ONCO-HEMATOLOGIA

CITOMETRIA DE FLUXO

UTILIZAÇÃO DA IMUNOFENOTIPAGEM POR CITOMETRIA DE FLUXO PARA IDENTIFICAÇÃO DE NEOPLASIA LINFOPROLIFERATIVA EM AMOSTRA DE CEREBELO: RELATO DE CASO

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Objetivos: Demonstrar a importância da associação da imunofenotipagem por citometria de fluxo com exames histopatológicos para o diagnóstico de neoplasia linfoproliferativa em amostra rara, por meio de um relato de caso de recidiva de linfoma de Burkitt em cerebelo. **Material e métodos:** Os dados clínicos foram coletados de registros do prontuário eletrônico e os resultados dos exames laboratoriais do sistema informatizado da Unidade de Laboratório de Análises Clínicas e do Serviço de Patologia de um hospital universitário. **Resultados:** Paciente do gênero feminino, 55 anos, HIV-soropositivo em tratamento com antirretrovirais e carga viral indetectável com diagnóstico prévio de linfoma de Burkitt estágio IV com acometimento extranodal, ósseo (mandíbula), mama e SNC por exames de imagem e anatomopatológico. Foi realizado tratamento com Hyper-CVAD com boa resposta. Após quatro anos, a paciente retorna ao serviço com tontura, sonolência, náuseas, vômitos e diarreia. Após avaliação de TC de crânio, foi observada lesão expansiva sólida no hemisfério cerebelar esquerdo de cerca de $5{,}0 \times 4{,}8 \times 3{,}0$ cm. A paciente foi encaminhada à cirurgia para coleta de biópsia da lesão. Na análise imunofenotípica por citometria de fluxo da amostra de biópsia foi evidenciada a presença de 72,8% de células linfoides B de grande tamanho, maduras e monoclonais com fenótipo sugestivo de linfoma de Burkitt (CD19+, CD20+FR, CD45++, Lambda+FR, CD38+ homogêneo, CD10+, CD81++, BCL-2+; CD5 e CD39 negativos). Morfologicamente, essas células apresentaram de médio a grande tamanho, citoplasma basofílico com vacúolos proeminentes e cromatina nuclear condensada. Esse resultado foi confirmado pela análise anatomopatológica, o qual foi compatível com linfoma de alto grau (CD20+, CD10+, BCL-2+, BCL-6+, MYC+ e EBV+). O esquema terapêutico escolhido na recidiva foi de rituximabe + HDMTX e citarabina. A paciente segue em acompanhamento ambulatorial. **Discussão:** A aplicabilidade clínica da citometria de fluxo para identificar, analisar e separar células em suspensão de amostras líquidas, como o líquido cefalorraquidiano, já está muito bem estabelecida. No entanto, com exceção de amostras de linfonodo, há limitações quando se trata de tecidos sólidos, devido à necessidade de amostras com elevada viabilidade celular para detectar anormalidades. Recentemente, artigos têm demonstrado a utilização da neurocitometria (citometria de fluxo aplicada ao tecido cerebral) para analisar uma variedade de populações de células cerebrais neuronais e não neuronais. Além disso, sabe-se que o método apresenta alta especificidade e pode confirmar o diagnóstico de linfomas mais rapidamente, quando comparado à análise histopatológica com imuno-histoquímica. A agilidade do diagnóstico é um fator muito importante, considerando que o linfoma de Burkitt apresenta rápida progressão e o prognóstico é fortemente influenciado pelo estágio de acometimento da doença. **Conclusão:** O caso relatado demonstra a importância da imunofenotipagem por citometria de fluxo para o auxílio no diagnóstico ágil de neoplasias linfoproliferativas em amostras de tecidos não convencionais, como o tecido cerebelar. Isso permite o início precoce do tratamento em casos de doenças agressivas como o linfoma de Burkitt.

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ACUTE MYELOID LEUKEMIA WITH CBFB: MYH11 FUSION AND PRESENCE OF ABERRANT MAST CELLS: A CASE REPORT

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Objectives: Acute myeloid leukemia (AML) with CBFB::MYH11 fusion is characterized by the clonal proliferation of monocytic and neutrophilic myeloid precursors and typically an abnormal eosinophilic component. Mastocytosis is an abnormal accumulation of clonal mast cells in organs or tissues and may not be detected when associated with another neoplasm. This article reports a rare case of AML with CBFB::MYH11 fusion with aberrant mast cells, in which the detection of mast cells occurred late, impacting the patient's first diagnosis and prognosis. **Materials and methods:** Clinical data were collected from medical records and laboratory test results were obtained from the Clinical Analysis Laboratory of the University Hospital information systems. **Results:** A 51-year-old male patient was forwarded to a university hospital with fatigue and exhaustion, night sweats, weight loss, loss of appetite, and loss of consciousness during physical effort. Laboratory exams showed: hemoglobin 6.5 g/dL, hematocrit 19.5%, leukocytes 42.770/μL with 43% blasts, and platelets 6.000/μL. The myelogram revealed blasts, immature myeloid cells, and aberrant eosinophils. Immunophenotypic analysis detected the presence of 44.6% blasts (CD34++, CD45+) committed (CD38+) to the myeloid lineage (MPO+, CD13++, CD33+, CD117+, HLA-DR+FR),

20.4% monocytic cells where 70% were mature monocytes (CD14+, CD300e+), 18% promonocytes (CD14+, CD300e-), and 12% monoblasts (CD14-, CD300e-), indicating acute myelomonocytic leukemia. The presence of mast cells (0.1%) was observed. Subsequently, genetic analysis and molecular biology confirmed AML with CBFB-MYH11 fusion and FLT3-D835 mutation. The treatment protocol chosen was ARA-C + Daunorubicin. One month after the beginning of induction chemotherapy, immunophenotyping of the bone marrow showed 0.6% myeloblasts with a phenotype like that found at diagnosis and 0.2% of mast cells (CD117+++), of which 53% have aberrant phenotype (CD2-/+FR, CD25+, CD64-/+, CD203c+, CD123+FR). The patient presented a serum tryptase dosage of 14.5 ug/L (reference value: 11 ug/L), and the KIT-D816 gene was not detected (performed on a blood sample). Six months after the initial diagnosis, the patient achieved remission by morphological and immunophenotyping analysis. **Discussion:** According to the latest classification of hematological tumors established by the WHO, some criteria must be met for the diagnosis of mastocytosis. Although the patient presented the presence of aberrant mast cells in the bone marrow expressing CD2 and CD25, no other criteria for the definition of mastocytosis were met. It is possible to suggest that the patient's diagnosis is systemic mastocytosis associated with a hematologic neoplasm, but more investigations will be necessary to conclude this diagnosis. Patients with mastocytosis and AHN have a worse prognosis, nevertheless, this patient achieved remission of AML with CBFB::MYH11 fusion after induction chemotherapy. **Conclusion:** It is crucial that the diagnosis and monitoring of hematologic neoplasms be carried out in a multidisciplinary manner, combining clinical and laboratory diagnostics using various techniques, particularly flow cytometry and molecular biology. In this case, these techniques were essential for diagnosing the aberrant mast cells, defining the prognosis, and monitoring minimal residual disease.

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DOES PERIPHERAL BLOOD MIRROR IMMUNE TUMOR MICROENVIRONMENT? FLOW CYTOMETRY QUANTIFICATION OF PERIPHERAL BLOOD LYMPHOCYTE SUBPOPULATIONS AND OUTCOMES IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Introduction: DLBCL presents clinical, biological, and prognostic heterogeneity. The composition of the tumor TME strongly contributes for its biological diversity, being able to predict treatment response and determine prognosis. DLBCL with TME enriched in CD8+T-lymphocytes and NK cells demonstrate increased OS compared to those with TME enriched in histiocytes. Data from patients with solid tumors have established associations between absolute quantification of lymphocyte subpopulations (LS) in peripheral blood (PB) and prognosis. Therefore, it is hypothesized that the cellular constituents of PB may mirror the TME, and their quantification may constitute an easy and fast tool to access biological biomarkers predictive of therapeutic response and prognosis in malignancies. This study aims to determine the absolute quantification of LS in the PB of DLBCL patients, seeking associations with outcomes and specific clinical phenotypes. **Methods:** This study involved 54 newly-diagnosed DLBCL patients. PB collection was performed before starting any therapy, for blood count and immunophenotyping on a *BD Facs Calibur* flow cytometer. End points included OS and EFS. Survival curves were constructed using the KM method. The Mann-Whitney test and the Kruskal-Wallis test were used to establish the relationship between the lymphocyte profile, clinical variables, therapeutic response and outcomes. The Contal O'Quingley test was used to determine cut-offs for each LS capable of discriminating outcomes. Univariate analysis was performed using the Cox method and a $p\text{-}value \leq 0.05$ was considered statistically significant. **Results:** The median age at diagnosis was 54 years and 55.6% were female. Clinical stage III/IV was observed in 77.8%, 57.4% had bulky ≥ 7 cm, 63.0% had B-symptoms, and 53.7% presented IPI ≥ 3. The ORR was 80.8%. With a median follow-up of 21.3 months, the estimated 2-year OS and EFS were 73.3% and 64.7%. The median absolute values obtained for the PB lymphocyte subpopulations were: 1026 cells for CD3+ T-lymphocytes, 591 cells for CD4+ T-helper lymphocytes, 412 cells for CD8+ T-cytotoxic lymphocytes, 1.6 for CD4/CD8 ratio, 240 cells for T-reg lymphocytes, 112 cells for LG T-lymphocytes, 173 cells for NK cells, and 70 cells for B-lymphocytes. In univariate analysis, using a cut-off value = 367 CD8+T-cells/mm^3 and 141 NK-cells/mm^3, cytotoxic T-lymphodepletion was associated with decreased OS ($p = 0.005$) and EFS ($p < 0.001$), as well as NK-lymphodepletion was associated with decreased EFS ($p = 0.033$), but not with shortened OS ($p = 0.240$). DLBCL patients presenting higher NK-cell counts had lower rates of chemoresistance to the R-CHOP ($p = 0.024$), and those with higher NK-cell counts ($p = 0.033$) and higher CD8+T-lymphocyte counts ($p = 0.039$) had lower probability to develop relapse, progression or death, confirming the central role of these PB cellular constituents in anti-tumor immunity. We observed that B-cell lymphodepletion was associated with an adverse clinical phenotype, including higher frequency of bulky disease ($p = 0.009$), B-symptoms ($p < 0.001$) and advanced clinical stage ($p = 0.033$). **Conclusion:** In newly-diagnosed DLBCL, the lymphodepletion of CD8+T-cells and NK cells quantified in PB were associated with decreased OS and EFS. Similarly, peripheral T-cytotoxic and NK lymphodepletion were associated with absence of response to the R-CHOP