

acute lymphoblastic leukemia, 21; T-cell lymphoma, 11; MDS, 4; granulocytic sarcoma, 1; and B-cell lymphoma, 1. Our study showed that only 4 (4%) patients developed CMV infection. Three of these patients had concomitant fungal pneumonia, and 1 had bacterial pneumonia. All 4 patients were CMV seropositive prior to the granulocyte transfusions. Thus recommending serologic screening for cytomegalovirus in granulocyte donors seems unwarranted.

A previous study in our institution, involving 34 patients who received granulocyte colony-stimulating factor (G-CSF)-stimulated granulocyte transfusions for bacterial, fungal, or both infections clearly showed that the granulocyte transfusions were effective in 16 patients (47%).<sup>5</sup> Therefore clinicians must individually weigh the risks and benefits obtained from granulocyte transfusions when confronted with cases of severe bacterial or fungal infections resistant to antimicrobial or antifungal agents alone.

We believe that CMV infection in these patients could be due to primary reactivation of latent CMV or perhaps secondary to infection with another CMV strain. Therefore, screening of potential granulocyte donors for CMV antibody may not provide

additional benefit to fungal- and bacteria-infected severely granulocytopenic patients with cancer. A more detailed investigation of each individual case needs to be done.

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## References

1. Meyers JD, Flournoy N, Thomas ED. Risk factors for cytomegalovirus infection after human marrow transplantation. *J Infect Dis.* 1986;153:478-488.
2. Forman SJ, Zaia JA. Treatment and prevention of cytomegalovirus pneumonia after bone marrow transplantation: where do we stand? *Blood.* 1994;83:2392-2398.
3. Hillyer CD, Emmens RK, Zago-Novaretti M, Berkman EM. Methods for the reduction of transfusion-transmitted cytomegalovirus infection; Filtration versus the use of seronegative donor units. *Transfusion.* 1994;34:931-934.
4. Andreu G. Role of leukocyte depletion in the prevention of transfusion-induced cytomegalovirus infection. *Semin Hematol.* 1991;28:26-31.
5. Pena E, Narvios A, Lichtiger B. Therapeutic granulocyte transfusions: retrospective study of 34 patients [abstract]. *Blood.* 96;11:63a.

## To the editor:

### Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome

We read with great interest the article of Stetler-Stevenson et al,<sup>1</sup> which brings surface-marker analysis in myelodysplastic syndrome (MDS) to the attention of the scientific community and provides evidence for its possible utilization. However, we have some comments and questions related to this study.

First, the authors seem to have relied on pattern recognition and "eyeballing" of flow cytometry scattergraphs to identify abnormalities without establishing objective criteria to differentiate MDS from other entities. Although this approach may work in obvious cases, borderline cases may be difficult to discern. Orthogonal light scatter (SSC), discrete clusters of blasts, CD11b/CD16 abnormal pattern, and CD13/CD16 abnormal pattern are examples.

Second, the authors may have used inappropriate control for comparison. For example, to identify abnormalities in SSC, the authors compared patients' bone marrows (BM) with normal peripheral blood (PB). Bone marrow granulocytes change their light scatter properties with maturation<sup>2,3</sup> and the use of PB granulocytes as a control for BM granulocytic scatter properties may not be appropriate.

Third, the definition of megakaryocytic dysplasia by flow cytometry is not persuasive. The authors used a numerical value derived from patients with aplastic anemia. Although the authors acknowledge that this value does not differentiate normal marrow from dysplastic marrow, they continue to apply this value to all patients to identify dysplastic megakaryopoiesis.

Fourth, the authors provide no indication that at least a semiquantitative correlation between BM morphology and flow cytometry maturation pattern was performed. CD64<sup>-</sup> granulocytes are normally present in the bone marrow since CD64 is lost at the band and segmented-neutrophil stages.<sup>4,5</sup> In our experience, approximately 30% of cells in the granulocytic gate of non-MDS marrows are CD64<sup>-</sup>.<sup>6</sup> Similarly, CD10 is usually detected in the bone marrow granulocytes at the segmentation stage and may not be observed with granulocytic left shift. We have encountered several non-MDS bone marrows showing virtually no segmented neutrophils on the marrow aspirate and no CD10<sup>+</sup> cells within the granulocytic gate. The pattern of increased CD11b and decreased

CD16 can be seen with granulocytic left shift and with G-CSF therapy as well.<sup>7(Fig2F)</sup>

Finally, the authors report an interesting finding of myeloid cells expressing lymphoid markers, such as CD22 and CD7, in as many as 38% of cases. Our recent literature review identified only rare reports with similar findings.<sup>8</sup> We will be interested in knowing the definition of positivity used by the authors.

We agree with the authors' conclusion that flow cytometry is more sensitive than morphology in assessing granulocytic dysplasia and that flow cytometry can support the diagnosis of MDS in equivocal cases.

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## References

1. Stetler-Stevenson M, Arthur DC, Jabbour N, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood.* 2001;98:979-987.
2. Terstappen LWMM, Safford M, Loken MR. Flow cytometric analysis of human bone marrow. III neutrophil maturation. *Leukemia.* 1990;4:657-663.
3. Lund-Johansen F, Terstappen LWMM. Differential surface expression of cell adhesion molecules during granulocyte maturation. *J Leukoc Biol.* 1993;54:47-55.
4. Fleitt HB, Wright SD, Durie CJ, Valinsky JE, Unkeless JC. Ontogeny of Fc receptors and complement receptor (CR3) during human myeloid differentiation. *J Clin Invest.* 1984;73:516-525.
5. Ball ED, McDermott J, Griffin JD, Davey FR, Davis R, Bloomfield CD. Expression of the three myeloid cell-associated immunoglobulin G Fc receptors defined by murine monoclonal antibodies on normal bone marrow and acute leukemia cells. *Blood.* 1989;73:1951-1956.
6. Elghetany MT, Martinez J, Patel J, Sackey K, Alter BP. Granulocytic surface marker abnormalities are detected only in patients with myelodysplastic syndrome and patients with Shwachman diamond syndrome but not other bone marrow failure syndromes [abstract]. *Blood.* 2000;96(suppl):357a.
7. Fujimoto H, Sakata T, Hamagushi Y, et al. Flow cytometric method for enumeration and classification of reactive immature granulocytic population. *Cytometry.* 2000;42:371-378.
8. Elghetany MT. Surface marker abnormalities in myelodysplastic syndromes. *Haematologica.* 1998;83:1104-1115.

## Response:

### Diagnostic flow cytometric immunophenotyping in myelodysplastic syndrome: the US-Canadian consensus recommendations on the immunophenotypic analysis of hematological neoplasia by flow cytometry apply

Elghetany's questions and comments concerning our recent study on the utility of flow cytometric immunophenotyping in myelodysplastic syndrome (MDS) highlight several important issues in flow cytometric evaluation of hematological diseases. His comments underline the differences between the immunology-based (eg, CD4/CD8 enumeration) approach to flow cytometry and the more hematopathology-based pattern-recognition approach recommended in the US-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry.<sup>1,2</sup> When one is studying nonneoplastic lymphoid cells, it is reasonable to set analysis parameters on the normal lymphoid cells (usually based upon light scatter) and report the number of cells staining more intensely than negative controls. However, in neoplasia, this type of information is not useful. Current state-of-the-art evaluation for hematological malignancies involves extensive knowledge of normal patterns as a basis for identification and characterization of abnormal patterns. What Elghetany refers to as "eyeballing of flow scatter graphs to identify abnormalities," rather than setting an analysis gate and generating numbers, is precisely what is done and what was recommended by the leaders in the US and Canada at the consensus meeting in 1997. Although one can argue whether or not MDS is a premalignant or malignant condition, the pattern-recognition-based approach is the one we find useful in MDS. If the clinician interpreting flow cytometric data knows normal patterns, abnormal patterns are easily detected with this approach, even in borderline cases.

There is another important point brought to light in Elghetany's comments. One cannot perform diagnostic flow cytometry in the absence of clinical history and morphologic evaluation. The need to correlate results with history and morphology is stressed in the US-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry.<sup>2</sup> Therefore, we review the patient's history and morphology before making a diagnosis. We exercise appropriate caution with recent granulocyte colony-stimulating factor (G-CSF) treatment and do not consider a diagnosis of MDS in a healthy individual with normal complete blood count results. For example, the discrete cluster of blasts seen based upon side scatter versus CD45 in MDS can also be residual malignant blasts in a patient with acute myelogenous leukemia following treatment. However, the history indicates the appropriate diagnosis. Elghetany's example of a bone marrow with a marked left shift in which no segmented neutrophils were observed would not be mistaken for MDS. Usually we find that the cases that benefit from flow cytometric immunophenotypic analysis are those in which the marrow is hypocellular and aspirates are not adequate to fully evaluate all lineages morphologically. Complete cytogenetic analyses may not be possible in these cases either. The history is often consistent with aplastic anemia or MDS. In these specimens the observed differences in numbers of megakaryocytes are useful. Since a diagnosis of MDS would not be entertained in a normal bone marrow, the inability to distinguish MDS from normal based upon numbers of megakaryocytes is irrelevant.

Elghetany points out that reports of myeloid cells expressing lymphoid markers in MDS are rare. Clearly this is due to the panels previously utilized in studying MDS. In general, lymphoid markers are paired with other lymphoid markers. As we were looking for myeloid abnormalities in our study, we designed our panel to detect expression of T- and B-cell antigens by myeloid cells. This is a more sensitive method to detect this abnormality.

Concerning the minor points raised in the letter, Elghetany is incorrect in stating that normal peripheral blood was used as a control. As stated in "Patients, materials, and methods," bone marrow aspirates from healthy volunteers were used as the normal controls.<sup>3</sup> Furthermore, bone marrow aspirates from patients with aplastic anemia and patients who have completed chemotherapy for nonmyeloid neoplasia were utilized, as we believe these are more useful. On another minor point, when we report the granulocytic cells as being negative for CD64 or CD10, we mean that all of the granulocytic cells are negative, not just a subset. This is a highly abnormal pattern. Because we stress that it is not a single abnormality but multiple ones in more than one lineage that are characteristic of MDS, whether one might observe one of these abnormalities in an individual without appropriate history or morphology is again irrelevant.

In summary, we utilize a pattern-recognition-based approach to diagnosis of MDS. Detection of abnormal patterns of antigen expression is a very powerful approach but requires an extensive knowledge of what is normal. We also feel correlation with clinical history and morphology is absolutely necessary in making a diagnosis of any hematologic disease utilizing flow cytometric immunophenotyping. Furthermore, we stress that it is a pattern of multiple immunophenotypic abnormalities in more than one lineage and in the appropriate clinical setting that leads to a diagnosis of MDS. Lastly, we would like to repeat our reported conclusion that flow cytometric immunophenotyping is not a screening test for MDS. An astute morphologist and access to an excellent cytogenetics laboratory is sufficient to render a correct diagnosis in the majority of cases. Flow cytometric immunophenotyping is useful when these fail.

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## References

1. Stelzer GT, Marti G, Hurley A, et al. U.S.-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures. *Cytometry*. 1997;30:214-230.
2. Borowitz MJ, Bray R, Gascoyne R, et al. U.S.-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: data analysis and interpretation. *Cytometry*. 1997;30:236-244.
3. Stetler-Stevenson M, Arthur DC, Jabbour N, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood*. 2001;98:979-987.



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