# Dissecting the sCD3-CD4+ T-cell population: a valuable screening tool for Angioimmunoblastic T-cell lymphoma

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Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma characterized by systemic symptoms, lymph nodes involvement by polymorphous infiltrate, and a prominent proliferation of follicular dendritic cells and endothelial venules.

According to the 2017 revision of the World Health Organization classification of lymphoid neoplasms, AITL now resides under the category of nodal T-cell lymphoma with follicular helper T (TFH) cell phenotype accounting for 15-20 % of peripheral T-cell lymphomas (PTCL) and 1-2% of all non-Hodgkin lymphomas.

The neoplastic cell in AITL is a TFH cell, surrounded by cellular components of the microenvironment including high endothelial venules (HEVs), EBV+ B cells and TFH. The dysregulation of the last mentioned cell leads to germinal center anarchy and successive development of AITL. The B cell activation often leads to autoimmune hemolytic anemia (AIHA) and hypergammaglobulinemia.

Gene expression profiling has indicated TFH origin of AITL, on top of a wide variety of described mutations in AITL: *RHOA* (60-70%), *TET2* (50-80%), *DNMT3A* (20-30%), *IDH2* (20-30%), *CD28* (5-10%), *PLCG1* (5-10%).

Although excisional biopsy among with clinical presentation remains the gold standard diagnoses approach, many other tools like multiparameter flow cytometry (MFC) has proven to be an extremely versatile and useful tool in the diagnosis and monitoring of AITL as its diagnosis can often be challenging, especially in the earlier phases of disease (1).

Inconsistent and not well-documented reports have discussed peripheral blood (PB) involvement by AITL, remaining a scientific debate. For instance, Bassegio et al., 2006 found PB involvement in all cases where PB sample was available. Singh et al., 2014 studied 17 cases of AITL, all of which showed PB involvement by malignant T lymphocytes. Loghavi et al., 2016 described PB involvement in 78% of their cases. According to the literature reviewed, median range of neoplastic cells in PB has been set around 8.5-23%(1).

While B-cell lymphomas in which one can reliably identify monotypic (or lack of) surface immunoglobulin light chain expression in neoplastic cell populations, altered/aberrant patterns of expression in antigens that are normally expressed in non-neoplastic T-cells is the most common diagnostic approach in identifying neoplastic T-cells. However, the recent advance identifying TRBC1 as a clonal marker has dramatically changed the scenario.

Circulating sCD3-CD4+ T-cells have been described in the setting of AITL. Singh and colleagues compared AITL with other forms of leukemic CD4+ PTCL; they showed that sCD3-CD4+ circulating T-cells were present in the PB of all patients with AITL whereas circulating cells with this aberrant immunophenotype were observed in only 2.5% of patients with other forms of peripheral T-cell lymphoma (one patient with mycosis fungoides). This feature alone was present in 80% of AITL patients in another series of cases(1). This mentioned population (sCD3-CD4+) has been described among AITL and lymphocytic-variant of hypereosinophilic syndrome (L-HES)(2). CD5 bright expression has been associated to the sCD3-CD4+ population among L-HES patients in different series of cases(1,2), however its discriminatory reliability in this setting has not been reported to date.

Moreover, pan-T antigen expression has also been studied; where CD2 and CD5 were the least commonly down-regulated antigens and CD7 may be absent in up to 28-67% of cases. Based on previous studies, several markers, particularly CD10, CD279 (PD-1) or CD200 have been associated to AITL, individually or in combination(1), but to the extent of our knowledge there are no reports gathering all of them in a single MFC tube. Herein we show a single 8-color FC tube as a reliable screening tool for AITL (Figure 1A). In our daily laboratory routine, we use a 3-tube 8-color MFC panel as a first diagnostic approach in suspected T-cell lymphoproliferative disorders

(Table 1). When AITL is suspected or sCD3-CD4+ population is detected, we add an additional 8-color-tube combining CD10, CD200, CD279 (PD-1) (tube 4, table 1).

From January 2014 to May 2021, 35 cases in which the sCD3-CD4+ population was detected were found. Among them, 12 AITL, 3 L-HES, and 3 Sézary syndrome were diagnosed. In 17 subjects no evidence of disease was shown after a median follow-up of 1.9 years (min 0.2-max 3.9 years). Some of these 35 cases were studied at different time points implying a total of 63 studies. During this time period, MFC lymphocyte population analysis was performed in a total of 3,675 subjects involving a sCD3-CD4+ population detection rate of 0.95%. We aimed for a sensitivity of at least $10^{-4}$ as a prerequisite for obtaining reliable detection rates, since this population was detected in an exceptionally low frequency (median 0.15% leukocytes, 0.01-2.9%). In tubes 1-3 and tube 4, we routinely acquire 100,000 events and 500,000 events, respectively. Reliable quantification of sCD3-CD4+ population required 50 clustered events, thus defining the limit of quantification as 50/leukocytes.

Then, we hypothesized whether the sCD3-CD4+ population could have a predictive and differentiating value as an indicator of tumor burden. We compared the medians of the percentage of sCD3-CD4+ population with respect to the total leukocytes between in AITL, L-HES, Sézary syndrome and in the group of patients who had not evolved to any malignant pathology during our follow up (the medians were 0.38, 0.25, 0.04 and 0.09, respectively). Intriguingly, we obtained significant differences comparing malignant cases (AITL, L-HES and Sézary syndrome; median 0.34) vs healthy subjects (P=0.012). On the other hand, we did not find significant differences regarding patients with AITL vs L-HES (P = 0.63), but we did find both groups AITL vs Sézary (P=0.031) and AITL vs healthy subjects (P=0.003). This data should be taken cautiously, however the idea that the sCD3-CD4+ tumor burden would be higher in the malignant group shall be considered.

Figure 1 depicts an AITL case whose sCD3-CD4+ population expressed CD10, PD-1, and CD200, recapitulating in a single tube different TFH markers related with AITL. Specifically, CD10 expression by MCF analysis has been suggested as an interesting marker when discriminating AITL from other forms of PTCL. CD10 expression is aberrantly expressed in 50-90% AITL cases, depending on the site of involvement. However, CD10 positive cutoff designation is necessary as

few cases of reactive hyperplasia, follicular lymphoma or marginal lymphoma may also harbor benign T cells showing positivity for CD10. High PD-1 expression by flow cytometry was a consistent finding in PTCL with TFH phenotype, as well as PD-1 bright T-cell population reliably discriminated morphologic mimics from AITL with great sensitivity and specificity. CD200 is a transmembrane glycoprotein expressed by different population subsets, such as B cells, activated T-cells, endothelial cells, or neurons. CD200 is expressed by follicular helper T-cells in reactive lymphoid environment and neoplastic cells in angioimmunoblastic T-cell lymphoma. In contrast, only some cases of T-cell neoplasms other than angioimmunoblastic T-cell lymphoma were immunoreactive for CD200.

Remarkably, CD5 bright was observed in our 3 patients diagnosed with L-HES whereas this finding was not detected among Sézary patients and only in one out of our 12 AITL series [L-HES 3/3 (100%); AITL1/12 (8,23%); Sézary syndrome 0/3 (0%)]. This points out as a notable discriminative feature of L-HES (Figure 1C).

Simultaneously, we performed a retrospective analysis among the AITL cases diagnosed after pathology examination. We found 81 cases codified as AITL by our Pathology Department from January 2014 to May 2021. In those cases, PB examination was performed in 45.7% of cases (37/81), evidencing the sCD3-CD4+ population in 32.4% of cases (12/37). Strikingly, 21.6% (8/37) of the patients who were initially diagnosed with AITL and who did not present the aforementioned sCD3-CD4+ population, displayed different atypical T-lymphocyte populations. We have studied this set of patients in a retrospective manner and for greater interest, the expression of CD10 and/or CD200 was positive in 5 out of them. A CD4+CD10+CD200+ phenotype was observed in 2 patients and CD4+CD200+CD10- in 3 of them. With regard to the remaining 3 out of 8 cases, these presented nonspecific immunophenotypic abnormalities. Two cases showed CD7- and 1 case CD7-CD2+dim, with no evidence of sCD3-CD4+ population, CD10 and/or CD200 expression. Thus, the inclusion of this Tube 4 could allow us to better typify these populations and study their evolution and diagnostic interest in the future.

Our findings support the role of flow cytometry immunophenotyping in the assessment of patients with AITL and illustrate how a single and feasible 8-color flow cytometry tube may rise as a highly reproducible and objective tool in T-cell lymphoproliferative disorders discrimination.

If this test could be included among other screening methods and applied in prospective studies or larger series of patients, it would strengthen the value of this assay as a new diagnostic/prognostic tool in clinical practice.

## CONFLICT OF INTEREST

Nothing to report.

## LITERATURE CITED


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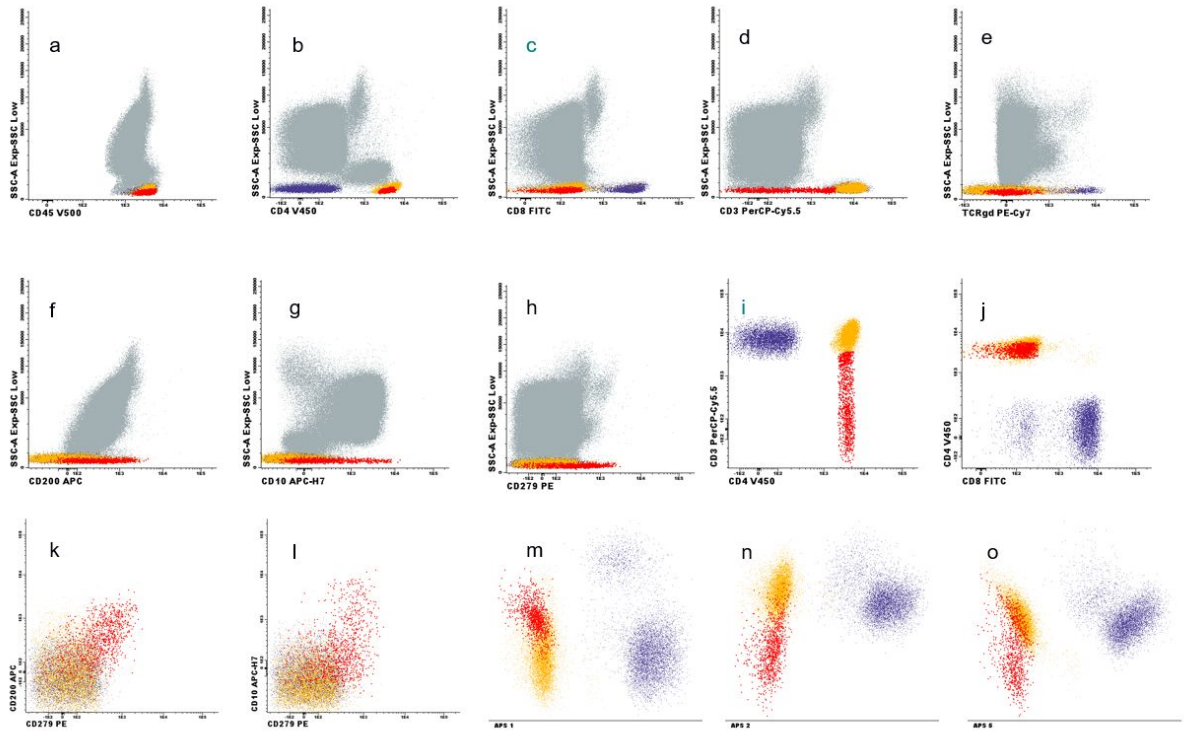


**FIGURE 1A.** Representative flow cytometry histograms of angioimmunoblastic T-cell lymphoma (AITL) patient. The figure shows the immunophenotypic profile of abnormal CD4+ T-lymphocytes, normal CD4+ T-lymphocytes and CD8+ T-lymphocytes (colored in red, yellow and blue, respectively) regarding the expression of CD45 (a), CD4 (b), CD8 (c), sCD3 (d), TCR gamma/delta (e), CD200 (f), CD10 (g), CD279/PD-1 (h) and CD4/CD8 (j) The presence of a sCD3-CD4+ population is better identified in a sCD3/CD4 biparametric histogram (i). The expression of the follicular T helper (TFH) cell markers CD200, CD279/PD-1 and CD10 is also showed in biparametric histograms on gated T-cells (k, l). The aberrant sCD3-CD4+ population is displayed as a different cluster in APS (Automatic Population Separator) graphs (m, n, o) from the Infinicyt software. This graphical representation is an automatic separation of events based on Principal Component Analysis, in which the represented parameter is defined by calculations of different percentages of each parameter for their contribution to the most optimized separation of clusters.

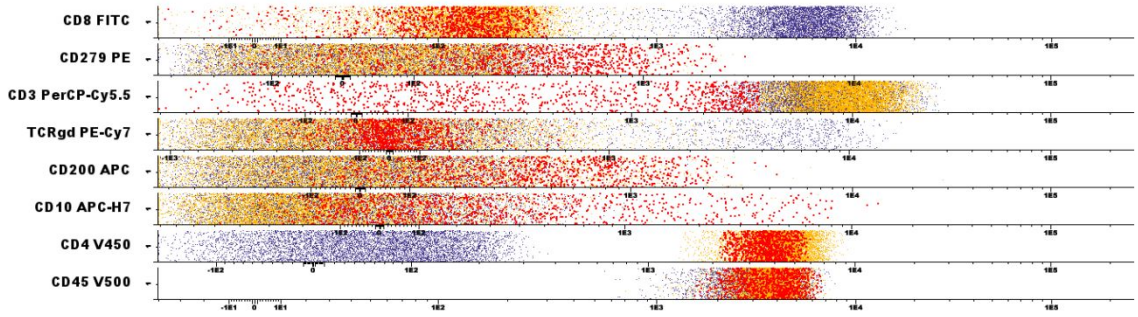


**FIGURE 1B.** Whole immunophenotypic profile of abnormal CD4+ T-lymphocytes, normal CD4+ T-lymphocytes and CD8+ T-lymphocytes using a multiparameter band dot plot from the Infinicyt software. The aberrant sCD3-CD4+ population is depicted as red dots, while normal residual CD4+ and CD8+ T-cells are shown as yellow and blue dots, respectively.

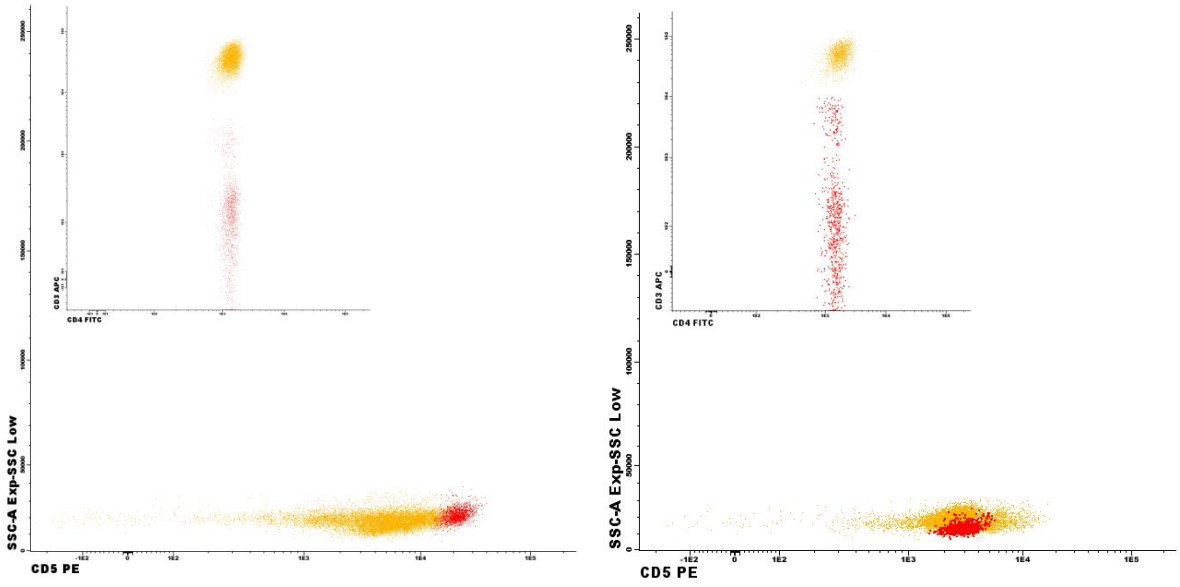


**FIGURE 1C.** CD5 flow cytometry analysis within the sCD3-CD4+ aberrant population (Left, L-HES showing CD5 bright expression; right, AITL case).

| Tube # | FITC | PE | PerCPCy5.5 | PECy7 | APC | APC-H7 | V-450 | V-500 |
|---|---|---|---|---|---|---|---|---|
| 1 | CD2 | CD7 | CD5 | TCR gamma/delta | CD3 | CD8 | CD4 | CD45 |
| 2 | CD57 | CD56 | CD8 | TCR gamma/delta | CD3 | CD38 | CD4 | CD45 |
| 3 | CD57 | CD26 | CD3 | TCR gamma/delta | CD7 | CD8 | CD4 | CD45 |
| 4 | CD8 | CD279 | CD3 | TCR gamma/delta | CD200 | CD10 | CD4 | CD45 |

**TABLE 1**. Composition of the 4-tube 8-colour MFC panel for detection of abnormal T-cells used in our laboratory daily routine. The first 3 tubes are used as a first diagnostic approach in suspected T-cell lymphoproliferative disorders. When AITL is specifically suspected or sCD3-CD4+ aberrant population is detected, the 4rth tube is systematically added.

Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCPCy5.5, peridinin–chlorophyll–protein–cyanin5.5; Cy7, cyanin7; APC, allophycocyanin; H7, hilite7; s, surface.