

***Education-Family Physician Corner***

**Application and Disadvantages of Flow Cytometry in Hematopathology: A Brief Review**

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**Flow cytometry (FCM) is an analytical method used for measurement of light scattering and emission of fluorescence produced by cells and chromosomes<sup>1</sup>. It is a laser-based technology used to measure characteristics of biological particles, particularly used to perform measurements on whole cells as well as prepared cellular constituents such as nuclei and organelles in the size range of 0.5  $\mu\text{m}$  to 40  $\mu\text{m}$  diameter<sup>1</sup>. Laser is most often used as a light source in flow cytometry<sup>2</sup>.**

**The first flow cytometry device was made by Wallace H. Coulter in 1953. A decade after Coulter's disclosure, German engineer Wolfgang Göhde introduced the first fluorescent flow cytometry. However, flow cytometry was commercialized in the late 60s by another German named Partec. The use of flow cytometers started to flourish through the 70s and 80s, and gained distinction for use in the laboratory in the 90s.**

**Flow cytometers are used in immunophenotyping, ploidy analysis, cell counting and GFP expression analysis<sup>3</sup>. Specifically, FCM measures volume and morphological complexity of cells, cell ligaments, DNA content, chromosomes, protein expressions and antigens, enzymatic activity, membrane potentials and fluidity, cell apoptosis and viability and HIV monitoring<sup>4-6</sup>.**

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Flow cytometers are very complex instruments composed of four closely related systems. The fluidic system transports particles from a suspension through the cytometer for examination by an illumination. The resulting light scattering and fluorescence is collected, filtered, and converted into electrical signals by the optical and electronics system. The data storage and computer control system saves acquired data and the user interface for controlling most instrument functions. These four systems provide a very unique and powerful analytical tool for researchers and clinicians because they analyze the properties of individual particles and thousands of particles can be analyzed in a matter of seconds. Therefore, data for a flow cytometric sample are a collection of many measurements of a single bulk measurement<sup>7</sup>.

One of the most common ways to study cellular characteristics is through FCM involving the use of fluorescent molecules such as fluorophore-labeled antibodies. These fluorescent molecules attach to a specific molecule on the cell when added to the specimen, either on the inside of the cell or on the cell's surface. Cell characteristics are identified by the flow

cytometer through the process of wavelength to fluorophore. The fluorescent light emitted from the cells sends signals to the detectors and mirrors inside the cytometer as the cells travel along its laser path. The magnitude of fluorescent signal that is generated will be analyzed and recorded by the cytometer and can be read through a graphical output.

## **Application of FCM in Hematopathology**

### **a. In Lymphoid Malignancies**

FCM is able to identify aberrant immunophenotypes, useful in identification and monitoring of  $\beta$ -cell lymphoma subtypes. Because of the heterogenous complexity of certain lymphoid neoplasms, clinicians and even oncologists find it hard to discern the diagnosis. FCM aids the clinicians because of its capability to analyze different characteristics of the cell, together with the antibodies especially in the diagnosis of leukemia and lymphoma<sup>8</sup>. In addition, FCM has efficacy and sensitivity<sup>9</sup>. It has been shown that single-cell phosphospecific FCM can detect and identify aberrant signaling patterns in malignant  $\beta$ -cells<sup>10</sup>. The expression of kappa and lambda light chains can also be detected by FCM<sup>11</sup>. Furthermore, the dependence on the detection of clonal immunoglobulin in the diagnosis of some  $\beta$ -cell lymphomas in contrast to hyperplasia based on the immunoglobulin light chain restriction may be difficult. In flow cytometry, there is a lower IgM expression in small lymphocytic lymphomas compared to what has been shown to have a high level of spa higher expression of these Ig in reactive processes<sup>12</sup>.

FCM could not only define abnormal natural killer cells in neoplasms involving the bone marrow and peripheral blood, but can determine the malignant classification of these neoplasms, tumor clones in T-cell lymphomas and detecting residual disease in  $\beta$ -cell non-Hodgkin lymphomas<sup>13</sup>.

When compared to immunohistochemistry, other studies show that the sensitivity of flow cytometry is almost equally sensitive as immunohistochemistry in diagnosing non-Hodgkin's lymphoma and hairy cell leukemia<sup>14</sup>. Immunophenotyping and purifying classical Hodgkin's lymphoma (CHL) cells from lymph nodes by FCM and flow cytometric cell sorting simplifies the diagnosis of CHL. It complements immunohistochemistry in the detection of light chain restriction in  $\beta$ -cell lymphomas<sup>15</sup>. Routine FCM parameters can aid in differentiating Burkitt's lymphoma and CD10+ diffuse  $\beta$ -cell lymphoma on their mean fluorescent intensity for CD20, CD10, CD38, CD79b, CD43 and CD71, percentage of neoplastic cells positive for CD 71 and even the identification of aberrant immunophenotypes and the rare CD 56 expression in diffuse large  $\beta$ -cell lymphoma<sup>16</sup>. In comparison to histopathology, flow cytometric immunophenotyping is more accurate in differentiating between benign lesions and lymphoma and in classifying non-Hodgkin's lymphoma<sup>14</sup>.

FCM is efficient in the diagnosis of mantle cell lymphoma, bone marrow staging in patients with mantle cell lymphoma after therapy, in lymphomas of the head and neck and in the diagnosis of lymphoproliferative processes of the breast<sup>17</sup>.

### **b. In Leukemias**

The use of multiparameter FCM in the diagnosis of acute leukemias has proved successful in many studies. Its rapid and detailed profiling of the antigens particularly in acute leukemias coupled with a careful investigation of the cellular characteristics often suggests an accurate

and definitive diagnosis in most of the cases<sup>18</sup>. FCM can differentiate malignant hairy cells from normal  $\beta$ -cells and other lymphoproliferative disorders. In differentiation of plasmacytoid dendritic cell leukemia from CD 4(+) CD 56(+/-) undifferentiated or monoblastic acute leukemia, the high intracellular intensity of T-cell leukemia 1 expression is currently considered as the best marker for plasmacytoid dendritic cell lineage assignment<sup>19</sup>.

Flow cytometry offers a quick and effective method in monitoring minimal residual disease in patients with T-cell acute lymphoid leukemia using 4-color 6-color combinations of fluorescent antibodies<sup>20</sup>. Multidimensional flow cytometry can identify leukemic patients with residual disease who are at risk of relapse and have poor outcome, and also on patients who had allogeneic stem cell transplantation for acute leukemia<sup>21</sup>. The combination of CD20, CD10, CD45, and CD19 plus CD38, CD22, CD11a and CD58 was shown to be a cost effective panel for minimal residual disease detection among patients with precursor B-lineage acute lymphoblastic leukemia. In virtually all AML patients, leukemia-associated immunophenotypes (LAIPs) are detectable with MFC<sup>22</sup>. This is also true among cases of chronic lymphocytic leukemia (CLL), where these advanced diagnostics have led to remarkable treatment response rates and complete hematological remissions<sup>23</sup>.

Early detection of neoplastic meningitis by flow cytometric antibody panels ensures rapid institution of therapy that may mitigate the course of the disease in contrast to cytology. Furthermore, the utility of flow cytometry in the investigation of antigens present on neoplastic hematopoietic cells has led to the resolution of certain complex mature lymphoid malignancies<sup>9</sup>. However, despite most of leukemia and lymphoma cases have their specific flow cytometric, cytogenetic and molecular genetic aberrations, chromosomal translocations, gene fusion, JAK2 mutation and other aberrations can occur even in healthy individuals<sup>24</sup>.

### **c. In Multiple Myeloma**

FCM immunophenotyping has been found to be very valuable in the diagnosis, therapy, prognosis and detection of multiple myeloma disease<sup>1</sup>. Flow cytometry immunophenotyping of multiple myeloma provides clinically significant information in the evaluation of peripheral blood and bone marrow diseases<sup>25</sup>. Multiple myeloma (MM) is characterized by plasma cell proliferation and osteolysis destruction. FCM is very useful in differentiating MM from other types of plasma cell disorders, lymphoplasmacytic lymphoma (LPL) and non-Hodgkins lymphoma. Moreover, FCM has also been found to aid the clinicians in the diagnosis and prognostosis of certain rare cases of MM, and in the determination of minimal residual disease (MRD) in a patient under treatment<sup>25</sup>. Immunophenotyping by multiparameter FCM provides significant information of prognosis and minimal residual disease detection in MM and can also distinguish between MM from monoclonal gammopathies of undetermined significance<sup>13</sup>. The differentiating characteristic between myeloma cells and normal plasma cells is the immunophenotypic characteristic of MM using the CD38/SSC gating method, and also on the diversity of the cell antigens of MM<sup>13,25</sup>. In long-term control of MM, immune surveillance can be improved by flow cytometry<sup>26</sup>. The quantification of regulatory T-cells (Tregs) can demonstrate the immune status among MM patients<sup>27</sup>.

### **d. Sickle Cell Disease and Red Cell Disorders**

The use of FCM in sickle cell disease and red cell disorders has gained attention in the 2000s due to FCM's capability to distinguish and analyze a multitude of cell populations in a

sample, particularly the blood cells maturation pathway in the bone marrow<sup>28</sup>. FCM helped clinicians to understand sickle cell disease. Therefore, further research studies have been initiated to understand the pathophysiology, diagnosis and treatment of patients with sickle cell disease<sup>28</sup>.

#### **e. Diagnosis and Monitoring of Paroxysmal Nocturnal Hemoglobinuria**

FCM became the method of choice in the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). The ability of FCM to monitor the outcome of management and follow-up of patients, including monitoring remission and relapse significantly is indispensable<sup>29</sup>.

#### **f. Monoclonal Gammopathies**

Because of its diagnostic, prognostic and predictive significance, there is a need for immunophenotyping in research of monoclonal gammopathy (MG)<sup>30</sup>.

#### **Disadvantages**

Despite the numerous advantages and utility of FCM in various disease entities enumerated above, FCM also presents with some pitfalls in its use. One particularly major disadvantage of FCM is its inability to characterize individual cell's features and activity. The output generated from a FCM analysis is from the whole cell population and not an individual analysis. Another major limitation FCM is the passage of the cell specimen through the fluid stream, thus making it a requirement that specimens for analysis should be in a suspension. Otherwise, specimens not in a suspension has to be subjected to enzyme lysis in order to release the cells from its solid state to a liquid state. The third disadvantage of FCM's use is its inability to process a larger volume of cell population. The cell sorting function of FCM is slow, and inconvenient if you are conducting a major experiment.

Apart from these major problems identified with the use of FCM, there are a few more reasons to disfavor the use of FCM: a highly skilled and well-trained technician should be hired to run and operate the flow cytometer, FCM are very expensive instrument ranging from 75,000 US dollars to a few hundred thousand dollars. They require superior maintenance from its operators, apart from experienced operators should only be the ones allowed to run the machine to have at least an acceptable result. Thus, an institution should not only focus on the purchase of such instrument, but rather consistently monitor the flow of its function and performance to get superior desired results.

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