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Molecular pathology 2020: Interpretation And Diagnosis of Chronic Lymphoproliferative Disorders by Flow Cytometry Using Four-Color Combinations for Immunophenotyping

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Abstract

Background:
"Multiparametric flow cytometry (MFC)" is a powerful tool for the diagnosis of hematological malignancies and has been useful for the classification of chronic lymphoproliferative disorders (CLPD) according to the WHO criteria. Following the purpose of Flow Cytometry , the aim of this report was to standardize the minimum requirements to achieve an accurate diagnosis in CLPDs, considering the different economic possibilities of the laboratories in our country. Most laboratories in India work with 4-fluorescence flow cytometers, which is why this study has been proposed 4-color monoclonal antibody (MoAb) panels .

Multiparametric flow cytometry (MFC) is a powerful tool for the diagnosis of hematological malignancies and has been useful for the classification of chronic lymphoproliferative disorders (CLPD) according to the WHO criteria. Following the purposes of the Brazilian Group of Flow Cytometry (GBCFLUX), the aim of this report was to standardize the minimum requirements to achieve an accurate diagnosis in CLPDs, considering the different economic possibilities of the laboratories in our country. Most laboratories in Brazil work with 4-fluorescence flow cytometers, which is why the GBCFLUX CLPD Committee has proposed 4-color monoclonal antibody (MoAb) panels.

Key terms: chronic lymphoproliferative disorders; flow cytometry; monoclonal antibody panel; lymphoma; multiple myeloma, Flow cytometry principles, Flow cytometry in hematology, immunophenotype characterization, immunophenotype characterization, Immunophenotype characterization for chronic lymphoproliferative disorders

Methods/Results: Panels for screening and diagnosis in B, T and NK lymphoproliferative disorders were developed based on the normal differentiation pathways of these cells and the most frequent phenotypic aberrations. Important markers for prognosis and for minimal residual disease (MRD) evaluation were also included. The MoAb panels presented here were designed based on the diagnostic expertise of the participating laboratories and an extensive literature review.

Conclusion: The 4-color panels presented to aid in the diagnosis of lymphoproliferative neoplasms by Flow Cytometry aim to provide clinical laboratories with a systematic, step-wise, cost-effective, and reproducible approach to obtain an accurate immunophenotypic diagnosis of the most frequent of these disorders.

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Conclusion: The 4-color panels presented to aid in the diagnosis of lymphoproliferative neoplasms by GBCFLUX aim to provide clinical laboratories with a systematic, step-wise, cost-effective, and reproducible approach to obtain an accurate immunophenotypic diagnosis of the most frequent of these disorders. © 2016 International Clinical Cytometry Society.

Introduction:
In modern diagnostics, flow cytometry has an important place as one of the basic and irreplaceable tools for diagnosis, classification, monitoring and prediction of malignant hematological disease . The extreme complexity of these diseases, on one hand, and the availability of the different therapeutic protocols for the different types of these diseases on the other, makes accurate and precise diagnosis imperative. Contributing to this is the fact that the World Health Organization (WHO), in the Classification of Tumours of Hemopoietic and Lymphoid Tissues, suggests a multiparametric approach in diagnosing these diseases; basic parameters required are morphological, immunophenotypic and genetic analysis for each entity of the disease, in addition to a detailed history of the disease and clinical examination. The clinical picture and cell morphology, as a well-known and traditionally-used means of examination, are insufficient in many cases; quite often, because of a similar clinical presentation and cell morphology, it is not possible to draw a diagnostic conclusion based on these findings or a wrong diagnosis may be reached in some cases.

Flow cytometry principles

Flow cytometry is a powerful technology that simultaneously measures many aspects of single particles, usually cells. Any suspended particle or cell from 0.2–150 μm is suitable for analysis. However, it can also measure soluble molecules if trapped onto a particulate surface and bound by fluorochromes. Virtually any component or function of a cell can be measured if the fluorescent probe can be made to detect it.

Sample preparation should provide a homogeneous suspension of cells with monoclonal antibodies conjugated with fluorochromes of a different emission spectrum. Depending on the sample, it most often includes incubation, erythrocyte lysis, centrifugation, washing and fixation.

Becoming more available in clinical laboratories, a wide range of clinical applications of flow cytometry are constantly expanding and the most common among them are in, for example, lymphoma and leukemia diagnosis, stem cell enumeration for transplantation, estimation of minimal residual disease, paroxysmal nocturnal hemoglobinuria diagnosis, immunodeficiencies, HIV infection.

Flow cytometry in hematology

Flow cytometric immunophenotyping enables examination of the phenotype of the separate cells in the suspension and summarizing of the results, which gives data about the presence or absence of antigen expression as well as the expression intensity [5]. Hence, an immunophenotypic pattern is obtained on the cell population of interest for the examined disease. Meanwhile, there are no separate antigens specific for the particular disease. Instead, their mutual relation is observed and analysed, which makes the analysis of the flow cytometry results very demanding and complex, but usually very useful and precise owing to the huge amount of data that can be collected from the cells [6]. Therefore, flow cytometry helps with determining the cell line, the degree of cell maturity, abnormal patterns of expression and provides a detailed immunophenotype of the pathological cell population [7]. From information on all the aforementioned factors, a diagnostic conclusion is drawn if there is a phenotype characteristic for some disease. In the case of an atypical phenotype, the disease is assigned to the appropriate group and additional tests should be done to gain a precise diagnosis (such as immunohistochemical, FISH, molecular tests).

immunophenotype characterization.

The antibody panel for the analysis of the sample to be tested by flow cytometry depends, to a large extent, on the available information of other findings made for that patient. According to the Bethesda Group recommendations from 2006, which were aimed at regulating a more systematic approach in this field (and are still valid today), before sending a sample to flow cytometry, a detailed history of the disease, clinical examination, microscopic examination of cell morphology, and other laboratory tests should be carried out, and based on this, diagnosis or differential diagnosis determined. In this way significant rationalization and cost reduction can be achieved.

Immunophenotype characterization for chronic lymphoproliferative disorders

For both of the two major groups of malignant hematologic diseases, those derived from mature and from immature cells, flow cytometry is of a great importance. Neoplasms of mature lymphoid cells, according to the WHO Classification, include chronic lymphoid leukemia and non-Hodgkin's lymphoma. Their basic characteristic is that they have an immunophenotype similar to mature lymphoid cells and, accordingly, they show an absence of immaturity indicators (CD34, TdT). According to the origin, in relation to the cell line, they can be divided into T, B and NK neoplasms.

In most cases of CLL, cell morphology is characteristic and typical for this disease. However, in a number of cases, flow cytometry has a huge and decisive significance for diagnosis (Fig. 3) [13]. CLL and MCL share many morphological and immunophenotypic features [14]. As a result of their partial overlap, a differential diagnosis of MCL is most considered when making a diagnosis of CLL. Because of the different therapeutic approach and prognoses of the diseases, their diagnostic differentiation is very important. For that purpose cyclin D1 testing is recommended [15, 16]. Unlike the other chronic lymphoproliferations, HCL cells do not match any stage of the normal lymphoid cells development. Morphologically typical HCL cells have fine, hair-like, cytoplasmic projections, which are sometimes difficult to find in the peripheral blood smear. Because of this and a very specific immunophenotype, flow cytometry is essential for HCL diagnosis.

Advantages

The possibility of combining more antibodies in the same tube and analysing their interactions on the population of interest for the given disease is the greatest advantage of multiparametric flow cytometry, which involves simultaneously collecting and analysing a large amount of data from cells or particles.

Considerations

Comprehensive analysis involves considering possible causes of false-positive or false-negative results, thus avoiding an incomplete or incorrect interpretation of flow cytometry data.

Other difficulties, such as non-standardized methods, particularly the issue of regulation in cytometry, different antibody panels, cut-off values, analysis subjectivity – recommended visual approach, result analysis complexity, report form, etc., are the subject of work by various associations dealing with cytometry in order to achieve harmonization.

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