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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

A COMBINED IMMUNOPHENOTYPIC AND MOLECULAR APPROACH TO DIFFERENTIATE T-CUS, LOW-COUNT T-LGLL AND T-LGLL

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Introduction: The identification of small T-cell clones sharing overlapping immunophenotypic and molecular features has blurred the distinction between reactive and neoplastic proliferations, including T-cell Clones of Uncertain Significance (T-CUS), Low-Count (LC) T-cell Large Granular Lymphocyte Leukemia (T-LGLL) and full blown T-LGLL. T-LGLL is a chronic clonal disorder of cytotoxic T lymphocytes often associated with neutropenia and activating *STAT* mutations. Traditionally, arbitrary thresholds (500/1,000 LGL/µL) separate indolent T-CUS and symptomatic LC T-LGLL from full blown T-LGLL. This study challenges numerical cut-offs and proposes an integrated approach combining immunophenotypic, molecular and clinical data to better define T-cell proliferations.

Methods: A cohort of 110 patients was consecutively enrolled and classified, according to clonal size and clinical features, as full blown T-LGLL (n=50; LGL>1,000/µL), LC T-LGLL (n=20; LGL≤1,000/µL and ANC<1,500/µL) and T-CUS (n=40; LGL≤1,000/µL and ANC≥1,500/µL), evaluated for immunophenotype, *STAT3/STAT5B* mutations (Sanger sequencing), TCR Vβ and TRBC1/2 expression (positivity ≥85% or ≤15%).

Results: Within full blown T-LGLL we identified mainly CD8+ T-LGLL subtype (56%), of which 43% were *STAT3-mutated* (*MUT*), and CD4+ T-LGLL (44%), including 23% *STAT5B-MUT* cases. Among LC T-LGLL patients, 80% (25% *STAT3-MUT*) and 20% showed CD8+ and CD4+ cell expansion, respectively. T-CUS subset comprised 65% CD8+ and 35% CD4+ cases, with no *STAT* mutations detected. Based on surface markers, distinct immunophenotypic patterns emerged among subgroups ($p<0.05$). In detail, most T-LGLL patients exhibited a terminal effector profile (CD28-/CD62L-), particularly in symptomatic CD8+ *STAT3-MUT* cases. LC T-LGLL showed heterogeneous profiles (CD28+/CD62L+: 35% CD8+, 67% CD4+). Conversely, most T-CUS displayed a less-differentiated phenotype, marked by CD28 (75% CD8+, 50% CD4+) and CD62L (64%) positivity. Across LGL clonal expansions, CD28 and CD62L expression showed a positive correlation ($r=0.70$, $p<0.01$). As expected, symptomatic T-LGLL was characterized by CD56-/CD57+ cell expansion and 60% of LC T-LGLL cases also displayed CD56-/CD57+ phenotype. In contrast, CD56+/CD57+ profile was frequent in T-CUS patients (84%), suggesting its association with indolent disease. Moreover, CD45RA+/CD45RO+ co-expression, indicative of intermediate memory-to-effector function or a less proliferative condition, was detected in 40% of T-LGLL, respect to LC T-LGLL (30% CD8+, 67% CD4+) and 64% T-CUS cases. All symptomatic T-LGLL patients displayed a CD45RA+/CD45RO- phenotype. Further, VB2 and VB13.1 were enriched in CD8+ T-CUS, compared with other subgroups ($p<0.05$). TRBC1/2 restriction confirmed clonality across all disease subsets.

Conclusions: We identified immunophenotypic and molecular patterns that help distinguish indolent T-CUS from LC T-LGLL and full blown T-LGLL, suggesting new insights to better classify T-LGL disorders.