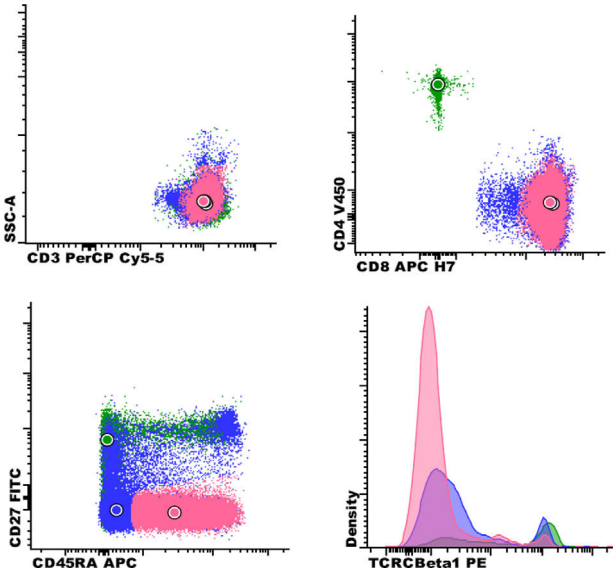
cells/μL - normal age range 2-323) CD45RA(-) CD27+ TCRCBeta1+5% (monoclonal). *4.9% TCD4+ TCRCBeta1+44% (polyclonal). **Immunophenotype:** CD45++ CD3+ CD8+ CD38dim CD45RA-/+ CD45RO-/+ CD2+ CD5++ CD7+FR TCR ALPHA-BETA+ PERFORIN+ TCRCBETA1 ++5%; Negative expression: CD25 CD26 CD27 CD28 TCL1 CCR7 TCR GAMMA-DELTA 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.

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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.

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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.

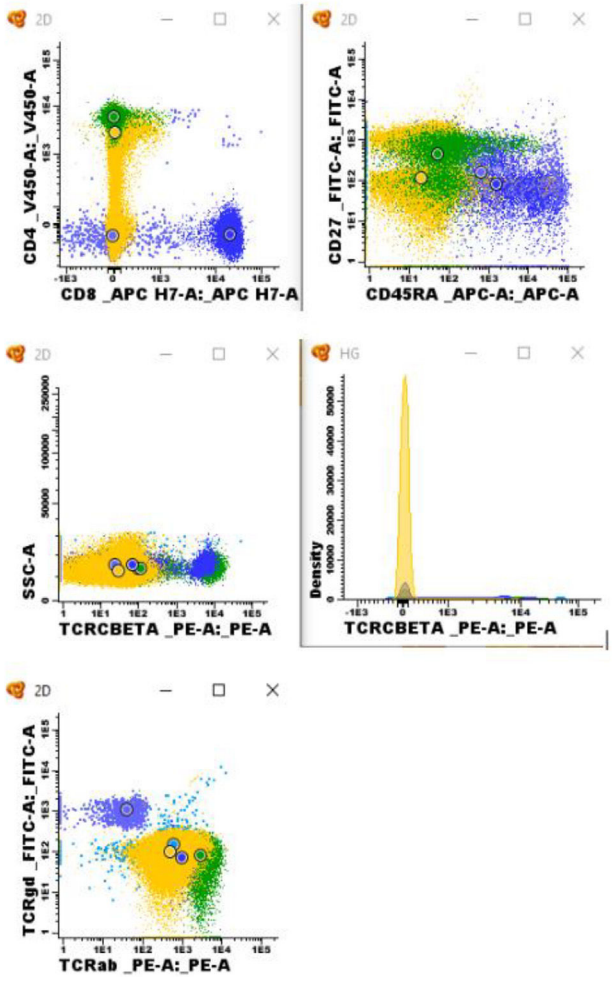

Figure 1 Sézary cells in yellow, normal TCD4+ cells in green and normal TCD8+ cells in dark blue.

References

1. Shi M, Jevremovic D, Otteson GE, Timm MM, Olteanu H, Horna P. 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.
2. 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;141:821-9. e2.
3. Okada R, Kondo T, Matsuki F, Takata H, Takiguchi M. Phenotypic classification of human CD4+ T cell subsets and their differentiation. Int Immunol. 2008;20:1189-99.
4. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, et al.; ISCL/EORTC. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110:1713-22.
5. 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;36:1720-48.
6. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C, et al. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): part I. Diagnosis: clinical and histopathologic features and new molecular and biologic markers. J Am Acad Dermatol. 2014;70:205.e1-16; quiz 221-2.
7. Horna P, Shi M, Olteanu H, Johansson U. Emerging role of T-cell receptor constant β chain-1 (TRBC1) expression in the flow cytometric diagnosis of T-cell malignancies. Int J Mol Sci. 2021;22:1817.

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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