REVIEW ARTICLE

Role of Flow Cytometry In Diagnostic Pathology

Nitin Gupta, Ram Kumar

Introduction

Flow cytometry is a qualitative and quantitative technology that simultaneously measures and then analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity and relative fluorescence intensity. Flow cytometry is used for immunophenotyping of a variety of specimens, including whole blood, bone marrow, serous cavity fluids, cerebrospinal fluid, urine and solid tissues.These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. Flow cytometric immunophenotyping is an indispensable tool for the diagnosis, classification, staging, and monitoring of hematologic neoplasms. The last 10 years have seen advances in flow cytometry instrumentation and availability of an expanded range of antibodies and fluorochromes that have improved our ability to identify different normal cell populations and recognize phenotypic aberrancies, even when present in a small proportion of the cells analyzed (1).

Principle

Inside a flow cytometer, cells in suspension are drawn into a stream created by a surrounding sheath of isotonic fluid that creates laminar flow, allowing the cells to pass individually through an interrogation point. At the interrogation point, a beam of monochromatic light, usually from a laser, intersects the cells. Emitted light is given off in all directions and is collected via optics that direct the light to a series of filters and dichromic mirrors that isolate particular wavelength bands. The light signals are detected by photomultiplier tubes and digitized for computer analysis. The resulting information usually is displayed in histogram or two-dimensional dot-plot formats (2).

Applications in Diagnostic Pathology

DNA Content Analysis: The measurement of cellular DNA content by flow cytometry uses fluorescent dyes, such as propidium iodide, that intercalate into the DNA helical structure. The fluorescent signal is directly proportional to the amount of DNA in the nucleus and can identify gross gains or losses in DNA. Abnormal DNA content, also known as "DNA content aneuploidy", can be determined in a tumor cell population. DNA aneuploidy generally is associated with malignancy.

DNA aneuploidy correlates with a worse prognosis in many types of cancer but is associated with improved survival in rhabdomyosarcoma, neuroblastoma, multiple myeloma, and childhood acute lymphoblastic leukemia (3,4). In multiple myeloma, ALL, and myelodysplastic syndromes, hypodiploid tumors cells portend a poor prognosis. In contrast, hyperdiploid cells in ALL have a better prognosis (3).

Immunophenotyping Applications in Hematology: Many surface proteins and glycoproteins on erythrocytes, leukocytes, and platelets have been studied in great detail. The availability of monoclonal antibodies directed against these surface proteins permits flow cytometric analysis of erythrocytes, leukocytes, and platelets. Antibodies against intracellular proteins such as myeloperoxidase and terminal deoxynucleotidyl transferase are also commercially available and permit analysis of an increasing number of intracellular markers.In Paroxysmal nocturnal hemoglobinuria antibodies to CD55 and CD59 are specific for decay-accelerating factor and membrane-inhibitor of reactive lysis, respectively, and can be analyzed by flow cytometry to make a definitive diagnosis of PNH (5,6). The flow cytometric enumeration of reticulocytes uses fluorescent dyes that bind the residual RNA, such as thiazole orange (7). Because the fluorescence intensity is directly proportional to the amount of RNA and related to the immaturity of the RBC, a reticulocyte maturity index has been used clinically to assess bone marrow engraftment and erythropoietic activity and to help classify anemias (7,8). Flow cytometry has been used to accurately identify and phenotype the recipient's red cells (9). Flow cytometry is being used increasingly in the blood bank to assess leukocyte contamination in leukocyte-reduced blood products.Immunologic monitoring of HIV-infected patients is a mainstay of the clinical flow cytometry laboratory. The absolute CD4 count provides a powerful laboratory measurement for predicting, staging, and monitoring disease progression and response to

From the Department of Pathology, ASCOMS & Hospital Sidhra, Jammu (J&K)-India
Correspondence to : Dr Ram Kumar, Professor of Pathology, ASCOMS & Hospital Sidhra, Jammu (J&K)-India

treatment in HIV-infected individuals.Flow cytometry has a major diagnostic role in the immunophenotyping of leukemias and lymphomas. Flow cytometry can recognize monoclonality in B cell NHL more often(98%) compared with PCR(68%).For detecting T cell clonality,PCR is more superior(71% vs 29%).

Flow cytometry is very effective in distinguishing myeloid and lymphoid lineages in acute leukemias and minimally differentiated leukemias. Flow cytometry can also be used to identify leukemias that may be resistant to therapy (10). Because of the wide availability and conceptual straightforwardness of immunophenotyping, flow cytometry is the most accessible method for Minimal residual disease detection (11,12). In ALL, phenotype has been shown to correlate strongly with outcome. Flow cytometry can be used to identify neutrophils that lack the CD11/CD18 antigen complex to establish the diagnosis of leukocyte adhesion deficiency syndrome type I that is otherwise difficult to make. Flow cytometry is an excellent method for direct analysis of platelet-bound antibodies, and it has also been shown to be of benefit in detection of free plasma antibodies (13). Flow cytometry is a rapid and useful method of obtaining a diagnosis of Glanzmann thrombasthenia and Bernard-Soulier disease. Flow cytometry is used for the quantification of soluble molecules like OKT3 which is used in treating transplant rejection. Flow cytometric enumeration of CD34+ cells has become widely accepted as the technique of choice to quantify HSCs for the clinical management of stem cell transplantation.

Conclusion

Flow cytometry is a powerful technique for correlating multiple characteristics on single cells. This qualitative and quantitative technique has made the transition from a research tool to standard clinical testingThe ability of flow cytometry to identify and quantify the T cell population subsets CD4 and CD8 lymphocytes identified it as a most important technology in the diagnosis and monitoring of AIDS patients. Similarly, the ability of flow cytometry to make complex multivariate analyses of bone marrow to identify the CD34+ cells and subsequently sort and purify them has been a vital resource in transplantation immunology.Flow cytometry technique requires the passing of the cells through a fluid stream and restricts the analysis to only cell suspension solutions. Since flow cytometry has very sophisticated instrumentation, only skilled and highly trained operators can run it and get any acceptable levels of performance from such an apparatus. Finally the technique is very expensive. Inspite of a few drwawbacks, it is clear that flow cytometry has made a significant impact in diagnostic pathology. There are few technologies that can evaluate so many and diverse parameters on such small samples rapidly.

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