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Flow Cytometric Immunophenotyping: Emerging as an Important Diagnostic Tool in the Evaluation of Cytopenic Patients

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Cytopenia is a commonly encountered laboratory finding requiring further medical evaluation. If medical review and laboratory testing fail to reveal a cause, bone marrow biopsy and aspiration are indicated to rule out an infiltrative process, such as lymphoma, or a stem cell disorder, such as myelodysplastic syndrome (MDS). While detection of an infiltrative process such as lymphoma or metastatic carcinoma is fairly straightforward, other diagnoses may be more elusive. Truong and co-workers, in this issue (1), have examined the utility of flow cytometric immunophenotyping in the diagnostic evaluation of cytopenic patients and their study indicates a role for this technology in determining the more subtle medical causes of this finding.

MDS is a prominent consideration in evaluating older patients with cytopenia. Although the diagnosis is relatively uncomplicated when obvious dysplasia, increased myeloid blasts and ringed sideroblasts are detected, in a subset of patients diagnosis of MDS can be challenging. For this reason laboratory scientists have investigated the use of flow cytometry (FC) to increase the sensitivity and specificity of diagnosis in such cases. Early studies found immunophenotypic evidence of atypical granulocytic maturation and aberrant antigen expression in myelodysplasia (2–5). Subsequent, multiparametric flow cytometric studies examining the full spectrum of granulocytic, monocytic and erythroid immunophenotypic abnormalities definitively demonstrated an increase in the sensitivity and specificity of diagnosis in MDS (3,5,6). FC diagnosis of MDS is based upon the knowledge that maturation of hematopoietic lineages is tightly controlled, leading to a predictable normal pattern of antigen expression at different stages of differentiation. The abnormal granulocytic, monocytic and erythroid differentiation in MDS results in deviations from the normal pattern of antigen expression. MDS is distinguished from other disease processes by a pattern of multiple myeloid immunophenotypic abnormalities (3–6). Earlier studies demonstrated that flow cytometric abnormalities are detected in multiple lineages (3–6) and correlate with morphology and cytogenetics (4,6). Wells et al (5) introduced the concept of a flow cytometric scoring system to quantitate the extent of the abnormalities. Scoring systems are not only useful in the diagnosis of MDS but also provide vital prognostic information. The finding of a high number of immunophenotypic abnormalities and specific immunophenotypic profiles (resulting in a high FC score) is associated with a poor International Prognosis Scoring System (IPSS) score and International Prognosis Scoring System risk category (4,5,7,8). Currently several scoring systems that correlate with IPSS score and prognosis exist (5,7,9). High numbers of FC

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abnormalities are associated with post-transplantation relapse and poor overall survival (5) in a manner that can be independent of the IPSS in predicting relapse and survival (5). Furthermore FC identifies patients at risk for transfusion dependency and progressive disease (7). Interpretation of the FC data in MDS is demanding as there is no single immunophenotypic profile that is pathognomonic of MDS; moreover some abnormalities may be observed in other disorders (such as paroxysmal nocturnal hemoglobinuria, megaloblastic anemia, and growth factor therapy). In addition, problems with sample integrity may produce FC results that artifactually mimic MDS-like abnormalities. Because of the high degree of complexity of FC analysis for MDS, standard diagnostic flow cytometry laboratories were slow at first to adopt this testing. However, FC analysis is now accepted as an adjuvant test to increase the sensitivity and specificity of diagnosis in evaluation of bone marrow for MDS. The inclusion of FC testing in the minimal diagnostic criteria for MDS developed at a 2006 international working conference reflects its important diagnostic role (10).

In this issue Truong et al further demonstrate the role of FC in evaluating cytopenic patients. They confirmed its utility in diagnosing MDS and myeloproliferative disorders (MPD). Of even greater interest, they demonstrate that a negative FC test result in cytopenic patients is useful in identifying secondary cytopenias and excluding a diagnosis of MDS/MPD. They evaluated bone marrow aspirates for myelomonocytic abnormalities by FC at the time of initial diagnostic bone marrow sampling in patients with unexplained cytopenias. Patients in whom morphology and cytogenetic testing were both negative for evidence of dysplasia were followed and further evaluated. Of the patients who ultimately developed evidence of MDS/MPD, the vast majority had myelomonocytic immunophenotypic abnormalities. Only 2 patients with a final diagnosis of MDS/MPD demonstrated negative flow cytometry at initial testing; subsequently one of these 2 patients did demonstrate a positive FC finding on a follow up bone marrow evaluation. In contrast, positive FC results were rarely detected in the patients with cytopenias that were eventually attributed to medical causes. In those patients where the causes of cytopenia remained undetermined during the follow up period, there was a high rate of positive FC testing in patients who eventually received a diagnosis of “clinical MDS”. Abnormal FC results were obtained in bone marrows from 50% of the patients meeting the criteria for idiopathic cytopenia of uncertain significance (ICUS). Further follow up in these patients will be of value as a subset of patients with ICUS may develop MDS. However, flow cytometry was negative in the patients who spontaneously recovered.

The important role of FC continues to strengthen and evolve in the evaluation of cytopenic patients. We recognize that a positive FC test assists in the diagnosis of MDS and that a high flow cytometry score portends a poor prognosis. This study also demonstrates the converse; that a negative FC test result indicates a high likelihood of a positive clinical outcome.

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