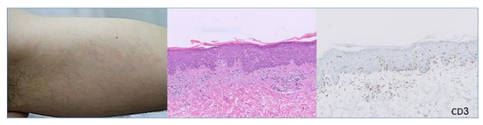
14/Hist-P-01

Evaluation of the PCR-based T Cell Receptor-β clonality test in the diagnosis of early Mycosis Fungoides

Aviv Barzilai [1, 2, 3], Orit Schachter [1], Assaf Debby [2, 3], Oz Segal [1], Sharon Baum [1, 3], Hila Tabibian-Keissar [2]

Department of Dermatology[1] and Institute of Pathology[2], , Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel
[3] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background
Diagnosis of early mycosis fungoides can be challenging both clinically and in interpretating the histopathology[1]. T-cell receptor (TCR) clonality is important for mycosis fungoides (MF) diagnosis[2]. Routine clonality analysis is performed using a polymerase chain reaction (PCR) TCR-γ assay; yet with this method 10%–50% of T-cell lymphomas escape detection. TCR-β gene rearrangement is an additional assay. Data about its efficacy is controversial.



Objective Evaluate the role of TCR-β assay in the diagnosis of early MF.

Methods
This is a retrospective cohort study. Cases were included if results of both TCR-ɣ and TCR-β clonality tests using BIOMED-2 primers and protocol[3] were performed on the biopsy specimens. The diagnosis of early MF was based on clinical, histologic, and immunohistologic criteria according to the ISCL criteria excluding monoclonality (score 4 and above). All patients were examined and followed up at the dermatology department, and biopsy specimens were available from the Pathology Institute at Sheba Medical Center. All medical files and biopsy specimens were reviewed. In all cases but tumors, where the infiltrate was mostly superficial, the sections were micro-dissected at the subepidermal interface of the papillary dermis, and only the upper half was submitted for DNA extraction. Statistical analysis was performed with SPSS, version 22.0, software (IBM, Armonk, NY). The association between TCR-ɣ and TCR-β results was assessed using an chi-square Fischer's exact test. Differences in clonality detection between TCR-ɣ and TCR-β assays were evaluated using a McNemar test. A P value of less than .05 was considered statistically significant.

Results
Sixty-one patients were included in the study (17 women, 44 men; age range, 18-88 years). These patients were categorized into 3 groups: 1. Twenty MF patients (4 or more on the ISCL score, excluding TCR monoclonality) (4 tumors (IIB) , 16 patch/plaque (IA); 4 women, 16 men; age range, 18-83 years). This group served to test the TCR assays in patients with definite MF. 2. Thirty patients suspected to have early MF (ISCL score of 3, excluding TCR monoclonality) (all with patches (I); 9 women, 21 men; age range, 19-88 years). This group was selected to assess the additional value of both tests in the diagnosis of early MF. 3. Eleven patients with chronic inflammatory disease (dermatitis, psoriasis, lupus erythematosus, or pityriasis lichenoides chronica) served as a negative control.

TCR clonality results for the whole cohort is described in Table I.

TCR gene rearrangement among patients with MF
Monoclonality by TCR-β and/or TCR-ɣ gene rearrangement was detected in 16 of 20 (80%) (15 (75%) TCR-β; 12 (60%) TCR-ɣ; 11 (55%) both) skin samples as described in Table II. All 4 tumors (100%) exhibited monoclonality in both assays. Despite the concordance rate of 75% between the 2 assays, an association between them could not be demonstrated (P = 0.1). Of the 16 patients with early MF, 12 (75%) exhibited monoclonality, 8 (50%) showed TCR-ɣ rearrangement, and 11 (69%) showed TCR-β rearrangement. One patient exclusively showed TCR-ɣ gene rearrangement. Therefore, in this group of patients with MF, TCR-β gene rearrangement assay detected clonality in more cases than TCR-ɣ gene rearrangement assay. However, this difference was not statistically significant.

TCR gene rearrangement among patients with suspected early MF
Fourteen out of the 30 patients (46%) showed either TCR-β or TCR-ɣ clonal gene rearrangement: all of them in TCR-β and only 5 (16%) in TCR-ɣ (Table III). According to the ISLC score combined score, these 14 patients were diagnosed with and treated for early MF. Therefore, when adding these patients to the 16 patients with MF in the group for which TCR was not required for the diagnosis, monoclonality was shown by TCR-β in 83% of patients with early-stage MF and by TCR-ɣ in 46%, as shown in Table I. Thus, TCR-β is significantly more sensitive in detecting early MF than TCR-ɣ (P = .002). This superior sensitivity of TCR-β was maintained when tumors were added, and all 34 patients with MF were included. For all patients with MF in the cohort, the sensitivity of these assays in diagnosing MF was 85% for TCR-β and 50% for TCR-ɣ. Fig 1 shows the distribution of TCR clonality for both assays among all patients with early MF. Figure 2 shows an example both TCR-ɣ and TCR-β monoclonality in a case of patch stage MF.

TCR gene rearrangement among patients with chronic inflammatory disease
None of the control samples showed monoclonality.

Thus, in our cohort, both assays showed a specificity rate of 100%. For the whole group of patients, the positive predictive value of both tests was 100%, and the negative predictive values were 84% and 61% for the TCR-β and TCR-ɣ assays, respectively.

Table I. Clonality detection results for T-cell receptor gene rearrangement in study population

	MF n=20	Suspected MF n=30	Total early-stage MF n=30	Chronic inflammatory dermatoses n=11
ß monoclonality	15	14	25	0
ß polyclonality	5	16	5	11
ɣ monoclonality	12	5	13	0
ɣ polyclonality	8	25	17	11

MF, mycosis fungoides;
Suspected MF- ISCL score=3;
Early-stage MF-ISCL score≥4

Table II. Concordance rate between TCR-β and TCR-ɣ in MF cases

	***TCR*-β**	
	Monoclonal	Polyclonal
***TCR*-γ** Monoclonal	11	1
***TCR*-γ** Polyclonal	4	4

Concordance rate: 75%

Table III. Concordance rate between *TCR*-β and *TCR*-γ in high suspicion of MF

	***TCR*-β**	
	Monoclonal	Polyclonal
***TCR*-γ** Monoclonal	5	0
***TCR*-γ** Polyclonal	9	16

Concordance rate: 70%

Figure 1. Clonality detection results for TCR gene rearrangement in early stage MF

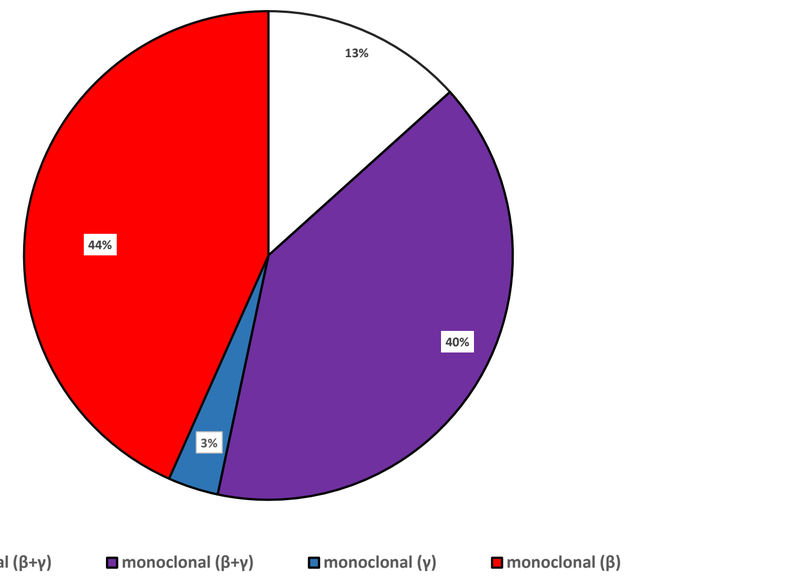
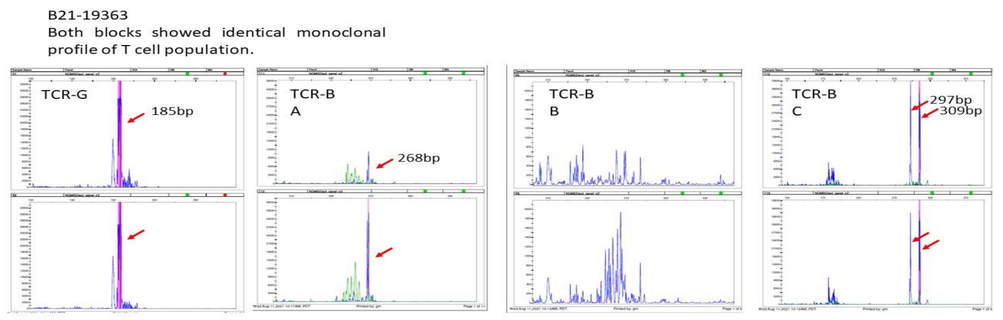


Figure 2: A case of patch stage MF showing clonality in both TCR-ɣ and TCR-β assays



Discussion
TCR clonality results have a role in the diagnosis of MF. Most publications have found no additive value in using the TCR-β clonality assay as a single test compared with the TCR-ɣ assay but have recommended the combination of these tests to maximize the negative predictive value[4-9]. Looking in detail at the published results, the TCR-β clonality test was more sensitive in early-stage MF (covering 10% of body surface area) compared to TCR-ɣ, increasing the detection rate of clonality. TCR-ɣ was more sensitive in cases of widespread disease. In the current study, the TCR-β clonality assay showed a significantly higher sensitivity rate compared to the TCR-ɣ assay in diagnosing MF (85% vs 50%, respectively, of all patients with MF and 69% vs 50%, respectively, of patients with early MF). Despite high concordance rates between the 2 assays, we could not show an association between them. Both of these findings favor performing a TCR-β assay to assess clonality in MF, at least in early or doubtful cases. Limitations of our study are its retrospective nature and the small cohort.

Conclusion

T-cell receptor β assay showed a higher sensitivity rate than T-cell receptor ɣ assay. Using both assays can improve the diagnosis rate of early-stage mycosis fungoides.

Refernces:
1. Willemze R, et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105(10): 3768-3785.
2. Pimpinelli N, et al. Defining early mycosis fungoides. J Am Acad Dermatol. 2005;53:1053-1063.
3. Sandberg Y, et al. Biomed-2 multiplex immunoglobulin/T-cell receptor polymerase chain reaction protocols can reliably replace Southern blot analysis in routine clonality diagnostics. J Mol Diagn. 2005;7:495-503.
4. Xu C, et al. Diagnostic significance of TCR gene clonal rearrangement analysis in early mycosis fungoides. Chin J Cancer. 2011;30(4):264-272.
5. Zhang B, et al. Combined use of PCR-based TCRG and TCRB clonality tests on paraffin-embedded skin tissue in the differential diagnosis of mycosis fungoides and inflammatory dermatoses. J Mol Diagn. 2010;12:320-327.
6. Kuo SY, et al. A parallel comparison of T-cell clonality assessment between an in-house PCR assay and the BIOMED-2 assay leading to an efficient and cost-effective strategy. J Clin Pathol. 2011;64:536-542.
7. Van Dongen JJ, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the Biomed-2 Concerted Action BMH4-CT98-3936. Leukemia 2003;17:2257-2317.
8. Assaf C, Hummel M, Dippel E, et al. High detection rate of Tcell receptor beta chain rearrangements in T-cell lymphoproliferations by family specific polymerase chain reaction in combination with the GeneScan technique and DNA sequencing. Blood. 2000;96:640-646.
9. Jawed SI, Myskowski PL, Horwitz S, et al. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome): part I. Diagnosis: clinical and histopathologic features and new molecular and biologic markers. J Am Acad Dermatol. 2014;70:205.