Peripheral Blood Flow Cytometry Testing – Clinician Handout

SYNOPSIS AND RELEVANCE

Flow cytometric analysis is a powerful tool for determining underlying causes of a subset of peripheral blood count abnormalities but is resource-intensive and not applicable to all peripheral blood abnormalities. Rational use of peripheral blood flow cytometry enables:

- Prompt, non-invasive diagnosis of some hematopoietic disorders.
- Triaging of patient care, such as need for bone marrow or lymph node biopsy.
- Reduction of over-utilization in situations where flow cytometry is unlikely to be informative.

BACKGROUND

Flow cytometric immunotyping (FCI) characterizes cells based on size, internal complexity, and immunophenotype, enabling identification of cell types and some cellular abnormalities. Because it uses cells in suspension, it is particularly useful in examination of peripheral blood, allowing for a relatively non-invasive method of diagnosing some hematologic abnormalities. FCI requires manual labor, sophisticated analyzers, and expensive reagents, and thus is resource intensive. This module will not address specialized FCI, such as FCI for minimal residual disease, or FCI performed on other specimen types such as body fluid, tissue (eg, lymph node), or bone marrow.

Routine peripheral blood flow cytometry immunotyping (PB FCI) is best used to characterize morphologically abnormal cells identified on a peripheral blood smear review, including blasts and lymphoma cells, or in clinical settings with a high pre-test probability for a hematologic disorder, such as in a patient with a history of hematologic malignancy. One of the best uses for FCI is to determine whether a lymphocytosis is reactive or represents a lymphoproliferative disorder (LPD).[1,2] B-cell LPD can be detected by monotypic kappa/lambda expression, and/or by expression of aberrant antigens (eg, CD5, CD123). T-cell LPD can be detected by skewed CD4/CD8 expression, monotypic TRBC1 expression, loss of pan-T cell antigens (eg, CD3, CD7), and aberrant antigen expression (eg, CD10, CD25). Another situation in which PB FCI is virtually always indicated is in the setting of increased peripheral blood blasts, in which the findings of lymphoblasts or immunophenotypically aberrant myeloblasts would indicate potential underlying acute leukemia or myeloid neoplasia to warrant further investigation, such as bone marrow examination and molecular/cytogenetic testing.[2,3] PB FCI may also be helpful in some cases of neutropenia or monocytopenia by detection of a T-large granular lymphocytic leukemia or hairy cell leukemia, respectively. Lymphocyte-variant hypereosinophilic syndrome is a rare cause of hypereosinophilia, but warrants use of PB FCI in the setting of persistently elevated eosinophils. Finally, a history of hematologic malignancy raises the pre-test probability of finding a significant abnormality on PB FCI, for which PB FCI is generally considered indicated when there is clinical concern for persistent or recurrent disease.

There are, however, other PB abnormalities in which PB FCI may have limited utility. PB FCI currently in use cannot distinguish normal mature neutrophils, basophils, red blood cells, or platelets from those arising from a neoplastic clone (with the exception of specialized testing for paroxysmal nocturnal hemoglobinuria). The 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry discussed the appropriate indications for flow cytometric evaluation and suggested that FCI was not indicated for cases of isolated neutrophilia, basophilia, polyclonal hypergammamglobulinemia, thrombocytosis or erythrocytosis.[4] Furthermore, the 2018 Choosing Wisely recommendation discouraged PB FCI in the settings of mature isolated neutrophilia, basophilia, erythrocytosis, thrombocytosis, isolated anemia and thrombocytopenia.[5]

Morphologic review of the peripheral blood, molecular studies, and bone marrow biopsy are some of the other laboratory tests available to investigate underlying causes of peripheral blood count abnormalities for which PB FCI may be low-yield.[6,7] Leukocytosis in the absence of blasts, lymphocytosis or eosinophilia is a non-specific finding seen in a variety of disease states. For example, left-shifted neutrophilia with basophilia is highly suggestive of chronic myeloid leukemia, which is best diagnosed by detection of *BCR::ABL1* gene fusion.[2,3] Detection of abnormalities by morphologic review of the peripheral blood smear also significantly correlates with detection of

abnormalities by PB FCI and requires fewer resources (less time for pathologists and technologists, no expensive flow reagents). According to Rivas et al., peripheral blood morphologic review for peripheral blood count abnormalities had a sensitivity of 80.3% and a specificity of 98.3% for diagnosing hematologic malignancy, compared with a sensitivity of 90.1% and specificity of 100% by FCI, and was superior to FCI in identifying chronic myeloid neoplasms.[8]

INSIGHTS

- Flow cytometry is very helpful in determining whether lymphocytosis is due to a lymphoproliferative disorder or reactive cause.
- Flow cytometry is useful in determining the lineage of atypical cells, such as blasts and atypical lymphocytes.
- Review of peripheral blood smear aids in determining whether or not peripheral blood flow cytometry may be informative.
- Flow cytometry can help to determine the underlying cause of a subset of cases of eosinophilia, neutropenia and monocytopenia.
- Flow cytometry has limited utility in mature neutrophilia, basophilia, erythrocytosis, thrombocytosis, isolated anemia, and isolated thrombocytopenia.
- Other ancillary tests, such as molecular studies, may be better tests to evaluate for abnormal peripheral blood count abnormalities in the appropriate clinical context.

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