Review Article

Flow Cytometry Immunophenotyping for the Evaluation of Bone Marrow Dysplasia

Matteo Giovanni Della Porta,[1]* Francesco Lanza,[2] and Luigi Del Vecchio[3] for the Italian Society of Cytometry (GIC)

[1]Department of Hematology Oncology, University of Pavia Medical School and Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

[2]Section of Hematology and Bone Marrow Transplantation, Ospedale di Cremona, Cremona, Italy

[3]CEINGE, Biotecnologie Avanzate, and Department of Biochemistry and Biotechnology University Federico II, Napoli, Italy

The pathological hallmark of myelodysplastic syndromes (MDS) is marrow dysplasia, which represents the basis of the WHO classification of these disorders. This classification provides clinicians with a useful tool for defining the different subtypes of MDS and determining individual prognosis. The WHO proposal has raised some concern regarding minimal diagnostic criteria particularly in patients with normal karyotype without robust morphological markers of dysplasia (such as ring sideroblasts or excess of blasts). Therefore, there is clearly a need to refine the accuracy to detect marrow dysplasia. Flow cytometry (FCM) immunophenotyping has been proposed as a tool to improve the evaluation of marrow dysplasia. Rationale for the application of FCM in the diagnostic work up of MDS is that immunophenotyping is an accurate method for quantitative and qualitative evaluation of hematopoietic cells and that MDS have been found to have abnormal expression of several cellular antigens. To become clinically applicable, FCM analysis should be based on parameters with sufficient specificity and sensitivity, data should be reproducible between different operators and the results should be easily understood by clinicians. In this report, we reviewed the most relevant progresses in detection of marrow dysplasia by FCM in MDS as defined by WHO criteria. © 2011 International Clinical Cytometry Society

Key terms: myelodysplastic syndromes; flow cytometry; diagnosis

How to cite this article: Della Porta MG, Lanza F, Del Vecchio L. Flow cytometry immunophenotyping for the evaluation of bone marrow dysplasia. Cytometry Part B 2011; 80B: 201–211.

THE CHALLENGE OF THE DIAGNOSIS OF MYELODYSPLASTIC SYNDROMES (MDS)

Myelodysplastic syndromes (MDS) are a group of disorders clinically characterized by peripheral cytopenia, followed by a progressive impairment in the ability of myelodysplastic stem cells to differentiate and an increasing risk of evolution into acute leukemia (1).

The clinical course of the disease is very heterogeneous, ranging from indolent conditions spanning years to forms rapidly progressing to leukemia (2,3). This heterogeneity reflects the complexity of the underlying genetic defects, which are still to be clarified (4).

According to the prevailing dogma, clonal transformation in MDS would occur at the level of a committed myeloid stem cell that can give rise to red cells, platelets, granulocytes and monocytes (4). The biologic hallmark of these stem cells is, rather, dysplasia, which indicates a defective capacity for self-renewal and differentiation and relies on various morphological abnormalities. Karyotypic aberrancies (involving loss of genetic material and less frequently balanced translocations) are detected in about 50% of primary MDS, and when present are a marker of clonal hematopoiesis (1,5–7). Although recurrent

*Correspondence to: Matteo Giovanni Della Porta, Department of Hematology Oncology, University of Pavia Medical School & Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy. E-mail: matteo@haematologica.org

Received 24 February 2011; Revision 6 May 2011; Accepted 17 May 2011

Published online 13 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/cyto.b.20607

cytogenetic defects were documented in MDS since several years ago, few specific gene abnormalities implicated in the development and/or progression of the disease were described until now (8–11).

The morphological evaluation of marrow dysplasia represents the basis of the World Health Organization (WHO) classification of these disorders (12,13). This classification provides clinicians with a very useful tool for defining the different subtypes of MDS and determining individual prognosis (3). The combination of overt marrow dysplasia and clonal cytogenetic abnormalities allows a conclusive diagnosis of MDS (13). However, this combination is found only in some patients, who tend to be those with more advanced disease. In many instances, cytogenetics is not informative so that the diagnosis of MDS is based entirely and exclusively on morphological evaluation (14).

The WHO proposal has raised some concern regarding minimal diagnostic criteria for formulating the diagnosis of MDS (12–14). Morphology may be difficult to evaluate, because cellular abnormalities of bone marrow cells are not specific for MDS and may be found in other pathological conditions. As a consequence, in clinical practice interobserver reproducibility for recognition of dysplasia is usually poor, particularly in patients who do not have robust morphological markers such as ring sideroblasts or excess of blasts (15,16). Moreover, poor technical quality of the specimen is a common obstacle in the accurate diagnosis of MDS. Finally, morphology may be difficult to evaluate in some patients either due to hypocellularity or fibrosis of the marrow (17,18). The implementation of WHO classification of MDS in clinical practice compels a refinement of the accuracy to detect marrow dysplasia (3,19).

RATIONALE TO THE APPLICATION OF FLOW CYTOMETRY (FCM) IMMUNOPHENOTYPING IN THE DIAGNOSIS OF MDS

Flow cytometry (FCM) immunophenotyping was introduced by WHO proposal for the classification of hematologic neoplasms as an indispensable tool for the diagnosis, classification, staging, and monitoring of several diseases, such as lymphoproliferative disorders and acute leukemias (12,13). In addition, immunophenotyping has been proposed as a tool to improve the evaluation of marrow dysplasia. Rationale for the application of FCM in the diagnostic work up of MDS is that immunophenotyping is an accurate method for quantitative and qualitative evaluation of hematopoietic cells and that MDS have been found to have abnormal expression of several cellular antigens (20,21).

To become clinically applicable, FCM analysis should be based on parameters with sufficient specificity and sensitivity, data should be reproducible between different operators, and the results should be easily understood by clinicians (22,23). With respect to this situation, the results of the studies that pointed out the feasibility of FCM analysis for the evaluation of marrow dysplasia raise some concerns (20,23). First, no single immunophenotypic parameter has been proved able to discriminate accurately between MDS and other conditions, and among laboratories that have experience in MDS FCM no consensus exists on which diagnostic parameters are the most appropriate (20,23). Moreover, FCM evaluation of erythroid dysplasia (which represents the milestone of morphological diagnosis of MDS) is particularly difficult, because of the limited availability of specific markers (24,25). Finally, published protocols are mainly based on a qualitative analysis of cytometric variables and are tested on very heterogeneous patient populations, thus limiting a wide clinical implementation (24,26,27).

For these limitations, at the time of introduction of the WHO classification (2001), FCM was not recommended as a screening procedure for MDS (12). More recently, many studies addressed the weak points of FCM immunophenotyping on the diagnosis of MDS and significant progresses were made. In 2006, a group of international experts met in Bethesda to formulate consensus recommendations for FCM testing based on the clinical presentation (28–30). Consensus was reached that immunophenotyping is indicated in the evaluation of patients with peripheral blood cytopenia: in this clinical situation, FCM can provide a sensitive screen for the presence of hematologic malignancy and assist in demonstrating the absence of disease. In addition, according to the report of 2006 Working Conference on MDS, FCM enables the detection of aberrancies in the differentiation of marrow cell populations by changes in antigen expression that are otherwise not detected by morphology, and therefore immunophenotyping may be useful to establish a definitive diagnosis of MDS in the absence of significant morphological dysplasia or increased blasts (7). More recently, the revised WHO classification (2008) recognized that FCM analysis of bone marrow cells may add important information to the diagnostic and prognostic evaluation of MDS patients (13). In this report, we reviewed the most relevant progresses in the evaluation of marrow dysplasia by FCM in MDS as defined by WHO criteria.

FCM EVALUATION OF MYELOID DYSPLASIA

Morphological myeloid dysplasia as defined by WHO criteria is present in about 60% of MDS patients at diagnosis (5). Most significant morphological alterations on granulocytic lineage included hypogranularity on myeloid cells, presence of pseudo-pelger neutrophils and increased prevalence in bone marrow of myeloid cells in the earliest stage of maturation. These abnormalities significantly affected the detection of physical parameters (i.e., side scatter, SSC and forward scatter, FSC) by FCM, as reported in different studies (24,27,31). Defective capacity for self-renewal and differentiation by myelodysplastic stem cells also relies on various abnormalities of antigen expression on granulocytic cells, which may be easily detected by FCM due to a large availability of specific antibodies for myeloid lineage (20,24). Reported aberrancies of granulocytic lineage include the presence of antigens that are not normally present, such as lymphoid antigens, and altered expression of myeloid antigens, either in a single population of cells, or within a

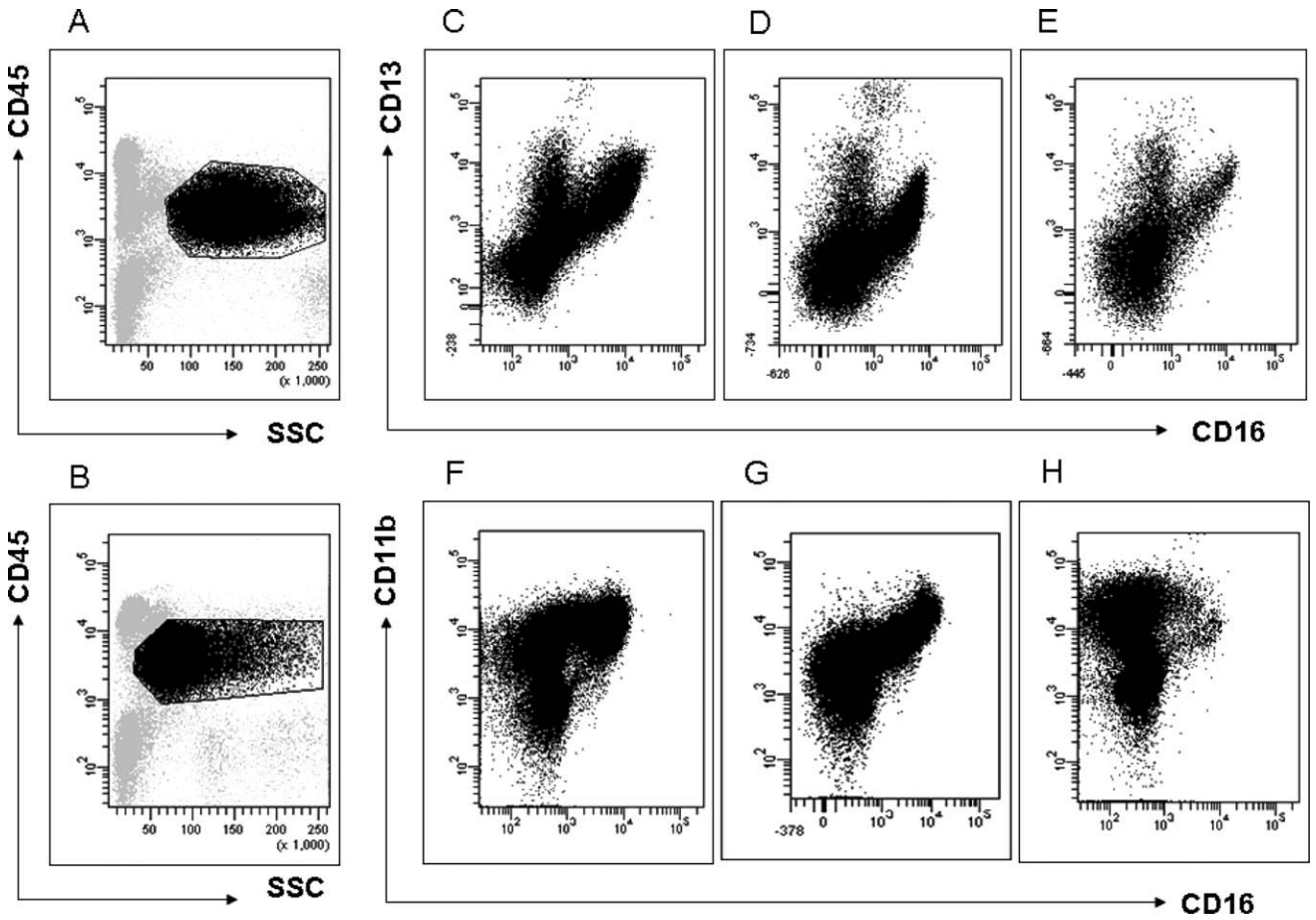

FIG. 1. Consistent immunophenotypic features of myeloid dysplasia in MDS. (**A**,**B**) Myeloid abnormalities in MDS demonstrated by CD45 versus side light scatter (SSC): (A) Healthy donor bone marrow: normal granulocytes in the boxed region; (B) MDS patient bone marrow with hypogranular neutrophils with low side scatter. (**C**–**E**) CD16 versus CD13 maturation pattern analysis on granulocytes (identified in CD45 versus SSC dot plot): (C) Healthy donor bone marrow; (D,E) bone marrow from representative MDS patients showing an increase of cells in myelocyte and metamyelocyte stages of maturation and a decrease in CD13+CD16+ segmented neutrophils. (**F**–**H**) CD16 versus CD11b maturation pattern analysis on granulocytes: (F) Healthy donor bone marrow; (G,H) bone marrow from representative MDS patients showing an increased percentage of granulocytic cells with low CD16 or both low CD16 and low CD11b.

generation of maturing cells (24,26,27,32–34). Furthermore, monocytic compartment is also affected in MDS (26,27) (Fig. 1 and Table 1).

Davis studied for the first time the pattern of CD16 and CD11b expression by maturing granulocytes in the bone marrow of patients with MDS and healthy controls (35). There was a highly consistent normal pattern of CD11b and CD16 expression in the granulocytic series in healthy subjects, while in MDS patients an increased percentage of granulocytic cells with low CD16 or both low CD16 and low CD11b was noticed (24). In addition, an altered granulocytic maturation pattern can be demonstrated by plotting CD13 versus CD16 (36). During maturation, myeloid cells normally acquire increasing levels of CD16 that are initially accompanied by a decrease in CD13 expression as cells mature from blasts through the myelocyte and metamyelocyte stages of maturation, followed by intermediate levels of CD13 in band forms and high levels in segmented neutrophils. Several abnormalities on CD13/CD16 maturation pattern were described in MDS patients, including an increase of cells in myelocyte and metamyelocyte stages of maturation and a decrease of CD13+CD16+ neutrophils (Fig. 1 and Table 1).

Although these investigations defined immunophenotypic abnormalities in MDS, they did not address the potential contribution of FCM to the diagnosis of MDS. The study of Stetler-Stevenson et al. published in 2001 (24) was the first to demonstrate that the identification of immunophenotypic abnormalities by FCM is useful in establishing a diagnosis of a MDS, especially when the results of morphologic evaluation and cytogenetic studies are indeterminate. In addition to maturation abnormalities, aberrancies in the expression of several antigens on granulocytes such as CD64, CD10, and CD56 were described in MDS (24,26,27,32,34,37). Lymphoid antigens, such as CD2, CD5, CD7, and CD19 may be abnormally expressed on myeloid progenitors and maturing myeloid cells. Moreover, a common finding in these patients is the atypical expression of

Table 1
Frequency and Reproducibility of Myeloid, Erythroid, and CD34-Related Abnormalities in MDS

Lineage	Immunophenotypic marker	Frequency in MDS	Frequency in controls	Analytical method	Reproducibility in MDS setting
Myeloid	Reduction of SCC on granulocytes	10–85%	Single myeloid abnormality reported in 30–40% of controls.	Quantitative	High
	CD11b vs. CD16 and CD13 vs. CD16 maturation patterns	25–80%		Qualitative	No data, good tool for expert operators
	Aberrant expression of CD64, CD10, CD56 and other on granulocytes	5–65%	Multiple abnormalities uncommon	Qualitative/ quantitative	No data, good tool for expert operators
Erythroid	Dys-synchronous expression of CD71 vs. Gly A on erythroblasts	>70%	20–30%	Qualitative	No data, good tool for expert operators
	Increased H ferritin and reduced CD71 on erythroblast	>80%	About 20%	Quantitative	Good, at least in a intralaboratory setting
	Increased CD105 expression on erythroblasts	20–30%	Few data	Quantitative	Few data
CD34+ cell compartment	Increased CD34+ myeloblasts	20–40% of low risk MDS	5–10%	Quantitative	High
	Reduced CD34+ B cell progenitors	40–70%	20–40%	Quantitative	High
	Aberrant expression of CD45 on myeloblasts	20–40%	10–20%	Quantitative	High
	Reduced CD38 expression on myeloblasts	>80%	5–10%	Quantitative	Good, at least in a intralaboratory setting
	Aberrant expression of CD11b, CD15, CD56, CD4, CD7, and other on myeloblasts	5–80% Multiple abnormalities are common	5–10% Multiple abnormalities uncommon	Qualitative/ quantitative	Few data, good tool for expert operators

antigens on immature myeloid cells that are normally expressed on mature myeloid cells, such as CD11b and/or CD15 (24,26,27,32,37). More recently, some intracellular markers, such as myeloid nuclear differentiation antigen were reported to be differentially expressed between MDS and controls (38). As far as monocytic compartment is concerned, most frequent abnormalities observed in MDS patients include altered expression of CD56, HLA-DR, CD36, CD33, CD15, CD14, CD13, and CD11b (26,27,32,33; Table 1). In general, the amount of abnormalities reported by FCM correlates with the degree of dysplasia assessed by morphology (27,37). Although most of the studies have evaluated bone marrow cells, there is some evidence that FCM analysis of peripheral blood could also assist in the diagnosis of MDS (39). Frequencies of single myeloid aberration in MDS patients are highly variable in different studies. In general, hypogranulation of neutrophils is observed in the great majority of MDS patients (frequency ranging from 10 to 84%) (26,27,31,37) and was found to be potentially highly reproducible if quantitatively expressed as ratio between SSC granulocytes to SSC lymphocytes (31). Abnormalities on CD11b versus CD16 and CD13 versus CD16 patterns were also reported with high frequency in MDS despite a wide variability in different studies (frequency ranging from 23 to 78%). Aberrancies in the expression of CD64, CD10, CD56, and other antigens on granulocytes were described in a percentage comprised between <5 and 66% of MDS cases (26,27,31,37). Scientific evidence suggests that aberrant antigen expression by myeloid cells is more frequent and carries more discriminant weight on detection of marrow dysplasia than altered expression of monocytic antigens (23,27,40). A single myeloid immunophenotypic abnormality was reported in about 30–40% of patients affected with nonclonal cytopenia (26,27,31,37). Therefore, a single myeloid immunophenotypic abnormality is not a definitive finding for MDS, and other abnormalities should be detected on granulocytic cells to conclude that myeloid dysplasia is present (23). Multiparametric evaluation of myeloid and monocytic maturation and antigen expression pattern leads to the identification of two or more aberrancies in the great majority of MDS cases (from 70% to more than 90% in different studies) (24,27,32,37,40). In general FCM is more sensitive in detection of myeloid dysplasia with respect to morphology, and immunophenotypic myeloid abnormalities are identified in a significant percentage of cases (from 20% to more than 90%) classified as refractory cytopenia with unilineage dysplasia or unclassifiable MDS (24,27; Table 1). In addition, FCM was found to be useful for detection of marrow dysplasia in a proportion of patients with marrow hypocellularity, fibrosis or inadequate specimen collection, suggesting that variables related to sample quality are less significant in immunophenotypic analysis than in morphological evaluation (24,37).

As discussed before, WHO classification provides clinicians with a very useful tool for determining individual

prognosis in MDS (3,5,19). In particular, among patients with MDS without excess blasts, isolated involvement of the erythroid lineage rather than bilineage or trilineage marrow dysplasia is associated with a significantly better prognosis (41). Interesting data have also been emerging on the ability of the WHO classification to guide clinical decision making regarding therapeutic choices. Patients with unilineage dysplasia have been shown to have a significantly higher probability of responding to treatment with hematopoietic growth factors compared to those with multilineage dysplasia, (42) while patients with 5q deletion were found to have a high response rate to lenalidomide (43). Finally WHO classification predict post-transplant outcome in patients receiving allogeneic stem cell transplantation (44). In this context, the possibility to recognize patients with unilineage versus multilineage dysplasia by FCM with higher sensitivity with respect to morphology might have important clinical implications.

The great variability on the percentage of reported immunophenotypic abnormalities in MDS patients reflect in part the biological heterogeneity within these disorders, but more likely, the lack of a standardized and reproducible procedure for the evaluation of these parameters (20,23). The most largely used approach to evaluate myeloid dysplasia by FCM is pattern recognition analysis (24,26,27,32). This is a qualitative method based on recognition of a deviation from normal antigen expression pattern. Although similarly to morphological evaluation this approach is a good tool for expert operators (i.e., people with extensive knowledge of changes in antigen expression in normal and pathological hematopoietic cell differentiation;45) pattern recognition analysis presents several weak points. The numerical description of the results is difficult, thus quantitative analysis is not possible; moreover the precise definition of the normal pattern of reference may be complex (24); finally, with rare exceptions, no reproducibility data in the setting of MDS are currently available (20,23). To overcome these limitations, other studies analyzed the expression of myeloid antigens as percentage of positive cells (37). This is a quantitative method (the results of FCM analysis are expressed as numbers) and has proved to be reproducible at least in an intralaboratory setting. However, the definition of the threshold distinguishing between positive and negative populations, which in most methods ultimately rely on arbitrary criteria, remains a major limitation of this approach (20). An alternative analytical method to express cytometric variables in a quantitative manner is the mean fluorescence intensity (MFI), defined as the ratio between the measured MFI of the marker tested and the measured mean autofluorescence of the cells (46). FCM analysis of marrow dysplasia by MFI appears particularly promising. In fact MFI has proved to be highly reproducible in both intralaboratory and interlaboratory settings (37,46,47). Indeed, fluorescence ratios depend both on the percentage of cells expressing the marker tested and on the intensity of expression, that may be important in MDS were marrow populations are typically heterogeneous for a particular marker (37,46; Table 1). Overall, FCM multiparametric approaches based on a quantitative evaluation of myeloid antigens allow to correctly classify about 90% of cases with suspected MDS (37).

A standardized application of FCM in detection of myeloid dysplasia also requires a minimal variability in sample processing, antibody combinations and data acquisition. In fact, the inherent variability of instrument set-up can give significant differences in the data collected between and within institutions. Similarly, differences in reagents, especially the fluorochromes used, will result in inconsistent sensitivity and specificity of data (23,30). In this context, the European LeukemiaNET (ELN) working group for FCM in MDS started a consensus process on how to standardize sample collection/preparation and data acquisition (23), that is expected to significantly improve the FCM accuracy in detection of marrow dysplasia.

FCM EVALUATION OF ERYTHROID DYSPLASIA

Erythroid dysplasia is the milestone of the morphological diagnosis of MDS (1,13). In fact, it is present in almost all patients with MDS and is the only morphological abnormality in those with refractory or sideroblastic anemia (13). Evaluation of erythroid dysplasia represents a challenge in the immunophenotypic analysis of myelodysplastic marrows: the precise identification of marrow erythroid precursors is problematic and there is a limited availability of specific markers (20,23,25).

The first critical issue of erythroid compartment immunophenotyping is the gating strategy to identify marrow erythroid precursors. Nucleated erythroid cells are characterized by reduced/absent CD45 and low SSC (25,37). To gate CD45dim to negative/SSClow cells is certainly simple and seems likely to be reproducible (23,24,37). However, this region also contains mature (anucleate) red cells, cellular debris, and nonhematopoietic cells, which are not discriminable on the basis of CD45 or scatter proprieties. Alternatively, an immunological gate based on the antigens expressed by erythroid cells can be performed (37). During physiological development from the basophilic erythroblast to the erythrocyte, there is a progressive decrease in CD45 expression (25). An increase in glycophorin A (Gly A) is observed early upon differentiation from the basophilic erythroblast to the orthochromic erythroblast. Finally, CD71 is one of the earlier antigens expressed during erythroid maturation (which anticipates Gly A expression), remains on the reticulocyte after enucleation and then is lost prior to the loss of the RNA (25). From a theoretical point of view, gating erythroblast on the basis of CD71 expression would be preferable, Gly A+ cells excluding a proportion of more immature erythroid precursors, which may be increased in MDS (23,24,29). However, a dysregulation of CD71 expression is reported in MDS, (24,37) and Gly A that has a very tight coefficient of variation of intensity from individual to individual, (25) should be preferentially adopted in gating erythroid precursors in the setting of MDS.

The lysis process is also critical, affecting nucleated as well as mature red blood cells to an unknown variable degree (23,30). Although a no-lyse, no-wash system would provide the most accurate estimate of nucleated red cell, a lyse no-wash approach is certainly simpler and more easily implementable in the diagnostic work-up of MDS patients (24,29,37,47).

The study by Stetler-Stevenson et al. demonstrated for the first time the feasibility of the evaluation of erythroid dysplasia by FCM (24). Abnormal erythroid precursors were detected based upon immunophenotype in the great majority of MDS cases studied (77%). However, the only consistent erythroid abnormality in this study was dys-synchronous expression of CD71 versus Gly A on red cell precursors.

A promising approach to overcome the limited availability of FCM markers specific for erythroid dysplasia is the analysis of proteins involved in cellular iron metabolism. It is well known that iron metabolism is essential in erythroid cells for heme production and is peculiarly perturbed in MDS, (48) as suggested by the abnormal expression in these patients of CD71, the cellular receptor for transferrin (24). At the cellular level, ferritin with H and L subunits plays a critical role in regulating intracellular iron homeostasis by storing iron inside its multimeric shell (49). It also plays an important role in detoxifying potentially harmful free ferrous iron to the less soluble ferric iron by virtue of the ferroxidase activity of the H subunit. Transferrin receptor CD71 is indispensable for cellular iron uptake. Cytoplasmic ferritin and transferrin receptor are mutually regulated by iron regulatory proteins, that bind to this stem loop structure and inhibit mRNA translation (48,49). MDS erythroid cells present an "iron-loaded" phenotype characterized by increased ferritin contents (in particular H subunit) and reduced transferrin receptor (47). Interestingly, both H ferritin and CD71 expression reflects the degree of dysplasia assessed by morphology. Moreover, in an erythroid culture model induction of H ferritin occur at very early stage of differentiation, confirming a strong relationship with the myelodysplastic phenotype (47) (Fig. 2 and Table 1). Preliminary data suggest that a multiparametric approach based on the evaluation of iron metabolism proteins allows to correctly classify more than 90% of MDS and pathological controls with a acceptable interobserver reproducibility (47).

In addition, FCM analysis might be helpful in characterization of the sideroblastic anemias, a distinct MDS subgroup characterized by the presence of ring sideroblasts, that is, red cell precursors with mitochondrial iron accumulation (50). Recent advances in our understanding of iron metabolism clarified that iron deposited in perinuclear mitochondria of ring sideroblasts is present in the form of a particular type of ferritin, called mitochondrial ferritin (MtF) (51) that might be a specific marker of sideroblastic anemia. By using a specific (not commercially available) marker against MtF, FCM analysis confirmed that MtF expression is closely related to the presence of ring sideroblasts in bone marrow (47). Since, the morphological recognition may be difficult in some cases, in prospective FCM evaluation of MtF might be considered a specific marker of these diseases (Table 1).

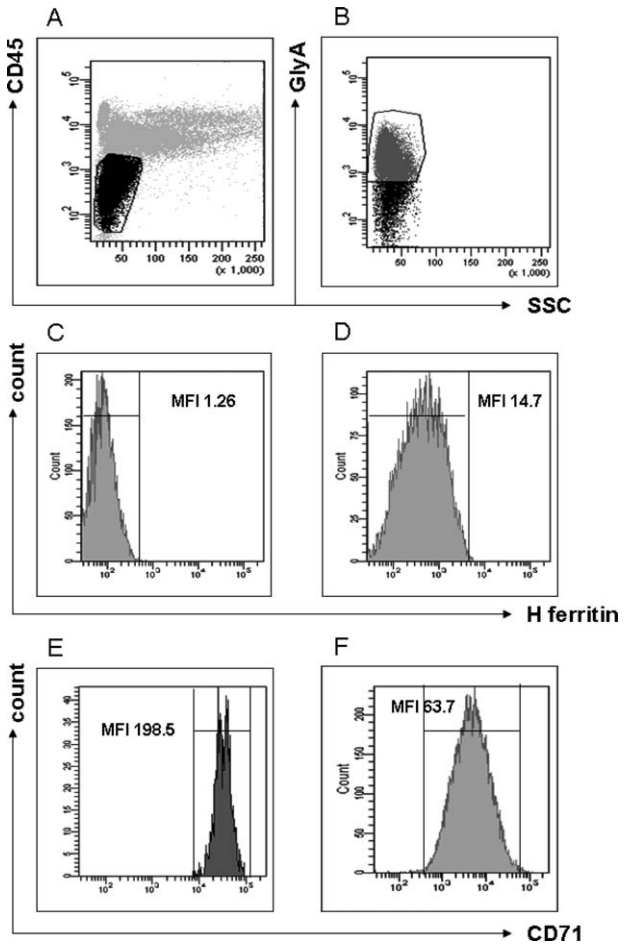

FIG. 2. Immunophenotypic analysis of erythroid dysplasia based on the evaluation of iron metabolism proteins. (**A**,**B**) Gating strategy to identify bone marrow erythroid precursors. Erythroblasts are defined as CD45low-/SSClow/Gly A+ cells. Cytosolic ferritin (H subunit) and transferrin receptor (CD71) expression on erythroid precursors are evaluated as mean fluorescence intensity (MFI, defined as the ratio between the measured MFI of the marker tested and the measured mean autofluorescence of the cells). Cytosolic H ferritin: (**C**) Healthy donor bone marrow; (**D**) MDS patient bone marrow showing a significantly increased expression of H ferritin. Transferrin receptor (CD71): (**E**) Healthy donor bone marrow; (**F**) MDS patient bone marrow showing a significantly decreased expression of CD71.

FCM EVALUATION OF BLAST CELLS AND CD34 COMPARTMENT

Clonal transformation in MDS occurs at the level of a CD34+ myeloid committed stem cell which has a competitive advantage over normal stem cell compartment (1). These hematopoietic precursors (blasts) are morphologically defined as "immature cells with uncondensed chromatin pattern, prominent nucleoli, low nuclear/cytoplasmic ratio, and no/few cytoplasmic granules" (13,16). The evaluation of blast compartment has diagnostic relevance in the WHO system, (13) and the

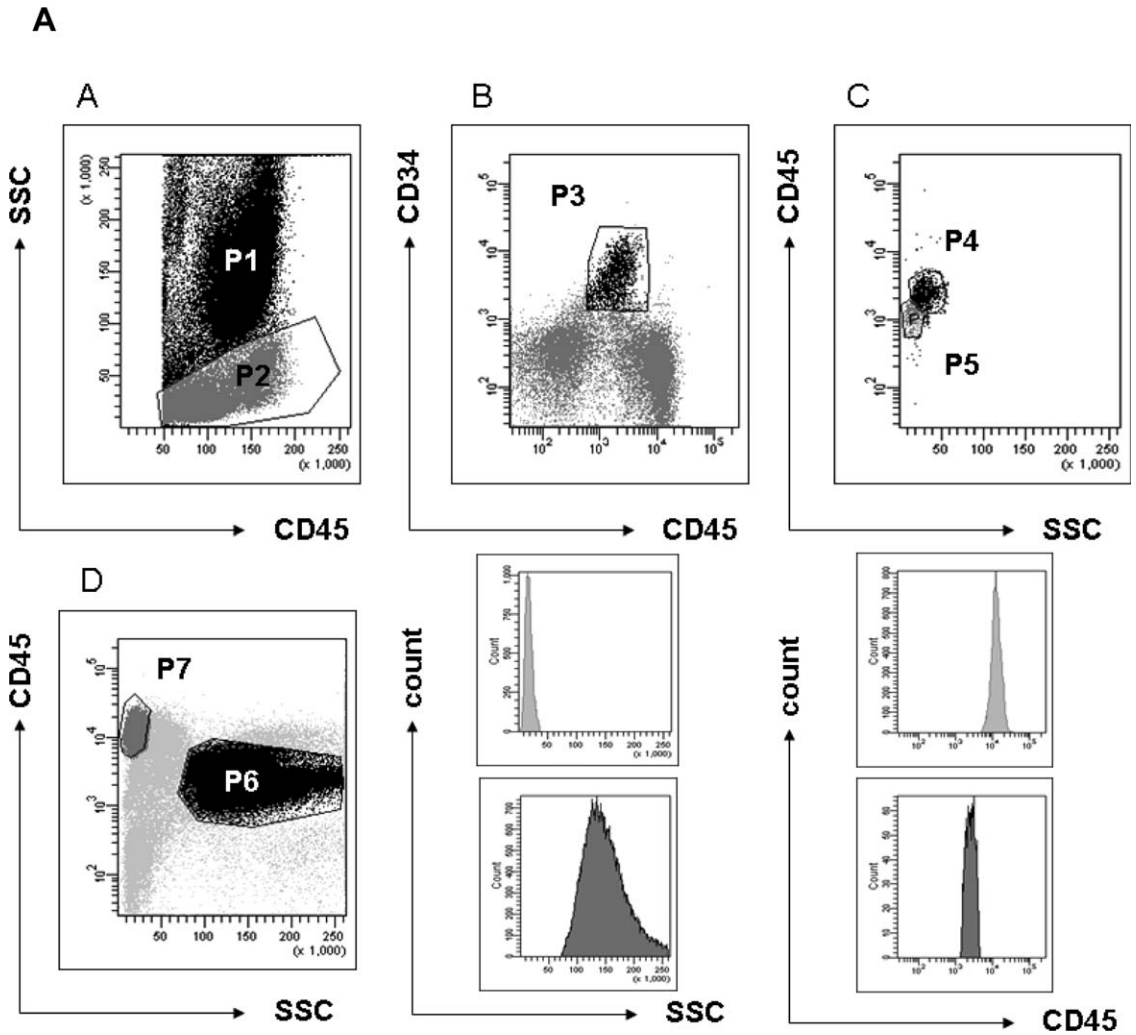

FIG. 3. (**A**) Detection of marrow dysplasia by analysis of four cardinal parameters of marrow dysplasia for from a single cell aliquot stained with CD34 and CD45 antibodies. Gating strategy. (**a**) All nucleated cells (P1) and cells with relatively low SSC (R2). (**b**) Cells in R2 in panel A were displayed on a CD34-versus-CD45 plot, and CD34+ cells with intermediate CD45 expression were gated (P3). (**c**) Cells in R3 in panel B were displayed on a CD45-versus-SSC plot. A cluster of CD34+ B-cell progenitors was identified in the lower left region of CD34+ cells (P5). Cells in P4 were composed mainly of myeloblasts and thus called CD34+ Myeloblasts. (**d**) Granulocytic cells (P6) and lymphocytes (P7) were gated on a CD45-versus-SSC plot. (**e**) SSC of lymphocytes (upper panel) and granulocytic cells (lower panel). SSC peak channel values (SSC channel number where the maximum number of cells occurs) of both fractions were computed using the software. (**f**) CD45 expression of lymphocytes (upper panel) and CD34+ Myeloblasts (lower panel). Mean fluorescence intensity (MFI) of CD45 of both fractions was computed. (**B**) Detection of marrow dysplasia by analysis of four cardinal parameters of marrow dysplasia for from a single cell aliquot stained with CD34 and CD45 antibodies. (**a**–**f**) Healthy donor bone marrow; (A,C,E) bone marrow from representative MDS patients showing an increase of CD34+ myeloblasts (b), a decrease of CD34+ B cell progenitors (b), a reduced SSC in granulocytic cells (d) and an aberrant expression of CD45 on myeloblasts (f).

percentage of marrow blasts has recognized to have prognostic effect by all the currently available prognostic scores (19,52). In the WHO guidelines, despite inaccuracies inherent in manual differential counting, morphological analysis is actually the gold standard for determining blast percentage (12,13). The first attempt of FCM immunophenotyping was to provide a quantitative estimation of bone marrow blasts with increased sensitivity and reproducibility with respect to morphological count (53). Unfortunately, the quantitative evaluation of marrow blasts in MDS by FCM presents both technical and intrinsic limitations (12,20). First, MDS blasts are not predominant cells in the bone marrow, making their reliable analysis difficult and in addition, blasts lack of a specific immunophenotypic markers (21). They are identified in the CD45 versus SSC dotplot as CD45lowSSClow cells (21,37); however, hypogranular more mature myeloid cells may have decreased SSC and fall in this region, and it may be difficult to distinguish monoblasts from more mature monocytes. The percentage of CD34+ cells determined by FCM has been tested as substitution for a visual blast count (37). However, although hematopoietic cells that express CD34 are blasts, not all blasts express CD34. It should be considered in addition that marrow samples for morphological evaluation can considerably differ form that

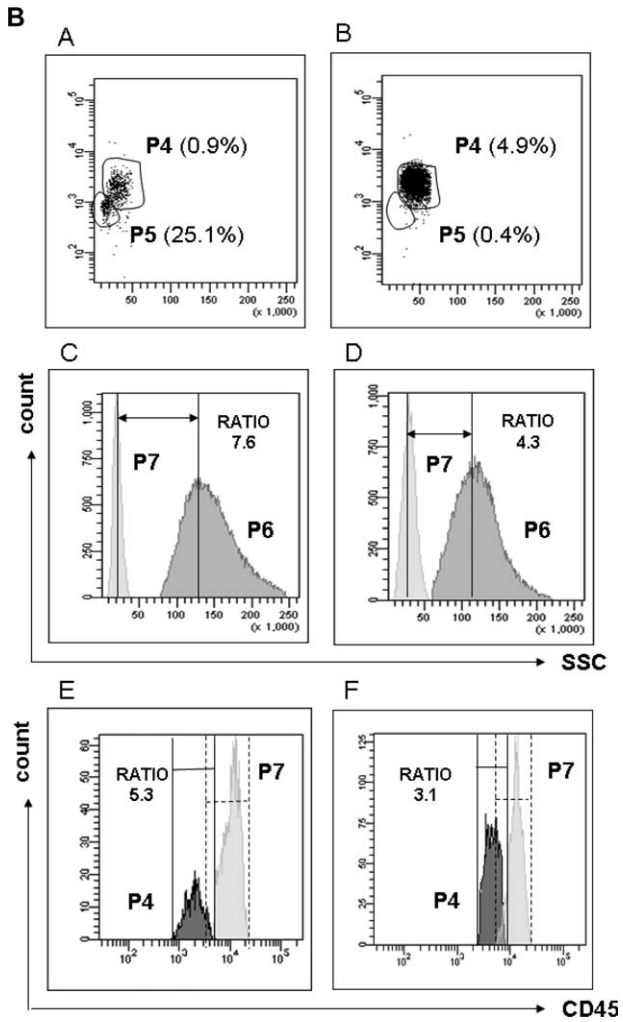

FIG. 3. (Continued)

for FCM analysis. Hence, the percent of CD34+ cells determined by FCM as substitution for a visual blast count in MDS is discouraged by current WHO classification (12,13).

More interesting results in the light of a diagnostic application of FCM in work-up of MDS patients derive from the analysis of immunophenotypic abnormalities of CD34+ cell compartment (21,53–55). As underlined above, CD34+ cell compartment is peculiarly perturbed in MDS and therefore CD34-related parameters are good candidates for the identification of diagnostic markers for these disorders (1,8,9). Clonal transformation in MDS occurs at the level of a CD34+ myeloid committed stem cell. Accordingly, the proportion of CD34+ cells is significantly higher in MDS with respect to healthy subjects, and the great majority of cells are committed to the myeloid lineage (CD34+CD38+HLA-DR+CD13+CD33+) (21,37,55,56). In addition, a significant down-regulation of B-cell lineage-affiliated genes was observed in CD34+ cells isolated from low-risk MDS with respect to healthy controls and patients with nonclonal cytopenia, and a reduction in stage I hematogones is one of most consistent immunophenotypic findings in MDS patients (57–59). In different studies, a significant decrease of CD34+ B cell progenitors was observed in 40–70% of subjects with a conclusive diagnosis of MDS and in 20–40% of patients with nonclonal cytopenia (56,57,59,60) The analysis of both percentage of CD34+ myeloblasts and CD34+ B cells was found to have little interobserver variability (31,56,60).

Several other immunophenotypic abnormalities on MDS CD34+ cell compartment were reported, including asynchronous co-expression of stem-cell and late-stage myeloid antigens (CD117, CD15, and CD11b) or abnormal expression of lymphoid markers (CD2, CD5, CD7, CD19, and CD56) (26,27,29,55,61). However, most of these parameters do not have adequate reproducibility in the MDS setting (31,60) with the exception of lymphocytes-to-myeloblasts CD45 ratio that ensures acceptable interobserver variability by adjusting data on target cells with those on lymphocytes in the same sample (31,60).

The analysis of percentage of CD34+ myeloblasts, CD34+ B-cell progenitors and myeloblast CD45 expression by FCM has little interoperator variability and appears to be applicable in many laboratories (56,60). When combined together with the evaluation of SSC on granulocytes, these parameters differentiate correctly the majority of MDS and pathological controls, sensitivity ranging from 30 to 70% and specificity ranging from 80% to more that 90% in different studies, (56,60; Fig. 3 and Table 1). A study evaluated the expression of CD38 on CD34+ cell compartment as a diagnostic test in MDS (58). B-cell progenitors have high CD38 expression, and a lower number of B-cell progenitors in MDS would reduce the mean fluorescence intensity of CD38 on CD34+ cells. In addition, the expression of CD38 on CD34+ cells would be further reduced in high-risk MDS as this condition has a higher number of immature CD34+CD38- progenitors. In this study, the reduced expression of CD38 on CD34+ cells below a threshold value diagnosed low-grade MDS with high sensitivity and specificity (>90%;58).

Interestingly, the evaluation of CD34-related parameters seems to be useful in establishing the diagnosis of MDS also in patients without specific markers of marrow dysplasia (such as ring sideroblasts and/or clonal chromosomal abnormalities) (60). As underlined before, a critical issue for the morphological evaluation of marrow dysplasia is that it may be hampered by the presence of hypocellularity, fibrosis, or inadequate specimen collection. In this context, the great majority of marrow samples even if diluted with peripheral blood, provides accurate data for most CD34-releted parameters (56,60). All these findings strongly suggest that CD34-related parameters are good candidates for the identification of diagnostic markers that not only can be used for the diagnosis of MDS patients but also are relatively stable and result in acceptable between-operator data variation.

CONCLUDING REMARKS

The implementation of WHO classification of MDS in clinical practice compels a refinement of the accuracy to detect marrow dysplasia. FCM immunophenotyping has been proposed as a tool to improve the evaluation of marrow dysplasia. To become clinically applicable, FCM analysis should be based on parameters with sufficient specificity and sensitivity, data should be reproducible between different operators, and the results should be easily understood by clinicians. With respect to this ideal situation, the results of the studies that pointed out the feasibility of immunophenotyping in diagnostic work-up of MDS patients raise some concerns: no single marker has proved able to discriminate accurately between MDS and other pathological conditions, no consensus exists on which diagnostic parameters are the most appropriate, and published protocols are mainly based on a qualitative analysis of cytometric variables thus limiting a wide clinical implementation. However, in recent years significant progresses were made. Clonal transformation in MDS occurs at the level of a CD34+ committed stem cell, and therefore CD34-related parameters are good candidates for identification of diagnostic markers for these disorders. More consistent immunophenotypic aberrations reported in MDS CD34+ cell compartment are increase of CD34+ myeloblasts, decrease of B cell progenitors, coexpression of stem cell—and late stage—myeloid antigens, expression of lymphoid antigens and abnormal CD45 expression. Increasing evidence suggests that these parameters have little interoperator variability and, when combined, are able in discriminating between MDS (including those subjects without robust markers of dysplasia) and patients with nonclonal cytopenia.

Evaluation of erythroid dysplasia represents a challenge in the immunophenotypic analysis of myelodysplastic marrows due to a limited availability of specific markers. A promising approach to overcome this limit is the analysis of proteins involved in cellular iron metabolism that is clearly important in erythroid cells for heme production and is peculiarly perturbed in MDS. MDS erythroid cells present an "iron-loaded" phenotype characterized by increased ferritin contents and reduced transferrin receptor (CD71). Both ferritin and CD71 expression reflects the degree of dysplasia assessed by morphology and is closely related to the myelodysplastic phenotype in *in vitro* models.

Several data also suggest that immunophenotypic aberrancies reflect the disease severity and in particular the degree of marrow dysplasia. WHO classification emerged as important prognostic tool and interesting data have been reported on the ability of the WHO classification to guide clinical decision making regarding therapeutic choices. In this context, the possibility to recognize patients with unilineage versus multilineage dysplasia by FCM with higher sensitivity with respect to morphology might have important clinical implications. Moreover, variables related to sample quality appear to be less significant in immunophenotypic analysis than in morphological evaluation.

A standardized application of FCM in the diagnosis of MDS also requires a minimal variability in sample processing, antibody combinations and data acquisition. In fact, the inherent variability of instrument set-up can give significant differences in the data collected between and within institutions. Similarly, differences in reagents, especially the fluorochromes used, will result in inconsistent sensitivity and specificity of data (23,30). The European LeukemiaNET (ELN) working group for FCM in MDS started a consensus process on how to standardize sample collection/preparation and data acquisition (23). It is expected to significantly improve the diagnostic accuracy of FCM in MDS.

According to the available evidence and published diagnostic guidelines (7,13,28–30), in clinical practice immunophenotyping is strongly indicated in the screening evaluation of patients with peripheral blood cytopenia: in this clinical situations, it can provide a sensitive screen for the presence of hematologic malignancy and/or assist in demonstrating the absence of disease. In addition, when morphology and cytogenetics are indeterminate, an abnormal phenotype determined by FCM can help to establish a definitive diagnosis of MDS.

LITERATURE CITED

1. Cazzola M, Malcovati L. Myelodysplastic syndromes-coping with ineffective hematopoiesis. N Engl J Med 2005;352:536–538.
2. Della Porta MG, Malcovati L, Strupp C, Ambaglio I, Kuendgen A, Zipperer E, Travaglino E, Invernizzi R, Pascutto C, Lazzarino M, and others. Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica 2011;96:441–449.
3. Malcovati L, Della Porta MG, Cazzola M. Predicting survival and leukemic evolution in patients with myelodysplastic syndrome. Haematologica 2006;91:1588–1590.
4. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 2011;29:504–515.
5. Malcovati L, Della Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, Passamonti F, Arcaini L, Maffioli M, Bernasconi P, and others. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: A basis for clinical decision making. J Clin Oncol 2005;23:7594–7603.
6. Rigolin GM, Bigoni R, Milani R, Cavazzini F, Roberti MG, Bardi A, Agostini P, Della Porta M, Tieghi A, Piva N, and others. Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype. Leukemia 2001;15:1841–1847.
7. Valent P, Horny HP, Bennett JM, Fonatsch C, Germing U, Greenberg P, Haferlach T, Haase D, Kolb HJ, Krieger O, and others. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leuk Res 2007;31:727–736.
8. Pellagatti A, Cazzola M, Giagounidis AA, Malcovati L, Della Porta MG, Killick S, Campbell LJ, Wang L, Langford CF, Fidler C, and others. Gene expression profiles of CD34+ cells in myelodysplastic syndromes: Involvement of interferon-stimulated genes and correlation to FAB subtype and karyotype. Blood 2006;108:337–345.
9. Pellagatti A, Cazzola M, Giagounidis A, Perry J, Malcovati L, Della Porta MG, Jadersten M, Killick S, Verma A, Norbury CJ, and others. Deregulated gene expression pathways in myelodysplastic syndrome hematopoietic stem cells. Leukemia 2010;24:756–764.
10. Malcovati L, Della Porta MG, Pietra D, Boveri E, Pellagatti A, Galli A, Travaglino E, Brisci A, Rumi E, Passamonti F, and others. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Blood 2009;114:3538–3545.

11. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, Raza A, Root DE, Attar E, Ellis SR, and others. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008;451:335–339.
12. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–2302.
13. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, and others. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 2009;114:937–951.
14. Vardiman JW. Hematopathological concepts and controversies in the diagnosis and classification of myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program 2006:199–204.
15. Ramos F, Fernandez-Ferrero S, Suarez D, Barbon M, Rodriguez JA, Gil S, Megido M, Ciudad J, Lopez N, del Canizo C, and others. Myelodysplastic syndrome: A search for minimal diagnostic criteria. Leuk Res 1999;23:283–290.
16. Mufti GJ, Bennett JM, Goasguen J, Bain BJ, Baumann I, Brunning R, Cazzola M, Fenaux P, Germing U, Hellstrom-Lindberg E, and others. Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. Haematologica 2008;93: 1712–1717.
17. Della Porta MG, Malcovati L. Myelodysplastic syndromes with bone marrow fibrosis. Haematologica 2011;96:180–183.
18. Della Porta MG, Malcovati L, Boveri E, Travaglino E, Pietra D, Pascutto C, Passamonti F, Invernizzi R, Castello A, Magrini U, and others. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. J Clin Oncol 2009;27:754–762.
19. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt B, Bernasconi P, Knipp S, and others. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–3510.
20. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008;111:3941–3967.
21. Ogata K, Nakamura K, Yokose N, Tamura H, Tachibana M, Taniguchi O, Iwakiri R, Hayashi T, Sakamaki H, Murai Y, and others. Clinical significance of phenotypic features of blasts in patients with myelodysplastic syndrome. Blood 2002;100:3887–3896.
22. Ogata K. Diagnostic flow cytometry for low-grade myelodysplastic syndromes. Hematol Oncol 2008;26:193–198.
23. van de Loosdrecht AA, Alhan C, Bene MC, Della Porta MG, Drager AM, Feuillard J, Font P, Germing U, Haase D, Homburg CH, and others. Standardization of flow cytometry in myelodysplastic syndromes: Report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica 2009;94:1124–1134.
24. Stetler-Stevenson M, Arthur DC, Jabbour N, Xie XY, Molldrem J, Barrett AJ, Venzon D, Rick ME. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood 2001;98:979–987.
25. Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow: I. Normal erythroid development. Blood 1987;69:255–263.
26. Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, Leisenring WM, Deeg HJ. 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 2003;102:394–403.
27. van de Loosdrecht AA, Westers TM, Westra AH, Drager AM, van der Velden VH, Ossenkoppele GJ. Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. Blood 2008;111:1067–1077.
28. Davis BH, Holden JT, Bene MC, Borowitz MJ, Braylan RC, Cornfield D, Gorczyca W, Lee R, Maiese R, Orfao A, and others. 2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: Medical indications. Cytometry Part B Clin Cytom 2007;72B (Suppl 1):S5–S13.
29. Stetler-Stevenson M, Davis B, Wood B, Braylan R. 2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia. Cytometry Part B Clin Cytom 2007;72B (Suppl 1):S3.
30. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: Optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Cytometry Part B Clin Cytom 2007;72B(Suppl 1):S14–S22.
31. Satoh C, Dan K, Yamashita T, Jo R, Tamura H, Ogata K. Flow cytometric parameters with little interexaminer variability for diagnosing low-grade myelodysplastic syndromes. Leuk Res 2008;32:699–707.
32. Kern W, Haferlach C, Schnittger S, Haferlach T. Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: Correlation to cytomorphology, cytogenetics, and clinical data. Cancer 2010;116:4549–4563.
33. Matarraz S, Lopez A, Barrena S, Fernandez C, Jensen E, Flores J, Barcena P, Rasillo A, Sayagues JM, Sanchez ML, and others. The immunophenotype of different immature, myeloid and B-cell lineage-committed CD34+ hematopoietic cells allows discrimination between normal/reactive and myelodysplastic syndrome precursors. Leukemia 2008;22:1175–1183.
34. Stachurski D, Smith BR, Pozdnyakova O, Andersen M, Xiao Z, Raza A, Woda BA, Wang SA. Flow cytometric analysis of myelomonocytic cells by a pattern recognition approach is sensitive and specific in diagnosing myelodysplastic syndrome and related marrow diseases: Emphasis on a global evaluation and recognition of diagnostic pitfalls. Leuk Res 2008;32:215–224.
35. Davis BH, Foucar K, Szczarkowski W, Ball E, Witzig T, Foon KA, Wells D, Kotylo P, Johnson R, Hanson C, and others. U.S.-Canadian Consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: Medical indications. Cytometry 1997;30:249–263.
36. Elghetany MT. Surface marker abnormalities in myelodysplastic syndromes. Haematologica 1998;83:1104–1115.
37. Malcovati L, Della Porta MG, Lunghi M, Pascutto C, Vanelli L, Travaglino E, Maffioli M, Bernasconi P, Lazzarino M, Invernizzi R, and others. Flow cytometry evaluation of erythroid and myeloid dysplasia in patients with myelodysplastic syndrome. Leukemia 2005;19:776–783.
38. McClintock-Treep SA, Briggs RC, Shults KE, Flye-Blakemore LA, Mosse CA, Jagasia MH, Shinar AA, Dupont WD, Stelzer GT, Head DR. Quantitative assessment of myeloid nuclear differentiation antigen distinguishes myelodysplastic syndrome from normal bone marrow. Am J Clin Pathol 2011;135:380–385.
39. Vikentiou M, Psarra K, Kapsimali V, Liapis K, Michael M, Tsionos K, Lianidou E, Papasteriades C. Distinct neutrophil subpopulations phenotype by flow cytometry in myelodysplastic syndromes. Leuk Lymphoma 2009;50:401–409.
40. Truong F, Smith BR, Stachurski D, Cerny J, Medeiros LJ, Woda BA, Wang SA. The utility of flow cytometric immunophenotyping in cytopenic patients with a non-diagnostic bone marrow: A prospective study. Leuk Res 2009;33:1039–1046.
41. Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, Hildebrandt B, Giagounidis A, Aul C, Gattermann N, Haas R. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica 2006;91:1596–1604.
42. Jadersten M, Malcovati L, Dybedal I, Della Porta MG, Invernizzi R, Montgomery SM, Pascutto C, Porwit A, Cazzola M, Hellstrom-Lindberg E. Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. J Clin Oncol 2008;26:3607–3613.
43. List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, Powell B, Greenberg P, Thomas D, Stone R, and others. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 2006;355:1456–1465.
44. Alessandrino EP, Della Porta MG, Bacigalupo A, Van Lint MT, Falda M, Onida F, Bernardi M, Iori AP, Rambaldi A, Cerretti R, and others. WHO classification and WPSS predict posttransplantation outcome in patients with myelodysplastic syndrome: A study from the Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Blood 2008; 112:895–902.
45. Scott BL, Wells DA, Loken MR, Myerson D, Leisenring WM, Deeg HJ. Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome. Blood 2008;112:2681–2686.
46. Maynadie M, Picard F, Husson B, Chatelain B, Cornet Y, Le Roux G, Campos L, Dromelet A, Lepelley P, Jouault H, and others. Immunophenotypic clustering of myelodysplastic syndromes. Blood 2002;100:2349–2356.
47. Della Porta MG, Malcovati L, Invernizzi R, Travaglino E, Pascutto C, Maffioli M, Galli A, Boggi S, Pietra D, Vanelli L, and others. Flow cytometry evaluation of erythroid dysplasia in patients with myelodysplastic syndrome. Leukemia 2006;20:549–555.

48. Cazzola M, Della Porta MG, Malcovati L. Clinical relevance of anemia and transfusion iron overload in myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program 2008:166–175.
49. Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood 2002;99:3505–3516.
50. Cazzola M, Invernizzi R, Bergamaschi G, Levi S, Corsi B, Travaglino E, Rolandi V, Biasiotto G, Drysdale J, Arosio P. Mitochondrial ferritin expression in erythroid cells from patients with sideroblastic anemia. Blood 2003;101:1996–2000.
51. Levi S, Corsi B, Bosisio M, Invernizzi R, Volz A, Sanford D, Arosio P, Drysdale J. A human mitochondrial ferritin encoded by an intronless gene. J Biol Chem 2001;276:24437–24440.
52. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, and others. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–2088.
53. Ogata K, Satoh C, Hyodo H, Tamura H, Dan K, Yoshida Y. Association between phenotypic features of blasts and the blast percentage in bone marrow of patients with myelodysplastic syndromes. Leuk Res 2004;28:1171–1175.
54. Ogata K, Yoshida Y. Clinical implications of blast immunophenotypes in myelodysplastic syndromes. Leuk Lymphoma 2005;46:1269–1274.
55. Westers TM, Alhan C, Chamuleau ME, van der Vorst MJ, Eeltink C, Ossenkoppele GJ, van de Loosdrecht AA. Aberrant immunophenotype of blasts in myelodysplastic syndromes is a clinically relevant biomarker in predicting response to growth factor treatment. Blood 2010;115:1779–1784.
56. Ogata K, Kishikawa Y, Satoh C, Tamura H, Dan K, Hayashi A. Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes. Blood 2006;108:1037–1044.
57. Sternberg A, Killick S, Littlewood T, Hatton C, Peniket A, Seidl T, Soneji S, Leach J, Bowen D, Chapman C, and others. Evidence for reduced B-cell progenitors in early (low-risk) myelodysplastic syndrome. Blood 2005;106:2982–2991.
58. Goardon N, Nikolousis E, Sternberg A, Chu WK, Craddock C, Richardson P, Benson R, Drayson M, Standen G, Vyas P, and others. Reduced CD38 expression on CD34+ cells as a diagnostic test in myelodysplastic syndromes. Haematologica 2009;94:1160–1163.
59. Maftoun-Banankhah S, Maleki A, Karandikar NJ, Arbini AA, Fuda FS, Wang HY, Chen W. Multiparameter flow cytometric analysis reveals low percentage of bone marrow hematogones in myelodysplastic syndromes. Am J Clin Pathol 2008;129:300–308.
60. Ogata K, Della Porta MG, Malcovati L, Picone C, Yokose N, Matsuda A, Yamashita T, Tamura H, Tsukada J, Dan K. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: A prospective validation study. Haematologica 2009;94:1066–1074.
61. Harrington A, Olteanu H, Kroft S. The specificity of immunophenotypic alterations in blasts in nonacute myeloid disorders. Am J Clin Pathol 2010;134:749–761.