

Polymerase Chain Reaction and Heteroduplex Analysis Based Detection of Clonal T Cell Receptor Gamma Gene Rearrangements in Paraffin-embedded Tissues of Cutaneous T Cell Proliferative Diseases

Un Cheol Yeo, M.D¹., Kyungho Park, M.D¹., Young-Hyeh Ko, M.D²., Eil Soo Lee, M.D¹., Kwang Ho Han, M.D³., Chul Woo Kim, M.D⁴., Kwang Hyun Cho, M.D¹.

Departments of Dermatology¹ and Diagnostic Pathology², Samsung Medical Center, Sunkunkwan University School of Medicine, Departments of Dermatology³ and Pathology⁴, Seoul National University College of Medicine, Seoul, Korea

Background : Recently, the molecular pathologic investigation for clonality in lymphomas has been introduced and has gained a role in the diagnosis of lymphomas. In fact, the clonality test using TCRGR phenomenon has been done by Southern blot analysis (SBA) and polymerase chain reaction (PCR) for molecular pathologic diagnosis of T cell lymphomas. However, it is difficult to perform SBA with paraffin embedded specimens or with samples of small skin biopsies.

Objective : We investigated the efficacy of PCR amplification of TCR gene in paraffin embedded cutaneous T cell lymphomas.

Methods : In this study, the clonality was assessed by polymerase chain reaction (PCR) analysis of T cell receptor gamma (TCR) gene from the DNA extracts obtained from paraffin embedded tissues (PET) of malignant T cells, B cell lymphomas, and benign cutaneous T cell proliferative disorders. Heteroduplex analyses were also performed to rule out the false positives.

Results : Among the total of 62 cases analyzed, monoclonality was observed in 4 out of 10 mycosis fungoides, 7 out of 9 cutaneous T cell lymphomas excluding mycosis fungoides, 1 out of 3 angiocentric lymphomas, 2 out of 2 lymphomatosis papulosis, 1 out of 7 large plaque parapsoriasis, and 1 out of 2 T cell lymphomas in other organs. No monoclonality was observed in 9 inflammatory cutaneous diseases, 5 small plaque parapsoriasis, 4 cutaneous B cell lymphomas, and 11 B cell lymphomas in lymph nodes.

Conclusion : The results suggest that the PCR method and heteroduplex analysis used in this study were not only practical but also efficacious for the diagnosis of cutaneous T cell lymphomas using tissues embedded in paraffins. (Ann Dermatol 13(3) 139~147, 2001).

Key Words : Cutaneous T cell lymphoma, Heteroduplex analysis, T cell receptor, PCR

Received November 1, 2000.

Accepted for publication January 3, 2001

Reprint request to : Kwang Hyun Cho, M.D. Department of Dermatology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-Gu, Seoul, Korea

Tel. 82-2-760-2412, Fax. 82-2-742-7344

Malignant tumors have characteristic features in the cells that comprise the tumors, namely, monoclonality of tumor cells. During T cell development in thymus, each T cell acquires a specific T cell receptor (TCR) gene rearrangement. A clone derived from a single T cell has the same rearrangement pattern in TCR gene. This T cell receptor gene rearrangement (TCRGR) phenomenon has

Table 1. Results of PCR for TCRg, b-globin, and heteroduplex analysis

Sample	Sex/Age	Diagnosis	TCRg	b-globin	Heteroduplex
1	F/72	MF tumor	band	ND	band
2	F/72	MF patch	smear	band	ND
3	F/44	MF tumor	band	band	band
4	M/19	MF patch	smear	band	ND
5	F/29	MF patch	smear	band	ND
6	F/55	MF patch	smear	band	ND
7	F/72	MF tumor	band	band	band
8	F/72	MF patch	smear	band	ND
15	M/58	MF patch	band	ND	disappear
16	F/35	MF patch	band	ND	band
9	M/24	subcutaneous panniculitic lymphoma	band	ND	band
11	M/65	LBTL	band	ND	band
12	M/41	LBTL	band	ND	band
13	M/38	PTCL	band	ND	band
17	M/38	PTCL	band	ND	band
18	F/60	ALCL	band	ND	band
21	F/61	ALCL	band	ND	band
19	M/36	angiocentric lymphoma	smear	ND	disappear
26	M/29	angiocentric lymphoma	smear	band	ND
20	M/54	angiocentric lymphoma	band	ND	band
22	F/34	CBCL	smear	band	ND
23	F/34	CBCL	smear	band	ND
24	M/48	CBCL	band	band	disappear
25	M/51	CBCL	smear	band	ND
27	M/48	lymphomatoid papulosis	smear	band	ND
29	M/70	lymphomatoid papulosis	smear	band	ND
10	F/37	parapsoriasis(large plaque)	band	ND	band
14	M/35	parapsoriasis(large plaque)	band	ND	band
43	M/40	parapsoriasis(large plaque)	smear	band	ND
44	F/29	parapsoriasis(large plaque)	smear	band	ND
45	M/49	parapsoriasis(large plaque)	smear	band	ND
46	M/48	parapsoriasis(large plaque)	smear	band	ND
47	F/44	parapsoriasis(large plaque)	band	band	band
48	F/54	parapsoriasis(large plaque)	smear	band	ND
49	F/29	parapsoriasis(large plaque)	smear	band	ND
50	F/65	parapsoriasis(small plaque)	smear	ND	disappear
54	M/24	parapsoriasis(small plaque)	smear	ND	disappear
58	M/32	parapsoriasis(small plaque)	smear	ND	disappear
59	F/40	parapsoriasis(small plaque)	smear	ND	disappear
60	F/32	parapsoriasis(small plaque)	smear	ND	disappear
28	M/31	EBV-associated lymphoproliferative disorder	smear	band	disappear
51	F/62	lichen planus	smear	ND	disappear
53	F/47	lichen planus	smear	ND	disappear
55	M/42	lichen planus	smear	ND	disappear
56	M/51	lichen planus	smear	ND	disappear
57	M/36	lichen planus	smear	ND	disappear

Sample	Sex/Age	Diagnosis	TCRg	b-globin	Heteroduplex
61	M/52	lichen planus	band	ND	disappear
62	M/43	lichen planus	smear	ND	disappear
52	M/42	pityriasis rosea	smear	ND	disappear
30	F/68	BCL in lymphnode	smear	band	ND
31	F/52	BCL in lymphnode	smear	band	ND
32	F/66	BCL in lymphnode	smear	band	ND
33	M/38	BCL in lymphnode	smear	band	disappear
34	F/37	BCL in lymphnode	smear	band	disappear
35	F/45	TCL in lymphnode	band	band	band
36	M/55	BCL in lymphnode	smear	band	disappear
37	M/52	BCL in lymphnode	smear	band	ND
38	F/47	BCL in lymphnode	smear	band	disappear
39	F/54	BCL in lymphnode	smear	band	disappear
40	M/48	TCL in bonemarrow	smear	band	ND
41	M/45	BCL in bonemarrow	smear	band	disappear
42	M/75	BCL in bonemarrow	smear	band	Nd

ND: not done

MF: mycosis fungoides

PTCL: peripheral T cell lymphoma

LBTL: lymphoblastic T cell lymphoma

ALCL: anaplastic large cell lymphoma

CBCL: cutaneous B cell lymphoma

BCL: B cell lymphoma

TCL: T cell lymphoma

been used for clonality test in T cell lymphomas. Investigation of TCRGR by molecular pathologic method comprises Southern blot analysis (SBA) and polymerase chain reaction (PCR). The sensitivity of SBA is much higher than that of the morphologic anyalysis or immunophenotyping, since only 1-5% of monoclonal cells are necessary for detection¹⁴. However, there are diverse pitfalls of SBA to be used in clinical settings for diagnostic purpose. Therefore, the PCR method, which is 10 times more sensitive than SBA, is becoming increasingly popular for the molecular pathologic investigation of T cell lymphoma³⁻⁸. The PCR technique for analysis of TCRGR has evolved much from usage of TCR β to TCR γ whose V region is structurally simpler than TCR β ⁹. The method using multiple or single primer sets for PCR of TCR γ has been developed¹⁰. The consensus primer set which recognizes group I region of TCR V γ and TCR J γ was used in the PCR of TCR γ as described in Bourguin et al. in 1990¹¹, Volkenandt et al.

in 1991¹², and Benhattar et al. in 1995¹³. Other investigators also verified these PCR methods¹⁴⁻¹⁹.

The TCRGR analyses for T cell lymphoma in the field of dermatology have been performed with SBA²⁰⁻²², PCR with multiple primer sets²³, and PCR using techniques described by Bourguin et al. or Volkenandt et al.^{15,16,18,19}, mostly with fresh frozen tissues. More recent application of PCR in the evaluation of cutaneous T cell lymphoma was expanded to include parattin embedded tissues²⁴⁻³⁰. DNA analysis using PET has been regarded as an indispensable technique in genetics or cancer research⁵. PETs are inadequate for SBA because of DNA damage during fixation and retrieval process^{4,6}.

The PCR amplification capacitates the usage of not only fresh tissues but of PETs as well, enabling a retrospective study⁶⁻⁸.

This study was performed to investigate the clonality of various cutaneous T cell lymphomas and T cell proliferative inflammatory diseases in

paraffin-embedded state.

MATERIALS AND METHODS

1. Materials

Total of 62 cases in PET from Seoul National University Hospital and Samsung Medical Center were included. They were diagnosed clinically, histopathologically and immunohistochemically. They comprised cutaneous T cell lymphoma, angiocentric lymphoma (T cell and NK cell lineage), cutaneous B cell lymphoma, lymphomatoid papulosis, parapsoriasis and other benign cutaneous T cell infiltrative diseases. Cutaneous T cell lymphoma was classified according to the revised European-American classification of lymphoid neoplasm (REAL) classification based on clinical and immunohistochemical diagnosis³¹. The present study included mycosis fungoides, subcutaneous panniculitic lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma and lymphoblastic T cell lymphoma. T cell and B cell lymphoma involving lymph node and bone marrow were also included to validate the specificity of the present study.

2. DNA extraction from PET

DNA samples were extracted from the deparaffinized tissue sections using phenol and chloroform/isoamyl alcohol. The extracted DNA was quantified with a spectrophotometer to determine the amount of template DNA for PCR and then stored at -20°C.

3. PCR for TCR γ -gene

Primers DNA extracted from confirmed T cell lymphoma was used as a positive control, whereas distilled water was used as a negative control in the analysis of PCR for TCR γ . Primer sequence used for this study was identical to that of Benhattar *et al.*¹³. Amplified PCR products sized from 160 to 190 bp.

PCR PCR for TCR γ gene was performed in a 50 μ l reaction tubes. Five hundred ng of template DNA, 25 pmoles of TCR V γ primer and TCR J γ primer and 2.5 unit of Taq DNA polymerase were used. The final concentrations of each reagents were as follows: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP. PCR was performed with the Perkin-Elmer DNA thermal cy-

cler model 480. After 3 minutes denaturation at 95°C, the mixture was subjected to 30 cycles. Each cycle consisted of 30 seconds of denaturation at 94°C, 30 seconds annealing at 58°C and 30 seconds extension at 73°C, followed by 10 minutes reaction at 73°C.

4. PCR for β -globin⁵

To assess the quality of DNA extracted from paraffin-embedded samples, human β -globin gene was amplified in a single round PCR reaction for 35 cycles. The nucleotide sequence of primer used for β -globin amplification were as follows:

GH20 5' -GAAGAGCCAAGGGCAGGTAC-3'
PC04 5' -CAACTTCATCCACGTTCCACC-3'

5. Electrophoresis

PCR products of β -globin and TCR γ were subjected to 8% polyacrylamide gel electrophoresis with a 100bp DNA ladder as a size marker. Electrophoresis was done with the Bio-Rad Power supply and vertical slab gel unit (Model SE 400, Hoeper) for 4.5 hours at 100 volts. Then PCR products were stained for 20 minutes with ethium-bromide, and washed for 20 minutes with distilled water, and visualized under UV illuminator. A distinct band of 160-190 bp size in the gel was regarded as a band whereas a PCR product, which did not form a vivid band on the gel, was regarded as a smear.

6. Heteroduplex analysis of PCR products for TCR γ gene³²⁻³⁴

Setting After the PCR products for TCR γ were denatured at 94°C, they were cooled for 1 hour at renaturation temperature and were subsequently placed on ice until electrophoresis for heteroduplex analysis. This renaturation step was performed at different temperatures (4°C, 10°C, 22°C, 30°C), which revealed similar results allowing us to perform heteroduplex analysis at the convenient room temperature of 22°C (Fig. 1). The positive control was a T cell lymphoma depicting distinct band on heteroduplex analysis. The negative control was a reactive cutaneous T cell infiltrative disease which showed a relatively dense smear on electrophoresis and total disappearance on heteroduplex analysis.

Heteroduplex analysis The PCR products for TCR γ showing distinct bands or relatively dense

Fig. 1. Heteroduplex analysis of TCR γ PCR products in different temperature settings
M: marker (100bp ladder)
P: positive control (T cell lymphoma)
N: negative control (benign T cell infiltration)
*: PCR products without heteroduplex preparation

Fig. 3. Results of PCR for TCR γ and heteroduplex analysis
(A: PCR for TCR γ , B: heteroduplex analysis)
M; marker (100bp ladder)
P: positive control (T cell lymphoma)
50,54,58-60: Small plaque parapsoriasis
51,53,55-57,61,62: Lichen planus
52: Pityriasis rosea

Fig. 2. Results of PCR for TCR γ and heteroduplex analysis
M: marker (100bp ladder)
P: positive control (T cell lymphoma)
N: negative control (benign T cell infiltration)
9,11-13,17-20: Cutaneous T cell lymphoma
10,14: Lymphomatoid papulosis
15,16: Mycosis fungoides

Fig. 4. Electrophoresis of β -globin PCR products on 8% polyacrylamide gel
M: marker (100bp ladder)
P: positive control (T cell lymphoma)
43-49: Large plaque parapsoriasis

smears on the electrophoresis were subjected to heteroduplex analysis to confirm monoclonality. 10 μ l of products, which had been subjected to denaturation and renaturation process, were mixed with DNA gel loading buffer and then loaded onto a 12% polyacrylamide gel prepared with 1X TBE (Tris-Boric acid-EDTA) buffer. The electrophoresis was performed for about 20 hours at 8mA with the Bio-Rad and vertical slab gel unit (model SE 400, Hoeper) at room temperature. The PCR products were stained for 20 minutes with ethium-bromide, washed for 20 minutes with distilled water, and visualized under UV illuminator.

RESULTS

Sixty-two cases of cutaneous T cell lymphomas, cutaneous B cell lymphomas, benign lymphoinfiltrative diseases, T and B cell lymphomas in other organs were evaluated with PCR for TCR γ and heteroduplex analysis (Table 1).

Four of ten cases of mycosis fungoides (40%) showed monoclonality. Of the 4 cases that revealed monoclonal band, 3 cases were from mycosis fungoides at a tumor stage whereas 1 case was from a patch stage. Six cases that failed to show monoclonal band were all from the patch stage. Nine cases of cutaneous T cell lymphoma excluding mycosis fungoides were evaluated. Among them, 7 cases (78%) showed monoclonality. They consisted of 4 cases of peripheral T cell lymphoma, 1 case of subcutaneous T cell lymphoma, 2 cases of lymphoblastic T cell lymphoma and 2 cases of anaplastic large cell lymphoma (Fig. 2). Only 1 of 3 cases of angiocentric lymphoma showed monoclonal band. All 4 cases of cutaneous B cell lymphoma failed to show monoclonality. One of seven cases of large plaque parapsoriasis and 2 of 2 cases of lymphomatoid papulosis exhibited monoclonality. None of the 5 cases of small plaque parapsoriasis displayed monoclonal band. Nine cases of inflammatory skin disease, consisted of 7 cases of lichen planus, 1 case of pityriasis rosea and 1 case of EBV-associated lymphoproliferative disorder, failed to show monoclonality (Fig. 3). Eleven cases of B cell lymphoma involving the lymph node, 1 case of T cell lymphoma involving the lymph node and 1 case of T cell lymphoma involving the bone marrow were also investigated to reveal monoclonality only in T cell lymphoma involving the lymph node. PCR for

β -globin confirmed successful DNA extraction from PETs (Fig. 4).

DISCUSSION

In cutaneous inflammatory diseases, mostly T cells, not B cells, infiltrate the lesions. Because of morphological similarity, the diagnosis of cutaneous T cell lymphoma is often confused with cutaneous lymphocytic infiltrative diseases. Thus, TCRGR analysis is frequently used and its clinical usefulness is already well known. Analysis of TCRGR in cutaneous T cell lymphoma has been performed mostly using freshly frozen tissues^{15,23}. More recently, PCR method was used to detect monoclonality in the PETs for mycosis fungoides and other CTCLs²⁴⁻³⁰. The overall detection rate of monoclonality in paraffin-embedded tissues mostly ranged between 70% and 90%. DNA in PET is damaged continuously depending on the fixation time and material. Therefore, amplification fragments larger than 200bp could not be amplified efficiently by PCR⁵. This is the reason for using the method described by Benhattar *et al.* of which PCR products ranged in size from 160 to 190bp in our study.

In our study, 7 of 9 cases of T cell lymphoma (excluding mycosis fungoides) showed distinct monoclonal bands. The sensitivity rate of our study was similar to those of other studies done with paraffin tissues. Cutaneous T cell lymphoma does not have favorable occurrence in a certain T cell that has arranged a specific TCR V γ ^{35,36}. Therefore, performing PCR with our method using the consensus primer for group I of TCR γ V region could elicit the possibility that TCR gene is not amplified if the lymphoma was derived from T cells that had rearranged gene from group II, III or IV of TCR γ V region. In certain circumstances, abnormal TCRGR, such as V γ gene region conjoined with J δ gene or oncogene, could result in a failure of amplification of TCR γ by PCR. In addition, when there is an excessive infiltration of background inflammatory cells, DNA from tumor T cell is diluted and exhibition of monoclonal band becomes difficult. These are considered to play a role as restrictive factors in sensitivity of PCR for TCR γ in cutaneous T cell lymphoma.

None of the 4 cases of cutaneous B cell lymphoma, none of 11 cases of B cell lymphoma in-

volving lymph node, and none of 9 cases of benign cutaneous lymphocytic infiltrative disease showed monoclonal band, which signifies high specificity of this method.

However, 4 of 10 cases of mycosis fungoides, all 3 cases of tumor stage mycosis fungoides and 1 of 7 cases of patch stage mycosis fungoides showed monoclonality. The results are remarkably lower in sensitivity rate than that of the studies done with frozen tissues, which is due to low sensitivity rate of patch stage mycosis fungoides. The number of malignant cells in patch stage is small compared to tumor stage mycosis fungoides. During DNA extraction from PETs, a small amount of malignant cell DNA clone might not be extracted enough to be amplified by PCR. Since the sharpness of monoclonal band have to be deciphered subjectively, the polyclonal products with negligible difference in size may mimic monoclonal product. Therefore, it is much more reliable to approve the monoclonality by sequence difference rather than size difference because polyclonal products have different sequence though they may have similar sizes. Heteroduplex analysis is a method in which homoduplex and heteroduplex, resulting from denaturation at 94°C and renaturation at lower temperature of PCR products, are separated in non-denaturing polyacrylamide gels based on their conformation. Amplified products of monoclonal TCR γ genes form homoduplex and persistently show a definite band after gel electrophoresis. In contrary, PCR products derived from polyclonal T cells form heteroduplex and disappear after electrophoresis. In the present study, Sample 15 (mycosis fungoides, patch stage), Sample 24 (B cell lymphoma) and Sample 61 (benign cutaneous disorder) were deciphered as monoclonal bands in electrophoresis after PCR for TCR γ , but perceived as negative when the distinctive band disappeared after heteroduplex analysis. As observed in our study, the adjuvant investigation, which discloses sequence differences, must accompany PCR for TCR γ to obviate false positivity. Heteroduplex analysis seems to be a simple and convenient method which does not necessitate special devices. In the present study, 4 of 10 cases of mycosis fungoides revealed monoclonality. We identified monoclonality in all 3 cases of tumor stage mycosis fungoides, and 1 of 7 cases of patch stage mycosis fungoides. Samples 1, 2, 7, and 8 were from identical patients. Samples 1 and 7

were from tumor stage mycosis fungoides and Samples 2 and 8 were from patch stage mycosis fungoides. The repeatability of our method was affirmed from the fact that all samples of tumor stage, but none of patch stage from this identical patient, exhibited monoclonality. Angiocentric lymphoma has hitherto been described to show phenotypical features of T or NK cells. One of three cases in our study showed monoclonal band and expressed CD3, CD8, CD45RO, CD 56 but not CD20, which supports that angiocentric lymphoma can be derived from T cell lineage. Analysis of TCRGR is useful not only for analyzing clonality, but also for studying the lineage of lymphocyte derived tumors. Both cases of lymphomatoid papulosis and 1 case of large plaque parapsoriasis out of 5 small plaque and 7 large plaque parapsoriasis disclosed a monoclonal band.

High specificity of our method in the present study was affirmed from the negative results observed in all 9 cases of benign lymphocytic infiltrative disorders. Yet, as observed in Sample 62 expressing a monoclonal band in electrophoresis of PCR products for TCR γ , the heteroduplex analysis should be performed to enhance specificity. The concept of EBV-associated lymphoproliferative disorder as the disease entity with persistent, chronic skin infection by EBV, eventually progressing to an overt malignant lymphoma has not been established until recently³⁷. We did not find monoclonality in this disorder but it was in vain. Among 11 cases of B cell lymphoma, 1 case of T cell lymphoma involving lymph node, and 1 case of T cell lymphoma involving bone marrow, only 1 case of T cell lymphoma involving lymph node showed monoclonality in the analysis of TCRGR, which again corroborates the high specificity of our method.

In our study, PCR was performed with some modification to the method by Benhattar et al. and this simple method was sufficient to achieve satisfactory results. In conclusion, we suggest that PCR technique and heteroduplex analysis used in the present study are not only simple and convenient but also efficient tools in evaluating TCRGR with PETs from cutaneous T cell lymphoma.

REFERENCES

1. Terhune MH, Cooper KD: Gene rearrangements

- and T-cell lymphomas. *Arch Dermatol* 129:1484-1490, 1993.
2. Wood GS, Haeffner A, Dummer R, Crooks CF: Molecular biology techniques for the diagnosis of cutaneous T-cell lymphoma. *Dermatol Clin* 12: 231-241, 1994.
 3. Sklar J, Longtine J: The clinical significance of antigen receptor gene rearrangements in lymphoid neoplasia. *Cancer* 70(suppl):1710-1718, 1992.
 4. McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E, Wiedemann LM: A simplified method of detection of clonal rearrangements of the T-cell receptor γ chain gene. *Diagn Mol Pathol* 1:173-179, 1992.
 5. Greer CE, Peterson SL, Kiviat NB, Manos MM: PCR amplification from paraffin-embedded tissues. Effects of fixative and fixation time. *Am J Clin Pathol* 95:117-124, 1991.
 6. Lorenzen J, Jux G, Zhao-Hohn M, Klockner A, Fischer R, Hansmann ML: Detection of T-cell clonality in paraffin embedded tissues. *Diagn Mol Pathol* 3:93-99, 1994.
 7. Weinberg JM, Rook AH, Lessin SR: Molecular diagnosis of lymphocytic infiltrates of the skin. *Arch Dermatol* 129:1491-1500, 1993.
 8. Lessin SR, Rook AH: T-cell receptor gene rearrangement studies as a diagnostic tool in lymphoproliferative skin diseases. *Exp Dermatol* 2:53-62, 1993.
 9. Rezuze WN, Abernathy EC, Tsongalis GJ: Molecular diagnosis of B-and T-cell lymphomas: fundamental principles and clinical applications. *Clin Chem* 43:1814-1823, 1997.
 10. Whittaker S: T-cell receptor gene analysis in cutaneous T-cell lymphomas. *Clin Exp Dermatol* 21:81-87, 1996.
 11. Bourguin A, Tung R, Galili N, Sklar J: Rapid, non-radioactive detection of clonal T-cell receptor gene rearrangements in lymphoid neoplasms. *Proc Natl Acad Sci USA* 87:8536-8540, 1990.
 12. Volkenandt M, Soyer HP, Kerl H, Bertino JR: Development of a highly specific and sensitive molecular probe for detection of cutaneous lymphoma. *J Invest Dermatol* 97:137-140, 1991.
 13. Benhattar J, Delacretaz F, Martin P, Chaubert P, Costa J: Improved polymerase chain reaction detection of clonal T-cell lymphoid neoplasms. *Diagn Mol Pathol* 4:108-112, 1995.
 14. Meyer JC, Hassam S, Dummer R, Muletta S, Dobbeling U, Burg G: A realistic approach to the sensitivity of PCR-DGGE and its application as a sensitive tool for the detection of clonality in cutaneous T-cell proliferations. *Exp Dermatol* 6:122-127, 1997.
 15. Haeffner AC, Smoller BR, Zepter K, Woods GS: Differentiation and clonality of lesional lymphocytes in small plaque parapsoriasis. *Arch Dermatol* 131:321-324, 1995.
 16. Bakels V, van Oostveen JW, van der Putte SC, Meijer CJ, Willemze R: Immunophenotyping and gene rearrangement analysis provide additional criteria to differentiate between cutaneous T-cell lymphomas and Pseudo-T-cell lymphomas. *Am J Pathol* 150:1941-1949, 1997.
 17. Lefranc MP, Forster A, Baer R, Stinson MA, Rabbitts TH: Diversity and rearrangement of the human T cell rearranging γ genes : nine germ-line variable genes belonging to two subgroups. *Cell* 45:237-246, 1986.
 18. Yu RC, Alaibac M: A rapid polymerase chain reaction-based technique for detecting clonal T-cell receptor gene rearrangements in cutaneous T-cell lymphomas of both the $\alpha\beta$ and $\gamma\delta$ varieties. *Diagn Mol Pathol* 5:121-126, 1996.
 19. Curco N, Servitje O, Lluca M, et al: Genotypic analysis of cutaneous T-cell lymphoma: a comparative study of Southern blot analysis with polymerase chain reaction amplification of the T-cell receptor- γ gene. *Br J Dermatol* 137:673-679, 1997.
 20. Zelickson BD, Peters MS, Muller SA, et al: T-cell receptor gene rearrangement analysis: cutaneous T cell lymphoma, peripheral T cell lymphoma, and premalignant and benign cutaneous lymphoproliferative disorders. *J Am Acad Dermatol* 25:787-796, 1991.
 21. Kikuchi A, Naka W, Harada T, Sakuraoaka K, Harada R, Nishikawa T: Parapsoriasis en plaques: its potential for progression to malignant lymphoma. *J Am Acad Dermatol* 29:419-422, 1993.
 22. el-Azhary RA, Gibson LE, Kurtin PJ, Pittelkow MR, Muller SA: Lymphomatoid papulosis: a clinical and histopathologic review of 53 cases with leukocyte immunophenotyping, DNA flow cytometry, and T-cell receptor gene rearrangement studies. *J Am Acad Dermatol* 30:210-218, 1994.
 23. Staib G, Sterry W: Use of polymerase chain reaction in the detection of clones in lymphoproliferative diseases of the skin. *Recent Results Cancer Res* 139:239-247, 1995.
 24. 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* 21:743-747, 1997.
25. Liebmann RD, Anderson B, McCarthy KP, Chow JW: The polymerase chain reaction in the diagnosis of early mycosis fungoides. *J Pathol* 182:282-287, 1997.
 26. Tok J, Szabolcs MJ, Silvers DN, Zhong J, Matsushima AY: Detection of clonal T-cell receptor gamma chain gene rearrangements by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE) in archival specimens from patients with early cutaneous T-cell lymphoma: correlation of histologic findings with PCR/DGGE. *J Am Acad Dermatol* 38:353-360, 1998.
 27. Signoretti S, Murphy M, Cangi MG, Puddu P, Kadin ME, Loda M: Detection of clonal T-cell receptor gamma gene rearrangements in paraffin-embedded tissue by polymerase chain reaction and nonradioactive single-strand conformational polymorphism analysis. *Am J Pathol* 154:67-75, 1999.
 28. Andersen WK, Li N, Bhawan J: Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR/DGGE)-based detection of clonal T-cell receptor gamma gene rearrangements in paraffin-embedded cutaneous biopsies in cutaneous T-cell lymphoproliferative diseases. *J Cutan Pathol* 26:176-182, 1999.
 29. Krafft AE, Taubenberger JK, Sheng ZM, et al. Enhanced sensitivity with a novel TCRgamma PCR assay for clonality studies in 569 formalin-fixed, paraffin-embedded (FFPE) cases. *Mol Diagn* 4:119-33, 1999.
 30. Dippel E, Assaf C, Hummel M, et al: Clonal T-cell receptor gamma-chain gene rearrangement by PCR-based GeneScan analysis in advanced cutaneous T-cell lymphoma: a critical evaluation. *J Pathol* 188:146-154, 1999.
 31. Sander CA, Kind P, Kaudewitz P, Raffeld M, Jaffe ES: The revised European-American classification of lymphoid neoplasm (REAL): a new perspective for the classification of cutaneous lymphomas. *J Cutan Pathol* 24:329-341, 1997.
 32. Bottaro M, Berti E, Biondi A, Migone N, Crosti L: Heteroduplex analysis of T-cell receptor γ gene rearrangements for diagnosis and monitoring of cutaneous T-cell lymphomas. *Blood* 83:3271-3278, 1994.
 33. Chhanabhai M, Adomat SA, Gascoyne RD, Horsman DE: Clinical utility of heteroduplex analysis of TCR gamma gene rearrangements in the diagnosis of T-cell lymphoproliferative disorders. *Am J Clin Pathol* 108:295-301, 1997.
 34. Langerak AW, Szczepanski T, van der Burg M, Wolvers-Tettero IL, van Dongen JJ: Heteroduplex PCR analysis of rearranged T cell receptor genes for clonality assessment in suspect T cell proliferations. *Leukemia* 11:2192-2199, 1997.
 35. Theodorou L, Delfau-Larue MH, Bigorgne C, et al: Cutaneous T-cell infiltrates: Analysis of T-cell receptor γ gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. *Blood* 86:305-310, 1995.
 36. Thorstraten P, Ralfkiaer E, Hendriks J, Sermrt T, Vejlsgaard GL, Zeuthen J: T-cell receptor variable region genes in cutaneous T-cell lymphomas. *Br J Dermatol* 138:3-12, 1998.
 37. Cho KH, Kim CW, Lee DY, Sohn SJ, Kim DW, Chung JH: An Epstein-Barr virus-associated lymphoproliferative lesion of the skin presenting as recurrent necrotic papulovesicles of the face. *Br J Dermatol* 134:791-796, 1996.