HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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ABSTRACTS FLOW CYTOMETRTY

1

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

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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: Cytophysicochemical (CFQ): cell count 1000/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 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.

https://doi.org/10.1016/j.htct.2025.103992

2

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

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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/mm^3, leukocytes 16,000/mm^3, neutrophils 6,864/mm^3, eosinophils 448/mm^3, monocytes 928/mm^3, and 7,696/mm^3 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.

https://doi.org/10.1016/j.htct.2025.103993

3

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

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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/mm^3 (80% small-sized cells, high nucleus-to-cytoplasm ratio, loose chromatin, evident nucleoli, and agranular basophilic cytoplasm); Platelets 5,000/mm^3. 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

and monosomy 7. **Conclusion:** The occurrence of immunophenotypic shifts in B-ALL Ph+ highlights the need for further studies to establish the prognostic value of molecular classifications in these leukemias. This, in turn, will help define the most appropriate therapeutic approach for each subtype.

https://doi.org/10.1016/j.htct.2025.103994

4

NEOPLASIA MIELOIDE COM REARRANJO NO GENE FLT3 REFRATÁRIA QUE APRESENTA COMPROMETIMENTO COM A SÉRIE GRANULOCÍTICA E ERITROIDE

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Paciente do gênero masculino, 20 anos de idade procurou atendimento com quadro de fadiga, tosse produtiva, astenia intensa de padrão progressivo e linfonodomegalias em região cervical bilateral. No hemograma foi evidenciado anemia (4,3 g/dL), leucocitose (52.820/uL) com presença de 84% de blastos e plaquetopenia (17.000/uL). A imunofenotipagem por citometria de fluxo da amostra de medula óssea detectou 51,8% de blastos (CD117+, HLA-DR+FR, CD45+) comprometidos (CD38+) com a linhagem mieloide/granulocítica (MPO+, CD13+, CD33+ +, CD64+FR); além de 2,0% de blastos com comprometimento à série eritroide (CD117+, CD36++, CD105++, CD71++; CD38, HLA-DR, CD42b, CD203c, CD13 e CD33 negativos) e 3,1% de basófilos (CD123+, CD203c+, HLA-DR negativo) com ausência de expressão de CD22. Além disso, foi detectada a presença da mutação FLT3-DIT pelo PCR qualitativo. O paciente realizou o tratamento quimioterápico de indução 7+3. Após 17 dias do início do tratamento, apresentou leucopenia (860/uL) com 6% de blastos em sangue periférico. A análise imunofenotípica da medula óssea evidenciou a presença de 49% de blastos (CD117 +, CD45+, CD34 negativo) comprometidos (CD38+) com a linhagem mieloide/granulocítica (MPO+, HLA-DR+, CD13+, CD33++; CD36, CD71, CD7, CD19 e CD56 negativos) e 9,9% de blastos (CD117+, CD45+, CD34 negativo) comprometidos com a série eritroide (CD36++, CD105++, CD71++; CD38, HLA-DR, CD42b, CD203c, CD13 e CD33 negativos) semelhantes ao diagnóstico. Além disso, foram observadas 2,6% de células da série basofílica (CD203c+, CD33+, HLA-DR negativo), com ausência de expressão de CD22. Do total de basófilos, 50% apresentam características de imaturidade (CD45+FR, CD117+FR, CD13+FR, CD11b+FR). Paciente evoluiu com quadro de neutropenia febril e sepse de provável foco cutâneo (lesões de acne e abscesso perianal) com hemocultura positiva para *Klebsiella pneumoniae* co-produtora de KPC e NDM. Assim, para atingir estabilidade clínica, iniciou protocolo com alta dose de ARA-C associado a inibidor de FLT3. O paciente foi encaminhado ao transplante de medula óssea. A neoplasia com rearranjo no gene FLT3 (fms-like tyrosine kinase 3) é uma doença de células-tronco hematopoiéticas que pode se manifestar com características de SMD, neoplasias mieloproliferativas, mielodisplásicas/mieloproliferativas ou leucemia aguda de linhagem mieloide, linfoide B ou T. Associa-se a um prognóstico desfavorável, com evolução rápida e com alta mortalidade. Os pacientes geralmente apresentam anormalidades em sangue periférico (leucocitose, anemia e plaquetopenia) e podem apresentar linfonodomegalias e esplenomegalia. A maioria dos pacientes é diagnosticado na fase crônica da doença e, os casos em que há mais de 20% de blastos no momento do diagnóstico, devem ser classificados como neoplasia mieloide ou linfoide com a mutação FLT3 em fase blástica. Considerando a necessidade de esclarecimento da linhagem celular afetada, a imunofenotipagem é essencial para um diagnóstico preciso e direcionamento do tratamento. No caso do paciente relatado, as células apresentam comprometimento mieloide/granulocítico/eritroide). O FLT3 é um receptor de tirosina quinase que desempenha um papel importante na proliferação, diferenciação e sobrevida celular e, quando mutado aumenta a proliferação celular e inibe a apoptose. Dessa forma, alguns pacientes obtêm respostas hematológica e citogenética com a utilização de fármacos inibidores de FLT3, no entanto, a resposta parece ser de curta duração e o transplante alogênico é indicado.

https://doi.org/10.1016/j.htct.2025.103995

5

T-CELL CLONES OF UNCERTAIN SIGNIFICANCE/ T-CUS

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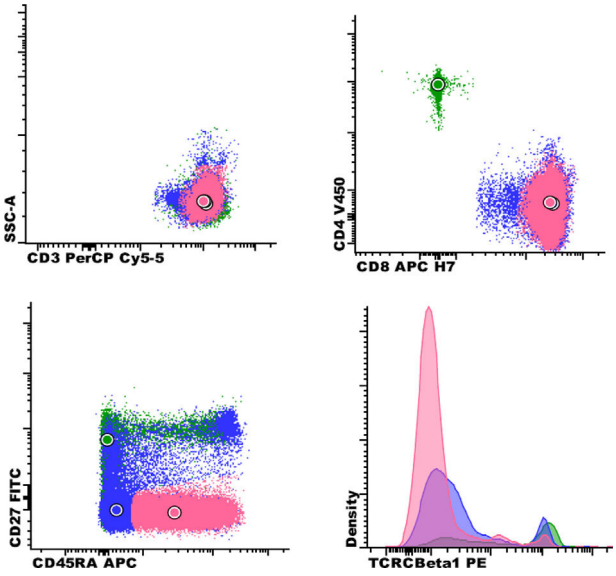
Female patient, 56 years old, with leukocytosis and lymphocytosis and Lymphoma suspicion - Peripheral blood sample with white blood count 23,200 cells / TCD8+ restriction (TCD4/ TCD8 ratio 0.1:1). Panel, Immunophenotyping and Gating Strategy. Triage of all the samples was analyzed in the LST tube. The TCRCBeta 1 tube was designed based on EuroFlow's Orientation Tube for Immunodeficiencies (PIDOT), using CD3, CD4, CD8 and CD45 as "backbones", CD27 and CD45RA to discriminate T-cell subsets, CD16 and CD56 to exclude NK cells, and including TCRCBeta1 in the PE channel. **Results:** 59% T Cells: *52.5% TCD8+: 39.4% TD (terminally differentiated) phenotype with increased absolute levels (9,141 cells/μL - normal age range 8-500) CD7dim CD27(-) CD45RA-/+ TCRCBeta1+5% (monoclonal); 0.7% *Naive* CD45RA+ CD27+ TCRCBeta1+30% (polyclonal), 1.2% CM/TM (central/terminal memory) CD45RA (-) CD27+ TCRCBeta1+19% (polyclonal); 9.7% EM (effector memory) phenotype with increased absolute levels (2,250

cells/μL - normal age range 2-323) CD45RA(-) CD27+ TCRCBeta1+5% (monoclonal). *4.9% TCD4+ TCRCBeta1+44% (polyclonal). **Immunophenotype:** CD45++ CD3+ CD8+ CD38dim CD45RA-/+ CD45RO-/+ CD2+ CD5++ CD7+FR TCR ALPHA-BETA+ PERFORIN+ TCRCBETA1 ++5%; Negative expression: CD25 CD26 CD27 CD28 TCL1 CCR7 TCR GAMMA-DELTA CD11C CD30 CD16 CD56 CD94. **Morphology:** In the analyzed smear, 32% of atypical medium-sized lymphoid cells were observed, with a globose nucleus, generally eccentric, with poorly condensed chromatin with an outline of a nucleolus, and a moderately basophilic, polarized and granular cytoplasm. The inicial diagnostic hypothesis was T-Lymphoma, NOS (the authors judged that there were no sufficient NK antigens expressed to characterize T-LGL). However, further investigation showed the patient had no identifiable T Cell lymphoma, and she eventually was diagnosed with Thrombotic Thrombocytopenic Purpura, with classic symptoms and laboratory confirmation. Since the implementation of TRBC1 for detection of abnormal T-cells, persistent clonal expansion of large granular lymphocytes (T-cell clones of uncertain significance/ T-CUS) have been reported in a variety of clinical conditions, such as hematological neoplasms and autoimmunity. The incidence of T-CUS increases with age, and probably represents reactive T-cell small clones (so called immunoclones), with less than 20% of total lymphocytes or 400 cells/mm3, highly prevalent in patients without T-cell malignancy. Most reactive T-CUS cases are CD8+ or CD4+/CD8+ double-positive.



T-CUS in pink, normal TCD4+ cells in green and normal TCD8+ cells in dark blue.

https://doi.org/10.1016/j.htct.2025.103996

6

POST-TRANSPLANT DOUBLE-POSITIVE T-CELL LYMPHOMA

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Male patient, 54 years old, post-transplant immune profile, bone marrow transplantation for B-cell Non-Hodgkin's-Lymphoma in 2019 - Peripheral blood sample with white blood count 1,800 cells post-transplant immune profile - marked (42%) Double-Positive proliferation. **Panel design, Immunophenotyping and Gating Strategy:** Triage of all the samples was analyzed in the LST tube. The TCRCBeta 1 tube was designed based on EuroFlow's Orientation Tube for Immunodeficiencies (PIDOT), using CD3, CD4, CD8 and CD45 as "backbones", CD27 and CD45RA to discriminate T-cell subsets, CD16 and CD56 to exclude NK cells, and including TCRCBeta1 in the PE channel. **Results:** 57% T Cells: *42% Double-positive CD3+ CD7(-) CD27(-) CD45RA+ TCRCBeta1+100% (monoclonal); *7.5% TCD4+ EM CD45RA(-) CD27(-) TCRCBeta1+ 38% (polyclonal); *7.5% TCD8+ EM CD45RA(-) CD27(-) TCRCBeta1+ 38% (polyclonal). **Immunophenotype:** CD45+ CD3+ CD4+ CD8+ CD26-/+ CD45RA+ CD2+ CD5+ CD11C+ CD57-/+ TCD ALPHA-BETA+ TCRCBETA1+. Negative expression: CD7, CD28, CD45RO, CD25, CD30, TCRGAMA/DELTA, CCR7, CD56, CD16, TCL1. The abnormal Double-positive T-cell population was subsequently detected with a bone marrow sample. The diagnostic conclusion was T-cell Lymphoma, NOS.

https://doi.org/10.1016/j.htct.2025.103997

7

CASE REPORT: BICLONAL SÈZARY SYNDROME

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Introduction: Sézary Syndrome (SS) is defined by the triad of erythroderma, generalized lymphadenopathy, and the presence of clonal T cells with cerebriform nuclei (Sézary cells) in peripheral blood. In addition, one or more of the following criteria are required: an absolute Sézary cell count $\geq$ 1000/μL, an expanded CD4+ T-cell population resulting in a CD4:CD8 ratio of $\geq$ 10, and loss of one or more T cell antigens. T cells have a CD3+, CD4+, CD8(-) phenotype, and characteristically lack CD7 and CD26. The normal counterparts of Sézary cells are circulating central memory T cells (CD27+, CD45RA(-), CD45RO+). According to the degree of circulating involvement, Staging B ("blood") for Sézary syndrome follow these

criteria: B0: circulating Sezary cells (Fig. 2A) below 250 cells/mm3 or < 5% atypical lymphoid cells in the smear; B1: circulating Sezary cells < 1000 cells/mm3 or 5-20% atypical cells in the smear; B2: circulating Sezary cells above 1000 cells/mm3 or > 20% atypical lymphoid cells in the smear. **Report:** Male patient, 88 years old, previous diagnosis of Mycosis fungoides, Peripheral blood sample with white blood count 18,000 cells, 25%TCD4+ and 9.3% Double-Positive Alfa/Beta populations with dim expression of TCD3. Flow Cytometry: 41.5% T Cells: *25% TCD4+ CD3dim CD7+/++ CD27+ CD45RA(-) TCRCBeta1(-)100% (monoclonal); *9.3% Double-negative TCRAlfa/Beta+ CD3dim CD7+ CD27+ CD45RA(-); TCRCBeta1(-) 100% (monoclonal); *3% TCD4+ TCRCBeta1+ 36.5% (polyclonal); *3.9% TCD8+ TCRCBeta1+28% (polyclonal). Negative expression: CD8 CD30 CD38 CD45RA CD56 CD57 CD94 TCL1 TCRCBETA1 TCR GAMMA/DELTA. **Comments:** The normal counterparts of Sézary cells are circulating central memory T cells (CD27+, CD45RA(-), CD45RO+); the phenotype profile in our case, with 2 identifiable clones (one TCD4+ clone and one Double-Negative Alfa/Beta clone), matches this description.

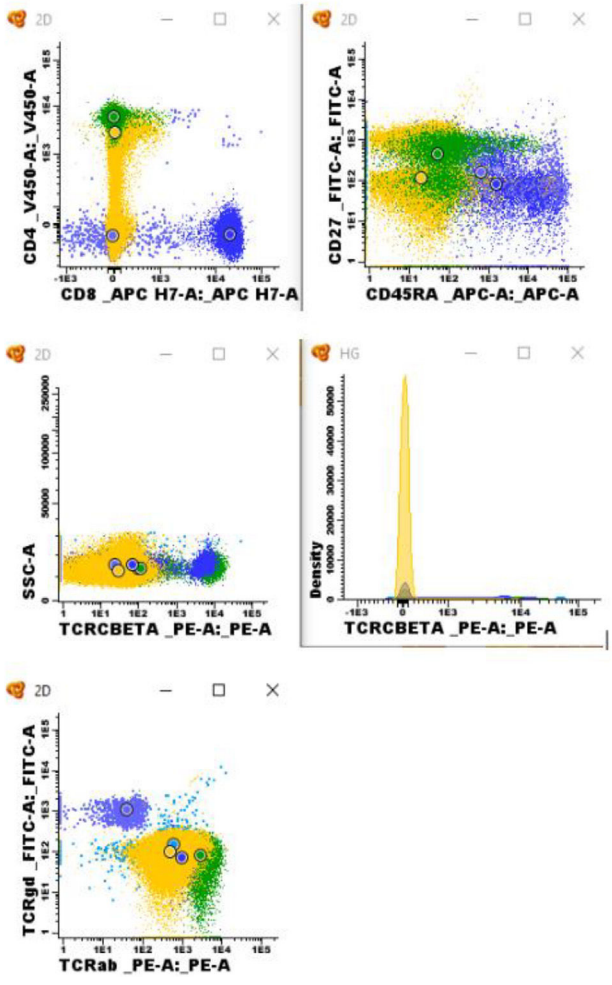

Figure 1 Sézary cells in yellow, normal TCD4+ cells in green and normal TCD8+ cells in dark blue.

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https://doi.org/10.1016/j.htct.2025.103998

8

CASE REPORT: DOUBLE-POSITIVE T-LARGE GRANULAR LEUKEMIA

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Introduction: T-LGL is a rare disease (accounts for 2 to 5% of chronic lymphoproliferative disorders), indolent and often

asymptomatic, and mainly characterized by cytopenias (primarily neutropenia, predisposing to infections). T-LGL leukemia patients may present with recurrent bacterial infections owing to (severe) neutropenia, anemia, and hepatosplenomegaly, but one-third of patients appear to be asymptomatic at diagnosis. T-LGL arrives from expansions of effectors T-cells, CD45RA+/CD28(-)/CD27(-)/CD94+/ – with variable expression of CD57, usually TCD8+ TCR Alpha/Beta. It is important to distinguish T-LGL from reactive LGL proliferation, which is frequent, particularly in the context of viral infections, autoimmune diseases, after splenectomy or in posttransplant patients. Diagnosis of LGL leukemia is based on two mandatory criteria which help to differentiate it from reactive LGL lymphocytosis: cytological identification of lymphocytes with granules >500 cells/mm3 observed at least over 6 months, and proof of clonality. **Report:** Female patient, 64 years old, leukocytosis, lymphocytosis and B-CLL suspicion, Peripheral blood sample with white blood count 35,500 cells - marked (93%) T Double-positive proliferation. **Results:** Flow Cytometry: 93% Double-positive CD4++ CD8+ CD3++ CD2++ CD5++ CD7-/+ CD27(-) CD45RA+ TCR Alpha/Beta+ TCRCBeta1+100% (monoclonal); dim expression of CD56 and CD57; Negative expression: CD25, CD26, CD27, CD28, CD45RO, CD94, CCR7, TCL1, TCR Gamma-Delta (Figure 1). **Morphology:** in the analyzed smear, predominance of atypical medium-sized lymphoid cells was observed, with a globose nucleus, generally eccentric, with poorly condensed chromatin with an outline of a nucleolus, and a moderately basophilic, polarized and granular cytoplasm (Figure 1).

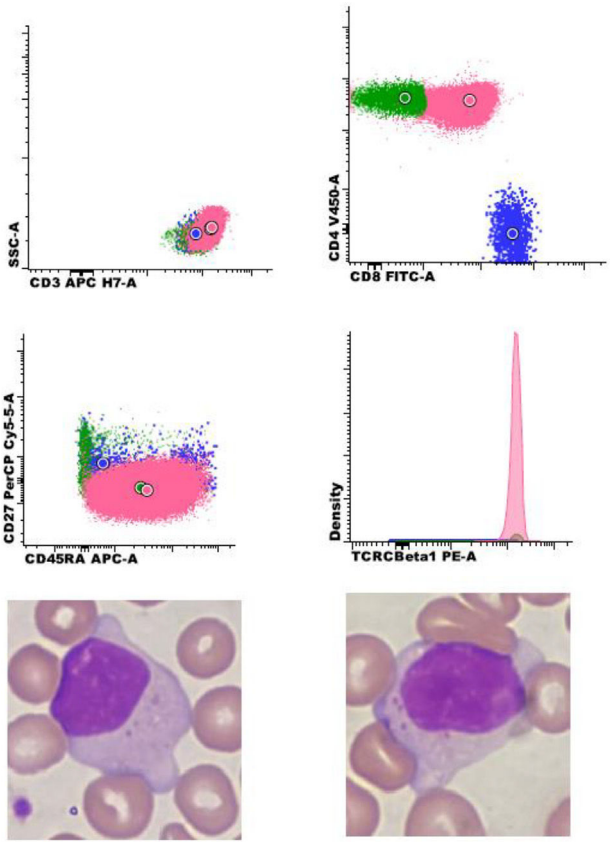

Figure 1 Flow Cytometry: Double-Positive LGL clone (pink; normal TCD4+ cells in green and normal TCD8+ cells in dark blue). Morphology: atypical medium-sized lymphoid cells was observed, with a globose nucleus, generally eccentric, with poorly condensed chromatin with an outline of a nucleolus, and a moderately basophilic, polarized and granular cytoplasm.

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https://doi.org/10.1016/j.htct.2025.103999

9

Case Report: TCD8 Proliferation Secondary To CMV In A Post Transplant Patient.

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Introduction: CD4+ and CD8+ lymphocytes regenerate within the first year after Bone Marrow Transplant (BMT). The survival and peripheral expansion of donor memory T-cells transfused with the graft is the dominant mechanism in the first year after the BMT, with the predominant expansion of TCD8+ cells. This explains the inverted CD4/CD8 ratio detected in post-BMT patients. That is the reason why it is not a surprise to find elevated TCD8 cells in an immune profile of a patient submitted to bone marrow transplant. However, post-transplant patients are prone to viral infections, and CD8 T cells display unique profiles depending on their viral specificity: Cells are predominantly CCR7+ CD27+ CD28+ during latent infection with HCV, CCR7(-) CD27+ CD28+ in EBV, CCR7(-) CD27+ CD28(-) in HIV, and CCR7(-) CD27(-) CD28 (-) in CMV. **Report:** Female patient, 25 years old, Previous diagnosis of B-Acute Lymphoid leukemia, D+180 after bone marrow transplantation, post-stem cell transplant immune profile, with progressive lymphocytosis, Peripheral blood sample with white blood count 6,900 cells. **Results:** 58% T Cells (4/8 = 0.2): *46.2% TCD8+ TCRCBeta1+36% (polyclonal) 21.75% TD phenotype with increased absolute levels (1501 cells/μL - normal age range 1-384) CD45RA+ CD27(-), 21.45% EM phenotype with increased absolute levels (1480 cells/μL - normal age range 5-69) CD45RA(-) CD27(-), 0.2% *Naive* CD45RA + CD27+, 2.8% CM/TM CD45RA(-) CD27+; *9.8% TCD4+ TCRCBeta1+36,5% (polyclonal) 0.6% *Naive* CD45RA+ CD27+, 2.5% CM/TM CD45RA(-) CD27+, 0.1% TD CD45RA+ CD27(-, 6.6% EM CD45RA(-) CD27+ (Fig. 1). **Conclusion:** peripheral blood sample showing a relative (58%) and absolute (4,005/mm^3) increase in mature (CD3+) T lymphoid cells (CD45++), with a predominant phenotype (42%) of polyclonal effector (terminally differentiated) CD8+ T cells / memory effector cells (CD27(-) CD28(-) CCR7(-) CD45RA+/- CD45RO-/+), with negative CCR7/CD27/CD28 expression, a phenotype suggestive of viral infection, most likely CMV, later confirmed with serologic tests.

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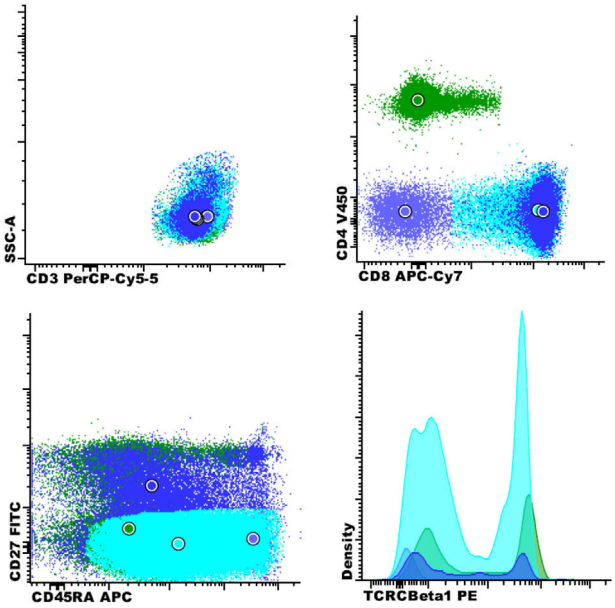

Figure 1 Normal double-negative Gamma/Delta cells in purple; normal TCD4+ cells in green; normal TCD8+ cells in dark blue; TD CD8+ cells in turquoise.

https://doi.org/10.1016/j.htct.2025.104000

10

CASE REPORT - T/NK LYMPHOMA

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Introduction: Less than 10% of chronic lymphoproliferative diseases are caused by T lymphocytes and NK cells. In addition to their rarity, the greatest obstacles to their identification have been the diversity and phenotypic complexity of these cells. Extranodal T/NK cell lymphomas are more common in adult men (mean age 44-54 years), originate from NK cells (or in some cases from cytotoxic T lymphocytes), and cause extensive vascular damage with prominent necrosis. They are strongly associated with Epstein-Baar virus (EBV) infection and involve the upper aerodigestive tract (mainly nasal cavity), skin, and lymph nodes. It is a highly aggressive lymphoma, with low survival rates and poor response to treatment. **Report:** Male patient, 42 years old, starts with cervical and inguinal lymph node enlargement. Underwent PET-CT

on 04/28/23: lymph node enlargement in the inguinal, iliac, pericaval, left paraaortic, retrocrural regions, in pulmonary hila and various compartments of the mediastinum, axillary and cervical, in addition to diffuse hypermetabolism in the bone marrow and splenomegaly. Performed inguinal lymph node biopsy on 05/05/23 – Immunohistochemistry released on 05/21/23 – Pathological cells positive for BCL2 CD10 CD4 CD5 CD7 CD8 Tdt, KI67+100%, diffuse CD3+, cytoplasmic pattern. Evolved with progressive worsening of the clinical condition and pancytopenia. Bone marrow aspirate collected on 05/26/23 for Immunophenotyping showing: 4.1% CD56+ CD3(-) CD8-/+ CD4(-) NK CELLS; 15% MATURE (CD45++) POLYCLONAL T LYMPHOID CELLS (CD3++): . 3.8% CD4+ TCRALFA/BETA+ (TCRCBETA1+ 50%); . 8.8% CD8+ TCRALFA/BETA+ (TCRCBETA1+ 20%); . 0.5% CD8+ TCRGAMA/DELTA+; . 1.3% CD4+CD8+ TCRALFA/BETA+ (TCRCBETA1+ 30%); . 0.6% DOUBLE-NEGATIVE: 0.1% TCRALPHA/BETA+ AND 0.5% TCRGAMA/DELTA+ 11.3% MATURE CD8+ LYMPHOID CELLS (CD45++), WITH PARTIAL EXPRESSION OF SURFACE CD4 AND CD3, EXPRESSION OF CYTOPLASMIC CD3+ (EPSILON CHAIN), T MARKERS (CD2+ CD5+ CD7+) AND CYTOTOXIC/NK ANTIGENS (CD56+ CD16-/+ CD94+ Perforin+), and effector T cell phenotype (CCR7(-) CD27-/+ CD28-/+ CD45RA+ homogeneous). NK profile stage 4: CD56++ CD16-/+ and CD57(-) (Figure 1).

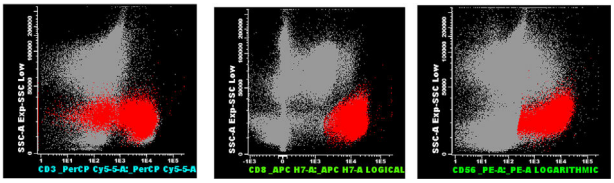

Figure 1: Mature CD8+ lymphoid cells, with partial expression of surface CD3, NK profile CD56++.

Negative antigens: CD25 CD57 CCR7 CD11C CD45RO CD1A CD117 CD30 TCRAlpha/Beta TCRCBeta1 TCRGama/Delta HLA-DR TDT and CD10. At that time, the patient presented skin lesions on the chest and face (Figures 2 and 3), his clinical condition worsened and he was admitted to the ICU. A cerebrospinal fluid sample was also infiltrated by T/NK cells. The phenotypic findings associated with the clinical condition led to the diagnostic conclusion of T/NK Cell Lymphoma. Serology tests for EBV were negative. He did not present clinical improvement, developed Mucormycosis and died.

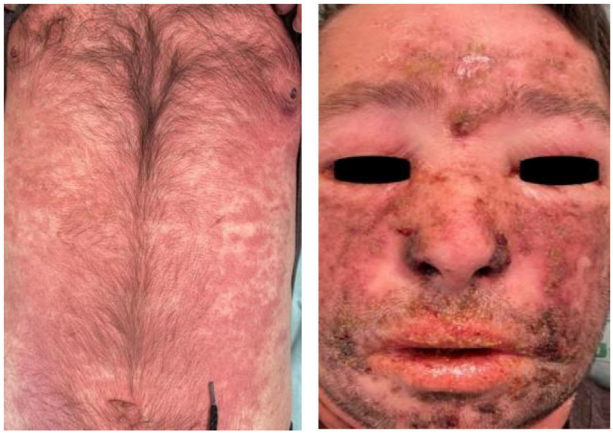

Figure 2: Skin lesions on the chest. Figure 3: Skin lesions on the face.

Comments: Flow cytometry was indispensable in the diagnostic elucidation, in the separation of normal and pathological T and NK cell subpopulations of the sample, and in the rapid definition of lymphoma with a severe and aggressive clinical course.

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https://doi.org/10.1016/j.htct.2025.104001

11

CASE REPORT: PLASMA CELL LEUKEMIA

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A 55-year-old female was referred to the medical hematology service for severe anemia and osteolytic lesions in the spine and skull. At the time of consultation, the hemogram showed "lymphocytosis," with the automated analyzer misidentifying plasma cells as lymphocytes, and the peripheral blood smear revealed cells resembling hairy cells, raising suspicion of hairy cell leukemia or plasma cell leukemia. The patient was requested to undergo a series of laboratory tests to perform a differential diagnosis between the two conditions. Laboratory findings were as follows: Hemoglobin: 6.7 g/dL (normocytic-normochromic anemia); Leukocytes: 48,620 cells/μL (leukocytosis); Platelets: 128,000 cells/μL (thrombocytopenia); Creatinine: 2.6 mg/dL; Calcium: 14.2 mg/dL (hypercalcemia); Beta-2-microglobulin: 42 mg/L; LDH: 146 U/L; Total proteins: 7 g/dL; Albumin: 4 g/dL. Peripheral blood morphology confirmed anemia and thrombocytopenia with 72% plasmacytoid cells. Serum protein electrophoresis revealed a monoclonal band in the mid-gamma region (1.5 g/dL). Immunofixation identified a monoclonal component of free Lambda light chains. Immunoglobulin levels were markedly reduced (IgG: 375 mg/dL, IgA: 10 mg/dL, IgM: 4 mg/dL), and no monoclonal bands were detected in urine. Peripheral blood was examined by multiparametric flow cytometry using first an LST tube and then plasma cell panel with the CytoFLEX BC cytometer. Using CD138/CD38 gating strategy identified 75% circulating leukocytes as plasma cells with a neoplastic phenotype. These cells expressed CD38 and CD138 but lacked CD19, CD45, CD56, CD117, and CD27, with cytoplasmic lambda light chain restriction. Bone marrow examination was deferred at the

time of diagnosis. FISH analysis for IGH/FGFR3 t(4;14), IGH/MAF t(14;16), and p53 gene deletions (17p13.1) showed no abnormalities. Based on international criteria for plasma cell leukemia (PCL)—defined as ≥ 20% and ≥ 2000/μL plasma cells in peripheral blood—the diagnosis of primary PCL was confirmed. The patient's clinical presentation, including hypercalcemia, anemia, and osteolytic lesions, supported the diagnosis. **Discussion:** PCL is a rare and aggressive form of multiple myeloma characterized by circulating plasma cells. Morphological variability of plasma cells can complicate diagnosis; cells may mimic lymphocytes, monocytes, or hairy cells. Key diagnostic tools include bone marrow studies, flow cytometry, and a comprehensive myeloma profile, including serum protein electrophoresis, immunofixation, free light chain assay, and 24-hour urine analysis. **Management and Conclusion:** Given the poor prognosis and aggressive nature of PCL, treatment was initiated promptly with a bortezomib-based regimen. PCL highlights the importance of accurate diagnostic methods to guide timely and appropriate therapy.

https://doi.org/10.1016/j.htct.2025.104002

12

COEXISTENCE OF CHRONIC MYELOMONOCYTIC LEUKEMIA AND LYMPHOPROLIFERATIVE DISORDER: THE DIAGNOSTIC ROLE OF IMMUNOPHENOTYPING IN PERIPHERAL BLOOD

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Introduction: The association of chronic myelomonocytic leukemia, with bone marrow infiltration by chronic lymphoproliferative diseases has been reported in the literature mainly by case reports, and documented through bone marrow biopsies. More recently, as the diagnosis of CMML can be made by peripheral blood immunophentyping, this association could be detected more frequently. This association has been included in the ICC classification of subtypes of CMML.[1] **Case Description:** Male patient, 72 years old, was hospitalized due to pneumonia. At that time, the peripheral blood (PB) count showed leukocytosis with monocytosis and thrombocytopenia, which did not resolve after the pneumonia was cured. Reviewing former PB counts, monocyte counts >10% could be detected. In 2024, no lymphadenopathy could be observed, but the spleen was 2 cm below the costal margin. **Tests during most recent hospitalization:** Creatinine: 0.9 mg/dL, Ferritin: 46 ng/mL, Serum iron: 67.7 μg/dL, ALT: 34 U/L, Vitamin B12: 794 pg/mL, Folic acid: 6.01 ng/mL, Beta-2 microglobulin: 2244 ng/mL (normal <2164), LDH: 320 U/L (normal), Protein electrophoresis: monoclonal peak of 1.46 g/dL; Serum immunofixation: IgM/Kappa. **Bone marrow aspirate:** Hemodiluted. M:E ratio: 4.28. Lymphocytes: 15%. **Bone marrow biopsy:** markedly hypercellular marrow tissue, granulocytic hyperplasia with maturation arrest. Scattered CD34+ cells. Interstitial infiltration by low-grade lymphoma (lymphocytes and plasma cells) with IgM Kappa on immunohistochemistry. Likely Waldenström macroglobulinemia, with marginal zone lymphoma as differential diagnosis. Immunophenotyping suggested. **Bone marrow immunophenotyping (October 2024):** Monocytosis of 9.1% composed of mature elements. Classical monocytes: 86.1%, Intermediate: 10.8%, Non-classical: 3.1%. Polyclonal plasma cells: 0.15%. Monoclonal B lymphocytes with the following phenotype: CD45+++, CD19++, CD20++, CD43+, CD79b++, CD200++, partial IgM (41%), and predominance of kappa light chain. **Peripheral blood immunophenotyping (November 2024):** Monocytosis of 22.3% composed of mature elements. Classical monocytes: 97.2%, Intermediate: 2.0%, Non-classical: 0.4%. 3.2% monoclonal B lymphocytes with phenotype: CD45+++, CD19++, CD20+++, CD79b++, CD200++, IgM+++, partial CD23 (30%), kappa light chain. **Comments:** In the last years, new strategies for the study of subtypes of monocytes using flow cytometry have been developed. This permitted to better understand the role of these cells in non-neoplastic inflammatory diseases as well as the detection of neoplastic clones. In the present case, the diagnostic work-up in a case with CMML using PB immunophenotyping by multiparametric flow cytometry permitted also the detection of a small lymphoid clone of Waldenström macroglobulinemia. The examination of bone marrow biopsy permitted to diagnose an extensive infiltration of this lymphoproliferative disorder. The patient was treated for macroglobulinemia, and is well and in observation. **Comments:** In this case, peripheral monocytosis detected during infections was known since 2015 but had never been thoroughly investigated. Only during the last infectious episode was the patient referred to a hematologist. After bone marrow immunophenotyping—of which the sample was very diluted—CMML was suspected, and peripheral blood testing confirmed the diagnosis. The monoclonal peak was an incidental finding, but immunophenotyping allowed the diagnosis of chronic lymphoproliferative disease. However, the true extent of this disease in the bone marrow was only assessable through biopsy. The co-existence of myeloproliferative neoplasms or CMML with chronic lymphoproliferative diseases is a rare event (1–2% of chronic myeloproliferative neoplasms). According to limited literature, these appear to be independent neoplasms, not derived from the same stem cell. Immunophenotyping (blood or bone marrow) is the most sensitive technique for detection.

Table 1 The evolution of PB counts.

	11/12/2025	04/16/2019	08/22/2021	01/05/2022	05/02/2024	08/2024	09/2024
RBC	3,81	3,82	3,18	3,89	3,73	3.63	3,7
HB	12,7	12,1	10,2	12,2	12	11,5	12,2
HCT	36,9	37,9	30,70	38,4	36	35,1	36,7
MCV	96,9	99,21	96,4	98,7	96,5	96,6	99,5
LEOCOCYTE	4200	6680	13940	7250	15660	26200	8100
BAND CELLS	42	67	0	73	626	1048	
SEGMENTED	1722	3073	10804	2826	9396	20436	4540
EOSINOPHILS	42	67	0	73	0	0	
BASOPHILS	0	0	42	73	0	0	
TYPICAL LYMPHOCYTES	1680	1536	1450	1740	3289	2620	14600
ATYPICAL LYMPHOCYTES	0	935	0	0	0	0	
MONOCYTES	714 (17%)	1002(15%)	1645 (11,8%)	2538 (35%)	2349 (15%)	2096 (8%)	1780
PLATELETS	140.000	150.000	88.450	121.000	53.280	59.140	101.000

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https://doi.org/10.1016/j.htct.2025.104003

13

UNEXPECTED FINDING IN THE PERIPHERAL BLOOD EVALUATION OF A HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) SEROPOSITIVE PATIENT

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Objective: The aim of this study is to report the unexpected finding of a clonal T-cell population in a carrier of HTLV-1 virus and review the pertinent literature. **Materials and methods:** Case report and review of the literature. **Case report:** Male patient, 55 years old, asymptomatic carrier of the HTLV-1 virus. A peripheral blood sample was sent for routine evaluation. The patient presented a normal complete blood count, with a hemoglobin level of 15 g/dL, leukocytes at 5,790/mm^3 (lymphocytes 2,310/mm^3) and 189,000/mm^3 platelets. In the immunophenotypic analysis of the peripheral blood, 30.8% of T lymphocytes were identified, with a CD4/CD8 ratio of 1.4:1. No T cells with the phenotype typically seen in Adult T-cell Leukemia/Lymphoma (ATLL), which was the reason for the sample being sent, were observed. No significant antigenic losses were found in CD4 T cells, and there was no expression of CD25. The TRBC1/TRBC2 ratio in CD4 T cells was found to be 0.85:1.0. Unexpectedly, a T cell population with moderate CD8 expression and a "Large Granular" T phenotype (CD2++/CD3++/partial CD5/CD7+++, CD8++/CD56+++/heterogeneous CD57/CD16 negative/TCR gamma-delta negative) and monoclonal for TRBC2, corresponding to 5.3% of the total leukocytes in the sample (306 cells/mm^3), was observed. **Discussion:** Small populations of monoclonal CD8 T cells can be observed in healthy individuals or during acute viral infections (such as HIV or viral hepatitis, for example), and oligoclonal patterns of TCR gene rearrangements have also been reported in patients with acute infectious mononucleosis (Epstein-Barr virus infection). With the introduction of studying the expression pattern of the constant region of the β1 chain of the T-cell receptor (TRBC1) within a subset of TCR$\alpha\beta$ T cells, it has become more common in routine laboratory practice to find T-cell clones of uncertain significance (T-CUS), which very often have a phenotype resembling T-cell Large Granular Lymphocytic Leukemia (T-LGLL). More recently, the concurrent study of the TRBC2 receptor, along with TRBC1 and a comprehensive T-cell panel, has enhanced the detection of T-cell clones, whether in healthy individuals or in patients with T-cell lymphoproliferative disorders, similar to the routine evaluation of kappa and lambda immunoglobulin light chains for the detection of clonal B cells. **Conclusions:** The diagnosis of T-cell malignancies is often challenging due to overlapping characteristics with reactive T cells and the limitations of currently available T-cell clonality assays. In this case, we suggest ongoing immunophenotypic monitoring, as the patient is asymptomatic, and we emphasize the important point that T-cell clonality by itself, in isolation, is not necessarily indicative of malignancy.

https://doi.org/10.1016/j.htct.2025.104004

14

LLA-B COM FENÓTIPO SUGESTIVO DE REARRANJO DO GENE DUX-4

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Relato de caso: A classificação de neoplasias hematológicas da Organização Mundial de Saúde (OMS) de 2022 incorpora em um grupo, novos subtipos de anormalidades genéticas presentes nas Leucemias Linfoides Agudas B (LLAs-B), incluindo os rearranjos dos genes DUX4, MEF2D, ZNF384 e NUTM1. Os rearranjos envolvendo o gene DUX4 são as lesões genéticas mais frequentes deste grupo e apresentam de forma mais característica correlação com um imunofenótipo específico. Sua identificação é fundamental para a estratificação de risco e, em alguns casos, para otimização da estratégia terapêutica. As LLAs-B com rearranjo envolvendo o gene DUX4 compreendem cerca de 20% das LLAs-B com cariótipo normal. São proporcionalmente mais prevalentes em adolescentes e adultos jovens. Estão associadas a um bom prognóstico mesmo na presença de outros fatores de risco adverso como deleção associada do gene IKAROS e pesquisa de Doença Residual Mensurável (DRM) positiva no início da terapia. É uma alteração genética que pode estar associada à

troca de linhagem celular durante o tratamento. O parceiro mais frequente nesta fusão é a IGH e menos comumente, o gene ERG, que resultam numa isoforma truncada do DUX4 nas células B. Estes rearranjos não são evidentes na análise citogenética convencional ou no FISH, sendo necessários estudos de perfil de expressão gênica ou sequenciamento de última geração (NGS), que não são facilmente disponíveis em nosso meio. Contudo, estudos prévios recentes evidenciaram forte associação entre esta alteração genética e perfil imunofenotípico específico, com forte expressão do antígeno CD371 (LLC-1) na superfície celular dos blastos, assim como fraca positividade para o antígeno T-associado CD2, com sensibilidade e especificidade em torno de 98%. Relatamos caso clínico de paciente de 39 anos, previamente hígido, masculino, etnia branca que apresentou quadro de hemoptise e cansaço aos esforços recente que o motivou a procurar atendimento médico. Hemograma inicial revelou Hb: 8.9 g/dl; Leucócitos: 17.540/mm^3; neutrófilos: 175/mm^3; 94% de blastos e Plaquetas: 124.000/mm^3. Foi realizada imunofenotipagem de sangue periférico que confirmou o diagnóstico de LLA-B Comum com a coexpressão de CD371 e CD2. Cariótipo masculino 46,XY, BCR-ABL negativo e líquor sem infiltração. Estudo de Nova GEração de Sequenciamento (NGS) foi realizado e detectou mutação dos genes NRAS (G12D - éxon 2) e KMT2D (Q2702Sfs*33 - éxon 33) com frequência de alelos menores (MAF) de 49,7% e 44,6%, respectivamente. O painel NGS estudado contempla pesquisa de 142 genes, no entanto, o gene DUX4 ainda não está disponível para estudo. Gene ERG foi contemplado e não encontrou-se mutado neste caso. Foi iniciado esquema quimioterápico GRAALL e a reavaliação de doença por citometria de fluxo no D33 foi negativa. **Conclusão:** A LLA-B com translocação DUX4 possui características clínicas, prognósticas e fenotípicas específicas e a imunofenotipagem por citometria de fluxo pode ser utilizada como teste alternativo e direcionador, para a identificação das LLAs-B com translocação DUX4.

https://doi.org/10.1016/j.htct.2025.104005

15

Leucemia Mielomonocítica Crônica (LMMC) com aparecimento simultâneo de um clone de Neoplasia Linfoproliferativa Crônica

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Paciente masculino, 72 anos, mecânico, internado por um quadro pneumônico que resolveu com amoxicilina. Na ocasião, hemograma com leucocitose/monocitose e plaquetopenia que não se resolveu após cura da pneumonia. Achados semelhantes no hemograma conhecidos desde 2015, sempre durante infecções. Internação por COVID em 2021. EF: BEG, ausência de adenomegalias, baço 2 cm RCE. Exames na última internação: creatinina 0,9, ferritina: 46 ferro sérico 67,7 TGP 34 vitamina B12 794, ácido fólico: 6,01 beta2 microglobulina: 2244 (normal até 2164) LDH: 320 (normal) eletroforese de proteínas: pico monoclonal 1,46 g/dl; imunofixação sérica: IgM/Kappa. **Mielograma:** Hemodiluído. RGE 4,28. Linfócitos 15% Biópsia de medula óssea: tecido medular acentuadamente hipercelular, aumento da série granulocítica com retardo maturativo. Esparsas células CD34+. Infiltração intersticial por linfoma de baixo grau (linfócitos e plasmócitos) IgM kappa na imunoistoquímica. Provavelmente macroglobulinemia de Waldenström, com diagnóstico diferencial de linfoma de zona marginal. Sugerida imunofenotipagem. Imunofenotipagem de medula óssea (10/2024): monocitose de 9,1% às custas de elementos maduros. Monócitos clássicos: 86,1%, intermediários: 10,8% e não clássicos: 3,1%. Plasmócitos policlonais 0,15%, linfócitos B monoclonais com fenótipo: CD45+++, CD19++, CD20++, CD43+, CD79b++, CD200++, IgM parcial (41%) e predomínio de cadeia leve Kappa. Imunofenotipagem de sangue periférico (11/2024): monocitose de 22,3% às custas de elementos maduros. Monócitos clássicos: 97,2%, intermediários: 2,0% e não clássicos: 0,4%. 3,2% de linfócitos B monoclonais com fenótipo: CD45+++, CD19++, CD20+++, CD79b++, CD200++, IgM+++, CD23 parcial (30%), cadeia leve Kappa. Conclusão: Leucemia mielomonocítica crônica (LMMC) (monocitose periférica detectada em 2015) e macroglobulinemia de Waldenström (detectada em 2024). **Comentários:** No presente caso, a monocitose periférica detectada durante infecções, já era conhecida desde 2015, mas nunca havia sido avaliada com mais detalhe. Apenas no último episódio infeccioso o paciente foi encaminhado ao hematologista. Após imunofenotipagem da medula, cujo material estava muito diluído se levantou a suspeita de LMMC, e se realizou também o exame no sangue periférico onde se pode confirmar este diagnóstico. O pico monoclonal foi um achado de exame, mas a imunofenotipagem permitiu diagnosticar a doença lifoproliferativa crônica. Apesar disso, a real extensão desta doença na medula óssea só foi possível avaliar na biópsia de medula. A co-existência de neoplasia mieloproliferativa ou LMMC com doenças linfoproliferativas crônicas é um evento raro (1-2% das NMPc). Segundo a escassa literatura existente, parecem ser neoplasias independentes, não originárias da mesma célula-tronco. A imunofenotipagem (sangue ou medula óssea) é a técnica mais sensível para a sua detecção.

https://doi.org/10.1016/j.htct.2025.104006