T-CELL CLONALITY ASSESSMENT BY FLOW CYTOMETRY USING ANTI-TCR-Cb-1: INITIAL EXPERIENCE OF A SINGLE CENTER

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Background: Clonality assessment in the diagnosis of reactive and neoplastic T-cell proliferations could be challenging in routine practice requiring expensive and time consuming molecular testing. The recent discovery of a monoclonal antibody specific for human TCRβC1 placed in our hands a new opportunity to detect clonality of immunophenotypically distinct T-cell populations by flow cytometry.

Aim: To develop a flow cytometric approach to establish clonality in mature T-cell lymphocytes by incorporating an anti-TCRβC1(JOVI-1) antibody specific for one of the two mutually exclusive β-chains.

Methods: Since March 2023 8 samples of peripheral blood and 3 of bone marrow from 11 patients (4 men; 7 women; mean age 56,5±18,43) with absolute lymphocytosis (71,19±184.2x10^9/L) were studied by antibodies against T-cell (CD3, CD4, CD8, CD2, CD5, CD7, CD26), B-cell (CD19, CD20), and NK-cell (CD56) markers as well as antibodies to characterise T-cell receptor (TCRα/β, TCRγ/δ, TCRβC1(JOVI-1) using 12-color stain-lysewash flow cytometry (FACSLyric, BD). TCRβC1 expression of <15% or >85% was considered monotypic.

Results: T-cell populations defined as clonal based on ≥85% positive or negative TCRβC1(JOVI-1) immunolabeling were identified in 6 patients: CD3(+)CD4(+) in two, CD3(+)CD8(+) in three and CD3(+)CD4(+)CD8(+) in one. The remaining T-cell subpopulations in these cases showed a polyclonal TCRβC1(+):TCRβC1(-) distribution - median TCRβC1-positivity was 41.7% (range 21.1–55.7%) . In 4 patients, the analysis showed negative clonality with polyclonal TCRβC1(+):TCRβC1(-) distribution maintained in all subpopulations (median TCRβC1-positivity 41.3.0%; range 30–49.9%). Monotypic cases showed significantly higher absolute lymphocyte counts (ALC) compared to those lacking clonality (mean ALC 137.4x10^9/L; range 5.6-527x10^9/L vs 4.5x10^9/L; range3.2-6.2x10^9/L, respectively) as well as aberrant morphology and/or phenotypes in 4/6 patients. In one case, the presence of oligoclonality was detected: there were 1.7x10^9/L CD3(+)CD4(+)CD7(-)CD26(-) T-cells with TCRβC1(JOVI-1) negativity in 90% and 0.3x10^9/L CD3(+)CD4(+)CD8(+) T cells, 95% of which were TCRβC1(JOVI-1)(-). However, no other clinical or laboratory abnormalities were present and the case raised the question of a T-CUS (T-cell clones of uncertain significance).

Conclusion: Concordant with published data, the incorporation of TCRβC1(JOVI-1) antibody in flow cytometric immunophenotyping panels allowed us rapid and reproducible determination of clonality in mature T-cell lymphoproliferations and facilitated the differentiation of reactive from neoplastic processes in the diagnostic process.

References

Shi M, Jevremovic D, Otteson GE, et al. Single antibody detection of T-cell receptor ab 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: 9