

ABSTRACTS FLOW CYTOMETRY

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IMPORTANCE OF FLOW CYTOMETRY IN THE RELATIONSHIP OF TOLOSA HUNT SYNDROME WITH LYMPHOMA: DIRECT CAUSE OR INDICATOR OF POOR PROGNOSIS?

Viviana Farino, Mariana Richard

Hospital "San Martin", Paraná-Entre Ríos, Argentina

Introduction: Hunt Syndrome (THS) is a rare pathology, defined as an idiopathic granulomatous inflammation of the cavernous sinus or the superior orbital fissure, which could act as a favorable microenvironment for lymphoproliferative development or, in patients with lymphoma, the involvement of the cavernous sinus could represent an early manifestation of aggressive systemic disease. Flow cytometry (FCM) emerges as a key tool for its study. **Clinical case presentation:** A 41-year-old male patient with a history of high blood pressure and no toxic habits consulted the ophthalmology department for unilateral painful ophthalmoplegia. On 8/28/2023, he was diagnosed with STH. On 10/18/2023, he was readmitted to the hospital to the Neurology department due to a worsening of the initial clinical presentation, with the addition of pain in the lower limbs for more than two weeks and facial paresis with sensory disorders. **Diagnostic Studies:** - Routine laboratory and immunological tests: no particularities; - Serological and virological studies: Negative; - Imaging study: CT scan of the head: no lesions. NMR: increased size of the previous right sellar-parasellar lesion with total occupation of the homolateral cavernous sinus; - CSF studies: Cyto-physicochemical (CFQ): cell count $1000/\text{mm}^3$ with mononuclear predominance. Bacteriological and VDRL: negative. -Cytological: positive for neoplastic cells with poor differentiation. Differential diagnosis with lymphoid origin. 07/11/2023: Consultation with Hematology, performance of CMF and Cytomorphology in CSF and bone marrow aspiration (BM). FCM in CSF: 97% of medium-sized cells with medium/low internal complexity, expressing: CD 45+, CD19++, heterogeneous CD 20++, CD79b+/-, co-expressing: CD10+, CD38++, CD81++. Negative for: CD5, CD11c, CD103, CD95, CD200, CD43 2531-1379/

and CD25. Clonal Lambda. FCM in MO: no evidence of infiltration due to a lymphoproliferative process. Cytomorphology: BM: hypercellular, polymorphic, megakaryocytes present. CSF: medium to large lymphocytes, round nucleus, fine chromatin and prominent nucleolus, basophilic cytoplasm with vacuoles, "starry sky" pattern. **Discussion:** Usually, STH presents with normal CSF CFQ, unlike this patient with an elevated mononuclear leukocyte count and negative bacteriology. Although the CSF pathology report was indicative but not conclusive, FCM turned out to be a high-impact test in diagnostic accuracy, allowing to demonstrate CNS involvement due to a lymphoproliferative process, clonal Lambda CD10+ CD95- B-NHL, concordant with Cytomorphology. It is worth mentioning that the FCM study was requested late due to the delay in the hematology consultation, which negatively influenced the diagnosis, prognosis and treatment of the patient, who died on 11/19/2023. Postmortem frozen biopsy B-NHL lymphoma (Burkitt). **Conclusion:** The association between STH and Lymphoma should be considered in cases of atypical presentation or suboptimal response to conventional treatment. FCM is a fundamental tool for early diagnosis due to its high sensitivity and diagnostic accuracy in CSF samples, allowing for the establishment of an effective treatment that changes the patient's prognosis. Although the results were consistent with the biopsy, the latter required a more representative sample for the diagnosis, which was obtained post-mortem.

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CASE REPORT: LARGE GRANULAR T-CELL LYMPHOMA/ LEUKEMIA WITH THE PRESENCE OF TWO CLONES

Flávia Arandas de Sousa, Elizabeth Xisto Souto, Laiz Cameirão Bento, Marília Sandoval Passaro, Nydia Strachman Bacal

Clinical Flow Cytometry Laboratory, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil

Introduction/Objective: The diagnosis of mature T-cell lymphoid neoplasms can be particularly challenging due to their overlapping features with reactive T cells. However, the development of specific antibodies targeting the mutually exclusive β -chain isoforms of the T-cell receptor, TRBC1 and TRBC2, has made it possible to assess the restriction of these chains and establish clonality evaluation. This report presents an uncommon case with an immunophenotype suggestive of "T-cell Large Granular Lymphocytic Leukemia," characterized by the presence of two clones: one in the CD4 compartment exhibiting TRBC1 monoclonality and another in the CD8 compartment exhibiting TRBC2 monoclonality. **Case Report:** A 66-year-old male patient with a normal physical examination. The complete blood count showed: hemoglobin 5.27 g/dL, platelets 207,000/mm³, leukocytes 16,000/mm³, neutrophils 6,864/mm³, eosinophils 448/mm³, monocytes 928/mm³, and 7,696/mm³ lymphoid cells with a mature appearance, nuclear irregularities, and abundant granular cytoplasm. A peripheral blood sample was submitted for immunophenotypic evaluation by flow cytometry, which revealed 33.8% T lymphocytes expressing CD2, CD3, CD5 (dim expression), CD56, CD57, and TCR alpha/beta. Of these cells, 20.9% exclusively expressed CD4 and TRBC1, while 12.9% exclusively expressed CD8 and TRBC2, identifying two distinct populations of monoclonal T lymphoid cells with an immunophenotype consistent with T-cell "Large Granular" lymphoid cells. **Discussion:** The diagnosis of mature T-cell lymphoid malignancies is complex and often challenging due to the immunophenotypic overlap between neoplastic T cells and non-neoplastic reactive T cells. The development and implementation of specific antibodies have led to significant advances in clonality assessment. Among these markers, TRBC1 and TRBC2 are especially useful in identifying specific neoplastic clones. The use of these markers has greatly improved the differentiation between neoplastic and reactive T cells, thereby increasing diagnostic accuracy. **Conclusion:** We report a rare case of two distinct mature T-cell lymphoid clones in a case suggestive of "T-cell Large Granular Lymphocytic Leukemia," with few prior cases documented in the literature. This highlights the importance of assessing T-cell clonality using TRBC1 and TRBC2 markers. Advances in this field have been crucial for achieving faster and more accurate diagnoses, which in turn facilitate more effective treatments and better clinical outcomes.

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CASE REPORT: B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (PH+) WITH IMMUNOPHENOTYPIC SHIFT TO MIXED-PHENOTYPE ACUTE LEUKAEMIA B/MYELOID

Laiz Cameirão Bento, Elizabeth Xisto Souto, Flávia Arandas de Sousa, Bruna Garcia Nogueira, Nydia Strachman Bacal

Clinical Flow Cytometry Laboratory, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil

Introduction/Objective: The shift in immunophenotype from B-cell Acute Lymphoblastic Leukemia (B-ALL) to Acute Myeloid Leukemia (AML) is a rare phenomenon known as a "phenotypic switch." The aim of this report is to describe a case of B-cell Acute Lymphoblastic Leukemia with an immunophenotypic shift to a Mixed-phenotype Acute Leukaemia B/myeloid. **Case Report:** A 37-year-old male patient presented with complaints of lesions on his tongue, right inguinal region, and buttocks, along with red spots on the skin. One day prior to seeking medical attention, he experienced gum bleeding lasting about an hour. The patient was obese (200 kg). Complete blood count: Hb 10.6 g/dL; Leukocytes 120,290/mm³ (80% small-sized cells, high nucleus-to-cytoplasm ratio, loose chromatin, evident nucleoli, and agranular basophilic cytoplasm); Platelets 5,000/mm³. Bone marrow aspiration was not performed due to technical difficulties. Peripheral blood immunophenotyping: Consistent with B-ALL, showing 65.9% low-complexity cells, dim/negative expression of CD45, and positivity for CD19/CD13/CD25/CD33/CD34/CD38/CD58/cyCD79a/CD123/CRFL-2(dim)/TdTnu, with absence of CD3sm/CD3cy/CD10/CD15/CD20/CD22/CD117/cyMPO/cyIgM. Cerebrospinal fluid: 1.6% positive for CD19/CD34/CD38/CD45 and negative for CD3/CD10/CD14/CD20/CD56. FISH: BCR::ABL1 rearrangement t(9;22). Karyotype: 46,XY,t(9;22)(q34;q11.2)[1]/45, idem, -7[19]. Lymphoid panel in peripheral blood: Presence of RUNX1 and BCR::ABL1. The patient was treated with Hyper CVAD + dasatinib and chemotherapy. On Day 20 of treatment, a bone marrow and cerebrospinal fluid reassessment was performed. Bone marrow: 78.0% small to moderately sized cells, high nucleus-to-cytoplasm ratio, nucleus with loose chromatin, and evident nucleoli, occasionally convoluted, basophilic cytoplasm with granules. Immunophenotype: Presence of two cell populations: one with 76.9% myeloid blast cells expressing CD4/CD11b/CD13/CD19/CD33/CD34/CD36/CD38/CD45/CD64/CD71/CD117/CD123/HLA-DR/cyMPO, and negative for CD2/smCD3/cyCD3/CD7/CD10/CD14/CD15/CD20/CD22/CD56/CD61/cyCD79a/IREM-2; and another with 8.3% lymphoid blast cells expressing partial CD10/CD13/CD19/CD22/CD34/CD38/CD58/cyCD79a/CD123/cyIgM (partial), and negative for smCD3/cyCD3/CD117/CD15/CD20/CD25/CD33/CD45/cyMPO/CRFL2. Cerebrospinal fluid immunophenotyping: 13.8% positive for CD34/CD38/CD45/CD64/CD117 and negative for CD3/CD10/CD14/CD19. The patient was treated with FLAG-IDA + Venetoclax + Ponatinib, but developed severe neutropenia, multi-drug-resistant Klebsiella pneumoniae infection, septic shock, and died on Day 17 of the chemotherapy cycle. **Discussion:** The shift in the leukemic cell lineage (lymphoid or myeloid) during the disease course is rare, and the mechanisms involved are not fully understood. These shifts may represent the expansion of a pre-existing clone prior to therapy, clonal evolution, or the development of a new clone. According to the International Consensus Classification of Acute Leukemias 2022, B-ALL Ph+ can be subdivided into two subtypes: "multilineage involvement" and "lymphoid-only involvement." However, there is still no consensus on how to classify these cases. In a large cohort of patients with B-ALL Ph+, Bastian et al. characterized two groups based on transcriptomic and genomic profiles. The "multilineage involvement" group exhibited a genomic pattern of HBS1L deletion