T-cell receptor γδ-large granular lymphocytic leukemia associated with an aberrant phenotype and TCR-Vβ20 clonality

Large granular lymphocytic (LGL) leukemia is a rare heterogeneous disorder of mature lymphocytes with a characteristic morphology, multiple autoimmune disorders and indolent clinical course. Most cases exhibit a T-cell phenotype of CD3, CD8 and CD57 positivity, while the minority exhibit a CD2, CD56, and CD16 positive NK-cell phenotype. We report a case of a 71-year-old female suffering from a TCRγδ positive T-cell leukemia with a morphology compatible to LGL leukemia. She referred to the hospital for investigation of mild anemia, lymphocytosis, neutropenia and hyperglobulinemia. Peripheral blood and bone marrow were occupied by mature large granular lymphocytes with abundant azurophilic granules. The immunophenotype was CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16-, CD56-, CD57- and the Vβ repertoire analysis showed clonal reactivity with Vβ20 mAb. The patient was diagnosed as having T-LGL and was treated with G-CSF. So far, she experiences an indolent clinical course. To our knowledge, this is a rare case of TCRγδ positive T-LGL leukemia with the aberrant immunophenotype of CD3+, CD4-, CD8-, CD16-, CD56-, CD57-.

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Large granular lymphocytic (LGL) leukemia is a rare heterogeneous disorder, but it is clinically, morphologically, and immunologically distinct. LGL leukemia cells have characteristic morphologic features of large lymphocytes with abundant cytoplasm and fine or coarse azurophilic granules. The updated criterion for the diagnosis of LGL leukemia is the demonstration of clonal expansion of a population of granular lymphocytes. Two phenotypes are commonly described. A T-cell phenotype (T-LGL) exhibits CD3, CD8, and some NK cell markers such as CD16 and CD57 with T-cell receptor gene rearrangements. This type is associated with chronic neutropenia, rheumatoid arthritis, splenomegaly, and humoral immune abnormalities. A less common phenotype is a more classical NK cell (NK-LGL) with CD2, CD56, and CD16. These cases do not exhibit TCR gene rearrangement and have distinct clinical features with more significant anemia, thrombocytopenia, and hepatosplenomegaly. The clinical course is indolent in most cases.

We report a case of T-cell leukemia with an indolent clinical course, who presented with mild neutropenia, lymphocytosis, autoimmune hemolytic anemia, hyperglobulinemia and antinuclear antibodies. The T-cells were TCRγδ positive with the aberrant immunophenotype of CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16, CD56, CD57-. The lymphocyte morphology was compatible to T-LGL leukemia.

Case report
A 71-year-old female was referred to the Department of Hematology of the University Hospital of Ioannina in October 2002 for investigation of mild anemia, lymphocytosis, neutropenia and hyperglobulinemia detected in a routine blood analysis. She had no previous medical history. Physical examination revealed neither hepatosplenomegaly nor lymphadenopathy.
The possibility of T-lineage T-prolymphocytic leukemia (T-PLL) with double-αβ repertoire for clonality assessment was studied using the FITC and PE mixtures of TCR Vα and Vβ domain expression was defined as the number of cells with specific fluorescence higher than the isotype control and autofluorescent samples. The expression of the CD phenotype in the population was performed on mononuclear cells (PBMCs) with scatter gate on the lymphocyte fraction and determined as follows: CD3+ 94%, CD19+ 5%, CD16+ 36%, 4%, TCRαβ+ 21%, TCRγδ+ 70%, CD8+ 10%, CD4+ 15%, CD38+ 94%, CD5+ 91%, CD7+ 89%, CD2+ 92%, HLA-DR+ 92%, CD1a+ 0%, CD20+ 4%, CD22+ 1%, CD23+ 2%, CD79b 4%, FMC-7+ 2%, slgM+ 4%, λ+ 0%, κ+ 0%, CD103-0%, CD10+ 0%, CD34+ 0%, CD25+ 3%.

In a further analysis using three different fluorochromes, the specific population was gated on CD3-PerCP fluorochrome and the Vβ domain expression was studied using the FITC and PE mixtures of TCR Vβ Repertoire Kit (Beckman Coulter). The results of immunophenotype suggested the existence of a clonality Vβ20mAb cells including: Vβ5.3+ 3,63%, Vβ7.1+ 1,97%, Vβ3+ 0,44%, Vβ9+ 3,41%, Vβ17+ 2,20%, Vβ16+ 0,20%, Vβ18+ 0,25%, Vβ5.1+ 5,25%, Vβ20+ 69,82%, Vβ13.1+ 0,92%, Vβ13.6+ 0,49%, Vβ8+ 2,28%, Vβ15.2+ 0,69%, Vβ2+ 2,94%, Vβ12+ 1,21%, Vβ23+ 0,22%, Vβ11+ 1,19%, Vβ21.3+ 2,54%, Vβ11+ 0,20%, Vβ22+ 1,34%, Vβ14+ 1,11%, Vβ13.2+ 1,42%, Vβ4+ 1,43%, and Vβ7.2+ 1,23% (figure 2).

In conclusion, the lymphocytes were TCRβ?-positive. The Vβ repertoire analysis showed clonal reactivity with Vβ20 mAb of 69% (min 0%-max 9,73%) as shown in figure 3.

The patient was diagnosed as T-LGL leukemia with the aberrant phenotype TCRβ+, CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16-, CD56-, CD57-. She was treated with G-CSF. The neutropenia resolved but lymphocytosis remained stable. The patient so far, has shown an indolent course.

**Discussion**

T-cell large granular leukemia is an heterogeneous disorder with morphological features of large lymphocytes with cytoplasmic granules. LGL leukemia cells have a mature T-cell immunophenotype. The majority of cases are TCRαβ+, CD3+, CD4+, CD8+, while rare variants have been observed: TCRαβ+, CD3+, CD4+, CD8- or TCRαβ+, CD3+, CD4+, CD8+. CD57 is often expressed in the common type. There is no unique karyotypic abnormality. For most cases the clinical course is indolent and non-progressive. Morbidity is associated with neutropenia. Although atypical immunophenotypes have been occasionally observed, T-LLGL leukemia with positive TCRγδ and negativity to CD4, CD8, CD16/56 and CD57 has not yet been reported. The present case was compatible with T-LLGL according to the clinical manifestation, the morphology of lymphocytes and the clinical course. However, the immunophenotype of TCRβ7-positive, double-negative T-lymphocytes was quite unusual. The possibility of hepatosplenic, nasal or enteropathy-type T-cell lymphoma is excluded, since in our case splenic, liver, nasal or gastrointestinal involvement was absent and the clinical course was indolent. The possibility of T-lineage acute leukemia was also excluded, due to completely different cell morphology, clinical manifestation and clinical course. T-prolymphocytic leukemia (T-PLL) with double-negative T-lymphocytes is a rare variant, but is usually TCRβ7-positive. Only one case of TCRβ7-positive T-PLL has been reported. However, T-PLL is characterized by morphological features of lymphocytes with prominent nucleous and cytoplasmic basophilia with no granules and an aggressive clinical course. Autoimmune lymphoproliferative syndrome is another entity that could be under consideration, since it exhibits common features with LGL. The syndrome is characterized by lymphocytosis due to defective lymphocyte apoptosis, hepatosplenomegaly, autoimmune disorders and an immunophenotype of double negative CD3+, CD4-, CD8- T-cells but is usually TCRαβ7-positive. However, the disorder is nonmalignant and clonal expansion of the population of lymphocytes is not determined.

Further accumulation of T-LGL cases is required to clarify whether T-LGL of this immunophenotype accounts for a rare variant of T-LGL, or this is an exceptional case.

**References**


