Peripheral Blood Flow Cytometry Testing

Version: 1.0
Date: August 27, 2025

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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.

OBJECTIVES

- Identify types of peripheral blood count abnormalities where peripheral blood flow cytometry is most applicable.
- Understand the limitations of peripheral blood flow cytometry.
- Recognize that there are some peripheral blood abnormalities in which other laboratory tests (cytogenetic, molecular, bone marrow examination) may be more informative than flow cytometry.

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.

INTERVENTIONS

- Establish electronic medical record alerts triggered by PB FCI orders which indicate what conditions PB FCI is generally uninformative: mature neutrophilia, basophilia, erythrocytosis, thrombocytosis, isolated anemia, and isolated thrombocytopenia.
- Consider re-naming flow cytometry panels to define the indication for ordering, such as “peripheral blood flow cytometry for lymphocytosis” or “peripheral blood flow cytometry for increased blasts.”
- Establish a triage process via review of peripheral blood smears and complete blood count results
    - PB FCI should be performed when ordered if absolute lymphocytosis or abnormal cells (eg, blasts, lymphoma cells) are present.
    - In the absence of such findings or a history of a hematologic malignancy, pathologists should be enabled to either cancel the test or contact the clinician to request cancellation.
    - Based on the PB morphologic findings, pathologists can contact the clinician to suggest more informative testing such as molecular analysis or bone marrow examination.

INTERVENTION ANALYSIS

The total number of orders for PB FCI testing can be compared prior to and after implementation of the intervention(s) selected. The number of appropriate indications among the total orders can be determined by review of provided clinical indication. Chart review for clinical scenarios and laboratory data can also be

performed for further information, as needed. Inappropriate testing can be evaluated by various filters, such as ordering providers, clinician specialties, or order indications, to provide targeted education.

1. Develop consensus ordering indications with clinical staff or the appropriate clinical laboratory utilization committee.
2. Perform a pre-intervention assessment of appropriate PB PCI orders based upon your established consensus indications.
3. Implement interventions as suggested above or those developed by your institution.
4. Perform a post-intervention assessment of appropriate PB PCI based upon your established consensus indications.
5. The impact can be calculated by comparing the change in appropriate PB PCI orders for your laboratory's pre-intervention and post-intervention performance.
6. This impact study can be repeated for each major intervention or guideline update.

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE

Understand the limitations of peripheral blood flow cytometry.

QUESTION 1

In which of the following is routine peripheral blood flow cytometry the most useful?

A. Determining if a mature neutrophilia is reactive or neoplastic.
B. Determining if a lymphocytosis is reactive or neoplastic.
C. Determining is a polycythemia is primary or secondary.
D. Finding low levels (<1%) of blasts in a patient with suspected myelodysplastic syndrome.
E. Determining the underlying cause of an isolated basophilia.

The correct answer is B. Lymphocytosis is one of the best uses for flow cytometry to determine whether the finding is reactive or represents a lymphoproliferative disorder based on monotypic light chain expression, skewed CD4/CD8 ratio, or aberrant antigen expression. Conversely mature cytophilias/cytoses of other cell types are not associated with immunotypic abnormalities and cannot be readily distinguished from a neoplastic clone.

A is incorrect. Routine flow cytometric panels cannot distinguish benign neutrophils from those associated with a neoplastic clone. Chronic leukemias are associated with cytogenetic or molecular abnormalities, such as *CSF3R* mutations in chronic neutrophilic leukemia which can be detected on next generation sequencing.

C is incorrect. Flow cytometry does not typically assess mature red blood cells. Molecular testing for *JAK2* mutations and other tests such as erythropoietin levels would be more helpful in this scenario.

D. is incorrect. Routine flow cytometric methods are best used to characterize discrete populations of cells, usually >1%. More specialized techniques such as minimal/measurable disease flow cytometry can detect <0.01% abnormal cells, but these are not used for diagnosis.

E. is incorrect. Routine flow cytometry panels cannot distinguish benign basophils from those associated with neoplastic clones.

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QUESTION 2 OBJECTIVE

Recognize that there are some peripheral blood abnormalities in which other laboratory tests (cytogenetic, molecular, bone marrow examination) may be more informative than flow cytometry.

QUESTION 2

Which of the following potential causes of persistent eosinophilia could be detected by peripheral blood flow cytometry immunotyping?

A. Lymphocyte-variant hypereosinophilic syndrome.
B. Myeloid neoplasm with *PDGFRA::FGFR1* rearrangement.
C. *Strongyloides stercoralis* infection.
D. Chronic eosinophilic leukemia.
E. Chronic myeloid leukemia.

The correct answer is A. Lymphocyte-variant hypereosinophilic syndrome demonstrates abnormal T cell populations (often sCD3-/CD4+) that can be detected with flow cytometry.
B is incorrect. In the absence of blasts, there are not typically immunophenotypic abnormalities on eosinophils or other circulating cells in these disorders. FISH for the *PDGFRA::FGFR1* rearrangement/*CHIC2* deletion is the best test to interrogate for this disease entity.
C is incorrect. Eosinophilia associated with reaction to infections does not show immunophenotypic aberrations.
D. is incorrect. Chronic eosinophilic leukemia does not show immunophenotypic aberrations. It is associated with abnormal bone marrow morphology and either a clonal cytogenetic abnormality and/or somatic mutation, thus bone marrow biopsy is generally required.
E. is incorrect. Molecular/cytogenetic analysis for the *BCR::ABL1* rearrangement is diagnostic for CML. Peripheral blood flow cytometry could be indicated if there are increased circulating blasts, but would not lead to the specific diagnosis without the molecular/cytogenetic testing.

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QUESTION 3 OBJECTIVE

Identify type of peripheral blood count abnormalities where peripheral blood flow cytometry is most applicable.

QUESTION 3

Which of the following would warrant the performance of peripheral blood flow cytometry immunotyping to interrogate for hematologic malignancy, even if no abnormal lymphocytes or blasts are detected on peripheral blood morphologic examination?

A. Lymphocytosis during viral infection.
B. Isolated thrombocytosis.
C. Leukemoid reaction during sepsis.
D. A history of hematologic malignancy.
E. Isolated microcytic anemia.

The correct answer is D. A history of hematologic malignancy increases the pre-test probability for a hematologic disorder potentially detectable by peripheral blood flow cytometry.
A is incorrect. Viral infection is often associated with benign reactive lymphocytosis.
B is incorrect. Thrombocytosis can be associated with myeloproliferative neoplasms; however these are not associated with immunophenotypic aberrancies which can be detected by flow cytometry. Molecular (*JAK2*, *CALR*, *MPL*, *BCR::ABL1*) tests and bone marrow evaluation may be more helpful in this scenario.
C. is incorrect. Neutrophilia, sometimes with left-shift, is a physiologic response to bacterial infection.

E. is incorrect. Flow cytometry does not assess mature erythrocytes. Microcytic anemia is typically associated with iron deficiency and thalassemias. Other laboratory tests which would be more useful in this scenario include ferritin/iron levels, RBC parameters (count, RDW, MCH), and potentially molecular testing of hemoglobin genes.

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