Using 4-Color Flow Cytometry to Identify Abnormal Myeloid Populations

Steven J. Kussick, MD, PhD; Brent L. Wood, MD, PhD

• ***Context.*—The diagnosis of myeloproliferative disorders (MPDs) and myelodysplastic syndromes (MDSs) has historically relied on combining clinical information with the morphologic features of the peripheral blood and bone marrow to reach a final diagnosis. Objective evidence of a myeloid stem cell neoplasm in the form of a clonal cytogenetic abnormality is provided in only 30% to 40% of the non-chronic myeloid leukemia (CML) chronic MPDs (non-CML MPDs) and in a similar percentage of the MDSs.**

***Objective.*—To identify normal patterns of antigen expression during myeloid maturation and to determine whether flow cytometric evaluation of myeloid maturation represents an additional objective way to assess the likelihood of a stem cell neoplasm.**

***Design.*—We retrospectively evaluated 4-color flow cytometry data from more than 400 bone marrow aspirates obtained since 1998 from patients suspected of having a non-CML MPD or an MDS.**

***Results.*—Reproducible patterns of antigen expression were seen in normal myeloid maturation as well as in benign reactive settings such as marrow regeneration. In addition, we summarize data, presented in detail elsewhere, from a retrospective comparison of the sensitivity of flow cytometry with conventional cytogenetics for a large number of bone marrow aspirates on which both types of studies were performed. These data indicate that more than 90% of non-CML MPD and MDS cases with a clonal cytogenetic abnormality will be identified as abnormal by 4-color flow cytometry, and they therefore validate the use of flow cytometry in the diagnosis of these disorders.**

***Conclusions.*—In experienced laboratories, 4-color flow cytometry represents a valuable addition to the workup of non-CML MPDs and MDSs.**

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The diagnosis of chronic myeloproliferative disorders (MPDs) and myelodysplastic syndromes (MPSs) has historically relied on combining the clinical history, the morphologic features of the peripheral blood and/or bone marrow, and the cytogenetic information. Whereas the t(9;22) translocation defines all cases of chronic myeloid leukemia (CML) under the new World Health Organization classification system,[1] clonal cytogenetic abnormalities are reportedly identified in only 30% to 40% of non-CML MPDs and in a similar percentage of MDSs.[2] Other techniques that can aid in the diagnosis of non-CML MPDs, such as the endogenous erythroid colony in the workup of polycythemia vera,[3] are highly specialized assays that cannot be performed by most hematopathology laboratories. Therefore, although the diagnosis of CML is straightforward by existing techniques, there is a pressing need for new sensitive and objective assays to aid in the diagnosis of both non-CML MPDs and MDSs.

Multiparametric flow cytometry represents a highly reproducible and objective way of assessing the expression of multiple antigens on a single cell. By comparing the patterns of antigen expression on a given cell population with the patterns identified on normal cells of that type, abnormalities can potentially be identified that, if sufficiently great, may substitute for clonality studies in identifying malignancy. Patterns of expression of a number of antigens during normal myelopoiesis have been described in relatively great detail.[4–6] However, only a relatively small number of studies over the past decade have applied flow cytometry to MPDs and MDSs, and most of these studies have investigated a relatively small number of antigens. Reported immunophenotypic abnormalities in CML have included decreased CD16,[7–9] decreased CD32,[8] decreased L-selectin expression on the $CD34^+$ cells,[10] aberrant expression of CD56 on the blasts and myeloid cells,[11,12] and aberrant expression of lymphoid antigens such as CD2, CD5, and CD7 on the blasts in a CML blast crisis.[13] Reported flow cytometric abnormalities among the non-CML MPDs have included the following: (1) increased expression of Bcl-X_L in polycythemia vera[14]; (2) decreased platelet GPIa/IIa,[15] decreased platelet GP1b and GPIIb/IIIa,[16] and elevated platelet P-selectin, thrombospondin, GPIV, and c-Mpl,[17,18] in essential thrombocytosis; and (3) aberrant coexpression of CD14 and CD66 on the myeloid cells in a subset of MPDs.[19]

Antigenic abnormalities have also been identified by flow cytometry in MDS patients, compared to normal controls. These abnormalities have included (1) loss of erythrocyte A, B, and H antigens in MDS[20]; (2) decreased ex-

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From the University of Washington Department of Laboratory Medicine, Seattle, Wash.

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Reprints: Steven J. Kussick, MD, PhD, Box 357110, University of Washington Medical Center, 1959 NE Pacific St, Seattle, WA 98195 (e-mail: skussick@u.washington.edu).

pression of c-Mpl, GPIIb/IIIa, and GPIb on platelets from patients with refractory anemia[21]; (3) dyssynchronous expression of CD11b and CD16 in the developing neutrophils of patients with MDS[22]; (4) decreased CD10 on neutrophils in MDS[23]; (5) changes in a variety of leukocyte activation antigens, including FcRI, FcRII, and FcRIII, in MDS[24]; (6) greater variability in the expression of CD38, CD71, CD13, and CD33 in refractory anemia versus either normal marrow or marrows involved by aplastic anemia[25]; and (7) aberrant coexpression of CD56 on myeloid blasts in MDS.[12,26] In addition, a variety of studies have used flow cytometry to document abnormalities in apoptosis and/or proliferation in MDS.[27–33] The latter studies have generally found abnormally increased apoptosis in refractory anemia/refractory anemia with ringed sideroblasts, with associated increases in caspase 3 activation and expression of pro-apoptotic Bcl-2 family members. In contrast, in refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia, there tends to be decreased apoptosis and increased proliferation.

Except for the study by Stetler-Stevenson and colleagues,[34] these studies have been limited in that they generally investigated small numbers of cases and relatively few antigens; even the study by Stetler-Stevenson and colleagues described a highly selected series of cases in which the diagnosis of MDS was equivocal on the basis of the morphologic and clinical features alone. Therefore, a thorough immunophenotypic evaluation of the blasts and maturing granulocytes and monocytes in unselected cases of possible MDS, as well as in cases of possible non-CML MPD, has not been described.

In the University of Washington Hematopathology Laboratory (Seattle, Wash), we have hypothesized that flow cytometric evaluation of myeloid and monocytic maturation is likely to represent an additional objective way to assess the likelihood of an underlying stem cell neoplasm, particularly when the blast percentage is low. Therefore, we have been using 4-color flow cytometry since 1998 to evaluate bone marrow aspirates from patients suspected of having a myeloid stem cell neoplasm, and we have accumulated a large data set of more than 400 cases. This data set has allowed us to identify reproducible patterns of antigen expression in normal myeloid and monocytic maturation, including changes seen in benign reactive settings such as marrow regeneration.

To determine the range of immunophenotypic alterations present in the non-CML MPDs and MDSs and to evaluate the utility of flow cytometry vis-à-vis conventional cytogenetics in the diagnosis of such cases, we recently completed a retrospective evaluation of all non-CML bone marrow and/or peripheral blood specimens evaluated by flow cytometry in our laboratory to rule out an MPD or MDS, on which the concurrent cytogenetic evaluation was also performed. The data, which will be presented in greater detail in manuscripts being prepared by the authors, demonstrate that significant flow cytometric abnormalities are identified in essentially all cases of cytogenetically abnormal non-CML MPDs and in nearly all cases of cytogenetically abnormal MDS. In addition, flow cytometric abnormalities are identified in a significant percentage of non-CML MPDs and MDSs with normal cytogenetics. Therefore, flow cytometry represents a useful addition to the workup of these cases.

MATERIALS AND METHODS

To refine our understanding of myeloid antigen expression in neoplastic and nonneoplastic myelopoiesis, 76 specimens evaluated in our laboratory between June 1998 and December 2001 to rule out non-CML MPD, along with 333 cases evaluated to rule out MDS, were identified retrospectively. Almost all of the specimens were bone marrow aspirates rather than peripheral blood, which is preferable for this type of analysis since the full range of myeloid maturation can only be evaluated in marrow. In no case was there cytogenetic or reverse transcriptase–polymerase chain reaction evidence of the t(9;22) translocation. In all cases, 4-color flow cytometry had been performed in our laboratory on Coulter XL instruments (Beckman-Coulter [BC], Hialeah, Fla) using commercially available reagents. Ammonium chloride lysis was used to remove the vast majority of the erythroid cells from the specimens prior to the addition of antibodies. In most cases, 100 000 to 150 000 viable cells were analyzed from each tube of cells and antibodies; however, as many as 400 000 cells were analyzed per tube in a minority of cases. One of the following 2 panels of antibodies was used in virtually all of the cases:

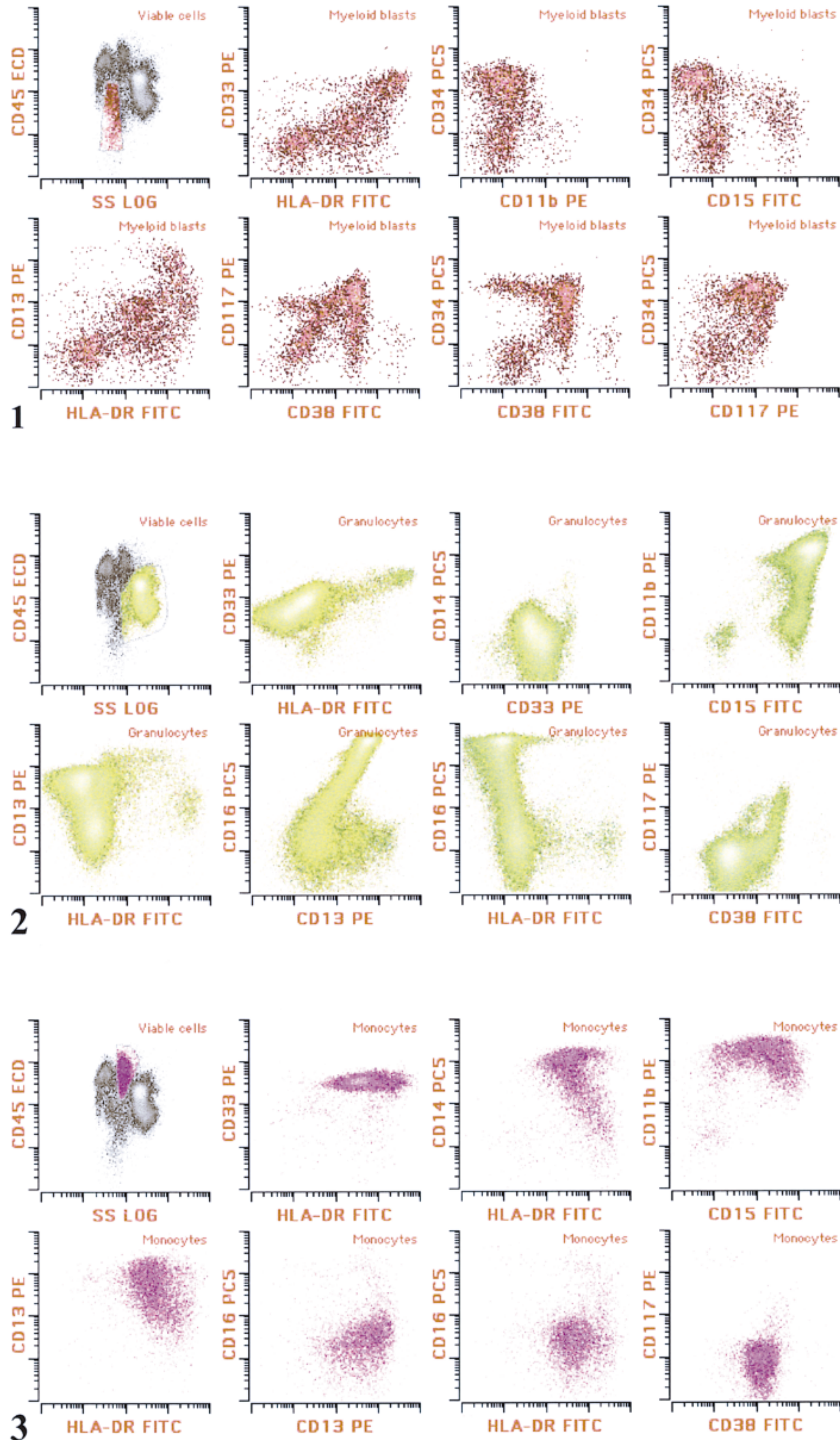
IgG2a-FITC/IgG1-PE/CD45-ECD/IgG2a-PE-Cy5
HLA-DR-FITC/CD33-PE/CD45-ECD/CD15-PE-Cy5
CD2-FITC/CD56-PE/CD45-ECD/CD14-PE-Cy5
CD10-FITC/CD19-PE/CD45-ECD/CD5-PE-Cy5
CD34-FITC/CD11b-PE/CD45-ECD/CD16-PE-Cy5
CD7-FITC/CD13-PE/CD45-ECD/CD117-PE-Cy5
CD2-FITC/CD7-PE/CD45-ECD/CD34-PE-Cy5

OR

IgG2a-FITC/IgG1-PE/CD45-ECD/IgG2a-PE-Cy5
HLA-DR-FITC/CD33-PE/CD45-ECD/CD14-PE-Cy5
CD15-FITC/CD11b-PE/CD45-ECD/CD34-PE-Cy5
HLA-DR-FITC/CD13-PE/CD45-ECD/CD16-PE-Cy5
CD38-FITC/CD117-PE/CD45-ECD/CD34-PE-Cy5
CD5-FITC/CD56-PE/CD19-ECD/CD45-PE-Cy5

The top panel shown above was used from approximately June 1998 through October 2000, while the bottom panel was used from November 2000 through the end of the study period in December 2001. An anti-CD45 antibody was included in each tube for gating purposes,[35] while the combinations of the other 3 antibodies were designed empirically to maximize our ability to identify antigenic abnormalities within the myeloid blasts, maturing granulocytes, and maturing monocytes. The specific clones used were obtained from BC, Becton Dickinson (BD, San Jose, Calif), or Dako Corporation (Carpinteria, Calif) as follows: CD45-ECD (BC, J.33), IgG2a-FITC isotype control (BD), IgG1-PE/isotype control (BD), IgG2a-PE-Cy5 isotype control (BC), HLA-DR-FITC (BD, L243), CD33-PE (BD, P67.6), CD14-PE-Cy5 (BC, RM052), CD15-FITC (BD, Leu-M1), CD15-PE-Cy5 (BC, 80H5), CD11b-PE (BD, Leu-15), CD34-FITC (BD, HPCA2), CD34-PE-Cy5 (BC, 581), CD13-PE (BD, Leu-M7), CD16-PE-Cy5 (BC, 3G8), CD38-FITC (BC, T16), CD117-PE (Dako, 104D2), CD117-PE-Cy5 (BC, 95C3), CD5-FITC (BD, Leu-1), CD5-PE-Cy5 (BC, BL1a), CD56-PE (BD, NCAM16.2), CD19-PE (BD, SJ25C1), CD19-ECD (BC, HD237), CD2-FITC (BC, T11), CD7-FITC (BD, Leu-9), CD7-PE (BC, 3A1), and CD10-FITC (Dako, SS2/36). In each case, the amount of antibody used was based on either the manufacturer's suggestion or titration experiments to optimize the signal-noise ratio.

Once the cases were identified, archival 4-color flow cytometry data were compensated and reanalyzed in a standardized fashion on MacIntosh G3 or G4 computers using software developed in our laboratory by one of us (B.L.W.). The individual bone marrow cell populations—myeloid blasts, maturing granulocytes, maturing monocytes, B lymphoblasts, and mature lymphocytes—were identified by CD45/sidescatter gating. The myeloid blast gate



Figures 1 through 3. *Four-color flow cytometric analysis of normal antigen expression among the myeloid blasts (Figure 1, in red), maturing granulocytes (Figure 2, in green), and maturing monocytes (Figure 3, in lavender). In each figure, CD45/sidescatter gating demonstrated in the upper left dot plot identifies the population of interest, and the other 7 dot plots show patterns of antigen expression in that population. Each tube evaluated contains anti-CD45 antibodies plus antibodies to 3 additional antigens. See ''Materials and Methods'' for the antibody combinations used. FITC indicates fluorescein isothiocyanate; PE, phycoerythrin.*

was frequently refined by largely limiting this gate to the $CD34^+$ cells, except in cases in which a $CD34^-$ (abnormal) blast population was identified.

The patterns of antigen expression on the myeloid blasts, maturing granulocytes, and maturing monocytes in the study cases were compared with the patterns typically seen in normal myeloid populations. A negative bone marrow from a patient with iron deficiency anemia was chosen as the ''gold standard'' for comparison with the case specimens (see Figures 1 through 4 for normal patterns), although the patterns of antigen expression in this control marrow were very similar to those we have observed in normal marrows in the 4 years we have been doing this assay.

Concurrent conventional cytogenetic results were available from local laboratories, particularly the University of Washington Cytogenetics Laboratory, on 67 of the 76 marrows evaluated for MPD (88%) and on 263 of the 333 marrows evaluated for MDS

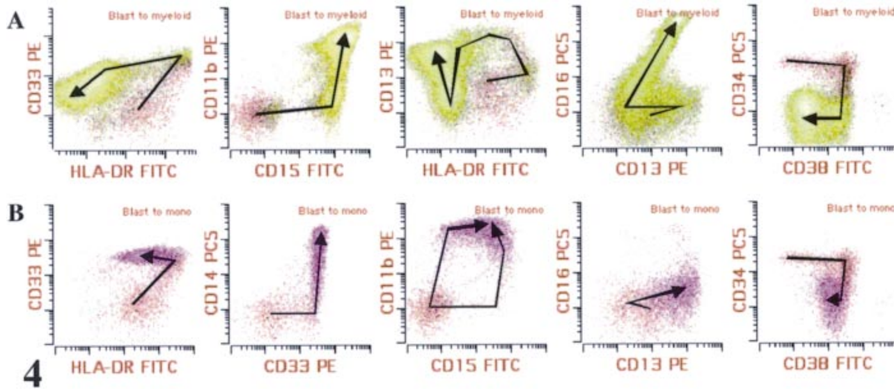


Figure 4. *Four-color flow cytometric analysis of the progression from blasts to mature neutrophils (part A, upper row of dot plots) and monocytes (part B, lower row of dot plots) during normal maturation in the bone marrow. Arrows denote the changes in antigen expression as maturation progresses from the early myeloid blasts (colored red) at the base of each arrow to the mature neutrophils (green) or monocytes (lavender) at the arrowheads. FITC indicates fluorescein isothiocyanate; PE, phycoerythrin.*

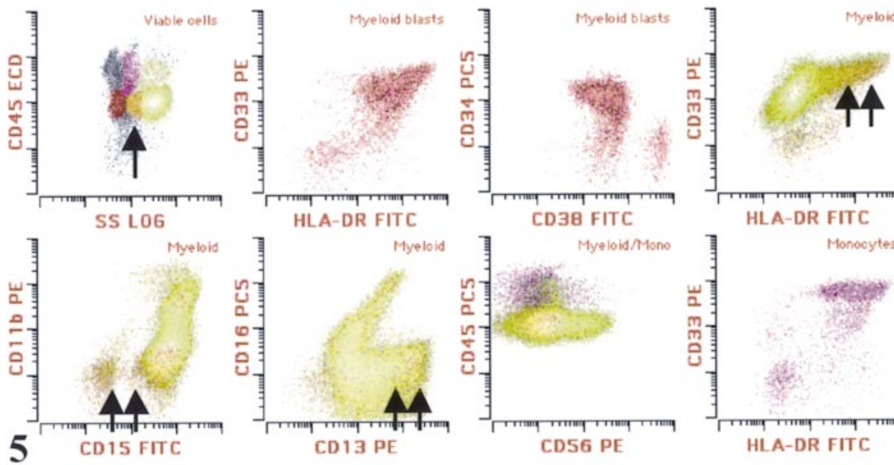


Figure 5. *Four-color flow cytometric analysis of a regenerating bone marrow following induction chemotherapy for acute myeloid leukemia, without granulocyte colony-stimulating factor treatment. CD45/sidescatter gating demonstrated in the upper left dot plot identifies the populations of interest. The myeloid blasts are colored red. The dot plots showing the maturing granulocytes are labeled "Myeloid," while the "Myeloid/Mono" dot plot includes both the maturing granulocytes (green) and monocytes (lavender). The single arrow in the CD45 versus sidescatter dot plot and the double arrows in the CD33 versus HLA-DR, CD11b versus CD15, and CD16 versus CD13 plots identify an expanded late myeloid blast/early promyelocyte population (colored yellow), reflecting the marked granulocyte left shift in this specimen.*

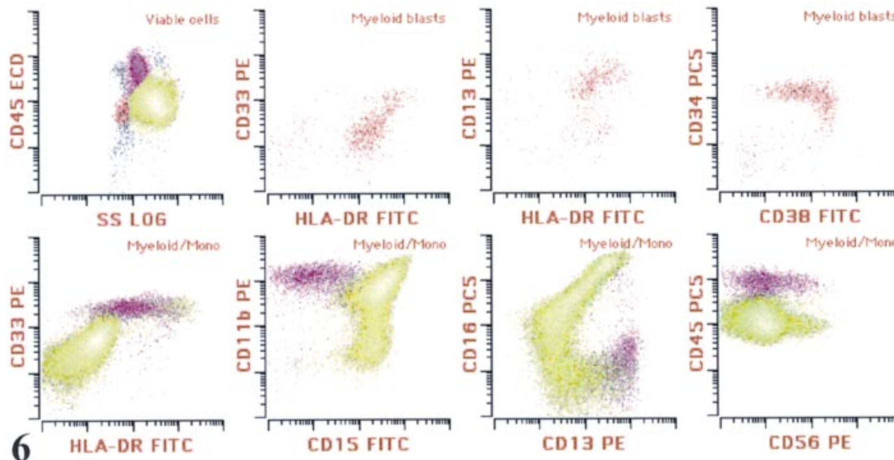


Figure 6. *Four-color flow cytometric analysis of a regenerating bone marrow following induction chemotherapy for acute myeloid leukemia, demonstrating the effect of granulocyte colony-stimulating factor treatment. The dot plots are labeled, colored, and gated as in Figure 5. The marrow in this figure is from a different patient than that in Figure 5.*

(79%). In most cases, the karyotypic analysis was based on 20 metaphases.

RESULTS

Normal Patterns of Myeloid Antigen Expression With Maturation

Patterns of antigen expression in a representative, immunophenotypically normal bone marrow aspirate are shown in Figures 1 through 4. Figures 1 through 3 demonstrate these patterns in the gated myeloblast, gated maturing granulocyte, and gated maturing monocyte populations, respectively. By focusing on antigens whose expression varies a great deal with maturation, we have been able to design combinations of antibodies to maximize our ability to distinguish normal from abnormal maturation. The combinations of antibodies shown in Figures 1

through 4 have been particularly useful and have remained the basis for our evaluation of myelopoiesis for nearly 3 years. For example, the coordinate variation in HLA-DR and CD33 that occurs as the myeloid blasts mature to both neutrophils and monocytes makes the combination of anti–HLA-DR and anti-CD33 antibodies particularly useful for identifying relatively subtle maturational abnormalities (see Figures 1 through 4). Similarly, the continuous variation in the expression of CD11b, CD13, and CD16 that occurs as the promyelocytes mature to neutrophils makes the combinations of CD11b and CD16, or CD13 and CD16, relatively sensitive for identifying abnormalities of granulocyte maturation (see Figure 2). In Figure 4, the arrows indicate the progression of maturation from the early myeloid blasts at the base of the arrow in each dot plot through the mature neutrophils or monocytes at the arrowheads. In both the granulocytic and monocytic series, the progression demonstrated in this figure occurs in parallel with an increase in CD45 expression.

Benign, Reactive Abnormalities of Myeloid Antigen Expression With Maturation

A critical requirement for using flow cytometry to identify myeloid stem cell neoplasms is the ability to distinguish benign, reactive abnormalities of myeloid maturation from neoplastic abnormalities. Figures 5 and 6 demonstrate characteristic reactive immunophenotypic abnormalities of postchemotherapy marrow regeneration in either the absence (Figure 5) or presence (Figure 6) of granulocyte colony-stimulating factor (G-CSF) therapy. These represent two of the most common settings in which we have seen reactive immunophenotypic abnormalities in myelopoiesis. Particularly striking abnormalities in the marrow in Figure 5 include (1) the abnormally homogeneous expression of HLA-DR, CD33, CD34, and CD38 among the myeloid blasts, which indicates the relatively synchronous maturation of the blasts in this setting; (2) an expanded late blast/early promyelocyte population shown in yellow that bridges the blast and maturing granulocyte populations on the CD45/sidescatter plot in the upper left of Figure 5 and is seen in only very small numbers in normal myelopoiesis (see Figure 4); (3) a marked myeloid left shift, as indicated by the predominance of granulocytes with low-to-negative expression of CD16, low CD11b, and relatively bright CD33; and (4) low-level aberrant CD56 expression on the maturing granulocytes and monocytes. The G-CSF–treated marrow (Figure 6) also demonstrates regenerative features, such as marked left shift and low-level CD56 on the maturing granulocytes and monocytes, as well as several unique abnormalities: (1) an expanded ''immature'' myeloblast population with relatively low CD33, CD38, and HLA-DR; (2) decreased sidescatter among the maturing granulocytes; and (3) characteristically dyssynchronous expression of CD13 and CD16 among the maturing granulocytes, with a right-angle–like shape to the pattern rather than the characteristic smoothly curved pattern. An additional abnormality that may be seen with G-CSF therapy is increased CD45 expression on the immature granulocytes.

Figure 7 shows marked myeloid left shift and an expanded immature blast population (low CD33 and HLA-DR) in an unusual case of T-cell large granular lymphocytic leukemia in which the presumed immunologic destruction of the neutrophil series involved earlier granulocyte precursors than is typically the case. Note that there is clearly a less aberrant expression of CD56 on the maturing granulocytes and monocytes in this setting than in the frankly regenerative marrows in Figures 5 and 6, which may reflect the different etiology of the left shift in T-cell large granular lymphocytic leukemia.

Other rare reactive marrow abnormalities we have evaluated by flow cytometry include transient myelosuppression of uncertain etiology and aplastic anemia (data not shown). In transient myelosuppression, there is typically some degree of granulocyte left shift and, at most, mild abnormalities of myeloid antigen expression, such as mildly decreased CD33 expression on the maturing granulocytes and monocytes. In aplastic anemia, the abnormalities tend to be quantitative rather than qualitative, with an across-the-board decrease in the percentages of blasts and maturing granulocytes and monocytes (relative to the lymphocytes) but relatively normal antigen expression on the myeloid cells that are present.

Application of Flow Cytometry to the Diagnosis of Non-CML MPD and MDS

To test our hypothesis that flow cytometry will represent a sensitive and specific way of assessing the likelihood of a myeloid stem cell neoplasm, we retrospectively compared the flow cytometric and cytogenetic findings for a large number of specimens on which both types of studies were performed. These studies, which will be described in detail elsewhere by the authors, separately compare flow cytometry and cytogenetics in the diagnosis of non-CML MPDs and MDSs. In these studies, the flow cytometry data were reanalyzed in a blinded, standardized fashion by software developed in our laboratory, and the patterns of antigen expression on the myeloid blasts, maturing granulocytes, and monocytes were compared in a blinded manner with the patterns typically seen in normal myeloid populations. Types of abnormalities included deviations in myeloid antigen intensity (defined as an increase or decrease of at least one third of a decade on a log scale), the abnormally homogeneous expression of 2 or more myeloid antigens, the dyssynchronous expression of 2 myeloid antigens, and the aberrant expression of nonmyeloid antigens (defined as the expression of the abnormal antigen by at least 10% of the cells in the population of interest). Cases were classified as normal, unequivocally abnormal, or indeterminate. The latter cases demonstrated mild antigenic abnormalities that did not strongly suggest a stem cell abnormality.

A representative example of abnormal bone marrow flow cytometry is shown in Figure 8. The final diagnosis in this case under the new World Health Organization classification was chronic eosinophilic leukemia/hypereosinophilic syndrome, and the specimen also contained a clonal cytogenetic abnormality. Flow cytometric abnormalities demonstrated in Figure 8 include (1) markedly decreased CD33 expression, abnormally homogeneous HLA-DR and CD13, and decreased CD38 on the myeloid blasts; (2) a markedly expanded eosinophil population with characteristically low CD33 and HLA-DR and lack of the neutrophil-associated antigen CD16; (3) mildly decreased CD33 expression on the relatively small neutrophil population; and (4) an expanded basophil population in its characteristic position at the upper end of the myeloid blast population on the CD45 sidescatter dot plot, which shows a mildly decreased CD33 expression as well

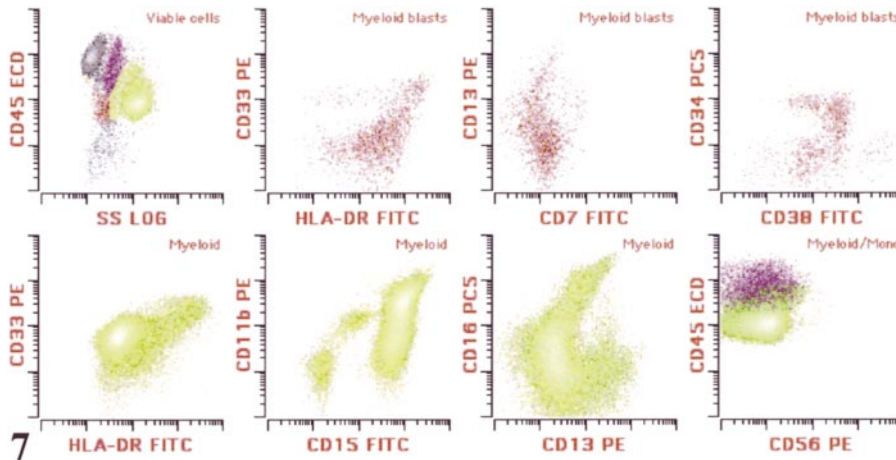


Figure 7. *Four-color flow cytometric analysis of the myeloid cells in an unusual case of T-cell large granular lymphocytic leukemia with marked granulocyte left shift. The dot plots are labeled, colored, and gated as in Figure 5. FITC indicates fluorescein isothiocyanate; PE, phycoerythrin.*

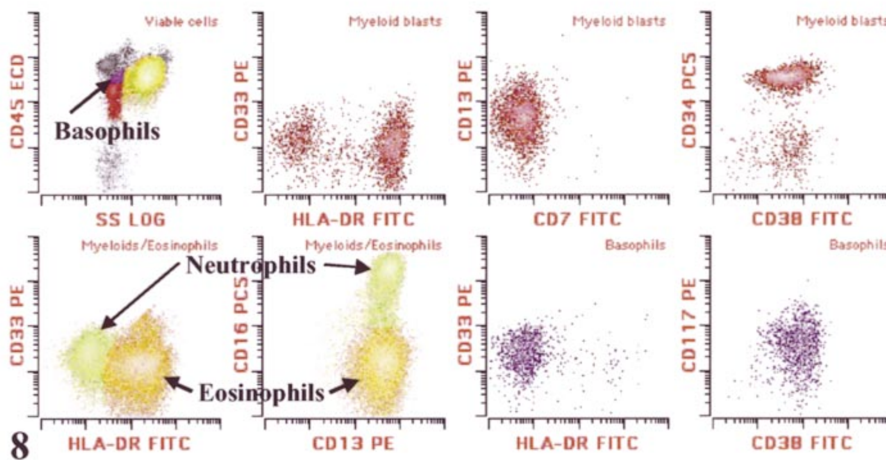


Figure 8. *Four-color flow cytometric analysis of a case of chronic eosinophilic leukemia/hypereosinophilic syndrome demonstrates multiple abnormalities of myeloid antigen expression. The markedly expanded eosinophil population (yellow) is included in the same gate as the smaller neutrophil population (green), while the basophil population (blue) is evaluated in a separate gate.*

Table 1. Flow Cytometry Versus Cytogenetics in Evaluating Non-CML MPD*

Flow Cytometry Result	n	Mean Age, y	Sex Ratio, M/F	Cytogenetics
Normal or indeterminate	37	52	16:21	0% abnormal
Abnormal	30	65	15:15	40% abnormal

* Non-CML MPD indicates non-chronic myeloid leukemia myeloproliferative disorders.

Table 2. Flow Cytometry Versus Cytogenetics in Evaluating MDS*

Flow Cytometry Result	n	Mean Age, y	Sex Ratio, M/F	Cytogenetics
Normal	101	57	46:55	3% abnormal
Indeterminate	46	64	21:25	6.5% abnormal
Abnormal	116	67	71:45	54% abnormal

* MDS indicates myelodysplastic syndrome.

as the aberrant expression of CD117. CD117 (c-Kit), which is strongly expressed by normal mast cells, is normally not expressed on basophils.[36] As in this case, we typically see similar levels of expression of the pan-myeloid antigens CD13 and CD33 on the basophils and neutrophils, so the mildly decreased CD33 expression on both populations in this case is not surprising.

Tables 1 and 2 summarize the comparisons between the results of flow cytometry and conventional cytogenetics in the workup of non-CML MPDs and MDSs. Among 67 non-CML bone marrows evaluated to rule out an MPD and for which concurrent cytogenetic information was available, clonal cytogenetic abnormalities were found in 12 of 30 (40%) of the flow-abnormal cases compared to 0 of the 37 (0%) flow-negative and flow-indeterminate cases ($P < .001$ for this comparison). Among 263 marrows evaluated to rule out an MDS, clonal cytogenetic abnormalities were found in 63 of 116 (54%) of the flow-abnormal cases compared to 3 of 46 of the flow-indeterminate cases (6.5%) and 3 of 101 (3.0%) of the flow-negative cases ($P < .001$

for the comparison of the flow-abnormal cases with the combined flow-indeterminate/flow-abnormal cases). In both study populations, the patients with abnormal flow cytometry tended to be older than the patients with normal or indeterminate flow cytometry.

CONCLUSIONS

There are a number of reasons why flow cytometry represents a very valuable technique in the evaluation of non-CML MPDs and MDSs. First, because normal hematopoiesis is characterized by highly reproducible patterns of antigen expression during myeloid maturation in the marrow, it is relatively easy to compare the patterns seen in patient specimens against the normal standards to determine the likelihood of an underlying stem cell neoplasm. Therefore, flow cytometry represents a new and objective method for diagnosing these disorders. By adding flow cytometry to the workup of these disorders, the historic reliance on potentially subjective morphologic criteria is likely to be lessened, which may be of particular benefit when the blast percentage is low and/or the morphologic features are equivocal. Second, our detailed comparison of flow cytometry and conventional cytogenetics in the evaluation of both non-CML MPDs and MDSs has demonstrated that flow cytometry will miss less than 10% of cases with clonal cytogenetic abnormalities, indicating that the assay has high sensitivity. Third, patterns of myeloid maturation can be easily followed in multiple marrow specimens from a patient over time. Such sequential evaluation can be helpful in supporting or excluding the possibility of an underlying myeloid stem cell neoplasm, as has been the case for a number of the patients in our MPD and MDS series. Fourth, unlike conventional cytogenetics, which is the current objective gold standard for diagnosing these disorders, flow cytometry does not require the cells of interest to grow in culture. This feature is particularly useful in evaluating low-grade MDSs, in which the abnormal blasts may be unlikely to grow in culture because of an increased rate of apoptosis.[28–30,32,33] Fifth, in contrast to the turnaround times of 1 week or more for conventional cytogenetics or esoteric tests such as the endogenous erythroid colony assay, flow cytometric evaluation of a specimen may be completed within hours of receipt. Therefore, clinical decision making is not significantly delayed by flow cytometry. Sixth, at our institution, the standard flow cytometric evaluation of myeloid maturation is significantly less expensive than conventional cytogenetics.

Note that the patterns of expression of CD45, CD34, HLA-DR, CD13, CD33, CD15, CD11b, and CD16 that we see in normal marrow are similar to the patterns described in the literature.[4–6] However, our work goes beyond many previously published flow cytometric studies of normal and abnormal myelopoiesis in a number of ways. First, we used a 4-color approach, allowing us to identify more complex relationships among the various antigens than could be identified by the 3-color approaches used in most of the studies in the literature. Second, by identifying reproducible patterns of antigen expression in reactive processes such as marrow regeneration and G-CSF therapy, we can distinguish benign from neoplastic abnormalities of antigen expression, which is particularly useful in light of the widespread use of G-CSF therapy today. Finally, and perhaps most importantly, by correlating our flow cytometry data with the cytogenetic data, we have validated the use of flow cytometry in the diagnosis of non-CML MPDs and MDSs.

Our study is probably most comparable to the 2001 study of Stetler-Stevenson and colleagues,[34] which made the important observation that flow cytometry can be very useful in distinguishing reactive from dysplastic bone marrows in morphologically equivocal cases. As we reported in our study, Stetler-Stevenson and colleagues found that abnormal patterns of expression of CD11b versus CD16, or CD13 versus CD16, among the maturing granulocytes were particularly useful in distinguishing benign from dysplastic processes, whereas the aberrant expression of nonmyeloid antigens and the loss of CD10 among the neutrophils were less common findings. Their study found that the loss of CD64 among the granulocytes was present in about two thirds of the MDS patients, although CD64 was not one of the antigens routinely evaluated in our panels. In addition, they attempted to evaluate maturing megakaryocytes by flow cytometry, which we have not attempted to do because of our concerns that platelet binding to the surfaces of nonmegakaryocytic cells may result in artifactual positivity for platelet-associated antigens.

However, our study goes beyond that study in several respects. First, we evaluated cases suspected of representing MPD rather than just MDS. Second, our cases were identified solely because of the clinical suspicion of an MPD or MDS, rather than being preselected following the morphologic characterization of the bone marrows. Third, we used 4-color flow cytometry rather than a 3-color approach.

In summary, we have demonstrated that 4-color flow cytometry is capable of distinguishing normal from abnormal myelopoiesis. Flow cytometry identifies significant antigenic abnormalities in essentially all non-CML MPDs possessing clonal cytogenetic abnormalities and in more than 90% of cytogenetically abnormal MDSs. By extension, the results suggest that antigenic abnormalities will be identifiable in many, if not most, cases of non-CML MPD and MDS that are cytogenetically normal. Indeed, our unpublished follow-up flow cytometry data on a number of patients initially found to have flow-abnormal but cytogenetically normal marrows have shown unequivocal evidence of a neoplastic process, including acquisition of cytogenetic abnormalities, or evolution of the blast percentage toward frank acute myeloid leukemia. Because the non-CML MPDs and MDSs (particularly low-grade MDSs such as refractory anemia) have historically lacked highly sensitive and objective diagnostic criteria, in experienced laboratories, 4-color flow cytometry is a desirable part of the diagnostic workup of these disorders.

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