***Case report***

# Unveiling T-Cell Prolymphocytic Leukaemia: A Comprehensive Case Study on Diagnostic Markers and Challenges



**Abstract**

Background

T-PLL is a rare T cell Chronic Lymphoproliferative disorder (T-CLPD), representing about 2% of mature lymphocytic leukaemia in adults over age of 30 years, with a median diagnosis age of 65 years. It commonly presents with symptoms like hepatosplenomegaly, generalized lymphadenopathyand occasionally skin infiltration or serous effusion.

Methods

The subject, a 40-year-old male, presented with symptoms such as high white blood cell counts, anemia, lymphadenopathy, and hepatosplenomegaly. Aperipheral blood sample was subjected to flow cytometric immunophenotyping to assess the expression of T-cell markers, including CD2, CD3, CD4, CD5, CD7, CD8 and CD38. The analysis focused on characteristic marker profiles consistent with T-PLL.

Results

Flow cytometry revealed a mature T-cell immunophenotype with CD2+, CD3 (dim to negative), CD4+, CD5+, CD7+, and CD38 (strongly positive). The CD4+/CD8- profile was noted, with rare co-expression of CD4 and CD8 observed in this case. These findings supported a diagnosis of T-PLL, aligning with known immunophenotypic features, including dim or absent surface CD3 expression which is commonly associated with T-PLL.

Conclusion:

Flow cytometry is essential in the accurate diagnosis of T-PLL, providing the detailed

immunophenotypic insights that enable differentiation from other lymphoproliferative disorders. This case underscores the importance of understanding T-PLL's characteristic markers in guiding diagnosis and management.

**Key words: Flow cytometry immunophenotyping, T-cell prolymphocytic leukaemia, T-PLL, T-CLPD**



## Introduction

"T-cell prolymphocytic leukemia (T-PLL) is an extremely rare T- Cell Chronic Lymphoproliferative disorder,accounting for approximately 2% of cases of mature lymphocytic leukaemias in adults aged >30 years"(1)."And the median patient age is 65 years. It is very uncommon among young individuals aged less than 30 years. It is characterized by the proliferation of small to mediumsized prolymphocytes with a mature post-thymic T- cell phenotype, involving the peripheral blood and bone marrow"(2,3).

"Most patients were present with hepatosplenomegaly and generalized lymphadenopathy.Skin infiltration is seen in 20% of casesand serous effusions in a minority"(4). "Absolute lymphocytosis is common in peripheral blood and bone marrow (often more than 100 $\times 10^3/\mu L$)"(5). Most patients were present with thrombocytopenia and anemia. A predominance of small to mediumsized lymphoid cells with non- granular basophilic cytoplasm; round, oval, or markedly irregular nuclei, and visible nucleoli are seen in the peripheral blood films.

The diagnosis of T-PLL is difficult to only based onperipheral blood and bone marrow histology.Flowcytometry immunophenotyping (FCI) plays an important role in the detection of T-PLL. Characteristic expression of Cluster of Differentiation (CD) markers arethe key factorswhich involving accurate diagnosis of T-PLL.

Peripheral T Lymphocytes in T-PLL are Terminal deoxynucleotidyl transferase (TDT) negative(6). And they are usually positive for CD2, CD5and CD7; the surface membrane expression of CD3 may be weak(2).

Malignant T cells in T-PLL are CD4-positive and CD8-negative in 60% of cases. In 25% of cases, they coexpress both CD4 and CD8, while 15% are CD4-negative and CD8-positive (2). This is an aggressive malignancy with a median survival of 1 to 2 years.

T-PLL is crucial for monitoring patients, evaluating their survival outcomes, and assessing their response to conventional therapies. Numerous studies have demonstrated that higher prolymphocytic counts are associated with poorer survival outcomes and increased resistance to treatment(7,8). T-PLL poses challenges in differentiation based solely on morphology. Flow cytometry is considered the gold-standard tool for the diagnosis and prognosis of haematological malignancies(9).

The aim of this case study is to describe an extremely rare case of T- PLL with the flowcytometry immunophenotyping pattern.

**Case Presentation**

This report was of a 40-year-old male patient. He was admitted to the Sri Jayewardenepura GeneralHospital, Colombo, Sri Lanka with the history of rapidly increasing leukocytosis, anemia, generalized lymphadenopathyand hepatosplenomegaly. Full Blood Count (FBC) andimmunophenotyping by flowcytometry were performed.

Investigations of peripheral blood revealed White Blood Cells (WBC) of $154\times10^3/\mu L$, platelet count of $19\times10^3/\mu L$, absolute count of lymphocytesis $138.6\times10^3/\mu L$ (90%).

Many atypical lymphoid cells characterized bysmall to medium cells with relativelyclumped chromatin and small nucleoli were noted in the stained blood film. Smudge cells were also

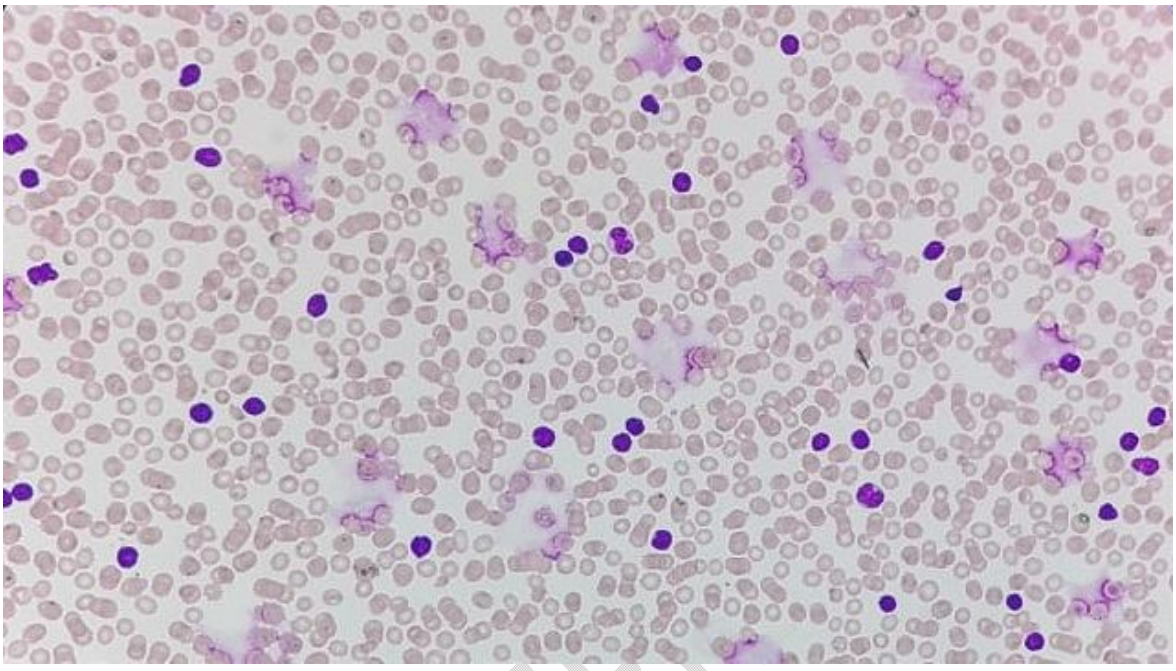

*Figure 1: Morphology of peripheral blood (High power)*

observed(10).

The flow cytometry immunophenotyping was performed on bone marrow aspirate. The reagents used to detect expression of CD markers were selected following the guidelines (2) and Euro Flow 8-color antibody panels for immunophenotyping of hematological malignancies and the validated protocols given by Becton Dickinson (BD) Bioscience, India(11).

The flow cytometer was configured with three lasers to detect up to eight colors. The computer workstation was equipped with a calibrated machine for quality control.

Live cells were gated by CD45 andtwo distinct populations as lymphocytes andgranulocytes were identified. Lymphocytes weregated by smCD3 and CD19.

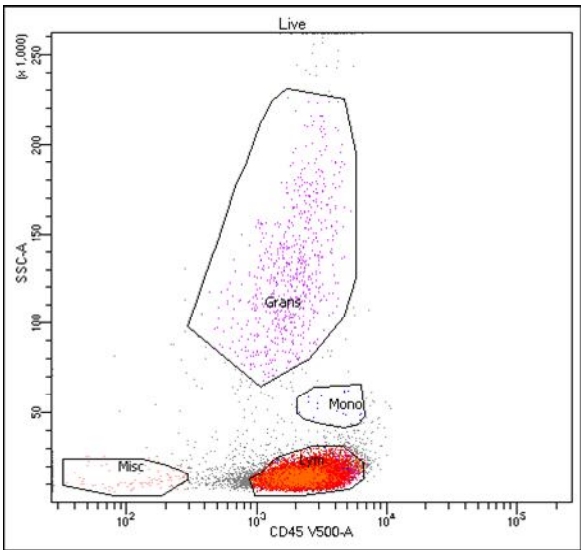


*Figure 2: CD45 gated cells*

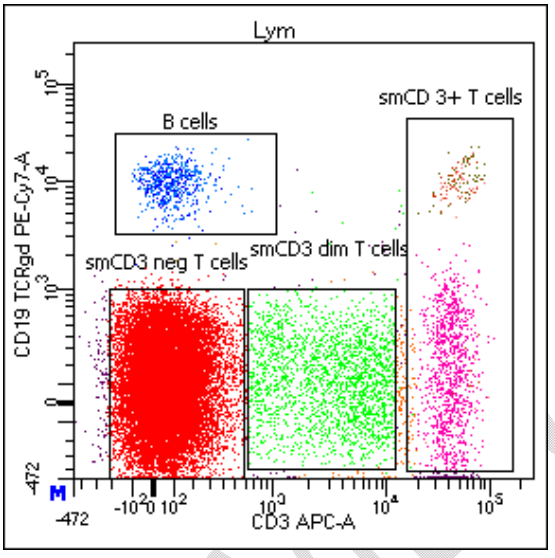


*Figure 3: CD3, CD19, TCRgd gated lymphocytes*

CD45 gated lymphoid cells (92.2%) revealed a non-clonal B cell population of 2.6% with anormal NK cell population of 2%.

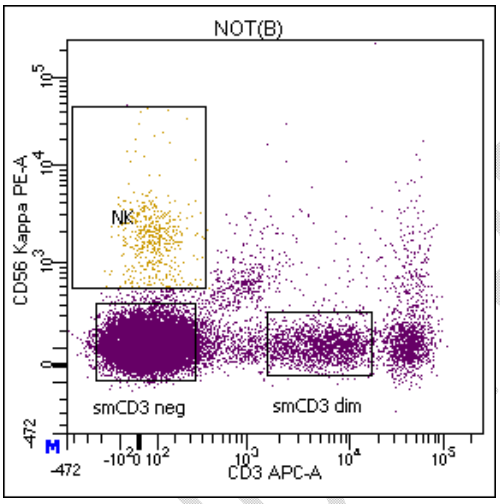


*Figure 4: CD3, CD56 gated T cells and NK cells*

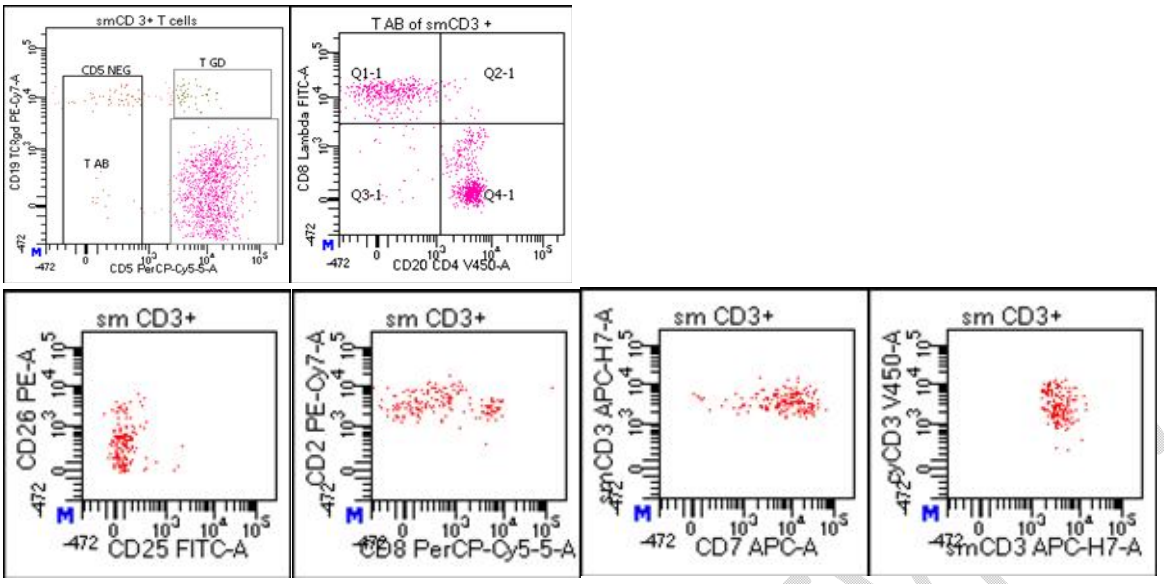


*Figure 5: CD markers expression of smCD3 positive T cells*

T cell population revealed,a small population of smCD3 positive cells (4%) with TCR αβ expression,positive panT cell antigens & positive CD4 and CD8 expression.

A population of T cells(6%) showed dim positivity for smCD3 with TCR αβ expression, positive expression for CD2, CD5, CD7, CD4 and dim expression of CD8.

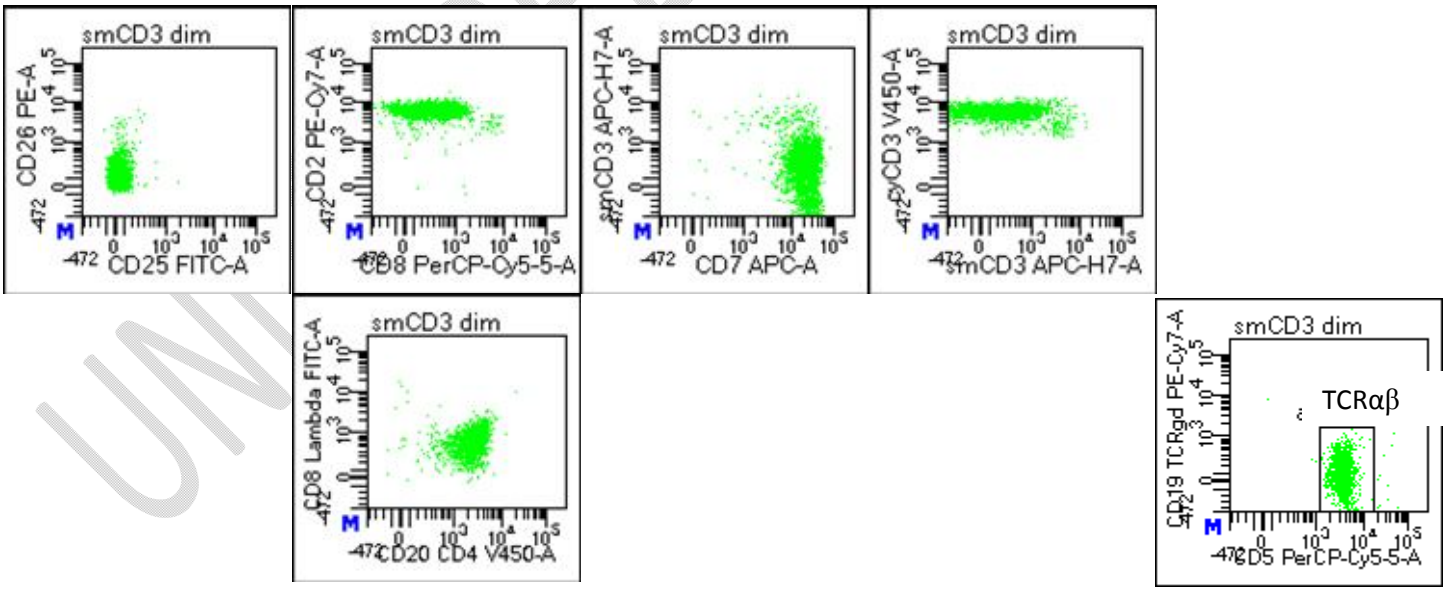


*Figure 6: CD markers expression of smCD3 dim positive T cells*

Strong positivity forCD38 noted indicating T cell activation.The larger T cell population of (76%) showed negativity for smCD3 with TCR αβ expression, positive expression for CD2,CD5,CD7,CD4 & dim expression for CD8.

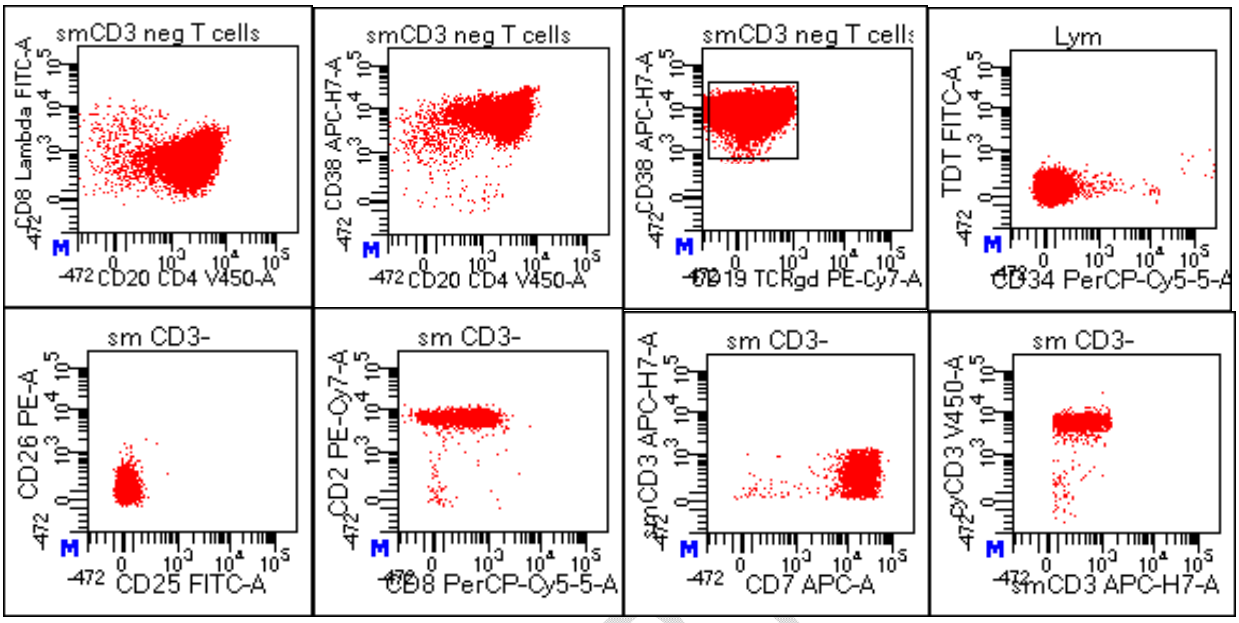


*Figure 7: CD markers expression of smCD3 negative T cells*

Strong positivity for CD38 noted indicating T cell activation in smCD3 negative T cells.The total T cell population was positive for cy CD3, negative for CD34 & TDT.

**Discussion**

"In our study T-PLL exhibits a TDT negative, mature T-cell immunophenotype (CD2+, CD5+, CD7+, CD16–, and CD56-) and co-expresses CD4 and CD8.The leukemic lymphocytes are generally CD4+/CD8- in majority of cases and a CD4-/CD8- phenotype has also been reported in very rare cases"(12). "CD7 is also generally highly expressed. The cells of T-PLL patients aremostly the TCR αβ phenotype. Only rare cases of TCR γδ expression have been reported"(3).

"Surface membrane CD3 may be lacking T-PLL cells, but they are always found in the cytoplasm. It causes to the positive of expression cyCD3.Such cyCD3 expression can be expected in immature T cells that do not yet express the CD3 antigen on the cell surface membrane"(5,13–15).

"In T-PLL, markers related to T-cell activation, such as CD25, CD38 may or may not be expressed"(16)."Our study showednegative expression of CD25 with bright positivity of CD38.

The weak expression ofsurface membrane CD3 and CD45, expression of both CD4 and CD8, and bright expression CD7 suggest that T-cell prolymphocytes might be at an intermediate stage of differentiation between thymic and post-thymic T cells"(5)."In our case a high CD45 expression is revealed yet some rare cases have reported with the lower expression of CD45"(16).

"T-PLL is a rare disease with a poor prognosis. Flowcytometry has led to better characterization of the cells involved in this disease and cytogenetics and molecular biology have revealed much about its pathophysiology"(5).

"The diagnosis of T-PLL presents several challenges due to the overlap of its symptoms with those of other conditions and the complexity of the diagnostic criteria involved. Initial assessments typically involve a comprehensive review of personal and family medical histories, physical examinationsand symptom evaluationincluding fatigue, night sweats, weight loss and other general signs common to various leukemias" (17,18).

"Symptoms of T-PLL can be quite nonspecific, often mirroring those of chronic lymphocytic leukemia (CLL) and other lymphoproliferative disorders. Common symptoms include swollen lymph nodes, an enlarged spleen or liver and abnormal blood counts, such as elevated white blood cell counts and low red blood cell counts"(18).

Consequently, distinguishing T-PLL from other heamatological conditions necessitates meticulous examination and additional testing like flow cytometry which is an evidence of T-cell clonality.

"Testing for genetic abnormalities plays a critical role in the diagnosis of T-PLL. Procedures like fluorescence in situ hybridization (FISH) are employed to identify specific genetic markers associated with the disease"(19). "Additionallymolecular profiling can reveal mutations that further support the diagnosis"(5).

**Conclusion**

In keeping with the morphological features and negativity for immature markers, the findingsfavour a diagnosis of T-chronic lymphoproliferative disorder.With a dim to negative smCD3 activity more in favours the diagnosis of **T-Prolymphocytic leukaemia**.

## Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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