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<p>Flow cytometric evaluation is considered a standard ancillary study for the diagnosis of most B-cell lymphoproliferative disorders. Establishing a neoplastic B-cell population depends on identification of light chain restriction or lack of light chain expression in mature neoplasms and demonstration of aberrant antigen expression in both immature and mature neoplasms, as compared with normal counterparts. The immunophenotypes of the most common B-cell neoplasms are herein described, with an emphasis on their immunophenotypic differential diagnosis and prognostic and therapeutic implications.</p>	
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<p>Flow cytometry plays an indispensable role in the diagnosis and subclassification of acute myeloid leukemia (AML). Using a multiparametric approach, flow cytometry immunophenotyping has the advantage of efficiency with high sensitivity. This article reviews the general gating strategy, antibody panels for routine analysis, and additional markers for lineage assignment in the subclassification of AML. Also discussed are diagnostic immunophenotypic features of hard-to-classify entities considered within the differential diagnosis of AML. Finally, briefly presented are the principles underlying the use of flow cytometry for minimal residual disease detection.</p>	
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of hematogones, the normal counterpart of leukemic B lymphoblasts. Assessment of multiple flow cytometry markers, in concert with each other in multidimensional histograms, is necessary to distinguish hematogones from malignant blasts. Emerging therapies targeting CD19 and other B-cell markers can disrupt the most frequently used MRD assessment, requiring a revised approach as the use of targeted therapies becomes widespread.

How Do We Use Multicolor Flow Cytometry to Detect Minimal Residual Disease in Acute Myeloid Leukemia?

787

Jie Xu, Jeffrey L. Jorgensen, and Sa A. Wang

Multicolor flow cytometry (MFC), combined with molecular and cytogenetic studies, is the most common method for detecting minimal residual disease (MRD) in acute myeloid leukemia (AML). Studies have shown that a positive MFC MRD study after induction and/or consolidation, or before allogeneic hematopoietic stem cell transplantation, correlates with risk of relapse and inferior survival. However, there is little information on technical and analytical details. This article shares the authors' experience using MFC for AML MRD detection, including antibody panel design, data analysis, and interpretation. It summarizes diagnostic pearls and pitfalls and provides practical information for pathologists or hematologists.

Flow Cytometric Assessment of Chronic Myeloid Neoplasms

803

Min Shi, Phuong Nguyen, and Dragan Jevremovic

Flow cytometry immunophenotyping of the hematopoietic cells from the bone marrow can help with diagnosis, prognosis, and therapy of chronic myeloid neoplasms. Unlike with B-cell neoplasms, there is no simple phenotypic test to substitute for clonality. Therefore, antigen panels to evaluate myeloid neoplasms are larger and the gating strategies more complex than for lymphoid neoplasms. The number of phenotypic abnormalities in hematopoietic cells correlates with disease severity and cytogenetic complexity and can be integrated into a scoring system for diagnostic and prognostic purposes. However, flow cytometry remains only an adjunct diagnostic modality.

Diagnosis of Plasma Cell Dyscrasias and Monitoring of Minimal Residual Disease by Multiparametric Flow Cytometry

821

Kah Teong Soh, Joseph D. Tario Jr, and Paul K. Wallace

Plasma cell dyscrasia (PCD) is a heterogeneous disease that has seen a tremendous change in outcomes as a result of improved therapies. Over the past few decades, multiparametric flow cytometry has played an important role in the detection and monitoring of PCDs. Flow cytometry is a high-sensitivity assay for early detection of minimal residual disease (MRD) that correlates well with progression-free survival and overall survival. Before flow cytometry can be effectively implemented in the clinical setting, sample preparation, panel configuration, analysis, and gating strategies must be optimized to ensure accurate results. Current consensus methods and reporting guidelines for MRD testing are discussed.

- Paroxysmal Nocturnal Hemoglobinuria Assessment by Flow Cytometric Analysis** 855
Mike Keeney, Andrea Illingworth, and D. Robert Sutherland
- Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon but frequently debilitating disease that, if untreated, may lead to death in up to 35% of patients within 5 years. Assessment of PNH clone size by flow cytometric analysis has increased in importance with the availability of therapeutic treatments, which prevent the hemolysis of red blood cells and, hence, the myriad symptoms that accompany the disease. This article addresses flow cytometric methodologies and highlights areas of importance in implementing testing, not only for classic PNH but also for other related bone marrow failure disorders, such as aplastic anemia and low-grade myelodysplastic syndrome.
- Mast Cell Disease Assessment by Flow Cytometric Analysis** 869
Jacqueline M. Cortazar and David M. Dorfman
- Mast cells are present at a low frequency in bone marrow, rendering high-sensitivity multiparametric flow cytometric analysis an ideal method to assess antigen expression on mast cells. This article discusses the normal antigen expression profile of mast cells, established criteria to identify neoplastic mast cells, and new immunophenotypic markers and approaches to identify the presence of neoplastic mast cells in cases of mastocytosis.
- Flow Cytometry in Pediatric Hematopoietic Malignancies** 879
Jie Li, Gerald Wertheim, Michele Paessler, and Vinodh Pillai
- Utility of flow cytometry in the evaluation of pediatric hematopoietic neoplasms and the differences from adult hematopoietic neoplasms are discussed in this article. Distinction of hematogones from B-lymphoblasts, detection of residual/relapsed disease after novel targeted therapies, and evaluation of pediatric myeloid neoplasms are discussed.
- Flow Cytometric Evaluation of Primary Immunodeficiencies** 895
Andreas Boldt, Michael Bitar, and Ulrich Sack
- Primary immunodeficiency diseases are genetic disorders that mostly cause susceptibility to infections and are sometimes associated with autoimmune and malignant diseases. For early detection and management of these diseases, flow cytometric procedures allow an encompassing assessment of cellular phenotypes and cellular functions. State-of-the-art cytometry is based today on 8- to 10-color staining and includes an assessment of lineage maturation and functional markers.
- Cost-effective Flow Cytometry Testing Strategies** 915
Catherine P. Leith
- Cost-effective flow cytometry (FC) requires development of FC panels focused to common diagnoses and strategies to identify cases in which limited FC testing is sufficient. Focused panels include sufficient antibodies to identify common diseases and appropriate analysis strategies

to identify rare diseases that need additional FC testing. Strategies to limit FC testing include the use of algorithms to predict disease probability, with limited FC performed if disease is unlikely. Successful algorithms use easily available parameters, have well-defined rules for use, and are periodically reviewed and updated to maximize efficiency while containing costs.

Automated Analysis of Clinical Flow Cytometry Data: A Chronic Lymphocytic Leukemia Illustration

931

Richard H. Scheuermann, Jack Bui, Huan-You Wang, and Yu Qian

Flow cytometry is used in cell-based diagnostic evaluation for blood-borne malignancies, including leukemia and lymphoma. The current practice for cytometry data analysis relies on manual gating to identify cell subsets in complex mixtures, which is subjective, labor intensive, and poorly reproducible. This article reviews recent efforts to develop, validate, and disseminate automated computational methods and pipelines for cytometry data analysis that could help overcome the limitations of manual analysis and provide for efficient and data-driven diagnostic applications. It demonstrates the performance of an optimized computational pipeline in a pilot study of chronic lymphocytic leukemia data from the authors' clinical diagnostic laboratory.

Applications of Mass Cytometry in Clinical Medicine: The Promise and Perils of Clinical CyTOF

945

Gregory K. Behbehani

Mass cytometry is a novel technology similar to flow cytometry in which antibodies are tagged with heavy metal molecules rather than fluorophores and then detected with time-of-flight mass spectrometry. This method enables the measurement of up to 50 simultaneous parameters with no autofluorescent background and little or no spillover or required compensation. Mass cytometry has tremendous potential for the analysis of highly complex clinical samples for the diagnosis and monitoring of malignant and autoimmune disorders. The technology also presents several unique challenges for clinical use and will require new approaches to analyze the large amounts of data generated.