Articles

The applicability of T-cell receptor γ gene rearrangement as an adjuvant diagnostic tool in skin biopsies for cutaneous T-cell lymphoma

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ABSTRACT

Objective: The diagnosis of cutaneous T-cell lymphoid infiltrates may be difficult based on clinical and routine immunohistologic findings. In this situation, an ancillary technique demonstrating the presence of a monoclonal cell proliferation could help to rule in or out cutaneous T-cell lymphoma (CTCL) in cases that clinically and histopathologically do not allow a definitive diagnosis. Southern blot analysis is a time-consuming method with low sensitivity that should not be considered for the routine diagnosis of cutaneous lymphoid infiltrates. Moreover, it can be used only when fresh tissue is available. New assays based on the amplification of the T-cell receptor gamma (TCRγ) chain gene rearrangement by polymerase chain reaction (PCR) have been proposed to overcome these limitations.

Methods: We retrospectively studied 124 biopsies from 104 patients (66 biopsies with the clinical and histological diagnosis or suspicious of CTCL and 58 biopsies with histological diagnosis of benign reactive dermatological conditions who presented to the Dermatology Unit at King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia between 1996 and 2004. The specimens were morphologically examined and then analyzed by PCR for the gamma chain of the TCRγ followed by gel electrophoresis.

Results: The results showed 87.1% sensitivity and 92% specificity in detecting clonal T-cell gene rearrangements among CTCL cases with a positive predictive value of 93.1% and negative predictive value of 85.2%. Therefore, negative TCRγ results in CTCL should be taken with caution.

Conclusion: The detection of clonal TCRγ gene rearrangement by PCR based method is an adjuvant diagnostic marker for CTCL, although it can be seen in some benign dermatoses.

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Detection of clonal T-cell receptor gamma (TCRγ) chain gene rearrangements by polymerase chain reaction (PCR) followed by gel electrophoresis has now become a valuable tool in the diagnosis of cutaneous T-cell lymphoma (CTCL).[1-4] Most laboratories use TCRγ PCR due to its early rearrangement during T-cell maturation, whereas TCRß PCR is rarely applied, due to its locus complexity.[5-6] Several studies have confirmed the improved sensitivity of PCR-based technique to detect clonal rearrangement of the TCRγ gene in skin biopsy specimens of CTCL. The technique has also been successfully applied to archival, paraffin-embedded skin tissues with variable sensitivity.[7] To determine the adjuvant diagnostic role of TCR gene rearrangement for the diagnosis of CTCL and its level of correlation with morphology and

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clinical findings, we studied 66 skin tissue samples with the clinical diagnosis of suspicious CTCL.

Methods. ***Patient samples.*** We retrospectively studied 66 skin biopsy specimens from patients being followed-up for CTCL diagnosis and management in the Department of Medicine, Dermatology Unit, King Faisal Specialist Hospital and Research Center between 1996 and 2004. The skin specimens were submitted for Histological and Molecular studies to the Department of Pathology and Laboratory Medicine. In addition, 58 skin biopsies with benign dermatological lesions were included in this study as negative controls. The majority of samples for molecular studies were from paraffin-embedded tissue blocks.

Deoxyribonucleic acid extraction from tissue (manual). The embedded material was removed from the paraffin block using xylene. Fresh tissue was minced in phosphate buffered saline (PBS) by manual manipulation with a scalpel. Cells were digested with detergent (10% sodium dodecyl sulfate [SDS]) and a proteolytic enzyme (Proteinase K-20 mg/ml) and Phenol: Chloroform: isoamyl alcohol added to the lysate. Nucleic acid in the aqueous phase was separated from protein in the organic phase. The nucleic acid is then precipitated by salting out, using 3M sodium acetate and absolute ethanol.[8]

Deoxyribonucleic acid extraction from tissue (automated). The embedded material was removed from the paraffin block using xylene. Fresh tissue was minced in PBS by manual manipulation with a scalpel. Cells were digested with detergent (10% SDS) and a proteolytic enzyme (Proteinase K-20 mg/ml). The resulting lysate was placed on the Magnapure (Roche) for automated DNA extraction using the Roche Magnapure LC DNA Isolation Kit I. The DNA bound to magnetic glass particles (MGP's) in the presence of chaotropic salts at a pH >7. The MGP's have a glass (silica) surface and a magnetic core. The DNA adsorbed to the silica surface of MGP's in the presence of isopropanol and a high concentration of chaotropic salts, which removed water from the hydrated molecules in solution. Polysaccharides and proteins that do not adsorb to the beads were removed by sequential washing steps. The complex of magnetic particles and bound DNA was separated from the solution by applying a magnet externally. Pure DNA is then eluted from the MGP's by applying low-salt concentrations and heat.[9]

T-cell receptor gene rearrangement. Enzymatic amplification was performed in a Perkin Elmer Gene Amp PCR System 9600 using consensus primers for TCRγ chain V regions and a single consensus primer for the J region as described previously with minor modifications. Briefly, the reaction mixture contained 0.5 ug of DNA; 10 mM of Tris-HCl, pH 8.3: 200 uM of each dNTP; 50 mM KCl; 1.5 nM $MgCl_2$; from 7-10 pmol/μL of each primer and 1 u Taq Polymerase (Fast start Taq; Roche) in a final volume of 25 ul. The consensus TCRγ chain V and J primers were synthesized by Gulf Biotech (Portland, Oregon, USA). Sequences were:

Vγ (1-8)II5'ACCAGGAGGGGA
AGGCCCCACAG3';
Vγ9 5'GGAAAGGAATCTGGCATTCCG3';
Vγ10 5'AATCCGCAGCTCGACGCAGCA 3';
Vγ 11 5'GCTCAAGATTGCTCAGGTGGG 3';
Vγ 12 5'CCTCTTGGGCACTGCTCTAAA 3';
Vγ1/2 5'ACCTGTGA CAACAAGTGTTGTTC 3'

The reaction mixture was subjected to 40 cycles of PCR following an initial 10 minute denaturation step of 94^{O}C. Each cycle consisted of a 30-second denaturation step at 94^{O}C, a 30 second annealing step at 62^{O}C, and a one minute elongation step at 72^{O}C. The last cycle was followed by a 15 minute elongation step at 72^{O}C. Ten μl of PCR amplified product was resolved by electrophoresis on a 6% polyacrylamide gel, stained with ethidium bromide and visualized under ultraviolet light. Discrete band(s) are seen in clonal T-cell processes within the predicted size range of 190-260 base pairs. All samples were subjected to amplification using primers for the erb-B2 gene to confirm that amplifiable DNA was present.[10]

Statistical analysis: The following measures of the T- cell receptor test performance were calculated: 1. the sensitivity, which is the probability of testing positive if the disease is truly present, 2. the specificity, which is the probability of testing negative if the disease is truly absent, 3. the predictive value positive, which is the probability that a person actually has the disease given that he or she tests positive, and 4. the predictive value negative, which is the probability that an individual is truly disease-free given that he or she tests negative.

Results. The detection of a clonal TCRγ gene rearrangement was defined by the presence of one or 2 bright, discrete bands, which resulted from the amplification of monoallelic and biallelic VJγ gene rearrangement. A polyclonal infiltrate was defined by the presence of a broad smear. One hundred twenty-four skin biopsies from 104 patients (including 58 cases with benign dermatological conditions and 66 cases with the clinical suspicion of CTCL were analyzed at the Molecular Hematology Laboratory,

Table 1 - Statistical analysis for TCR sensitivity and specificity.

Test	Percent	CTCL		Total
		1	2	
TCR 1	Count	54	4	**58**
	within TCR (%)	(93.1)	(6.9)	**(100)**
	within CTCL (%)	(87.1)	(8)	**(51.8)**
TCR 2	Count	8	46	**54**
	within TCR (%)	(14.8)	(85.2)	**(100)**
	within CTCL (%)	(12.9)	(92)	**(48.2)**
Total	Count	62	50	**112**
	within TCR (%)	(55.4)	(44.6)	**(100)**
	within CTCL (%)	(100)	(100)	**(100)**

Gold standard is CTCL: Sensitivity 87.1%, Specificity 92%, Predictive value positive 93.1%, Predictive value negative 85.2%
TCR - T-cell receptor gene rearrangement,
CTCL - cutaneous T-cell lymphoma

Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia between 1996 and 2004. The TCRγ chain gene rearrangement method sensitivity and specificity in predicting the diagnosis of CTCL have reached to 87.1% and 92% with a positive predictive value of 93.1% and a negative predictive value of 85.2% (**Table 1**).

Discussion. T-cell receptor gamma chain gene rearrangement is a tool that helps in differentiating CTCL from the benign inflammatory dermatoses by the demonstration of clonal T-cells in skin biopsy. As a marker of the T-cell clonality, the rearrangement of the TCR genes is amplified by PCR and subsequently analyzed by several electrophoresis techniques.[11] Previous studies that used PCR-based technique such as PCR/ denaturing gradient gel electrophoresis (DGGE,) PCR/temperature (gradient gel electrophoresis), and PCR/single-strand conformational polymorphism (SSCP), to investigate TCRγ gene rearrangement from the skin biopsy specimens have demonstrated clonality in 52-90% of cases with established CTCL.[12] In these studies, frozen rather than paraffin-embedded tissue was used. However, tissue available for molecular assessment of clonality is often limited to archival paraffin blocks. As a result, PCR technique has been optimized for detecting TCRγ gene rearrangement using paraffin-embedded tissue. Such studies, showed 59-73% of clonality both in established and borderline lesions, which is within the range of positivity found in fresh frozen tissues.[13] In this retrospective study, the majority of cases were extracted from paraffin-embedded tissue yet has shown 87.1% sensitivity and 92% specificity.

It has been previously known that Vγ 1-8 and Jγ1/Jγ2 segments are involved in approximately 60-70% of clonal TCRγ gene rearrangements detected by southern blot analysis in various T-cell malignancies.[14] Using primers for amplifying Vγ 1-9 and Jγ1/Jγ2 segments, clonality has been demonstrated in up to 90% of CTCL cases investigated by PCR/DGGE (80% for Vγ 1-8, 35% for Vγ 9) by Wood et al.[15] In that study, the 10% of CTCL cases negative by PCR/DGGE analysis presumably was due to clonal rearrangements in other Vγ genes (Vγ 10-11).

Signoretti et al[16] studied the TCRγ gene family entirely for detection of clonality in lymphoproliferative disorders by PCR/SSCP in paraffin-embedded tissue, using the primers Vγ1-8, Vγ9, Vγ10, Vγ11 and Jγ1/ Jγ2. Their result is similar to Wood et al[15] by showing 83.3% (20/24) cases with clonal TCRγ gene rearrangement involving Vγ1-8 and Jγ1/ Jγ2 regions. The additional 3 cases showed clonality of 4.2% (1/24), 8.3% (2/24) and 0% when subsequently analyzed with Vγ9, Vγ10 and Vγ11 primers. The overall sensitivity for detection of clonality in T-cell lymphoma cases was 95% (23/24 cases). In our study, we confirm the frequent detection of Vγ1-8, Vγ9 and Jγ1/ Jγ2 regions in neoplastic clones of CTCL, which account for 88.8% of positive cases, and 11.2% of Vγ10 in positive cases, representing the majority of CTCL, which have clonal gene rearrangement in our cases.

In conclusion, this is one of the largest series of patients studied by TCRγ with 87.1% sensitivity and 92% specificity among CTCL cases. However, performing this molecular assay on fresh skin tissue will reduce the risk of false negative results and using Gene Scan analysis might improve the level of interpretation, but not necessarily the detection rate of monoclonality. It is well known that clonality does not necessarily equate with malignancy,[17] this fact has been confirmed by the presence of TCRγ gene rearrangement in 4 cases with lymphocytic vasculitis, granulomatous dermatitis with the hyper-immune response, pityriasis lichenoides et varioliformis acuta and chronic superficial deep dermatitis.

Clonal populations of lymphocytes could be found in cutaneous lymphoid hyperplasia,[18] clonal dermatitis,[19] which in up to 25% of cases, may progress to lymphoma within 5 years, drug-induced dermatoses,[20] small plaque parapsoriasis (SPP),[21] dysregulated immunity[22] and in healthy elderly subjects.[23] By using molecular PCR assays employing comprehensive sets of primers and highly sensitive methods, such as Nested-PCR, a higher incidence of clonal rearrangements can be expected in reactive benign conditions.[24] Therefore, molecular data

should never be interpreted in isolation from the other clinicopathological findings in each particular case. Our observation suggests that some of CTCL might not be monoclonal de novo, but oligoclonal instead, and the malignant clone may develop eventually from one of the oligoclonal populations during the disease progression. These phenomena might explain the repeatedly negative CTCL case by TCRγ PCR and TCRß Southern blot confirmed at a reference laboratory.

Other factors which might contribute to the false negative results may include the presence of high number of polyclonal gene rearrangements masking the presence of a monoclonal band, formation of heteroduplexes during gel electrophoresis and sampling errors due to uneven involvement of tissue by the disease process.[25] Therefore, we emphasize that Physicians, Dermatologists and Pathologists should be aware of the sensitivity, specificity and the pitfalls of molecular techniques used to interpret their results.[26]

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