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.

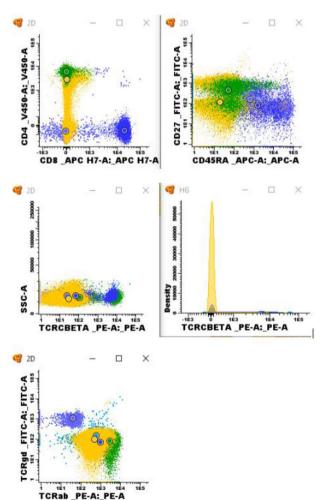


Figure 1 Sézary cells in yellow, normal TCD4+ cells in green and normal TCD8+ cells in dark blue.

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.
- 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.
- 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.

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

8

CASE REPORT: DOUBLE-POSITIVE T-LARGE GRANULAR LEUKEMIA

Maria Daniela Holthausen Perico, Renata da Silva Kalfeltz, Andressa de Oliveira Martin Wagner, Anise Osório Ferri, Franciani Turra Costella Delagnello, Liziani Crestani Bortoluzzi, Paula Gomes Back Prim

Hematology and Hemotherapy Center of Santa Catarina (HEMOSC), Cell Markers Laboratory, Florianopolis, SC, Brazil

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).

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.
- Horna P, Shi M, Jevremovic D, Craig FE, Comfere NI, Olteanu H. Utility of TRBC1 expression in the diagnosis of peripheral blood involvement by cutaneous T-cell lymphoma. J Invest Dermatol. 2021;141:821-829.e2.
- 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
- 4. Devvit KA, Kern W, Li W, Wang X, Wong AJ, Furtado FM, et al. TRBC1 in flow cytometry: Assay development, validation, and reporting considerations. Cytometry B Clin Cytom. 2024;106:192-202.
- 5. 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.

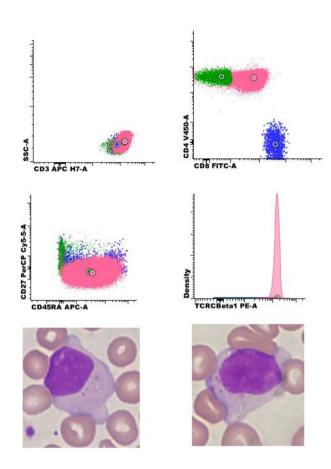


Figure 1 Flow Cytometry: Double-Positive LGL clone (pink; normal TCD4+ cells in green and normal TCD8+ cells in dark blue). Morphology: atypical medium-sized lymphoid cells was observed, with a globose nucleus, generally eccentric, with poorly condensed chromatin with an outline of a nucleolus, and a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

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

9

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

Maria Daniela Holthausen Perico, Renata da Silva Kalfeltz, Andressa de Oliveira Martin Wagner, Anise Osório Ferri, Franciani Turra Costella Delagnello, Liziani Crestani Bortoluzzi, Paula Gomes Back Prim

Hematology and Hemotherapy Center of Santa Catarina (HEMOSC), Cell Markers Laboratory, Florianopolis, SC, Brazil