DETECTION OF INTRACELLULAR MYELOPEROXIDASE, CD13, CD79a, CD22, CD3 AND TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE BY FLOW CYTOMETRY DIAGNOSIS OF ACUTE LEUKEMIAS


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Background. Detection of intracellular myeloperoxidase (MPO), cCD13, cCD79a, cCD22, cCD3 and Terminal deoxynucleotidyl transferase (TdT) has become the most specific tool for the assignment of myeloid (MPO and cCD13), B (cCD79a and cCD22) and T lymphoid lineage (cCD3) in acute leukemias. TdT is a useful marker for lymphoid precursor cells and is known as a useful marker for the diagnosis of acute lymphoblastic leukaemia/lymphoma by flow cytometry (FC). It is a DNA polymerase located in the cell nucleus which catalyses the polymerization of deoxynucleotides at the 3'hydroxyl end of oligo- or polydeoxynucleotide initiators without a template. The detection of intracellular cell markers by FC usually requires previous permeabilization of fresh cell suspensions. The aim of this study was to demonstrate the importance of this cell markers detection by FC in the differential diagnostic of acute leukemias.

Methods. Bone marrow and/or peripheral blood leukemic cells from 50 cases of acute leukemia: 16 acute lymphoblastic leukemia (ALL) and 34 acute myeloblastic leukemia (AML). The cells were fixed and permeabilized in briefly exposed to Becton & Dickinson Lyse Solution at concentration of 10%, and subsequently labeled with mouse monoclonal antibodies anti MPO, TdT, CD3, CD13, CD22 and CD79a.

Results. The MPO expression was observed in (35/36) and cCD13 in all cases of AML and in none ALL cases. Three cases of MPO-positive ALL (FAB-L2) could be reclassified as M0-AML. These cases were CD34+/HLADR+/CD33−/CD13−/CD7+ and cCD13+. The intensity of TdT expression was observed in 15/16 (93.8%) ALL, 5/36 (13.9%) of AML. The CD22 and CD79a were positive in 15/16 (93.8%) and 13/16 (81.3%) pre-B ALL respectively and cCD3 was expressed in one case of Pre-T ALL that initial phenotype was CD34+/HLADR+/TdT+/CD7+ and sCD3−).

Conclusions. These results indicate that monoclonal antibodies anti-MPO, cCD13, cCD79a, cCD22, cCD3 and TdT were excellent cell markers for the diagnosis and classification of acute leukemia and can be reliably detected by flow cytometry. This rapid technique should be a valuable addition to routine immunophenotyping of acute leukemia.
CONTRIBUTION OF IMMUNOPHENOTYPING BY MONOCLONAL ANTIBODY BY FLOW CYTOMETRY IN DIAGNOSIS OF ACUTE AND CHRONIC LEUKEMIAS

Background. Today the immunophenotyping by flow cytometry is a useful adjunct to cyt morphological analysis to correct diagnosis of leukemias. It provides objective and quantitative data allowing for a high level of sensitivity of detection and better characterization of acute and chronic leukemias. The purpose of this study was to demonstrate the contribution of the immunophenotyping by monoclonal antibodies (Mo.Ab.) in leukemic cells from patients with acute and chronic leukemias.

Methods. We analysed 76 patients with leukemias before the treatment. The diagnosis of leukemias was based on the morphological and immunophenotyping analysis of leukemic cells from peripheral blood and bone marrow. The cyt morphological analysis was based on French - American - British criteria (FAB classification) in blood and bone marrow films stain by Leishmann and the immunophenotyping by flow cytometry with a panel of Mo.Ab. specific to acute leukemias as: CD1a, CD2, CD3, CD4, CD5, CD8, CD7, CD10, CD13, CD22, CD33, CD34, CD38, HLADR, TdT, anti-myeloperoxidase, TdT, anti-IgM and anti-kappa and lambda light chain. Further clinical and laboratory information as age, sex, presence of tumoral mass, lymphadenopathy, hepatosplenomegaly, hemoglobin determination, total and specific white cell count and cyt morphological analysis of blood film and bone marrow smear.

Results. Thirty four patients presented acute myeloid leukemia (AML), twenty acute lymphoblastic leukemia (ALL), nineteen B cell chronic lymphocytic leukemia (B-CLL), two T cell chronic lymphocytic leukemia and one case was hairy cell leukemia (HCL). Males were more frequently found in all types of leukemias. ALL was more observed in children (age < 15 years old) and AML however was more frequently observed in adult patients. The chronic leukemias were presented in patients with 50 years old or more in all cases. The correlation between the immunophenotyping and clinical pathological profile of these leukemias demonstrated that ALL was more associated to lymphadenopathy, AML to hepatosplenomegaly, and CLL to lymphadenopathy and high count of white cells in peripheral blood.

Conclusions. This date suggest that today the immunophenotyping by flow cytometry is an important methodology to diagnostic and classification of leukemias.
COEXPRESSSION OF P53 PROTEIN AND MULTIDRUG RESISTANCE PROTEINS IN DIFFERENT TYPES OF LEUKEMIAS: THE PREDOMINANT ASSOCIATION IN ADVANCED STATUS OF DISEASE

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Background. One of the best characterized resistance mechanisms of leukemias is multidrug resistance (MDR) mediated by P-glycoprotein (Pgp) and multidrug-resistant related protein (MRP). In addition to Pgp and MRP cell expression, mutation of TP53 gene or inactivation of p53 protein might play a relevant role in therapeutic failure. Some studies have demonstrated that Pgp and MRP may be activated in association with overexpression of mutant or inactivated p53 protein. The aim of this study was to demonstrate the importance of the association between p53 expression and MDR proteins Pgp and MRP by flow cytometry (FC) expression in cells from patients with different types of leukemias.

Methods. Leukemic cells were obtained from 223 patients registered at the Hematology Service, Cancer Hospital, National Cancer Institute (Brazil). This series comprised 64 samples from patients with chronic lymphocytic leukemia (CLL): 34 at diagnosis (CLL/AD) and 30 samples from patients previously treated (CLL/PT) being 9 from patients with Richter’s syndrome (CLL/SR); forty three samples were from patients with acute myeloid leukemia (AML): 27 of which were from de novo, 7 were from relapsed and 9 were secondary ones; forty four samples were from patients with acute lymphoid leukemia (ALL): 36 of which were from at diagnosis and 8 of them in relapse and 72 in chronic myeloid leukemia (CML): 54 in chronic phases, 7 in accelerated phases and 11 in blastic crisis. The p53, Pgp and MRP proteins expression were performed by flow cytometry using monoclonal antibodies stain.

Results. Variable levels of p53 expression were observed in leukemic cells: 17 out of 72 (23.6%) in CML, 14 out of 64 (21.9%) in CLL, 19 out of 43 (44.2%) in AML, and 28 out of 59 (36.4%) in ALL samples. The Pgp expression was: 28 out of 56 (50.0%) in CML, 28 out of 43 (62.8%) in CLL, 27 out of 43 (62.8%) in AML, and 07 out of 35 (20%) in ALL samples. The MRP expression was observed in 23 out of 72 (31.9%) in CML, 22 out of 64 (40.1%) in CLL, 19 out of 43 (44.2%) in AML, and 20 out of 44 (45.5%) in ALL samples. A significant association between p53 and GpP proteins was observed in CML (p=0.00066) and AML (p= 0.0013) but not in CLL (p= 0.15) and ALL (p= 0.32). The association between p53 and MPR was significant in CML (p= 0.000066), CLL (p= 0.0079) and AML (p= 0.00044) but not in ALL (p= 0.83). Also we observed that the phenotypes p53+/Pgp + and p53+/MRP+ were more present in advanced status of disease in all types of leukemias.

Conclusions. The present results demonstrate that Pgp and MRP expression is closely associated with p53 protein accumulation in the advanced stages of leukemias. These data provide evidence to support the idea that mutant p53 protein activates the MDR genes in vivo.
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MONOCLONALITY DETERMINATION BY FLOW CYTOMETRY IN B CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES


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Background. Previous studies have suggested that analysis of the distribution of surface immunoglobulin light chain kappa and lambda by flow cytometry (FC) provides evidence for monoclonality of B cell tumors in patients with B cell chronic lymphoproliferative diseases (B-CLD). The objective is this study is to investigate the efficiency of FC in the determination of the monoclonality in B-CLD through the analysis of kappa and lambda light chain of immunoglobulin expression.

Methods. Samples of peripheral blood from 43 patients with B-CLD were analyzed. They were immunophenotyped by FC with the monoclonal antibody panel composed of CD3, CD4, CD5, CD8, CD10, CD19, CD22, CD23, CD25, CD38, CD45, CD16/CD56, HLADR, anti-heavy chain of immunoglobulin (IgH), anti-heavy chain μ of immunoglobulin (IgM) and anti-light chains (kappa and lambda) of immunoglobulin antibodies. In addition, information about sex, age and clinical and laboratorial data from the patients were also evaluated.

Results. A relation between kappa / lambda higher than 10:1 was found in all samples, suggesting a malignant B cells proliferation with 86% of the samples displayed kappa light chain of immunoglobulin expression and 14% displayed lambda light chain of immunoglobulin expression. The immunophenotyping data associated with the hematological and clinical ones, confirmed 35 cases of B-cell chronic lymphocytic leukemia, 3 cases of B-cell prolymphocytic leukemia, 3 of hairy cell leukemia, 1 case of Waldenström macroglobulinemia and 1 of mantle cell lymphoma.

Conclusions. The results showed that the kappa and lambda light chain of immunoglobulin expression analysis by FC is a simple and effective methodology in the malignant B- cell proliferation identification.