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.

References:

1. Purnama C, Camous X, Larbi A. An overview of T cell subsets and their potential use as markers of immunological ageing. Int Trends Immunity. 2013;1:21-32.
2. Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T−cell differentiation: human memory T−cell subsets. Eur J Immunol. 2013;43(11):2797-809.
3. van der Burg M, Kalina T, Perez-Andres M, Vlkova M, Lopez-Granados E, Blanco E, et al. The EuroFlow PID orientation tube for flow cytometric diagnostic screening of primary immunodeficiencies of the lymphoid system. Front Immunol. 2019;10:246.
4. Ogonek J, Juric MK, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune reconstitution after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:507.
5. Shi M, Jevremovic D, Otteson GE, Timm MM, Olteanu H, Horna P, et al. Single antibody detection of T−cell receptor $\alpha\beta$ clonality by flow cytometry rapidly identifies mature T−cell neoplasms and monotypic small CD8−positive subsets of uncertain significance. Cytometry B Clin Cytom. 2020;98:99-107.
6. Horna P, Shi M, Jevremovic D, Craig FE, Comfere NI, Olteanu H, et al. Utility of TRBC1 expression in the diagnosis of peripheral blood involvement by cutaneous T-cell lymphoma. J Invest Dermatol. 2021;141:821-829.e2.

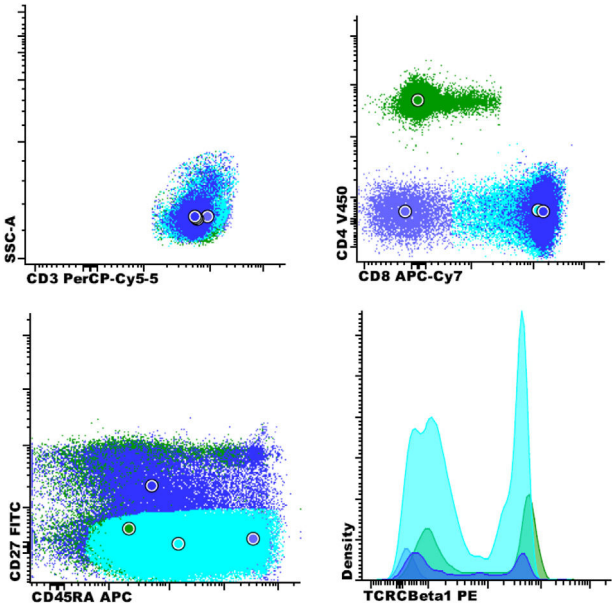

Figure 1 Normal double-negative Gamma/Delta cells in purple; normal TCD4+ cells in green; normal TCD8+ cells in dark blue; TD CD8+ cells in turquoise.

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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).

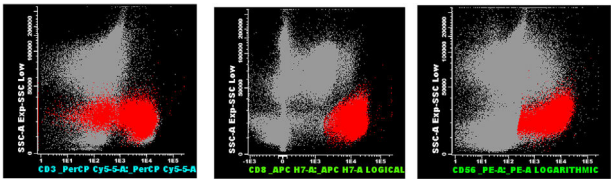

Figure 1: Mature CD8+ lymphoid cells, with partial expression of surface CD3, NK profile CD56++.

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.

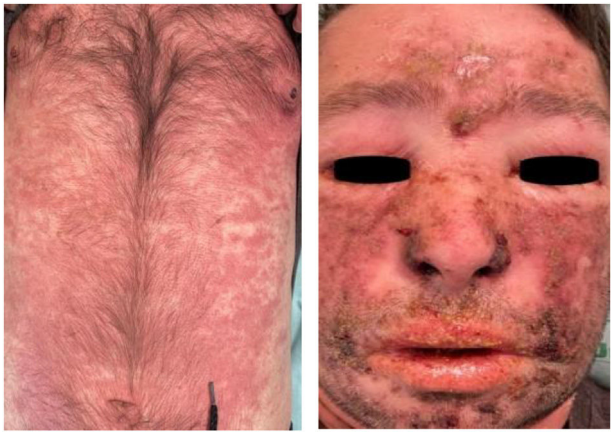

Figure 2: Skin lesions on the chest. Figure 3: Skin lesions on the face.

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.

References

1. Bárcena P, Jara-Acevedo M, Tabernero MD, López A, Sánchez ML, García-Montero AC, et al. Phenotypic profile of expanded NK cells in chronic lymphoproliferative disorders: a surrogate marker for NK-cell clonality. Oncotarget. 2015;6:42938.
2. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia. 2022;36:7:1720-48.

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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