Immunophenotyping of Acute Leukemias and Myelodysplastic Syndromes

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Immunophenotyping of acute leukemias (AL) and myelodysplastic syndromes (MDS) was one of the first areas where monoclonal antibodies were applied (1–3). Initially, indirect immunofluorescence techniques evaluated by fluorescence microscopy were used (4); later immunocytochemistry methods on fixed cells were developed (5). During the last 15 years, multiparameter immunophenotypic approaches using direct immunofluorescence stainings analyzed by flow cytometry have become widely used and the preferred method for the immunophenotypic analysis of AL and MDS (6). The extended use of flow cytometry immunophenotyping and its involvement in routine diagnosis were facilitated by the unique characteristics of this technology that allows an objective analysis of high numbers of cells in a relatively short period of time—information which is simultaneously being recorded about two or more monoclonal antibody stainings for single cells (7). Further development of other alternative or complementary immunophenotypic approaches, such as those based on laser scanning cytometry, never reached the same rate of success (8).

Initially, the rationale for the clinical use of immunophenotypic techniques was based on the need for more objective criteria to support the morphological diagnosis and classification of AL and MDS. The underlying hypothesis was that neoplastic cells from patients with these hematological malignancies corresponded to the leukemic counterpart of normal hematopoietic cells usually committed into one, or less frequently more than one, cell lineages, blocked at a specific maturation stage (9). Thus, a detailed analysis of the phenotypic characteristics of these cells would provide useful information to classify them according to their lineage and maturation stage. Classification of AL and MDS according to both parameters had already proven to be clinically useful on morphological grounds (10,11).

Since then, immunophenotyping has provided information that contributed to the refinement of already existing morphological classifications of AL and the definition of new prognostic entities among these patients (12–14). More recently, it has also proven to be of great help for the screening of genetic abnormalities (14–22), the fol-

low-up of minimal residual disease (MRD) (23–25), monitoring of patient-specific therapies (26,27), and the study of MDS (28,29). These new applications of flow cytometry immunophenotyping mainly rely on the concept that even if neoplastic cells show a great similarity to normal hematopoietic precursors, they frequently display aberrant phenotypes that allow their specific identification and discrimination from normal cells, even when present at very low frequencies (23–25). To a large extent, such aberrant phenotypes would be a consequence of the genetic abnormalities accumulated by the neoplastic cell (14–22).

In this paper, we will briefly review the most outstanding contributions of flow cytometry immunophenotyping for the management of patients with AL and MDS and provide a perspective for future developments.

IMMUNOPHENOTYPING OF ACUTE LEUKEMIAS
Contribution of Immunophenotyping to the Diagnosis and Classification of Acute Lymphoblastic Leukemias

Acute lymphoblastic leukemias (ALL) were the first group of hematological malignancies in which immunophenotyping proved to be clinically useful. More than 20 years ago, ALL was already classified as B, T, or null ALL (non-B, non-T) depending on whether leukemic cells expressed surface immunoglobulins (sIg), formed rosettes with sheep erythrocytes, or lacked on both markers (30). Later on, the identification of the CD10 antigen, present in around two-thirds of all ALL patients, provided the basis for the more recent classifications through the definition of a new subgroup of patients that included most non-B, non-T cases (the common ALL phenotype) (31). The phenotypic immaturity of these morphologically-appearing lymphoid-lineage cells was supported on immunophenotypic grounds by their positivity for the terminal deoxynucleotidyl transferase enzyme (nTdt) (32). Thereafter,

the availability of an increasingly high number of mono-clonal antibody clones that detected antigens present in lymphoid cells and their precursors, together with the parallel development of the multiparameter capabilities of flow cytometry, contributed to definitively prove that most ALL cases showing either a common or a null phenotype derived from a B-cell precursor (33). In this regard, multidimensional analysis of the immunophenotypic profiles of normal bone marrow (BM) B-cell precursors was crucial. These studies provided a detailed definition of the exact sequence of expression of multiple antigens along the normal B-cell maturation pathways in the BM (34–38). Accordingly, at present it is well accepted that the first B-cell associated antigens to be expressed after commitment of an early CD34+ hematopoietic precursor into the B-lymphoid lineage are CD22, CD10, and CD19 (on the cell membrane), nTdt, and cytoplasmic CD79a (cCD79a) (35–38). Immediately after, the B-cell precursors sequentially start losing CD34 and nTdt, decrease CD10 expression, and display reactivity for CD20 (35–37). Later on, the B-cell precursors produce Ig μ heavy chains which accumulate in the cytoplasm until Ig light chains are produced (37,39). When this occurs, IgM molecules are expressed on the cell surface of a functionally immature B-lymphocyte (37,39). Based on the maturation sequence of the normal BM B-cells, precursor-B-ALL patients are currently classified into four major groups (40): BI or null ALL (CD19+, cCD79a+), BII or common ALL (CD10+), BIII or pre-B ALL (cIgμ+), and BIV or B ALL (sIg+).

Similar to precursor B ALL, T-ALL is currently divided into four groups (40): pro-T (or TI), pre-T (or TII), cortical or (TIII), and mature (or TIV) ALL. Pro-T ALL typically shows coexpression of two early T-cell markers -CD7 and cCD3− in the absence of other T-cell-associated antigens. In addition to CD7 and cCD3, Pre-T ALL cases express surface CD2, CD5, and/or CD8. As cortical thymocytes (41), leukemic cells from cortical T-ALL display reactivity for CD1a. The TIV/mature T-ALL phenotype (sCD3+, CD1a−, CD4+, or CD8+) is more often observed among patients presenting with T-lymphoblastic lymphomas than a pure T-ALL. In both TIII and TIV T-ALL, surface expression of CD3 may be associated with expression of TCR of either the TCRα/β or TCRγ/d type.

Despite the clear association initially reported between the phenotypes of leukemic and normal lymphoid precursors, further studies demonstrated that both groups of cells do not display identical and overlapping phenotypes (42). As an example, accumulating evidence supports the notion that during B-cell ontogeny, CD10 is expressed at a very early stage even prior to CD19 (36,38). In this case, BI or null ALL, which typically display a cCD79a+, CD19+, CD10− immature (CD34+, Ig−) phenotype (43,44), would not fit into the normal B-cell maturation scheme (36,38). Also, the absence of reactivity for CD10 would represent an aberrant phenotype. In fact, during the last decade it has been shown (23,24,42,45–47) that both precursor-B and T ALL display aberrant phenotypes in more than 95% of the cases. This allows for an unequiv-

ocal discrimination between normal and leukemic lymphoid precursors in the BM (45–47), peripheral blood (PB) (45), and other body fluids (23,48). The occurence of these aberrant phenotypes can only be explained because of the existence of underlying genetic abnormalities in leukemic blast cells. Accordingly, CD10− blast cells from pro-B ALL frequently are CD15+, 7.1+, and/or CD65+ (43,44), a phenotype which has been shown to be closely related to the presence of t(4;11) and other cytogenetic abnormalities involving chromosome 11q23 (43,44). This concept can also contribute to the understanding of the associations observed between a common-ALL phenotype and hyperdiploidy (49), t(9;22) (18,49), and t(12;21) (17,20), as well as the additional correlations reported in adult and childhood common-ALL between the latter two translocations and a CD34high, CD38dim (18), and a CD20−/partial+, CD9− /partial+, CD34−/+heterogeneous phenotype (17,20), respectively. Moreover, in CD34−, CD20+ pre-B ALL patients, t(1;19) is frequently present (21) and slg+ B-ALL with a bcl2− /dim phenotype commonly display t(8;14), t(2;8), or t(8;22) (16,22) (Table 1).

Altogether, these associations between the phenotype and the genotype of blast cells contribute to explain the prognostic impact and clinical relevance of the immunological classification of precursor-B-ALL (50). At the same time, they also contribute to understanding the apparently controversial associations initially reported in precursor-B ALL, between the expression of individual markers and the prognosis of the disease (e.g., the expression of both CD34 and myeloid-associated antigens has been associated with adverse prognostic features in adults whereas in childhood ALL CD10 and CD34 were considered as favorable prognostic features) and why they have lost their prognostic relevance once the genetic subgroups of precursor-B ALL are separately considered (reviewed in 14,50).

In contrast to what is described above for precursor-B ALL, no clear association between the immunological classification of T-ALL and specific T-cell genotypes or prognosis, have been clearly established in the past (16,50). Despite this, it should be noted that recent reports (51) suggest that with current treatment strategies, cortical T-ALL patients could have a better outcome, which is probably due to a higher susceptibility of leukemic cells from these patients to undergo apoptosis.

Contribution of Immunophenotyping to the Diagnosis and Classification of Acute Myeloblastic Leukemias (AML)

Immunophenotypic studies are apparently less useful in AML than in ALL; this probably has a multifactorial explanation related to the higher complexity of the former group of leukemias. First, the so-called myeloid cells include up to seven different lineages (neutrophilic, basophilic, eosinophilic, monocytic, mast cell, erythroid, and megakaryocytic) plus dendritic cells (52–56). Moreover, from the phenotypic point of view, leukemic cells from

Table 1
Immunophenotypic Patterns of Both AML and Precursor-B-ALL Patients Classified According to Recurrent Specific Cytogenetic Abnormalities

	AML				Precursor-B-ALL			
	t(8;21)[+]	t(15;17)[+]	Inv(16)[+]	11q23[+]	11q23[+]	t(9;22)[+a]	t(12;21)[+]	t(1;19)[+b]
MPO	+/++	+/++	++	+dim	-	-	-	-
CD13	+dim/+	+het	++/+++	-/+dim	-/+dim	+dim	+dim	-
CD33	+	++	++	+	NR	NR	NR	NR
CD34	p[+]	-/p[+]	p[+]	-/+	+	++	-/+het	-
CD117	p[+]	-/+	p[+]	-/+	-	-	-	-
CD65	+	+	+	-/+	+	-	-	-
HLADR	+	-/p[+]	+	+	+	+	+	+
CD15	+het	-/+dim	p[+]	+	+	-	-	-
CD14	-	-	p[+]	-/p[+]	-	-	-	-
CD4	-	-	p[+]	+dim	-	-	-	-
CD11b	+	-	p[+]	-/+	-	-	-	-
CD2	-	-/+dim	-/+	-/+	-	-/p[+]	-	-
CD19	+	-	-	-/+dim	+	+/++	+	+
CD56	+/++	-/+	-	+	-	-	-	-
7.1	-	-	-	p[+]/+	+	-	-	-
CD10	-	-	-	-	-	+	++	+
CD20	-	-	-	-	-/+dim	-/+	-/p[+]	+
CD38	NR	-/+het	+	+	+/++	+dim	+/++	+/++
CD45	+	+	+/++	+/++	+	+	-/+dim	+
cIgμ	-	-	-	-	-	-	-/p[+]	+

[a]Adult ALL.
[b]Associated with CD19 partially positive.
p[+], partially positive; +het, heterogeneously expressed; NR, not reported.

AML patients are significantly more heterogeneous both in phenotypic and cytogenetic grounds, the presence of two or more subpopulations of blast cells being found in most cases (57,58). Apart from this, information about the normal maturation pathways of different myeloid cell lineages, especially about those less represented in BM, is limited (29,56). Finally, there is no specific and universal single myeloid marker that would identify early commitment of hematopoietic precursors into any of the myeloid lineages (29,52–56).

CD117 together with CD13 and CD33, is considered the earliest antigen to be detected during differentiation of hematopoietic precursors into myeloid cells (29,52–56). However, when individually considered, none of these markers is specific to myeloid leukemic cells (46,47,59,60), and their combined expression is also found in the more immature, uncommitted CD34+ hematopoietic precursors (38,61). At present, cytoplasmic expression of myeloperoxidase (MPO), lisozyme, and tryptase (with the B12 clone) are considered as the most characteristic markers of myeloid cells (40,62,63). Despite this, the expression of these markers is typically restricted to a few myeloid lineages. Accordingly, in normal myeloid cells, reactivity to MPO and lisozyme is restricted to the granulomonocytic precursors while B12 (tryptase) appears to be highly characteristic of maturation into the mast cell and basophilic lineages (29). CD15 and CD14 are strongly expressed in mature neutrophils and monocytes, respectively (29,52,56). However, these two markers are coexpressed during maturation of myeloid cells into both cell lineages (29,55,56), which limits their utility in distinguishing between AML containing neutrophil-(M1, M2, and M3 FAB morphological subtypes) and monocytic-lineage (M5 FAB subtype) blast cells (64). Glycophorin A is a highly specific erythroid marker (29,56,65); however, it is only expressed at relatively late stages of maturation of erythroid cells (29,65), which limits its utility in AML. In contrast, CD36 is expressed early during erythroid maturation, but it is not specific to erythroid cells, since it is also positive in precursor cells of the monocytic, dendritic, and megakaryocytic lineages (29). Regarding the magakaryocytic lineage, CD61, CD41, and CD42 (which recognize gpIIIa, IIb/IIIa, and IX/Ib, respectively) are considered as excellent markers for the detection of megakaryocytic leukemias (AML M7 FAB subtype) (12,66).

Altogether, these results indicate that the utility of individual markers in identifying commitment of leukemic cells into the different myeloid lineages is limited. In fact, it is generally accepted that positivity for two or more myeloid-associated antigens is necessary for the diagnosis of AML (14,40) and that the utility of immunophenotyping for further classification of AML is almost restricted to the identification of megakaryocytic leukemias, poorly differentiated AML, the microgranular variant of acute promyelocytic leukemia (APL) (14,40), and a rare subtype of dendritic cell neoplasias that is characterized by coexpression of CD123high, HLADRhigh, CD4+, CD56+, and 7.1+ in the absence of other lineage-specific markers (cMPO-, cCD3-, cCD79a-) (67,68). In other subtypes of AML, it is frequently claimed that immunophenotyping just stands for confirmation of morphological, cytochem-

ical, and genetic diagnoses (69). In line with this and in contrast to what was described above for ALL, there is still no accepted immunological classification for AML (70). In summary, these results point out the relatively limited utility of individual markers in AML as well as the need for more powerful multiparameter immunophenotypic analytical approaches as also discussed below for MDS.

In line with what has been described for ALL, most AML patients (>75%) also display aberrant phenotypes (23,71–76). These aberrant phenotypes are highly suggestive of the presence of underlying specific genetic abnormalities. Accordingly, leukemic cells from APL patients frequently show an immunophenotype similar to that of normal promyelocytes (CD34−/+heterogeneous, CD117−/+dim, HLADR−, CD13+/++, CD11b−) (29). In contrast to normal promyelocytes, however, these leukemic cells display abnormally low expression of CD15 (CD15-/dim versus CD15high) (Fig. 1, Table 1), a phenotype that is characteristically associated with the presence of t(15;17) (15). Other associations between immunophenotype and genotype in AML are less clearly defined (Table 1) and include CD56 expression in the context of either an immature monocytic (CD13+, CD33+, CD117+, CD64+, HLADR+) or a granulomonocytic (CD34+, CD15+, HLADR+) aberrant (CD19+) phenotype and 11q23 abnormalities (16,77,78) or t(8;21) (16,79–81), respectively. FLT3 internal tandem duplications have also been more recently associated with relatively mature (CD34−, CD117−) monocytic (CD36+, CD11b+) immunophenotypic features or APL (82).

Although it has been suggested that some individual antigens such as CD9, CD11b, CD14, and CD34 could be associated with an adverse prognosis in AML, their independent prognostic value could not be definitively confirmed (reviewed in 14).

Biphenotypic Acute Leukemias

For more than one decade, immunophenotyping of AL has pointed out the existence of a small proportion of cases (<5%) that show simultaneous coexpression of immunophenotypic characteristics highly specific of two different lineages: myeloid and lymphoid (e.g., MPO+/CD13+ and cCD3+/CD7+) (40,83). Such coexpression may occur in a single cell population (biphenotypic leukemias) or in two separate groups of blast cells (bilineal leukemias) in the same individual. Biphenotypic and bilineal acute leukemias should be specifically identified as different from both ALL with expression of myeloid associated markers and AML showing reactivity for lymphoid-related antigens. These latter cases may represent more than 20% of all AL (40,46,47,59). In addition, they should also be separately considered from ALL patients with precursor-B/T phenotypes and from AML cases in which blast cells display phenotypic features characteristic of more than one cell lineage.

Despite the fact that the most recent classification of AL proposed by the WHO (83) includes biphenotypic AL as a new entity, the information currently available about their
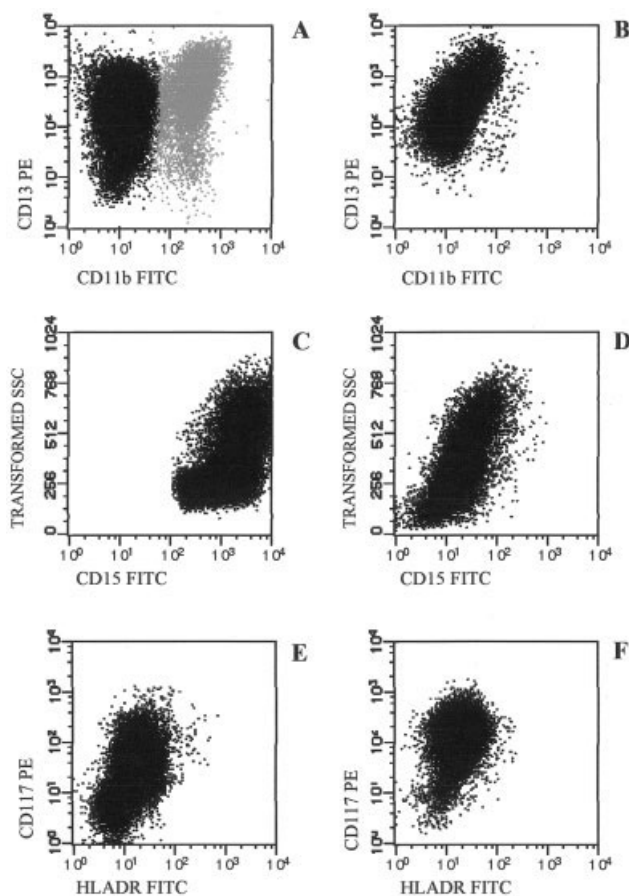

Fig. 1. Immunophenotypic characteristics of normal/reactive promyelocytes (**A, C, E**) as compared to leukemic promyelocytes from a patient with a t(15;17)+ acute promyelocytic leukemia (**B, D,** and **F**). The black dots correspond to normal and leukemic promyelocytes and the gray events to other CD45dim/SSChigh-gated bone marrow neutrophil-lineage cells. Please note that distinct expression of CD15 is observed in normal and leukemic promyelocytes, both being HLADR-negative.

clinical behavior and the most appropriate treatment strategies for their management is still limited and poorly documented (84).

DETECTION OF MINIMAL RESIDUAL DISEASE AND MONITORING OF THERAPY IN ACUTE LEUKEMIAS

In the last decade, the investigation of the presence of residual leukemic cells after treatment, using immunophenotypic approaches, has proved to be feasible and moved from the research laboratories into clinical diagnosis. For that purpose, it is required that leukemic cells display aberrant phenotypes, since with a few exceptions, the detection of tumor specific antigens cannot be applied routinely (23,24). Aberrant phenotypes are present in most ALL (>95%) (23,24,45–47,85) and AML cases (>75%) (23,24,71–76). They are typically defined by: 1) cross-lineage antigen expression (e.g., expression of CD5 in AML or CD33 in ALL); 2) asynchronous antigen expres-

sion (e.g., coexpression of CD34 and CD3 or CD34 and CD11b); and 3) ectopic phenotypes (e.g., TdT+ and/or CD34+ cells found in spinal fluid or Tdt+/cCD3+/CD34+ T-cell precursors in the BM) (23,24). MRD studies have contributed to the establishment of new concepts in onco-hematology such as that of immunological remission (23,24). At the same time, these studies allow a better prognostic stratification of AL at an early stage after initiation of therapy, and they permit a closer follow-up of treatment efficacy in individual patients (23,24).

In parallel, the availability of new treatment strategies, based on the use of monoclonal antibodies specific for proteins expressed by leukemic cells (e.g., anti-CD33) (27), has provided a stimuli for the use of immunophenotyping in the evaluation of the number of molecules expressed by the antibody-targeted cells as a highly valuable tool for predicting response to therapy (26).

IMMUNOPHENOTYPING OF MYELODYSPLASTIC SYNDROMES
Immunophenotypic Characteristics of MDS

It has been known for many years that MDS patients display BM changes that are morphologically recognizable. The identification, classification, and quantification of these alterations, especially those involving erythroid, megakaryocytic, neutrophil, and monocytic cells, together with the enumeration of ringed sideroblasts and blast cells, are of great utility in the diagnosis and classification of the disease (11,83,86).

As mentioned above, the availability of an increasingly high number of monoclonal antibody clones and the success of their application in the characterization of hematopoietic cells have suggested that these morphological abnormalities could also be studied by immunophenotypic approaches. For many years, the use of single stainings analyzed by fluorescence microscopy or flow cytometry have restricted the routine applications of immunophenotyping in MDS to the characterization of blast cell populations after transformation into AL (24,25). These studies confirmed that almost every AL following an MDS corresponded to an AML, B- and T-lymphoid blast crisis being either rare or exceptional, respectively (87). During this period, attempts to phenotypically characterize MDS at diagnosis were limited in number and their results were discouraging (28,29). This was probably a consequence of the great heterogeneity of the pathological cells present in the BM of MDS and the highly variable numbers and phenotypes of the cell subpopulations detected in different patients, which can not be properly identified with single or even double stainings (29,88). However, these studies clearly demonstrated the occurence of changes in the expression of individual antigens both in PB and BM of MDS patients (reviewed in 28). Accordingly, PB neutrophils from a variable proportion of all MDS patients (26–80%) display decreased expression of CD35, CD11b, CD15, CD16, CD11a, CD54, and CD116. In addition, the existence of abnormally high numbers of

CD33+, CD87+, CD14+, CD44+, and CD64+ cells is also frequently (18–54%) observed in the PB of these individuals. The latter three antigens being preferentially increased in high-risk MDS. Although phenotypic abnormalities of PB monocytes have been reported in the literature less frequently, the existence of decreased reactivity for CD54 and CD116, together with increased expression of CD15, CD64, CD87, and CD35, have been found to recur in these cells. In a similar way, a variable increase in the reactivity for antigens expressed on normal myeloid precursors (e.g., CD34, CD117, HLADR) and immature neutrophil-lineage cells (e.g., CD33, CD13, and CD66a), together with decreased expression of markers that are characteristic of the last stages of the neutrophil maturation (e.g., CD11b, CD16, CD11c, and NAT-9), have also been reported in the BM of MDS patients. Later studies have confirmed that these changes in the expression of individual antigens frequently reflect the existence of underlying abnormalities in the distribution of different BM cell compartments. In line with this, it has been shown that changes in the frequency of CD34+ cells are directly related to the proportion of blast cells by morphology (89). Accordingly, the number of CD34+ cells progressively increases from refractory anemia (RA) and RA with sideroblasts (RAS) to RA with excess of blasts (RAEB) and RAEB in transformation (RAEB-t) (89–91). In a similar way, decreased expression of neutrophil-associated markers is frequently found in cases showing decreased numbers of mature neutrophils; these abnormalities also translate in a progressive decrease in the BM neutrophil/monocyte ratio from RA and RAS to RAEB and RAEB-t (92).

Other immunophenotypic abnormalities reported in a significant proportion of all MDS patients refer to the expression of aberrant phenotypes. These include: 1) asynchronous antigen expression in the neutrophil (e.g., CD14+/CD66a− or CD11b+/HLADR+) and monocytic cell lineages (e.g., CD14+/CD54−, CD45dim/CD14+), as well as in the blast cell compartment (e.g., CD34+/CD117−, CD34+/CD56+, and CD34+/CD15+/HLADR−); 2) innapropriate expression of lymphoid associated antigens (including CD56) on myeloid cells, and 3) overexpression of individual antigens such as CD95 in erythroid cells, CD95L in CD34− cells, and P-gp in CD34+ blast/precursor BM cells (28,91,92,93).

Altogether, these results indicate that the phenotypic alterations present in MDS are highly complex and that they include abnormalities in the relative distribution between cells from different lineages and between different maturational compartments within a lineage, together with the expression of aberrant phenotypes (90–93). Because of this, immunophenotypic analysis of MDS at diagnosis requires more sophisticated multiparameter analytical approaches. In line with this, the most recent studies devoted to the immunophenotypic characterization of MDS (90–93) have utilized new analytical strategies. First, they focus on the identification of specific cell populations defined by light scatter and CD45 expression; second, they search for the potential presence of phenotypic
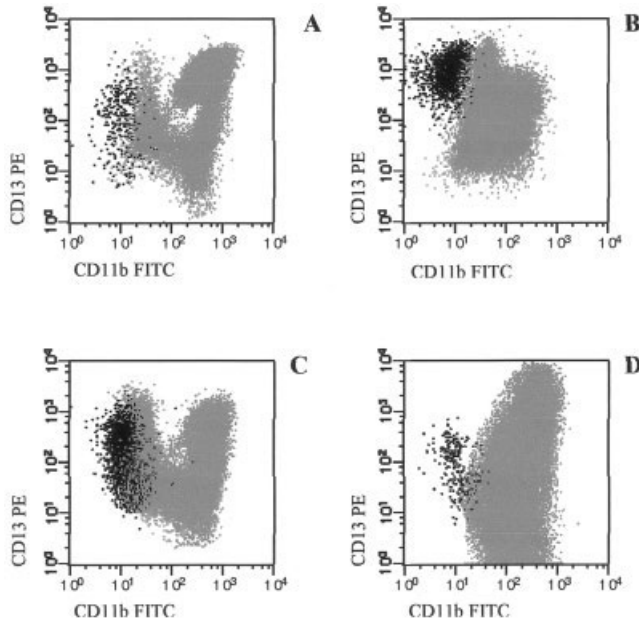
FIG. 2. Representative bivariate dot plots of the neutrophil maturation in a normal bone marrow (**A**) as compared to three different MDS patients with disgranulopoiesis (**B, C,, D**). In all dot plots, gated neutrophil-lineage cells are displayed. Black dots correspond to CD34+ cells.

abnormalities inside the regions/cell populations initially identified, through the use of different objective and/or subjective criteria (90–95) as exemplified in Figure 2 for the neutrophil compartment. To summarize, in these latter publications, it is suggested that in the future, immunophenotypical analysis of MDS will require multiple stainings for four or more antigens. In addition, the analysis of these stainings needs to be based on sequential steps aimed at: 1) the specific identification of the different cell compartments present in the sample, 2) the analysis within each cell compartment of the maturational distribution of the cells, 3) the objective characterization of the phenotypic patterns of each of the maturation stages identified, and 4) the enumeration of the abnormalities observed. Table 2 lists the immunophenotypic abnormalities found to be clinically useful in some of these analyses (91,93). As a consequence of the potential utility of these latter strategies, in the last few years there has been an increasingly high interest on the search for new phenotypic parameters that could be of clinical relevance in MDS (90–93).

Clinical Utility of Immunophenotyping in MDS

Despite the fact that a high number of antigens have been studied and many phenotypic abnormalities detected, the clinical utility of immunophenotyping of MDS remains marginal and it has still not become routine (28,29). This is probably the result of multiple circumstances. Several studies have reported the existence of characteristic immunophenotypic abnormalities in MDS,

but few have analyzed its real diagnostic utility. In addition, many of the reported abnormalities rely on an altered expression of individual antigens that are not constantly present in MDS at the same time they are also found in other conditions (28,29). On the other hand, abnormalities of the white cell precursors are more easily recognized on immunophenotypic grounds than those of the erythroid and megakaryocytic lineages (91).

From the prognostic point of view, the abnormal expression of several individual antigens has been associated with the clinical behavior of MDS (reviewed in 28). Accordingly, decreased reactivity for CD11b and increased expression of CD34, HLADR, CD13, and CD33 in the BM have been associated with both a higher risk of transformation into AL and a shorter survival. In addition, adverse cytogenetic features are also more frequently found among cases displaying an increased reactivity for CD33 on the BM neutrophil lineage cells, a greater expression of Pgp on blasts, and a higher number of phosphatidyl serine residues on the surface of CD34+ precursors (28). However, few of these individual markers retain an independent prognostic value.

Despite these results, recent studies in which the expression of several antigens is simultaneously evaluated in different BM cell lineages and their maturational compartments, according to updated immunophenotypic analytical criteria, show that immunophenotyping is of great utility for the diagnosis of MDS patients in whom inconclusive morphological and cytogenetic features are found (91). At the same time, it shows independent value from the IPSS (International Prognostic Scoring System) for predicting patients' outcome (93). Moreover, it is suggested that this new methodology together with the use of new scoring classifications as well as new patient clustering systems based on phenotypic information will contribute to improve the diagnosis, classification, and prognostic stratification of the disease (90,92).

FUTURE PERSPECTIVES

Despite recent advances, there is still plenty of room for immunophenotypic studies of both AL and MDS patients. In the future, these studies should address questions that remain either unexplored or unanswered using new tools. Apart from testing new markers and combinations of markers, these future studies should take advantage of recent technological developments in multicolor stainings and multiparameter analyses. In addition, more global approaches aimed at the analysis of all cell populations present in a patient sample, including mature nucleated cells and even the platelets, will be welcome since they will probably contribute to improve the differential diagnosis between de novo and secondary AML and the identification of dysthrombopoiesis, respectively. Also, a more detailed analysis of the phenotypic heterogeneity of the neoplastic cells is required for a sensitive identification and characterization of leukemic progenitors and stem cells. In parallel with this, more detailed studies of normal myeloid differentiation are also necessary, especially in

Table 2
Incidence and Type of Immunophenotypic Abnormalities Detected by Multiparameter Flow Cytometry in MDS Patients: Summary of the Results From Two Recent Studies*

Immunophenotypic abnormalities	Stetler-Stevenson et al. (91)[a]	Wells et al. (93)[b]
Myeloblast		
Abnormal number of myeloblasts	24/42 (53%)	72/115 (62%)
$CD2^{++}$ myeloblasts	12/45 (27%)	NR
Maturing neutrophil cells		
Abnormal SSC	35/45 (84%)	9/115 (8%)
Abnormally low CD45	NR	4/115 (3%)
Abnormal CD13/CD16 pattern	21/27 (78%)	27/115 (23%)
Abnormal HLADR/CD11b ratio	NR	6/115 (5%)
Abnormal CD11b/CD16 pattern	19/27 (70%)	NR
Asynchronous "left" shift	NR	26/115 (23%)
$CD56^{+}$	7/33 (21%)	18/115 (16%)
$CD33^{-}$	NR	6/115 (5%)
$CD34^{+}$	NR	7/115 (6%)
Presence of lymphoid antigens	17/45 (38%)	4/115 (3%)
Monocytes		
Abnormal SSC	NR	1/115 (1%)
Lack of CD13 and/or CD16	NR	1/115 (1%)
Abnormal HLADR/CD11b ratio	NR	5/115 (4%)
$CD56^{+}$	11/33 (33%)	19/115 (17%)
$CD33^{-}$ or $CD14^{-}$	NR	3/115 (3%)
$CD34^{+}$	NR	14/115 (12%)
Presence of lymphoid antigens	NR[c]	4/115 (3%)
Altered lymphoid/myeloid ratio	NR	38/115 (33%)
Erythroid cells		
Altered CD71, Glycophorin A and/or CD45 expression	34/44 (77%)	NR
Megakaryocytic cells (MKC)		
Increased number of MKC	26/44 (59%)	NR

*Results expressed as number of cases/total cases and percentage in brackets.
[a]This study includes 65 MDS patients studied at diagnosis.
[b]This study includes 115 MDS patients referred for consideration of a transplant.
[c]Not specifically reported.
NR, not reported.

the case of those cell lineages less represented in BM, to better understand the impact of specific genetic abnormalities in the altered patterns of protein expression and cell functionality. Finally, clinical studies in which the value of immunophenotypic parameters is prospectively analyzed in large series of patients should be performed. These studies must take advantage of new statistical approaches for multiparameter clustering of patients.

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