T-Cell Receptor Gene Rearrangement Detection in Suspected Cases of Cutaneous T-Cell Lymphoma

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Primary cutaneous T-cell lymphomas (CTCLs) are a heterogeneous group of extranodal non-Hodgkin's lymphoma that present on the skin with no evidence of extracutaneous disease at the time of diagnosis (Willemze *et al.*, 2005). The general consensus is that CTCLs are typically monoclonal in origin, developing from a single T cell that forms a malignant clone of T cells in the skin (Kandolf Sekulović *et al.*, 2007). Mycosis fungoides (MF) and Sézary syndrome (SS) are the two most common types of CTCL, accounting for almost 65% of CTCLs. In the early stages of disease, MF can pose a diagnostic quandary for physicians and often leads to a delay in diagnosis despite multiple skin biopsies. This occurs because the neoplastic T cells in the skin in the early stages of MF are far outnumbered by the body's immune response through tumor-reactive T cells and other effector cells in the skin. Together with the neoplastic T cells, these resemble a nonspecific inflammatory infiltrate on microscopy, making it difficult to distinguish from other benign dermatoses such as eczema or psoriasis (Mishra and Porcu, 2011). SS often has nonspecific features histologically or they are less marked changes than are typically seen in MF, thus also posing diagnostic difficulties from skin biopsy alone (Willemze *et al.*, 2005).

T-cell receptor (TCR) gene rearrangement studies demonstrated by PCR are used to detect clonality and can be a highly reliable method to aid in diagnosing CTCL (Ponti *et al.*, 2008). The technique is dependent on the fact that T cells bear a unique antigen receptor on their cell surface that serves as a specific marker for that cell and its clonal progeny. If a cell undergoes neoplastic transformation, its TCR becomes a tumor marker specific to that cell lineage (Wood, 2001). Using PCR and high-resolution electrophoresis, a TCR clone can be demonstrated in up to 90% of skin biopsies in MF cases (Bottaro *et al.*, 1994).

This article describes how the TCR gene rearrangement analysis is carried out and interpreted, as well as its advantages, limitations, and use in the diagnosis of CTCL.

WHAT TCR GENE REARRANGEMENT ANALYSIS DOES

- TCR gene rearrangement studies, as demonstrated by PCR and gel electrophoresis, are used to detect malignant T-cell clones in CTCL.
- It is an excellent tool to aid in the diagnosis of CTCL, although it cannot be used to make a diagnosis of CTCL on its own.

LIMITATIONS

- Lack of amplification of the TCR-γ gene may occur owing to loss of representative gene segments or cells, variations in section thickness and cell size, or degradation of DNA in the histological processing of tissue.
- It can produce false-negative results due to factors such as poor tissue sampling, a biopsy specimen that has a small number of malignant T cells, or the possibility that the primers used may not cover all possible TCR-γ gene rearrangements.
- The high sensitivity of PCR means it can also produce false-positive results by detecting a dominant clone in other benign chronic dermatoses.

HOW IS TCR GENE REARRANGEMENT ANALYSIS PERFORMED?

TCRs are protein heterodimers expressed on the cell surface of T lymphocytes. They are composed of either α and β chains (the vast majority) or γ and δ chains. Each of these TCR chains is composed of several distinct regions called variable (V), joining (J), diversity (D), and constant (C). TCRs are unique to individual T lymphocytes and are formed by the rearrangement and assembly of the TCR genes during T lymphocyte development in the thymus, a process called somatic recombination. The gene segments that encode the separate distinct regions of the TCR protein exist as multiple nonidentical sets. There are, for example, 12 TCR-γ V-region gene segments.

During T-cell maturation, the TCR genes undergo rearrangement. Thus, in TCR-γ chain assembly, a single V

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region from several V gene segments is positioned next to a single J region that has also been selected from several J gene segments to make a highly exclusive rearranged V/J gene region that is eventually expressed on the TCR-γ protein. To further add to the exclusivity of the TCR, there is then random loss of the original nucleotides at the ends where the V and J segments join. This loss is replaced by the insertion of N nucleotides at the joins. The result is a unique V/N nucleotide/J structure that serves as a "fingerprint" for that particular T cell and all its clonal progeny (Wood, 2001; Wood *et al.*, 1994).

TCR gene rearrangement analysis with PCR can be performed on any TCR gene. The TCR-γ gene is preferred because it is rearranged early in T-cell development both in α/β and in γ/δ T cells, and T cells generally retain intact rearranged TCR-γ genes although the protein may no longer be expressed on mature T cells. The γ gene structure is also simpler, with only 12 variable segments compared with other TCR genes such as the TCR-β gene, which has over 60 variable segments. Thus, fewer sets of primers are needed to detect most of the possible gene rearrangements (Kandolf Sekulović *et al.*, 2007; Wood, 2001).

The process involves three main steps: (1) extraction of genomic DNA from the skin specimen; (2) PCR-based amplification of the TCR-γ gene rearrangements from the genomic DNA; and (3) detection of the PCR products, most commonly by fractionation of the resulting products by gel electrophoresis (Figure 1) (Wood *et al.*, 1994).

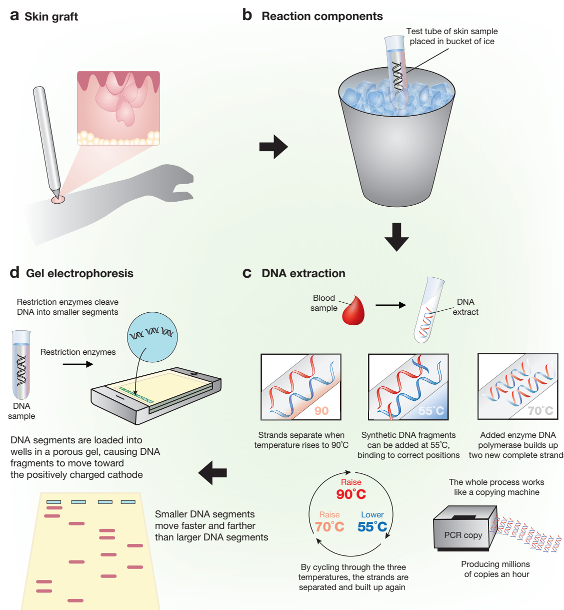

Figure 1. Method of TCR-γ gene rearrangement. (**a**) A skin biopsy specimen is taken from active lesions. (**b**) The skin specimen is placed into a container, which is placed on ice for transport. (**c**) The genomic DNA is isolated, and PCR amplification is started. (**d**) The PCR products are analyzed using gel electrophoresis. (**c**, Concept by E.K. Martin, Svenska Dagbladet. **d**, Adapted from http://cg.scs.carleton.ca/~morin/teaching/compbio/electro.html.)

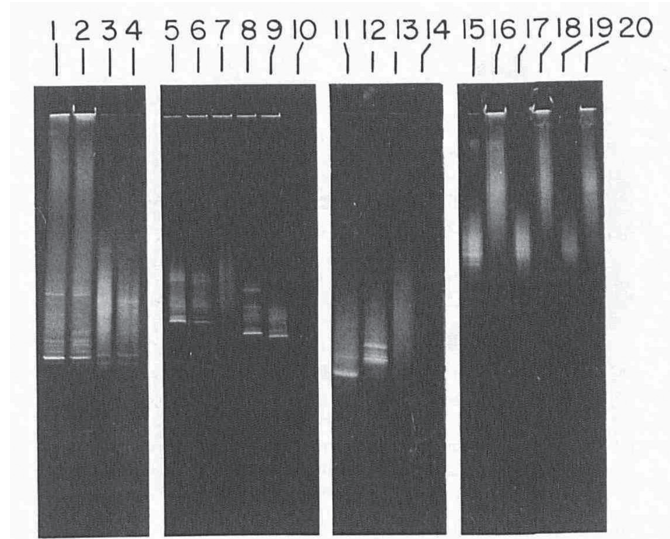

Figure 2. PCR/denaturing gradient gel electrophoresis (DGGE): dominant T-cell clonal patterns. Discrete sharp bands are present in lanes 1–6, 8, 9, 11, 12, and 15. These bands reflect the presence of dominant TCR-γ gene rearrangements and contrast with the diffuse smears present in Figure 3 and also in lanes 7, 13, and 16–20, which correspond to the absence of detectable dominant TCR-γ gene rearrangements. Reprinted with permission from Wood *et al.* (1994).

1. Extraction of genomic DNA from the skin specimen. Genomic DNA can be isolated from both fresh and formalin-fixed paraffin-embedded tissue. Although both methods are acceptable, fresh-frozen samples are preferred because of the higher sensitivity associated with isolating genomic DNA from them. If the specimen is in formalin-fixed paraffin, then DNA is isolated from paraffin blocks by deparaffinizing specimens by incubation with xylene, 100% ethanol, and acetone. After this, a mixture including proteinase K is added to the specimen and incubated overnight at 37 °C or for 3 hours at 55 °C (Sprouse *et al.*, 2000).

2. PCR-based amplification of the TCR-γ gene rearrangements from the genomic DNA. Once the genomic DNA is extracted, the PCR-based amplification process is started. Amplification is performed by adding genomic DNA as the template, combined with primer,

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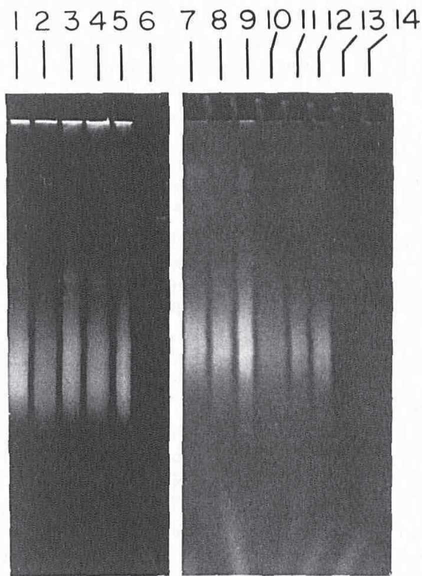

Figure 2. PCR/DGGE: Polyclonal T-cell pattern. Vγ1-8 PCR products *(lanes 1–6)* and Vγ9 PCR products *(lanes 7–13)* were subjected to DGGE, stained with ethidium bromide, and photographed using UV illumination. *Lanes 1–5* and *7–12* contain diffuse smears lacking discrete bands. This is the typical result in the absence of detectable dominant TCR-γ gene rearrangements. *Lane 1*, lesional skin, cutaneous lymphoid hyperplasia. *Lane 2*, lesional skin, B-cell lymphoma. *Lanes 3* and *4*, lesional skin and reactive lymph node, respectively, cutaneous lymphoid hyperplasia. *Lane 5*, lesional skin, cutaneous lymphoid hyperplasia. *Lane 6*, Vγ1-8 carry-over negative control containing all reagents except target DNA. *Lane 7*, lesional skin, MF. *Lanes 8–10*, two lesional skin specimens and peripheral blood mononuclear cells, respectively, MF. *Lanes 11* and *12*, two normal skin specimens. *Lane 13*, Vγ9 carry-over negative control. *Lane 14*, empty lane. Although the MF specimens were polyclonal for Vγ9, they showed dominant clonal bands for Vγ1-8 (for example, see *lane 8* in Fig 3).

Figure 3. PCR/denaturing gradient gel electrophoresis (DGGE): polyclonal T-cell pattern. Vγ1-8 PCR products (lanes 1–6) and Vγ9 PCR products (lanes 7–13) were subjected to DGGE, stained with ethidium bromide, and photographed using UV illumination. Lanes 1–5 and 7–12 contain diffuse smears lacking discrete bands. This is the typical result in the absence of detectable dominant TCR-γ gene rearrangements. Lane 6 shows Vγ1-8 carryover–negative control containing all reagents except target DNA. Reprinted with permission from Wood *et al*. (1994).

nucleotides including all four bases (adenine, thymine, cytosine, and guanine), and DNA polymerase to a test tube. Next, the test tube is placed in a thermocycler machine that raises the temperature to denature the DNA, then lowers the temperature to allow primers and DNA to anneal, and finally raises the temperature again to allow the DNA polymerase to extend the primers by adding nucleotides to the DNA template strand (Sprouse *et al*., 2000; Guitart and Kaul, 1999). (See the recent Research Techniques Made Simple article on PCR (Garibyan and Avashia, 2013) for a detailed description of the process.)

3. Detection of the PCR products. The final step involves analysis of the PCR products by gel electrophoresis to separate the PCR products according to their nucleotide sequence and size. The gel contains a urea/formamide-gradient that results in partial melting of the PCR products and therefore reduces the mobility at a point in the gradient determined by the nucleotide sequence. If there are enough numbers and fragments that are of identical size and sequence, as is produced by PCR of TCR genes from the genomic DNA of a monoclonal T-cell population, an intense clonal band is visible under UV light after the gel is stained with ethidium bromide (Figure 2). If there are heterogeneous fragments amplified from polyclonal cells, then only a diffuse smear is seen (Figure 3) (Bottaro *et al*., 1994; see Figure 1).

ADVANTAGES

Prior to the use of PCR and gel electrophoresis to detect clonality in suspected cases of CTCL, the Southern blot technique was commonly used. In comparison to the above technique, the Southern blot analysis is time consuming, requires large skin samples, and can detect only 5–10% of clonal subpopulations of cells (Kandolf Sekulović *et al*., 2007).

PCR has several additional advantages over Southern blot analysis. It is a much more sensitive technique that can be done with standard-size punch and shave biopsy specimens; in addition, it is less expensive than Southern blot analysis, and it takes only 2–3 days to complete. Unlike Southern blotting, which typically requires radiolabeled hybridization probes, PCR does not require radioactive agents. In addition, PCR can analyze lesions with sparse lymphocytic infiltrates, as is often the case in early-stage MF. Despite the low numbers of tumor cells, amplification ensures they are detected (Bottaro *et al*., 1994).

LIMITATIONS

Although the advent of PCR and gel electrophoresis has increased the sensitivity of detecting TCR-γ gene rearrangement, several limitations to this technique can lead to false-negative results. The TCR-γ gene of selected T lymphocytes may not amplify owing to factors such as section thickness and cell size variations or the degradation of DNA in the histological processing of tissue (Gellrich *et al*., 2000). Other limitations include poor tissue sampling (i.e., a biopsy taken from a skin sample with a small number of malignant T cells) and the possibility that the primers used may not cover all possible TCR-γ gene rearrangements. Finally, in the later stages of the disease, TCR gene deletion is possible as the cell undergoes malignant transformation leading to nonamplification of TCR-γ gene sequences (Kandolf Sekulović et *al*., 2007). False positives can also occur because of the presence of rare benign lymphoid infiltrates, leading to “pseudoclonal” results. Another cause of false positives is cross-contamination of specimens by clonal DNA from positive controls. For these reasons, it is not possible to rely on the results of the TCR gene rearrangement analysis solely to diagnose a patient with CTCL.

WHEN IS TCR GENE REARRANGEMENT ANALYSIS USED IN DERMATOLOGY?

The most common use of TCR gene rearrangement analysis is to aid in the diagnosis of CTCL. In the case of SS, the leukemic

form of CTCL, the presence of a T-cell clone in the skin and blood, together with certain cytomorphologic features and immunophenotypic features in the blood, is helpful in making a diagnosis and differentiating from benign inflammatory conditions that can mimic this condition. TCR gene rearrangement has not been shown to improve with resolution of disease and thus cannot be used as a marker of disease activity.

It is important to note that a positive clonality result does not necessarily equate to malignancy, as several benign dermatoses can produce a similar pattern, including lymphomatoid papulosis, pityriasis lichenoides et varioliformis acuta, lymphomatoid drug eruptions, actinic reticuloid, and lichen planus (Gellrich *et al.*, 2000). It is essential that clinical, histological, and immunological data be taken into consideration when making a diagnosis of CTCL and that the TCR gene rearrangement analysis is used as an adjunct to help make the case for or against a diagnosis of CTCL, rather than as the sole diagnostic feature.

IS TCR GENE REARRANGEMENT ANALYSIS USED FOR ANYTHING ELSE?

TCR gene rearrangement is an important event in T-cell ontogeny, and any disruption in this cycle may result in disease. T cells have been implicated in the pathogenesis of immunodeficiency, autoimmunity, and atopy. One of the most pivotal uses of TCR gene rearrangement analysis is in the diagnosis of lymphocytic disease and in distinguishing it from reactive lymphoid proliferations. It has been used in monitoring minimal residual disease after bone marrow transplantation, aiding in the diagnosis of immunodeficiency states, and characterizing T cells at disease sites in patients with allergy or autoimmune disease (Bottaro *et al.*, 1994).

SUMMARY AND FUTURE DIRECTIONS

TCR gene rearrangement analysis with PCR and gel electrophoresis is a widely used adjunct in the diagnosis of CTCL. Most studies on TCR gene rearrangement have focused on the diagnosis of CTCL or response to therapy. Few have addressed whether these molecular methods are a useful staging tool for predicting prognosis. The technical advantages of PCR make it the method of choice for detecting clonality, and it will continue to be the method of choice until newer methods are shown to have advantages over this technique.

MF disseminates to blood, lymph nodes, and viscera in the advanced stages. A positive clone in the blood is detected more frequently with progressive stages of cutaneous disease, such as those with cutaneous tumors or erythroderma. Studies have shown that the detection of a clone in dermatopathic lymph nodes (i.e., early-stage MF) can be associated with a worse prognosis. T-cell clones have even been found in the blood of a significant proportion of patients with early-stage MF, and the presence of a peripheral blood clone was shown to be associated with a worse outcome (Bottaro *et al.*, 1994). To date, such studies have involved small numbers of patients, and studies with a larger patient population will be needed to confirm these observations and to evaluate how best to incorporate these data in the staging of MF.

QUESTIONS

This article has been approved for 1 hour of Category 1 CME credit. To take the quiz, with or without CME credit, follow the link under the "CME ACCREDITATION" heading.

1. **In what way does the TCR gene rearrangement study aid in CTCL?**
   - A. A TCR gene is lost in CTCL and thus serves as an indicator for diagnosis of CTCL.
   - B. A TCR gene serves as a marker for the monoclonal T cell, thus aiding in diagnosis.
   - C. A TCR gene rearrangement study, if positive, is diagnostic of CTCL.
   - D. A TCR gene rearrangement test is not useful because it is nearly always negative in patients with CTCL.

2. **A major advantage of using PCR compared with Southern blotting is:**
   - A. Low sensitivity.
   - B. Low specificity.
   - C. High specificity.
   - D. High sensitivity.

3. **A positive TCR gene rearrangement test is interpreted positive when there is a:**
   - A. Polyclonal band detection on DGGE.
   - B. Smear detected on DGGE.
   - C. Monoclonal band detected on DGGE.
   - D. Blank space on DGGE.

4. **TCR gene rearrangement studies should be used:**
   - A. To help confirm a clinical suspicion of CTCL.
   - B. As a screening tool for all pruritic patients with no histologically confirmed diagnosis.
   - C. To help confirm a histological and clinical suspicion of CTCL.
   - D. To rule out CTCL in a pruritic patient.

5. **All of the following are limitations of PCR in TCR gene rearrangement studies, except:**
   - A. Loss of gene as the cell undergoes malignant transformation.
   - B. Poor tissue sampling.
   - C. Inadequate number of primers.
   - D. Inability to use paraffinized tissue for the test.

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MF is often preceded by a chronic nonspecific dermatitis that does not have diagnostic features of MF histologically, yet harbors a dominant T-cell clone. This observation has been termed "clonal dermatitis." Molecular studies have demonstrated that both diseases can share the same monoclonal population. Thus, clonal dermatitis may represent a subgroup of patients who are at increased risk of developing clinically overt MF. Future studies could help determine their long-term risk of MF. It is necessary to closely follow these patients to diagnose lymphoma at the earliest possible time and to institute the correct treatment (Bottaro *et al*., 1994).

What does the future hold for this molecular technique? This highly sensitive tool enables earlier and more accurate diagnosis of CTCL. With further studies and greater understanding of the pathogenesis of cutaneous lymphomas, these tools may be used for more precise disease staging and the ability to provide better prognostic information for patients, thus allowing for more tailored treatment (Wood, 2001; Gellrich *et al*., 2000).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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http://www.classmarker.com/online-test/start/?quiz=xh352d98e54d1a8a

SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for journal club or other teaching exercises is available at http://dx.doi.org/10.1038/jid.2014.73.

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