High incidence of clonal CD8+ T-cell proliferation in non-malignant conditions may reduce the significance of T-cell clonality assay for differential diagnosis in oncohematology

Y.V. Sidorova¹, K.A. Sychevskaya¹, N.G. Chernova¹, S.Ju. Smirnova¹, N.V. Ryzhikova¹,
V.R. Gorodetskiy², E.V. Naumova³, H.L. Julhakyanyan¹, E.E. Zvonkov¹, A.B. Sudarikov¹

¹ National Research Center for Hematology, Moscow, Russia ² V.A.Nasonova Scientific and Research Institute of Rheumatology, Moscow, Russia

³ Department of clinical laboratory diagnostics Russian Medical Academy of postgraduate continuous education, Moscow, Russia

Background

PCR analysis of rearranged T-cell receptor (TCR) genes is essential for differential diagnosis between reactive and malignant lymphoid proliferation. However, age-related narrowing of T-cell repertoire, the expansion of reactive or autoimmune clones may sometimes lead to false positive results.

Objective

To evaluate the specificity of PCR-based methods of T-cell clonality determination and applicability of clonal assays for the differential diagnosis between T-LGL leukemia and reactive conditions.

Materials & Methods

We have compared T-cell clonality data obtained from a large cohort of healthy individuals (HI) including the elderly (n-62), patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (n-14), patients with transitory reactive CD8+ LGL-lymphocytosis (n-17) with the appropriate data obtained for patients diagnosed with T-LGL leukemia (n-42). The median age of the patients with T-LGL leukemia was 57 years (29-79) and the median age of other participants was 48 years (22-77). T-cell clonality based on the rearrangements of the TCRG and TCRB genes was assessed using BIOMED-2 protocol. Total peripheral blood lymphocytes, CD8+ and CD57+ cells were analyzed.

Results

Monoclonal TCRG/TCRB rearrangements in blood were identified in 11.3%/4,8% of healthy individuals (7/3 of 62); 21.4%/14,3% of patients with rheumatic diseases (RA/SLE) and 17,6%/11,8% of patients with reactive CD8+ lymphocytosis (Table 1). Immunomagnetic selection of lymphocytes in this group (n 33) showed that the clonal T-cells were CD8+ and CD57+, except two patients with rheumatoid arthritis who had clonal expansion of CD4+ lymphocytes (Fig. 1). No clonal V β -J β TCRB rearrangements were revealed in control group, only D β -J β TCRB and TCRG (Fig. 2). Given the high detectability of monoclonal rearrangements of V β -J β TCRB (96.7%) in patients with $\alpha\beta$ -T-LGL leukemia, this marker had the greatest (100%) specificity and accuracy (99.2%) for T-cell clonality detection. Also good specificity and accuracy was achieved when monoclonality was detected in TCRG and TCRB loci simultaneously (100%; 91.2%).

Table 1. Monoclonal results for TCRG/TCRB genes in different groups of patients

Patient group	TCRG	TCRB D β -J β	TCRB V β -J β	TCRB total	TCRG + TCRB
T-LGL $\alpha\beta$ leukemia (n-30)	25 (83.3%)	17 (56.7%)	29 (96.7%)	30 (100%)	30 (100%)
T-LGL $\gamma\delta$ leukemia (n-12)	11 (91.7%)	5 (41.7%)	4 (33.3%)	7 (58.3%)	11 (91.7%)
Healthy (n-62)	7 (11.3%)	3 (4.8%)	0 (0%)	3 (4.8%)	9 (14.5%)
RA / SLE (n-14)	3 (21.4%)	2 (14.3%)	0 (0%)	2(14.3%)	5 (35.7%)
Reactive CD8+ lymphocytosis (n-17)	3 (17.6%)	2 (11.8%)	0 (0%)	2 (11.8%)	5 (29.4%)

Figure 1. The result of T-cell clonality testing in healthy individuals in the bulk population of lymphocytes, in selected CD57+ or CD8+ cells, and depleted fraction (CD57- or CD8-).

a) TCRG rearrangements, volunteer 41 years old, female, b) TCRB V β -J β rearrangements, donor 53 years old, male, c) TCRB D β -J β rearrangements, donor 45 years old, female, d) TCRB V β -J β rearrangements, donor 31 years old, male.

Figure 2. Frequency of different T-cell clonal patterns in control group: A) in healthy individuals of different ages, RA/SLE and CD8+reactive lymphocytosis; B) for TCRG V γ -J γ , TCRB D β -J β and TCRB V β -J β genes rearrangements. Mono-monoclonal; dbt/oligo – doubtful or oligoclonal; poly – polyclonal result.s

Summary

The presence of clonal CD8+CD57+ cells is common for healthy individuals and patients with reactive conditions and may not associate with any malignancy. Patients with T-LGL leukemia may have T-cell clonality profiles similar with healthy individuals, patients with rheumatic diseases or reactive LGL proliferation. Therefore, interpretation of T-cell clonality should be conducted with caution.

Discussion

Many diseases can occur with clinical manifestations similar to that of T-LGL leukemia. Prolonged neutropenia, proliferation of CD8+/CD16+, CD56+, or CD57+ lymphocytes, unsubstantial splenomegaly could be observed in connective tissue diseases, myelodysplastic syndrome, paraneoplastic syndromes and infections. Positive result of T-cell clonality test may thus be the only indication of the possible T-cell malignancy. However, based on the data presented, T-cell clonality itself cannot perceive as an exclusive argument in favor of T-LGL leukemia diagnosis.

Contact Information

Sidorova Yulia: iouliavl@rambler.ru

Chernova Natalia: ngchernova@mail.ru

Julhakyanyan Hunan : oncohematologist@mail.ru,
julhakyanyan.h@blood.ru