Article published online: 2025-03-07

Diagnostic Utility of Molecular Analysis in Cutaneous T-Cell Lymphoma: Tunisian Series on Clonality of TCRG Gene Rearrangement

Sarra Klai[1] Imen Helal[1] Raja Jouini[1] Houda Hammami[2] Maher Kharrat[3] Sami Fenniche[2] Fatma Khanchel[1] Aschraf Chadli-Debbiche[1]

[1] Department of Pathology, Habib Thameur Hospital, Tunis, Tunisia
[2] Department of Dermatology, Habib Thameur Hospital, Tunis, Tunisia
[3] LR99ES10 Human Genetics Laboratory, Faculty of Medicine of Tunis (FMT), University of Tunis El Manar, Tunis, Tunisia

Address for correspondence Sarra Klai, PhD, Department of Pathology, Habib Thameur Hospital, 1008 Mont Fleury, Tunis, Tunisia (e-mail: gksarrah@yahoo.fr).

South Asian J Cancer

Abstract

Introduction The diagnosis of cutaneous T-cell lymphoma (CTCL) is sometimes difficult. Detection of monoclonal T-cell receptor gamma (TCRG) gene rearrangement by polymerase chain reaction (PCR) has become an important adjunct to the diagnosis of CTCL. This study was designed to explore the concordance in terms of the diagnostic value of BIOMED-2 TCRG PCR protocol with the histological diagnosis.

Methods Confirmed and doubtful CTCLs were included in this descriptive cross-sectional study performed in the Habib Thameur Hospital in 2021. These cases were followed in the department of dermatology from 2012 to 2021. PCR tests were performed with TCRG BIOMED-2 clonality methods followed by capillary electrophoresis and GeneScan analysis. Clonality and statistical results were analyzed.

Results Monoclonality was identified in 51% of confirmed CTCL cases (16/28 cases with confirmed mycosis fungoides and 2/7 other CTCL cases) and in 63% of doubtful cases, which were converted to malignant diagnosis. The results of TCRG clonality demonstrated a significant correlation with histopathology diagnoses of specimens. A moderate concordance was found between histology and molecular clonality.

Conclusion Results from this molecular clonality emphasize the importance of interpreting data in association with histopathological features of the lesions.

Keywords
- cutaneous T-cell lymphoma
- mycosis fongoides
- clonality
- TCRG
- BIOMED-2 protocol
- histology

Introduction

Cutaneous T-cell lymphomas (CTCLs) demonstrate a variety of clinical, histological, and molecular features and can follow an indolent or very aggressive course.[1] While the term CTCL includes a number of rare disease entities, the most common subtypes are mycosis fungoides (MF) and Sézary's syndrome (SS), accounting for approximately 70 to 75% of all cases.[2] However, the underlying pathogenetic mechanisms of CTCL are not fully understood.[1,3] Therefore, accurate evaluation in each individual case is crucial for an adequate, stage-adapted therapeutic approach.[4] In a significant number of suspected T-cell lymphoproliferative disorders, regarded as doubtful cases, a definitive diagnosis of malignancy cannot be made without additional evidence of a clonal process.[4] Clonality may establish a form of molecular

DOI https://doi.org/10.1055/s-0045-1805056 ISSN 2278-330X

How to cite this article: Klai S, Helal I, Jouini R, et al. Diagnostic Utility of Molecular Analysis in Cutaneous T-Cell Lymphoma: Tunisian Series on Clonality of TCRG Gene Rearrangement. South Asian J Cancer 2025;00(00):00–00.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

confirmation in instances in these doubtful cases, in which the malignity of a lymphoid lesion is not certain based on histopathological or immunophenotypic assessment. T-cell receptor gamma (TCRG) assays provide molecular evidence of clonality in malignant or doubtful lymphoproliferative disorders and can facilitate minimal residual disease assessment.[5] This study aimed to explore the concordance in terms of the diagnostic value of BIOMED-2 TCRG polymerase chain reaction (PCR) protocol with the histological diagnosis.

Methods

Doubtful and confirmed CTCLs were included in this descriptive cross-sectional study performed in the Habib Thameur Hospital in 2021. These cases were followed in the department of dermatology from 2012 to 2021. Patients were excluded if there was lack of information on clinical data, or if the block was exhausted. In all cases, histopathological and immunophenotypic studies were performed in the pathology department. Molecular clonality analysis was done in the Molecular Biology Laboratory of the Pathology Department and the Human Genetics Laboratory of the Faculty of Medicine of Tunis. Demographic data, number of lesions per patient, and histopathological and immunophenotypical findings were collected. We grouped the lesions into MF, other CTCL, and doubtful cases with uncertain clinical presentation, noncontributory histology, and nondemonstrative immunohistochemistry (IHC) of the T-cell infiltrate.

DNA Isolation

Genomic DNA was extracted using the QIAamp DNA formalin-fixed paraffin-embedded tissue kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). In cases with insufficient DNA, a vacuum centrifugal concentration was carried out using an Eppendorf Vacufuge plus Vacuum Concentrator (ID: 22820109). In all cases, before proceeding to PCR, the DNA concentration and purity were assessed to ensure high-quality genomic DNA in the samples.

Clonality Assessment via Analysis of TCRG Gene Rearrangements

TCRG gene rearrangements were assessed using the standardized BIOMED-2 multiplex PCR clonality assays (InVivoScribe Technologies, San Diego, California, United States). Genomic DNA was amplified with a single master mix that contains primers that target the Vγ2, 3, 4, 5, 8, 9, 10, and 11 and Jγ1/Jγ2, JγP, and JγP1/JγP2 regions, generating PCR amplicons with an expected size range between 159 and 207 nucleotides. The reaction included both a clonal and a polyclonal control. The PCR components and standard program were set up to follow the provider's protocols. After TCR amplification, 8 µL of PCR products was loaded on 1% agarose gels to check whether PCR products had been formed. PCR amplification was followed by a capillary electrophoresis, fluorescence-labeled PCR products were run on an ABI Prism 3500 Genetic Analyzer, and the resulting data were analyzed using the GeneMapper V 5.0 software (Applied Biosystems, Foster City, California, United States).

Statistical Analysis

Statistical analysis was performed using IBM SPSS (v. 18.00; IBM Corp., Armonk, New York, United States). Data were summarized using frequencies, percentages, medians, and ranges. Fisher's exact test was used to determine whether a statistically significant relationship exists between TCRG monoclonality and confirmation of the preliminary diagnosis. The cutoff level for statistical significance was set at 0.05. Concordance was analyzed by the use of kappa coefficient. It was valued between 0 and 1 (0.93–1.00: excellent; 0.81–0.92: very well; 0.61–0.80: well; 0.41–0.60: moderate; 0.21–0.40: below the middle; and 0.01–0.20: poor concordance).

Results

In total, 113 skin biopsies from 54 patients were included in this study. The lesions were solitary in 35 cases, and multiple lesions were observed in 19 cases. Thirty-seven patients were male, with a median age at diagnosis of 56.5 years (18–73 years). Based on clinical, histological, and immunohistochemical (CD3, CD4, CD8, CD2, CD5, CD7) criteria, cases were divided into (35/54, 65%) confirmed CTCL cases with MF (28/35, 80%) and other CTCL (7/35, 20%) and (19/54; 35%) doubtful CTCL cases including 10 doubtful MF, 4 eczematous dermatoses, and 1 angioimmunoblastic T-cell lymphoma (AITL).

TCRG Clonality Interpretation

Results were interpreted according to the latest EuroClonality/BIOMED-2 guidelines for interpretation and reporting of TCR clonality testing in suspected lymphoproliferations. ►Fig. 1 shows the presence of a clear monoclonal TCRG rearrangement.

Among the 54 cases, 30 (56%) were monoclonal for TCRG rearrangement, and 24 (44%) were polyclonal; monoclonality was found in 18 of 35 (51%) cases of confirmed CTCL, in 16 of 28 MF (57%), in 2 of 7 other CTCL, and in 12 of 19 (63%) doubtful cases. ►Fig. 2 shows the number of clonal peaks that were detected. Among the cases with monoclonality results, 9/30 cases (30%) had two clonal peaks, and 1 case (3%) had three peaks.

Among the 19 doubtful cases, 12 were converted to malignant diagnosis (11 MF and 1 AITL) and 7/19 remained doubtful (►Table 1). Analysis of TCRG clonality as a discriminator between the confirmation and the rejection of the preliminary diagnosis was performed using Fisher's exact test. There was a significant correlation ($p = 0.038$) between TCRG monoclonality and confirmation of the preliminary histopathological diagnosis (►Table 2).

The results of TCRG clonality assay and the histology diagnosis were concordant in 14 cases (74%) and discordant in 5 cases (26%). A moderate concordance was found between histology and molecular clonality (kappa = 0.41).

Discussion

Our results showed a concordance in terms of the diagnostic value of BIOMED-2 TCRG PCR protocol with the histological diagnosis. In order to unify results and overcome technical problems, the BIOMED-2 protocol is considered the gold

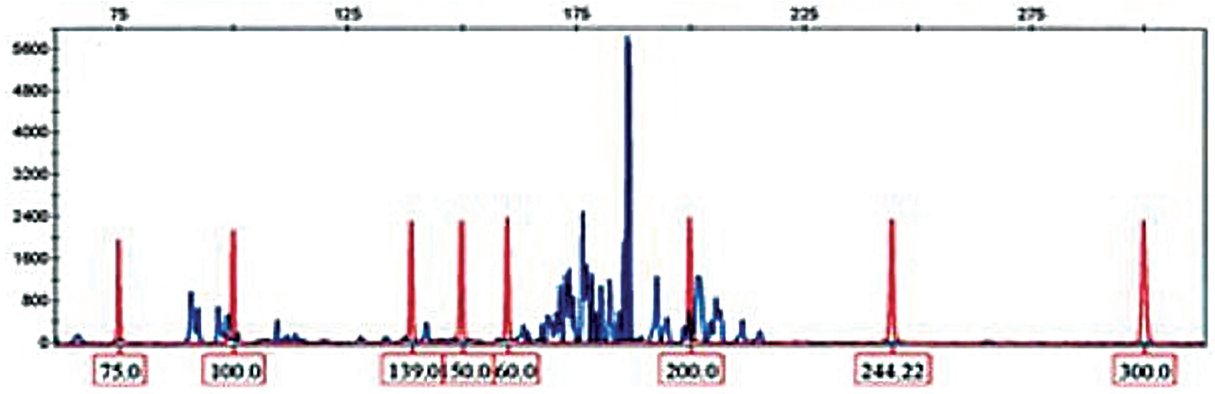

Fig. 1 T-cell receptor gamma gene rearrangement in an angioimmunoblastic T-cell lymphoma case showing a monoclonal peak with a size of 185 bp in a polyclonal background.

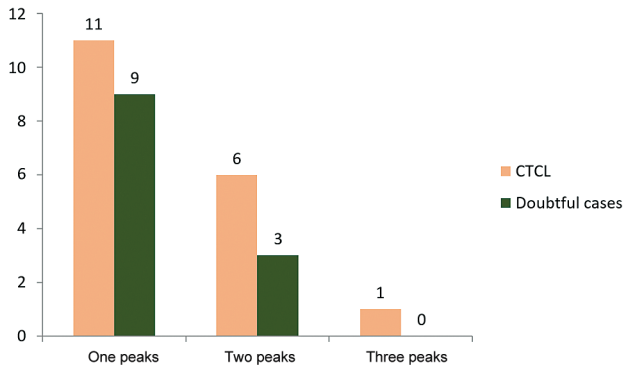

Fig. 2 Number of clonal peaks for confirmed and doubtful CTCL cases in the monoclonal group. CTCL, cutaneous T-cell lymphoma.

standard for clonality assessment in suspected lymphocytic infiltrations.[6–8] Another recent development in the area of T- and B-cell clonality testing by PCR is the use of capillary electrophoresis, which has significantly improved the resolution and quantitation of PCR products.[9,10]

Of the 54 patients included in this study, 30 (56%) were monoclonal for TCRG rearrangement. Multipeak monoclonality was observed in 10 cases, and the explanation for double-peak cases is either the presence of two dominant clones in the same sample or a clone with TCRG biallelic rearrangements. Three peaks in cases could indicate the presence of a minor tumor clone.[11] We detected monoclonality in 16/28 (57%) patients with confirmed MF. Similar detection rates were found in a large study by Massone et al.[12] Also, these findings are in concordance with many other studies, which demonstrated that in well-established CTCL cases, TCR clonality has been shown to detect T-cell monoclonality in 52 to 90% of established CTCL biopsy specimens.[13] Kirsch et al revealed superior frequencies using high-throughput sequencing of established CTCL cases.[3]

While MF and SS are the most common CTCLs, there are limited data about the other CTCLs. In the present study, among all the seven cases of other CTCL, two were monoclonal for a TCRG rearrangement. Analysis of clonal TCRG gene rearrangement plays an important role in the diagnosis of lymphoproliferative disorders. The identification of T-cell monoclonality within the lymphoid infiltrate can provide strong evidence of the diagnosis of CTCL.[13,14] Combining this approach with histopathologic and immunophenotypic

Table 1 Preliminary and final diagnoses of TCRG monoclonal cases among the doubtful cases

Preliminary diagnosis	Monoclonal TCRG rearrangement	Polyclonal TCRG rearrangement	Final diagnosis
Doubtful MF cases ($n = 14$)	10	4	MF ($n = 10$)
Eczematous dermatoses ($n = 4$)	1	3	MF ($n = 1$)
Suggestive of AITL ($n = 1$)	1	0	AITL ($n = 1$)

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; MF, mycosis fungoides; TCRG, T-cell receptor gamma.

Table 2 Correlation between the preliminary diagnosis and clonality among doubtful cases

TCRG clonality	Change of diagnosis ($N = 7$)	No change of diagnosis ($N = 12$)	p-Value[a]
TCRG monoclonality	1	11	0.038
TCRG polyclonality	4	3	

Abbreviation: TCRG, T-cell receptor gamma.
[a]Significant at $p \leq 0.05$.

examinations may aid in the diagnosis of doubtful cases. Twelve of 19 (64%) of doubtful cases revealed monoclonal TCRG gene rearrangement. Our finding is comparable with the results of many studies.[11,13,15] In a series of borderline lesions from 22 patients who subsequently developed MF and 32 newly suspected CTCL patients, TCRG monoclonality was detected in 50% (10/22) and 19% (6/32) of cases, respectively.[15] Regarding our study, the positive rate of TCRG gene rearrangement in suggestive MF cases was 71% (10/14). Similar rates were found by Hsiao et al.[16] However, different detection rates were found in other studies.[17] Several factors accounting for the variability include different sensitivity of PCR detection methods, different stages and duration of early lesions, the inclusion of nonspecific lesions, the subjectivity of interpretation by pathologists, and the protocol followed in IHC.

In 74% of doubtful cases (12/19), TCRG molecular analysis allowed the confirmation of the malignant nature of lymphoid proliferation. We demonstrated concordant TCRG molecular analysis and histology results, with moderate kappa score (kappa = 0.41). Eleven (58%) doubtful cases: 10 MF and 1 AITL demonstrated TCRG monoclonality, confirming their clonal origin and thus the diagnosis of CTCL. We have shown that the results of TCRG clonality demonstrated significant correlation with histopathologic diagnoses ($p = 0.038$). Similar results were found by Sandberg et al; in 17 samples, the molecular findings correlated with the histopathologic diagnosis of malignant T-cell proliferations.[18] In contrast, a previous study reported that differences in histologic parameters between PCR positive and PCR negative were not statistically significant. A polyclonal proliferation is not equivalent to benign lesions. This was justified by the fact that molecular studies are not very useful when the histopathologic findings show a more severe inflammation because inflammation dilutes the malignant T-cell clones. Even if there is a negative PCR result, it is important to consider the dilution of clonal T cells by reactive lymphocytes (in the case of a small clonal T-cell population) and more similar lesions need to be included.[16]

Conclusion

Detection of monoclonal TCRG gene rearrangement is a very powerful supplement to the diagnosis of CTCL. It is especially useful in the diagnosis of doubtful cases with atypical histopathologic features. BIOMED-2 PCR TCR clonality protocol has wide availability and produces satisfactory results, and thus, it is a useful tool in routine clinical practice. Results of molecular clonality testing in this study emphasize the importance of interpreting data in association with morphologic features as well as immunophenotypic characteristics of the lesions. New multicenter prospective studies are mandated to incorporate new possible markers related to given treatments.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Ethical Approval

This study was approved by the ethical committee of Habib Thameur Hospital.

Conflict of Interest

None declared.

Acknowledgments

This project is carried out under the MOBIDOC scheme, funded by the EU through the SWAFY Project and managed by the National Agency for the Promotion of Scientific Research.

References

1 Dummer R, Vermeer MH, Scarisbrick JJ, et al. Cutaneous T cell lymphoma. Nat Rev Dis Primers 2021;7(01):61
2 Wong HK, Mishra A, Hake T, Porcu P. Evolving insights in the pathogenesis and therapy of cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). Br J Haematol 2011;155(02): 150–166
3 Kirsch IR, Watanabe R, O'Malley JT, et al. TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL. Sci Transl Med 2015;7(308):308ra158
4 Jonak C, Tittes J, Brunner PM, Guenova E. Mycosis fungoides and Sézary syndrome. J Dtsch Dermatol Ges 2021;19(09):1307–1334
5 Mahe E, Pugh T, Kamel-Reid S. T cell clonality assessment: past, present and future. J Clin Pathol 2018;71(03):195–200
6 Zamò A, Bertolaso A, van Raaij AW, et al. Application of microfluidic technology to the BIOMED-2 protocol for detection of B-cell clonality. J Mol Diagn 2012;14(01):30–37
7 Langerak AW, Groenen PJTA, Brüggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 2012;26(10):2159–2171
8 van Dongen JJM, Langerak AW, Brüggemann 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(12):2257–2317
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(03):320–327
10 Walia R, Yeung CCS. An update on molecular biology of cutaneous T cell lymphoma. Front Oncol 2020;9:1558
11 Miyata-Takata T, Takata K, Yamanouchi S, et al. Detection of T-cell receptor γ gene rearrangement in paraffin-embedded T or natural killer/T-cell lymphoma samples using the BIOMED-2 protocol. Leuk Lymphoma 2014;55(09):2161–2164
12 Massone C, Kodama K, Kerl H, Cerroni L. Histopathologic features of early (patch) lesions of mycosis fungoides: a morphologic study on 745 biopsy specimens from 427 patients. Am J Surg Pathol 2005;29(04):550–560
13 Gibbs JD, Ma S, Kim A, et al. Utility of flow cytometry and gene rearrangement analysis in tissue and blood of patients with suspected cutaneous T–cell lymphoma. Oncol Rep 2021;45(01): 349–358
14 Xu C, Wan C, Wang L, Yang H-J, Tang Y, Liu W-P. Diagnostic significance of TCR gene clonal rearrangement analysis in early mycosis fungoides. Chin J Cancer 2011;30(04):264–272
15 Ashton-Key M, Diss TC, Du MQ, Kirkham N, Wotherspoon A, Isaacson PG. The value of the polymerase chain reaction in the diagnosis of cutaneous T-cell infiltrates. Am J Surg Pathol 1997;21 (07):743–747

16 Hsiao P-F, Hsiao C-H, Lin Y-C, Tseng MP, Tsai TF, Jee SH. Histopathologic-molecular correlation in early mycosis fungoides using T-cell receptor gamma gene rearrangement by polymerase chain reaction with laser capture microdissection. J Formos Med Assoc 2007;106(04):265–272
17 Kamarádová K, Hrochová K, Salavec M, Belada D. T-cell receptor antibodies expression in benign and malignant cutaneous lymphoid infiltrates in comparison with T-cell receptor gene rearrangement and its diagnostic utility in borderline cases. Pathol Res Pract 2020;216(12):153279
18 Sandberg Y, van Gastel-Mol EJ, Verhaaf B, Lam KH, van Dongen JJM, Langerak AW. 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(04):495–503

