Flow Cytometric Clonality Assessment of T-lymphocytes by T-cell Receptor β-Chain Constant Region 1 Expression

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Objectives

Diagnosing peripheral T-cell lymphomas (PTCLs) with conventional flow cytometry is subject to considerable drawbacks. The recent discovery of a new single monoclonal antibody targeting specific T-cell Receptor β-chain constant region 1 (TRBC1, clone JOVI-1) could be of added value in the differentiation of neoplastic (monotypic) versus non-neoplastic (polytypic) T-cells. In this study, we aimed to verify diagnostic performance characteristics of TRBC1 expression.

Methods

A total of 47 samples were prospectively evaluated in the laboratory of the AZ Sint-Jan Hospital, Bruges, Belgium, including 22 selected non-neoplastic samples from healthy volunteers (n=12), healthy neonates (n=5) and post-hematopoietic stem cell transplantation patients (n=5) and 25 non-selected routine samples with suspicion of PTCL. Results were correlated with molecular T-cell Receptor (TCR) gamma gene rearrangements and final clinical diagnosis.

Results

TRBC1 showed a polytypic (15-85%) expression in 22/22 selected non-neoplastic samples and in 14/25 non-selected routine samples, all clinically assessed negative for PTCLs. 6/25 samples showed a monotypic (<15% or >85%) TRBC1 expression, all clinically confirmed with PTCLs (sensitivity 100%) (Figure 1, 2).

In contrast, 5/25 routine samples showed a monotypic expression, in the absence of an overt clinically confirmed PTCL (specificity towards clinical diagnosis 88%) but in accordance to a monoclonal molecular TCR gamma gene rearrangements result (specificity towards molecular assay 100%). Final clinical diagnosis of these 5/25 samples included myelodysplastic syndrome (n = 1), lymphocytic-variant hypereosinophilic syndrome (n = 1), Epstein-Barr virus-driven post-transplant lymphoproliferative disorder (n = 1) and asymptomatic lymphocytosis with no indication for therapy (“T-cell clones of uncertain/undetermined significance”, T-CUS, n = 2). Since the presence of T-cell clones in these diseases has been recognized earlier and is confirmed by molecular analysis,

specificity of TRBC1 expression towards clinical diagnosis would increase when expanding true positive results beyond stringent WHO classification of PTCLs.

Figure 1. Percentage of TRBC1-positive events in CD4+ and CD8+ T-lymphocytes in various clinical conditions. *Both CD4+ and CD8+ T-lymphocytes showed polytypic TRBC1 expression (15-85%) in healthy controls, neonates & post-transplant patients (young T-lymphocytes) and in various infectious conditions. Patients with clinically and molecularly confirmed PTCL showed monotypic TRBC1 expression (<15% or >85%).*

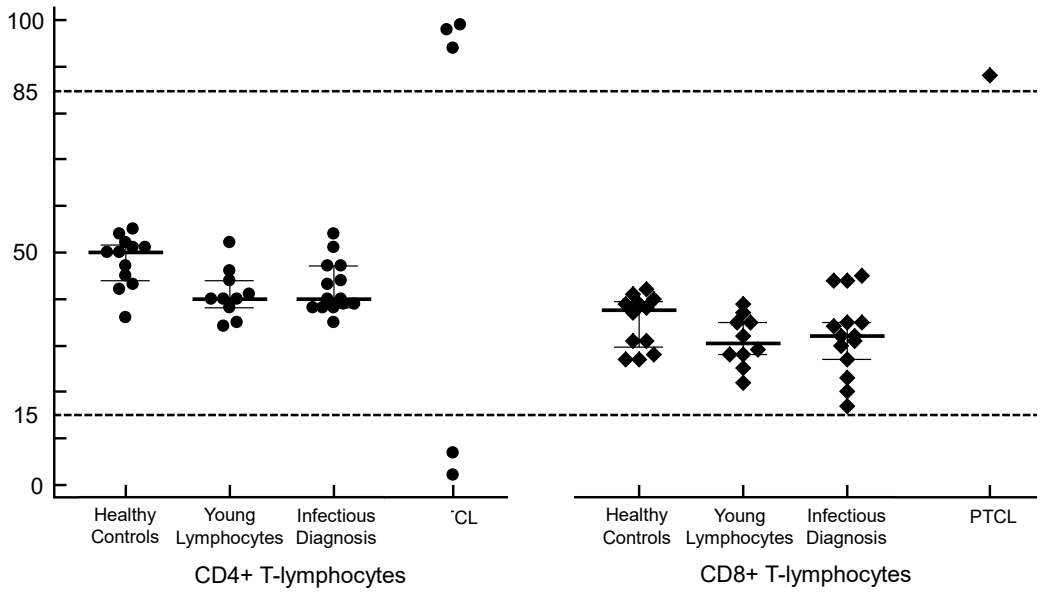
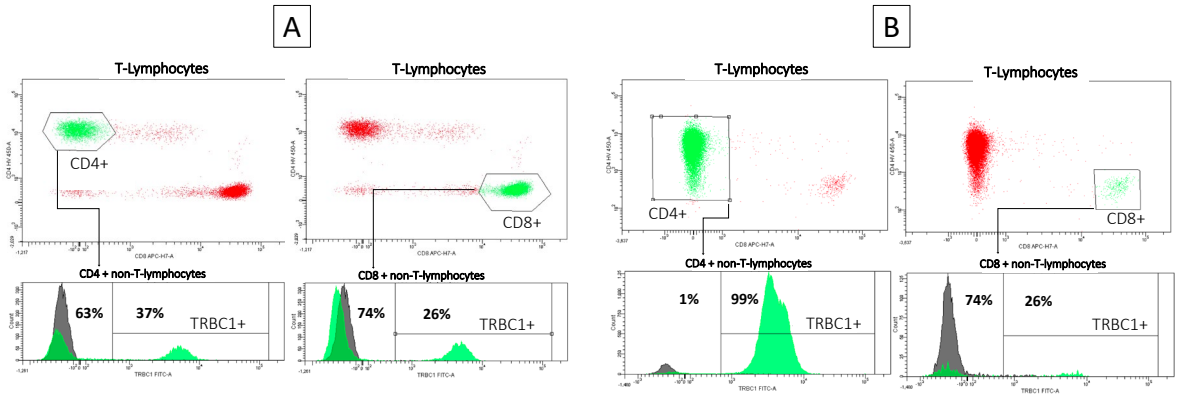


Figure 2. Flow cytometric dot plots after TRBC1 staining. *(A) Peripheral blood of a healthy donor showing both polytypic CD4+ and CD8+ T-lymphocytes. (B) Peripheral blood sample of a patient with known Sézary syndrome showing monotypic CD4+ T-lymphocytes.*



Conclusion

TRBC1 expression in the assessment of clonal CD4+/CD8-, CD4+/CD8+ or CD4-/CD8+ T-lymphocytes by flow cytometry is characterised by a high sensitivity and specificity. This assay can be of great value in the routine diagnostics of PTCLs besides TCR gene rearrangements in clinical laboratories and is endorsed by a faster turnaround time compared to the latter technique. However, a well-considered panel design is required to gate the TRBC1 expression on the population of interest.