Evaluation of the polymerase chain reaction–based T-cell receptor β clonality test in the diagnosis of early mycosis fungoides

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Background: T-cell receptor (TCR) clonality may help establish a diagnosis of mycosis fungoides (MF). Routine clonality analysis is performed by using a polymerase chain reaction TCR- gamma assay, yet with this method, 10% to 50% of T-cell lymphomas escape detection. TCR- beta gene rearrangement is an additional assay. Data about its efficacy are controversial.

Objective: To evaluate the role of TCR-β assay in the diagnosis of early MF.

Methods: A retrospective study of 61 skin biopsies, 20 from patients with MF, 30 from patients suspected to have early MF, and 11 from patients with chronic inflammatory skin disease.

Results: Monoclonality was detected in 16 of 20 (80%) MF cases: 15 (75%) with TCR-β and 12 (60%) with TCR-γ assay. Of the 30 suspected cases of early MF, 14 showed monoclonality with TCR-β, and only 5 of 14 showed monoclonality with TCR-γ assay. None of the chronic inflammatory condition samples showed monoclonality. Therefore, TCR-β clonality assay was more sensitive than TCR-γ in early MF (83% vs 43%; P = .002).

Limitations: This was a retrospective, relatively small study.

Conclusion: TCR-β showed a higher sensitivity rate compared with TCR-γ in early-stage MF. The combined use of the TCR-β and TCR-γ clonality tests can significantly improve the diagnosis rate of early-stage MF. (J Am Acad Dermatol 2020;83:1400-5.)

Key words: clonality analysis; CTCL; mycosis fungoides; PCR; T-cell receptor; TCR-β.

Cutaneous T-cell lymphomas (CTCLs) represent a heterogeneous class of non-Hodgkin lymphomas characterized by the clonal expansion of neoplastic T lymphocytes in the skin.[1] CTCLs are typically monoclonal in origin, developing from a single T cell derived from malignant clones of identical rearranged T-cell receptor (TCR) genes.[2] Mycosis fungoides (MF) is the most common type of primary CTCL, comprising approximately 50% of cases. It is characterized by an indolent clinical course with slow, if any, progression over years to decades through 4 main stages—patch, plaque, tumor, and visceral involvement.[2,3]

The diagnosis of MF is based on the combination of clinical manifestations and histopathologic findings, including immunohistochemistry. The differentiation of early MF from chronic inflammatory

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skin conditions may sometimes pose a diagnostic dilemma. TCR clonality testing has been commonly performed for more than 15 years, providing additional evidence for the diagnosis of MF.[2,4,5] The International Society for Cutaneous Lymphoma (ISCL) developed an algorithm for diagnosing early-stage MF involving a 4-point scoring system that integrates clinical, histologic, immunophenotypic, and molecular criteria to help in the detection of a dominant T-cell clonal pattern using polymerase chain reaction (PCR)—based analysis.[5,6] The overall sensitivity of PCR TCR methods varies according to the disease stage. The monoclonal pattern can be found in up to 100% of patients with disease in the tumor stage, in 83% to 100% of patients with erythrodermic MF, and in 52% to 83% of patients with disease in the patch and plaque stages.[7-13]

In recent years, PCR clonality has mainly been performed in routine practice using a TCR-γ assay.[3,14] Monoclonality does not necessarily indicate malignancy, because various benign dermatoses can produce this pattern.[2]

With the emergence of EuroClonality (BIOMED-2)—specific primers for TCR-β gene rearrangement testing, TCR-β has been studied as an additional/alternative approach to detect clonality in T-cell malignancies.[8,11,15,16] The BIOMED-2 study showed a 94% gene rearrangement rate of the TCR-β gene in T-cell malignancies compared to an 89% rearrangement rate of the TCR-γ gene.[11] However, to date, there are limited data on its sensitivity and specificity in patients with MF. In this retrospective study, we evaluated the role of TCR-β clonality using the BIOMED-2 panel in diagnosing early MF.

CAPSULE SUMMARY

- T-cell receptor analysis is an important diagnostic test for mycosis fungoides. Currently, T-cell receptor γ assay is performed. With this method, many cases, particularly in the early stage, escape detection.
- T-cell receptor β assay showed a higher sensitivity rate than T-cell receptor γ assay. Using both assays can improve the diagnosis rate of early-stage mycosis fungoides.

MATERIALS AND METHODS

Case selection

This is a retrospective cohort study. Cases were included if, in the process of diagnosis, both TCR-γ and TCR-β clonality tests using BIOMED-2 primers were performed on the biopsy specimens. Clonality results did not serve as an inclusion criterion. The diagnosis of early MF was based on clinical, histologic, and immunohistologic criteria according to the ISCL criteria (scoring 4 and above).[6] The tumor stage was classified according to the World Health Organization—European Organization for Research and Treatment of Cancer classification.[1] 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 records and biopsy specimens were reviewed. The study was approved by the institutional review board of Sheba Medical Center.

Clonality assessment

DNA was extracted from 20 to 25 4-μm sections of formalin-fixed, paraffin-embedded cutaneous punch biopsies using Qiagen (Hilden, Germany) columns (catalog number 51306). Minor changes were introduced to fit the protocol to formalin-fixed, paraffin-embedded cutaneous biopsy specimens. Next, 50 μL of proteinase K (40 mg/mL) were added to ATL buffer, and samples were incubated at 56°C overnight. The same amount of proteinase K was added in the morning, and samples were incubated at 56°C for 1 hour. The samples were then incubated at 90°C for 1 hour. DNA was precipitated by adding AL and alcohol, and the samples were incubated at 20°C for 1 hour. The rest of the protocol was performed as described by the manufacturer. DNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Next, 1 to 50 ng DNA was used for PCR. In all cases but tumors, where the infiltrate was mostly superficial, the sections were microdissected at the subepidermal interface of the papillary dermis, and only the upper half was submitted for DNA extraction. As previously reported,[17] it is our experience that with this procedure, the extract contains a higher proportion of malignant T-cell DNA.

PCR tests for TCR-γ and TCR-β gene rearrangements were performed in duplicate following the BIOMED-2 protocol using Identiclone kits (Invivoscribe, San Diego, CA) and Maxima HotStart Taq polymerase (Thermo Fisher Scientific) according to the manufacturers' protocols.[11] Next, 1 μL of each PCR product was analyzed by capillary electrophoresis (3500XL; Applied Biosystems, Foster City, CA) using 0.5 μL ROX (Applied Biosystems) as a size standard and 8.5 μL formaldehyde (Applied Biosystems, Foster City). Positive (monoclonal) and negative (polyclonal) controls were included in each run. Interpretations of polyclonal and monoclonal

Abbreviations used:

CTCL: cutaneous T-cell lymphoma
ISCL: International Society for Cutaneous Lymphoma
MF: mycosis fungoides
PCR: polymerase chain reaction
TCR: T-cell receptor

profiles of T-cell populations were performed as described in the literature.[11,18] It is our practice to define a monoclonal profile in a stringent way, that is, when the intensity of the bold peak is 2.5 to 3 times higher than the background. Performance characteristics were determined for each test individually and in combination. An oligoclonal pattern was classified as polyclonality.

Statistical methods

Statistical analysis was performed with SPSS, version 22.0, software (IBM, Armonk, NY). The association between TCR-γ and TCR-β results was assessed using an chi-square Fischer's exact test. Differences in clonality detection between TCR-γ 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. The first group comprised 20 patients (4 women, 16 men; age range, 18-83 years) diagnosed with MF (meeting 4 or more on the ISCL score, excluding TCR monoclonality): 16 had early MF (patch/plaque stage, stage I), and 4 had tumor MF (stage IIB); none were erythrodermic. This group served to test the TCR assays in patients with definite MF. The second group comprised 30 patients (9 women, 21 men; age range, 19-88 years) evaluated for suspected early MF (ISCL score of 3, excluding TCR monoclonality; T1 or T2, stage I). This group was selected to assess the additional value of both tests in the diagnosis of early MF. The third group comprised 11 patients with chronic inflammatory disease (dermatitis, psoriasis, lupus erythematosus, or pityriasis lichenoides chronica) and served as a negative control.

TCR gene rearrangement among patients with MF

Tables I to III summarize the monoclonality distribution according to the clinical findings and MF staging. Monoclonality by TCR-β and/or TCR-γ gene rearrangement was detected in 16 of 20 (80%) skin samples of patients diagnosed with MF. All tumors (4/4; 100%) exhibited monoclonality in both assays. Of the 20 cases, 15 (75%) showed TCR-β gene rearrangement, 12 (60%) showed TCR-γ rearrangement, and 11 (55%) showed both clonal TCR-β and -γ rearrangements (Table II). Despite the concordance rate of 75% between the 2 assays, an association between them could not be demonstrated (P = .1). Of the 16 patients with early MF, 12 (75%) exhibited monoclonality, 8 (50%) showed TCR-γ rearrangement, and 11 (69%) showed TCR-β rearrangement. One patient only showed TCR-γ gene rearrangement. Therefore, in this group of patients with MF, TCR-β gene rearrangement assay detected clonality in more cases than TCR-γ gene rearrangement assay. However, this difference was not statistically significant.

TCR gene rearrangement among patients with suspected early MF

Of the 30 patients with suspected MF, 14 (46%) showed either TCR-β or TCR-γ gene rearrangement: all of them showed TCR-β clonal rearrangement (46%), and only 5 (16%) showed TCR-γ clonal rearrangement. According to the ISLC score combining clinical, histopathologic, immunologic, and molecular results, 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 nontumor/early-stage MF and by TCR-γ in 46%, as shown in Table I. Thus, TCR-β is significantly more sensitive in detecting early MF than TCR-γ (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-γ. Fig 1 shows the distribution of TCR clonality for both assays and their relation among all patients with 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-γ assays, respectively.

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

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

MF, Mycosis fungoides.
*Suspected MF: International Society for Cutaneious Lymphoma score of 3.
†Early-stage MF: International Society for Cutaneious Lymphoma score of 4 or greater.

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

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

MF, Mycosis fungoides; *TCR,* T-cell receptor.
*Concordance rate: 75%.

DISCUSSION

MF can be a challenging diagnosis, particularly in the early stages, because it can simulate benign inflammatory dermatosis. The ISCL proposed a diagnostic algorithm to detect early MF that incorporates TCR monoclonality to support the diagnosis.[6] In recent years, a PCR assay of TCR-γ gene rearrangement has been the prevalent molecular test in diagnosing MF.[3,14] To unify results and overcome technical problems, the BIOMED-2 protocol has been adapted by most laboratories to assess monoclonality.[7-11] The BIOMED-2 protocol is considered the criterion standard for clonality assessment in suspected lymphocytic infiltrations.

Nevertheless, one of the drawbacks of this test is false negative results. Beyond the nature of the disease (in the early stage, there are a relatively small number of tumor cells), failure to show clonality can result from technical limitations such as section thickness, poor tissue sampling, cell size variations, or the degradation of DNA in the histologic processing of the tissue.[16] To overcome these false negative issues, it was suggested that the TCR-β gene be tested as an alternative approach. Assaf et al[12] found T-cell clonality in 100% of 24 samples from patients with advanced-stage CTCL when using the PCR-based TCR-β assay.[12] In 2010, Zhang et al[9] proposed an algorithm for the combined use of PCR-based TCR-γ and TCR-β clonality tests in diagnosing MF in patients with intermediate probability of MF. In this algorithm, Zhang et al proposed the TCR-γ assay as the primary clonality test and the TCR-β assay as an ancillary test.[9] In the current study, TCR-β showed a higher diagnostic sensitivity compared to TCR-γ among patients with MF, exhibiting a significant difference in the sensitivity rate among patients with early MF. The detection rate of TCR-γ primers (60%) was lower compared with previous studies, whereas TCR-β showed similar (75%) and even higher sensitivity rates.[7-11] This can be accounted for by the fact that, unlike most previous reports, our study deals only with MF and not with other CTCLs and by the fact that most patients enrolled were clinically and histologically determined to be in the early stage of disease, when oligoclonality is more prevalent.[2,9] In reviewing the literature, monoclonality assessed by TCR-γ assay was observed in 52% to 75% of patients with disease in the patch stage of MF, 73% to 83% with disease in the plaque stage of MF, and up to 100% with disease in the tumor stage of MF.[2] The polyclonal profile of early-stage MF cases can be explained by the low percentage of malignant cells in the infiltrate, whereas the majority of the cells are reactive T cells. Thus, the higher polyclonal peaks of the reactive T cells mask the small peak that represents malignant T cells. A rare clone that is not part of the BIOMED-2 primer mixes is also another possible explanation for the polyclonal result of early-stage MF, although it is less probable. Vega et al[19] described clonal heterogeneity in a subset of patients with MF, suggesting that early lesions emerge from polyclonal or oligoclonal activation of T cells, with independent outgrowth of several clones at different sites.

In the current study, the TCR-β clonality assay showed a significantly higher sensitivity rate compared to the TCR-γ assay in diagnosing MF (85% vs 50%, respectively, of all patients with MF and 69% vs 50%, respectively, of patients with early MF when the diagnosis is made irrespective of clonality). The concordance rate for the whole MF group was 75% and was 70% for those with early-stage disease and 100% for those with tumors. Furthermore, in our cohort, we could not show an association between the 2 assays. Both of these

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

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

MF, Mycosis fungoides; *TCR,* T-cell receptor.
*Concordance rate: 70%.

findings favor performing a TCR-β assay to assess clonality in MF, at least in early or doubtful cases. So far, there are mixed data in the literature about the utility of the TCR-β clonality assay in CTCL diagnosis. Unlike our study, most publications have found no additive value in using the TCR-β clonality assay as a single test compared with the TCR-γ assay but have recommended the combination of these tests to maximize the negative predictive value.[7-12] In contrast, Zhang et al[9] found similar sensitivity and specificity rates between TCR-γ and TCR-β assays. 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-γ, increasing the detection rate of clonality. TCR-γ was more sensitive in cases of widespread disease.[9] This aforementioned data are similar to our observation that in early-stage MF, TCR-β is more sensitive than TCR-γ (83% vs 43%), with a lower concordance rate between the 2 tests.

Different gene primers can be one explanation for the differences between the TCR-γ and TCR-β analyses. We sampled TCR gene rearrangement in 30 patients with a high suspicion of MF due to clinical, histopathologic, and immunophenotype characteristics but without a definitive diagnosis. TCR-γ gene rearrangement was detected in 5 patients (17%) compared with 14 (47%) with TCR-β. All cases that showed clonality based on the TCR-γ assay also exhibited clonality based on the TCR-β assay. Thus, based on the TCR-β assay results, the 14 patients who fulfilled the ISCL criteria were diagnosed as having early MF and were therefore treated accordingly. This higher sensitivity of the TCR-β assay did not come at the expense of specificity, because monoclonality was not detected among chronic inflammatory skin diseases.

Manual microdissection of the tissue specimens is not commonly performed when assessing TCR, thus posing the question of whether this procedure contributed to the higher sensitivity reported for TCR-β assay. However, this does not seem to apply to the current study. Manual microdissection used to enrich the sample with the cells of interest has

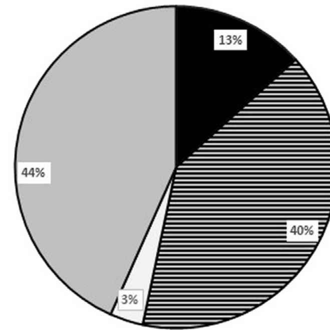

Fig 1. Clonality detection results for T-cell receptor gene rearrangement in total early-stage mycosis fungoides cases.

proven itself not only in MF[17] but also in general pathology.[20,21] We used the same DNA extracted from the microdissected specimens for both PCR reactions (TCR-γ and TCR-β). Therefore, no preference is given to one chain over the other, as was the case in previous studies, in which the assays were performed on the same DNA extracted from the whole biopsy.[7-12]

The study has certain limitations, mainly its retrospective nature and limited sample size. The latter may explain why clonality was not shown in any of the chronic dermatosis cases, such as pityriasis lichenoides, for which the rate is approximately 8%.[22] Furthermore, one may argue that in an era of next-generation sequencing/high-throughput sequencing techniques to asses clonality, studies such as ours are not beneficial. Nevertheless, in a recent abstract,[23] when high-throughput sequencing of the TCR-β gene was used in early MF, a clone was detected in 24 of 36 (67%) cases of early MF, a rate that is not dissimilar from what we have found (69%/83%). In addition, these techniques are still very expensive, are not standardized, and are not available in many laboratories. All these leave room to improve the old techniques.

In conclusion, our findings indicate that the detection of monoclonality using the TCR-β gene rearrangement assay has a role in diagnosing MF, mostly in the earlier stages and in doubtful cases. Whether it should replace the simpler, more commonly used TCR-γ gene rearrangement assay or whether it should be used only in conjunction with the TCR-γ assay in specific cases needs to be further studied. In any case, one must remember that monoclonal assays should always be interpreted as an adjunct assay to the clinical, pathologic, and immunophenotype characteristics in diagnosing patients suspected of having MF.

REFERENCES

1. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. 2005;105(10):3768-3785.
2. Kandolf L, Cikota B, Stojadinović O, et al. TCR gamma gene rearrangement analysis in skin samples and peripheral blood of mycosis fungoides patients. *Acta Dermatovenerol Alp Pannonica Adriat*. 2007;16(4):149-155.
3. Sufficool KE, Lockwood CM, Abel HJ, et al. T-cell clonality assessment by next-generation sequencing improves detection sensitivity in mycosis fungoides. *J Am Acad Dermatol*. 2015;73(2):228-236.
4. Santucci M, Biggeri A, Feller AC, et al. Accuracy, concordance, and reproducibility of histologic diagnosis in cutaneous T-cell lymphoma: an EORTC Cutaneous Lymphoma Project Group Study. European Organization for Research and Treatment of Cancer. *Arch Dermatol*. 2000;136:497-502.
5. Santucci M, Biggeri A, Feller AC, et al. Efficacy of histologic criteria for diagnosing early mycosis fungoides. *Am J Surg Pathol*. 2000;24:40-50.
6. Pimpinelli N, Olsen EA, Santucci M, et al. Defining early mycosis fungoides. *J Am Acad Dermatol*. 2005;53:1053-1063.
7. Xu C, Wan C, Wang L, et al. Diagnostic significance of TCR gene clonal rearrangement analysis in early mycosis fungoides. *Chin J Cancer*. 2011;30(4):264-272.
8. Sandberg Y, van Gastel-Mol EJ, Verhaaf B, 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.
9. Zhang B, Beck AH, Taube JM, 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.
10. Kuo SY, Liu H, Liao YL, 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.
11. Van Dongen JJ, Langerak AW, Bruggemann M, 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.
12. Assaf C, Hummel M, Dippel E, et al. High detection rate of T-cell 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.
13. 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.
14. Ponti R, Fierro MT, Quaglino P, et al. TCRγ-chain gene rearrangement by PCR-based GeneScan: diagnostic accuracy improvement and clonal heterogeneity analysis in multiple cutaneous T-cell lymphoma samples. *J Invest Dermatol*. 2008;128(4):1030-1031.
15. Sandberg Y, Heule F, Lam K, et al. Molecular immunoglobulin/T-cell receptor clonality analysis in cutaneous lymphoproliferations: experience with the BIOMED-2 standardized polymerase chain reaction protocol. *Haematologica*. 2003;88:659-670.
16. Brüggemann M, White H, Gaulard P, et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies: report of the BIOMED-2 Concerted Action BHM4 CT98-3936. *Leukemia*. 2007;21:215-221.
17. Dereure O, Levi E, Vonderheid EC, et al. Improved sensitivity of T-cell clonality detection in mycosis fungoides by hand microdissection and heteroduplex analysis. *Arch Dermatol*. 2003;139(12):1571-1575.
18. Lukowsky A, Muche JM, Möbs M, et al. Evaluation of T-cell clonality in archival skin biopsy samples of cutaneous T-cell lymphomas using the biomed-2 PCR protocol. *Diagn Mol Pathol*. 2010;19(2):70-77.
19. Vega F, Luthra R, Medeiros LJ, et al. Clonal heterogeneity in mycosis fungoides and its relationship to clinical course. *Blood*. 2002;100:3369-3373.
20. Ren ZP, Sällström J, Sundström C, et al. Recovering DNA and optimizing PCR conditions from microdissected formalin-fixed and paraffin-embedded materials. *Pathobiology*. 2000;68(4-5):215-217.
21. Hunt JL, Finkelstein SD. Microdissection techniques for molecular testing in surgical pathology. *Arch Pathol Lab Med*. 2004;128:1372-1378.
22. Weinberg JM, Kristal L, Chooback L, et al. The clonal nature of pityriasis lichenoides. *Arch Dermatol*. 2002;138(8):1063-1067.
23. De Masson A, Battistella M, Gruber A, et al. High throughput sequencing of the T-cell receptor beta gene in lesional skin in the diagnosis of primary cutaneous T-cell lymphoma: analysis of 76 patients. Poster presented at: 4th World Congress of Cutaneous Lymphoma, Barcelona, Spain, February 2020.