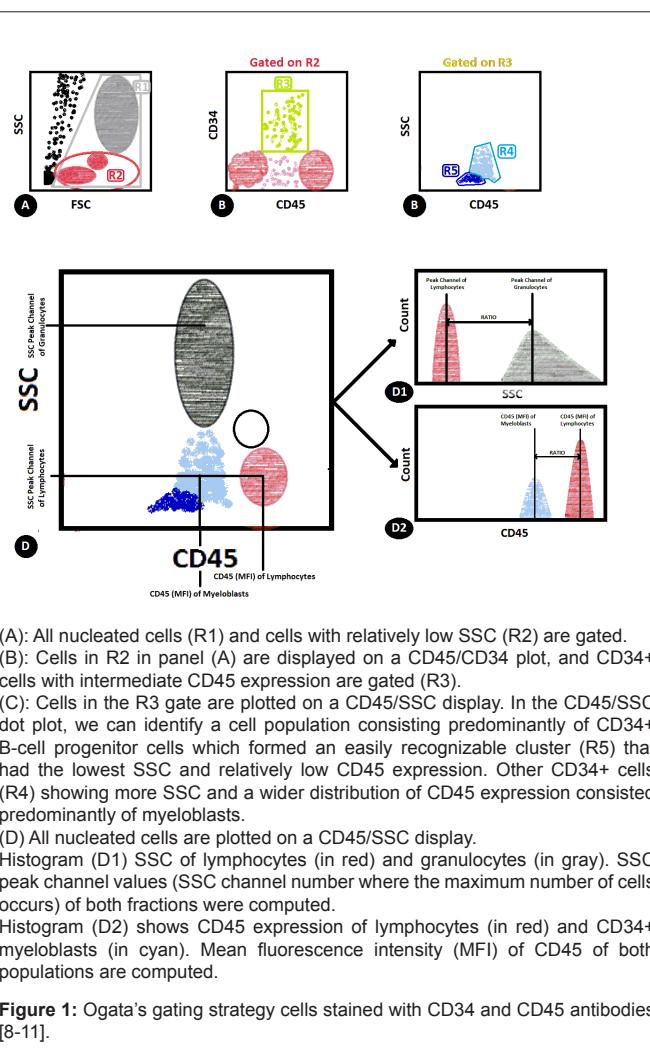
Qualitative changes are mainly manifested as aberrant expression of nonmyeloid markers, namely, T-cell, B-cell, and natural killer cell markers. CD7 and CD19 can be detected on maturing myeloid cells or monocytes [7]. CD56 may be found on myeloblasts and maturing myeloid cells and monocytes [7]. Abnormal 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



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Parameters	Cut-off values	Variable weighted score
CD34+ Myeloblast cluster size (%)*	≥ 2	1
CD34+ B-progenitor cluster size (%)**	≤ 5	1
Lymphocytes 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 myeloblasts 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.

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