Clinical significance of T-cell clonality in mycosis fungoides and other cutaneous T-cell lymphomas

Muche, J.M.

1

General introduction

Chapter 1 - General introduction

1.1 Primary cutaneous T-cell lymphoma

Primary cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of mature T-cell neoplasm, clinically originating in the skin and subsequently disseminating into lymph nodes, blood, and other visceral organs. After the gastrointestinal tract, the skin is the second most common site of extranodal non-Hodgkin lymphoma, with an estimated annual incidence of 1:100,000[1]. The primary cutaneous occurrence of neoplastic cells distinguishes CTCL from histologically similar lymphomas that develop skin lesions as sign of metastatic dissemination and aggressive behavior.

WHO-EORTC classification	%*	5-DS*
Indolent clinical behavior		
Mycosis fungoides	44	88
Folliculotropic MF	4	80
Pagetoid reticulosis	<1	100
Granulomatous slack skin	<1	100
Primary cutaneous anaplastic large cell lymphoma	8	95
Lymphomatoid papulosis	12	100
Subcutaneous panniculitis-like T-cell lymphoma	1	82
Primary cutaneous CD4+ small/medium pleomorphic T-cell lymphoma†	2	75
Aggressive clinical behavior		
Sézary syndrome	3	24
Primary cutaneous NK/T-cell lymphoma, nasal-type	<1	NR
Primary cutaneous aggressive CD8+ T-cell lymphoma‡	<1	18
Primary cutaneous γ/δ T-cell lymphoma‡	<1	NR
Primary cutaneous peripheral T-cell lymphoma, unspecified‡	2	16

Table 1. Relative frequency (%) and disease-specific 5-year survival (5-DS) of CTCL classified according to the WHO-EORTC classification, adapted from Willemze et al.[7].

*, data are based on 1905 patients with a primary cutaneous lymphoma registered at the Dutch and Austrian Cutaneous Lymphoma Group between 1986 and 2002; †, primary cutaneous peripheral T-cell lymphoma, unspecified excluding the three provisional entities indicated with a double dagger (‡); NR, not reached.

In 1806, Alibert introduced the name mycosis fungoides (MF) for a disease, which forms the prototype of CTCL at present day.[2] Since that time, various attempts have been taken to classify this heterogeneous group of malignant lymphoproliferations. In 1997, the EORTC task force on cutaneous lymphoma proposed the EORTC classification for primary cutaneous lymphomas[3]. The proposal was based on clinical, pathological, and immunohistochemical characteristics, and subgrouped CTCL into indolent (MF, MF-associated mucinosis follicularis, pagetoid reticulosis, large cell CD30+ CTCL, lymphomatoid papulosis), aggressive (Sézary syndrome, large cell CD30- CTCL), and some provisional entities. It has been clinically validated by several large studies, including follow-up data of more than 1300 patients suffering from primary cutaneous lymphoma[3,4,5]. The scheme has been widely used in clinical and research settings until the WHO classification of tumors of hematopoietic and lymphoid tissues has been published in 2001[6]. In contrast to the EORTC proposal, the WHO classification primarily focuses on T-cell lineage and immunohistochemical characteristics. Clinical data, particularly clear prognostic and therapeutic implications, were mainly lacking. Furthermore, differences between both schemes with regard to the classification of cutaneous B-cell lymphomas

and of CTCL other than MF, Sézary syndrome, and primary cutaneous CD30+ T-cell lymphoproliferative disorders lead to considerable debates and confusion. As a result of consensus meetings, representatives of both classification systems developed the WHO-EORTC classification for cutaneous lymphomas, which was published in 2005[7] (see table 1). In the meantime, this WHO-EORTC classification for cutaneous lymphomas has become the golden standard and most of its proposals have been adopted by the revised WHO classification of 2008[8].

1.2 Mycosis fungoides

MF is characterized by a proliferation of epidermotropic small- to medium-sized T-lymphocytes with cerebriform nuclei. With a relative frequency of 44%, it represents the most common entity of primary cutaneous lymphomas. The term MF should be reserved to the classical entity, which is defined by the subsequent evolution of patches, plaques, and tumors, and to variants with similar clinical behavior as bullous and hyper- or hypopigmented MF. Folliculotropic MF (i.e. MF-associated mucinosis follicularis), pagetoid reticulosis, and granulomatous slack skin show distinct clinical and pathological features and, therefore, are separated from classical MF.

MF typically affects elderly males (median age at diagnosis: 55-60 years, male-to-female ratio: 1.6-2.0:1) and has an indolent clinical course[9,10,11]. With a predilection for buttocks and other sun-protected areas, initial patches slowly progress into more infiltrated plaques and eventually into tumors within a period of years of sometimes decades. Typically, the individual patient shows a variegated pattern of variably infiltrated patches and plaques (and tumors). In cases with occurrence of tumors without concurrent patches and plaques, another type of CTCL must be considered. Only a minority of patients develops involvement of lymph nodes or visceral organs[7].

Histopathologically, early patches are characterized by superficial band-like or lichenoid infiltrates, mainly consisting of lymphocytes and histiocytes. Only a few small- to medium-sized atypical cells can be identified by their cerebriform nuclei and their epidermotropism. In typical plaques, epidermotropism is more pronounced, and intraepidermal accumulations of atypical cells (Pautrier microabscesses) occur as highly characteristic, but infrequent feature[12]. In tumor lesions, the dermal infiltrates become more diffuse and epidermotropism disappears. Atypical cells increase in number and size with variable proportions of cerebriform cells, blast cells with prominent nuclei, and intermediate forms.

Generally, the neoplastic cells express a mature memory T-cell phenotype (CD3+ CD4+ CD45RO+ CD8-). Demonstration of an aberrant phenotype (loss of pan–T-cell antigens as CD2, CD3, CD5) is often seen and represents an important adjunct in the diagnosis of MF[13]. In rare cases of otherwise classical MF, a CD4- CD8+ phenotype may be found.

The prognosis of patients with MF depends on type and extent of skin lesions and the presence of extracutaneous manifestations, as reflected by the revised TNM-classification of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization (EORTC, see table 2)[14]. In recent studies, 10-year disease-specific survival rate was 97%-98% for patients with limited patch/plaque disease (MF IA), 83% for generalized patch/plaque

disease (MF IB), 42% for tumor stage MF (IIB), and about 20% for histologically proven lymph node involvement (MF IVA)[9,10,11]. Patients with lymph node or visceral involvement, and cases with transformation into a large T-cell lymphoma show an aggressive clinical course. Death usually occurs due to systemic involvement or infections.

TNMB Definitions	**TNM Stage Groupings**
Skin (T)	Stage IA
T1 Limited patches, papules, and/or plaques, covering < 10% of the skin surface	T1, N0, M0, B0-1
T2 Patches, papules and/or plaques covering ≥ 10% of the skin surface	Stage IB
T3 One or more tumors, ≥1 cm diameter	T2, N0, M0, B0-1
T4 Confluence of erythema, covering ≥80% body surface area	
Regional lymph nodes (N)	Stage IIA
N0 No clinically abnormal peripheral lymph nodes, biopsy not required	T1-2, N1-2, M0, B0-1
N1 Clinically abnormal peripheral lymph nodes, histopathology Dutch grade 1 or NCI LN0-2, N1a = clone negative, N1b = clone positive	Stage IIB
N2 Clinically abnormal peripheral lymph nodes, histopathology Dutch grade 2 or NCI LN3, N2a = clone negative, N2b = clone positive	T3, N0-2, M0, B0-1
N3 Clinically abnormal peripheral lymph nodes, histopathology Dutch grades 3-4 or NCI LN4, clone positive or negative	
Nx Clinically abnormal peripheral lymph nodes; no histologic confirmation	Stage IIIA
	T4, N0-2, M0, B0
Distant metastasis (M)	Stage IIIB
M0 No visceral organ involvement	T4, N0-2, M0, B1
M1 Visceral involvement, must have pathology confirmation, organ involved should be specified	
Blood (B)	Stage IVA_1
B0 Absence of significant blood involvement: ≤ 5% of peripheral blood lymphocytes are atypical (Sézary) cells, B0a = clone negative, B0b = clone positive	T1–T4, N0-2, M0, B2
B1 Low blood tumor burden: > 5% of peripheral blood lymphocytes are atypical (Sézary) cells, but not criterion B2, B1a = clone negative, B1b = clone positive	Stage IVA_2
B2 High blood tumor burden: ≥1000/µL Sézary cells with positive clone	T1–T4, N3, M0, B0-2
	Stage IVB
	T1–T4, N0-3, M1, B0-2

Table 2. ISCL/EORTC revision of the TNM-classification of mycosis fungoides and Sézary syndrome.[14] For primary cutaneous lymphomas other than MF and Sézary syndrome, which show different clinical behavior, another TNM classification system should be applied, as published recently.[15]

In the present thesis, the TNM stages applied refer to the old classification of Bunn et al.[16], which differs in the definition of the N stadia: In the revised ISCL/EORTC version, the old, clinically not relevant N2 stadium (no clinically abnormal lymph nodes, pathology positive for CTCL) is removed and all N stadia are more precisely defined. Furthermore, TCR clonality is added as suffix to the N and B stadia.

1.3 Clonal T-cell receptor rearrangement

In early stages, an unspecific clinical and histopathological picture often hampers definitive diagnosis of MF. To overcome this problem, different approaches have been introduced: Calculation of a nucleus-contour-index of the cerebriform nuclei of the atypical cells by electron microscopy[17] showed high sensitivity and specificity in the differentiation between reactive and neoplastic T-cell infiltrates[18] but is time and labor consuming. Flow-cytometric estimation of the ratio of diploid and aneuploid cells[19] could increase diagnostic specificity[20], but is not suited for daily practice. Cytogenetic studies identified many structural and numerical chromosomal abnormalities, in particular in the advanced stages of MF, but recurrent, MF-specific chromosomal translocations have not been found[21,22]. By immunophenotyping, the neoplastic cells show a (non-specific) mature memory T-cell phenotype (CD3+ CD4+ CD45RO+ CD8-). However, loss of pan–T-cell antigens as CD2, CD3, CD5 represents an important adjunct in the diagnosis of MF[13].

It is generally accepted that carcinogenesis is initiated by a mutation event in a single cell, which leads to uncontrolled growth (or defects in cell death) of that cell. By subsequent cell division, genetically identical cells arise and form a neoplastic cell clone. During their maturation, T-lymphocytes rearrange the genes encoding the T-cell receptor (TCR)[23]. This rearrangement leads to a unique gene sequence, which defines every single T-cell (see figure 1A). Consequently, every single CTCL can be defined by the unique sequence of its TCR genes, and demonstration of the predominance of a single TCR rearrangement (i.e. clonal TCR rearrangement) identifies the presence of a T-cell clone.

Two different techniques have been developed to detect clonal TCR rearrangements:

1) Southern blotting (SB): Genomic DNA is digested by endonucleases, restriction fragments are separated by electrophoresis en blotted on nylon membranes. Hybridization of the blots with gene probes for the constant region of the TCR genes results in germ line bands (derived from cells without TCR rearrangement), a smear (derived from the multiple different TCR rearrangements of the T-cell background), and clonal (non-germ line) bands derived form the predominant TCR gene. Clonal bands become visible if clonal cells comprise 2-5% of all analyzed cells[24,25]. The technique is costly in labor and requires relatively high amounts of intact DNA. SB analysis of early stage MF often fails to detect a clonal TCR rearrangement[26].

2) PCR techniques: TCR genes of a given sample are amplified by PCR. Due to its limited combinatorial diversity, the TCRγ locus allows for simplified PCR primers and is often preferred for this analysis. PCR amplicons are subsequently separated on high-resolution gels. Predominant TCR rearrangements appear as sharp bands or high peaks (see figure 1 B-D). The diagnostic sensitivity of this approach has been estimated at 1-5%[27]. Both steps of the technique are prone to give pseudoclonal results: Low T-cell numbers in the sample or the use of (semi-) nested PCR assays may result in imbalanced amplification of the TCR genes. Differentiation between sharp (clonal) band and non-clonal smears after electrophoresis remains very subjective, especially when low-resolution techniques are applied. False-positive PCR results can be reduced by application of standardized primer sets (as the Biomed-2 approach[28]), by the use of more objective separation techniques (fluorescence fragment analysis[30]) and by analysis of several skin samples per patient[29]. PCR analysis

detects clonal TCR rearrangements in most MF cases[21]. In our hands, detection of TCR clonality succeeds in three-fourths of MF stage I patients (see table 3).

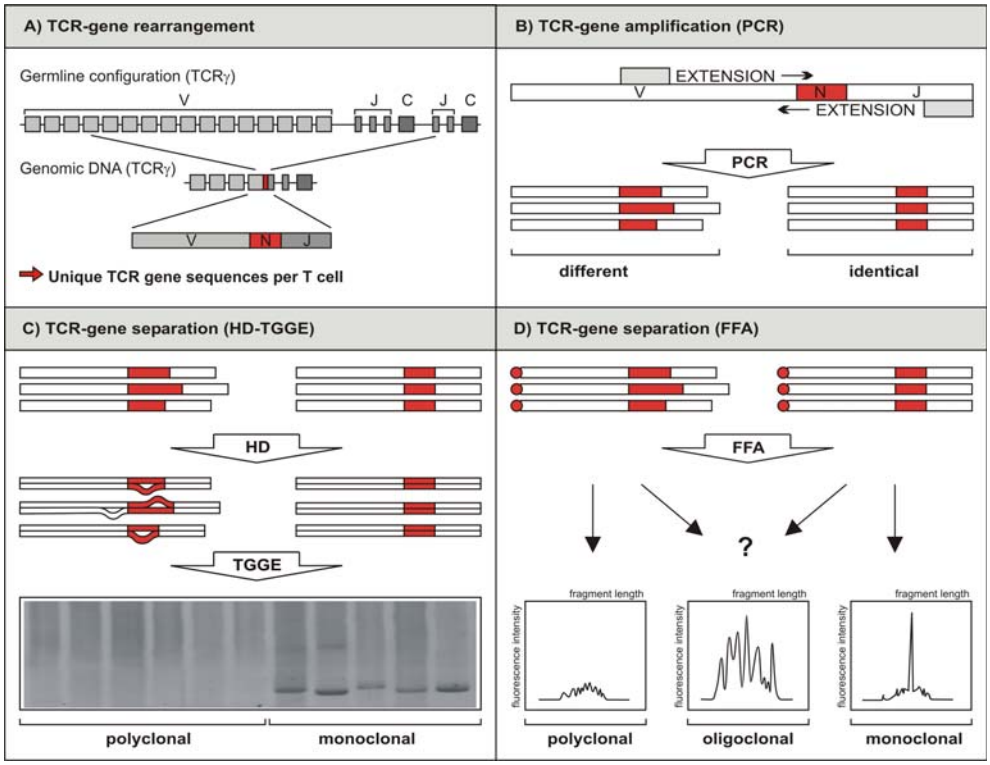

Figure 1. Detection of clonal TCR rearrangements. A) TCR rearrangement. During T-cell maturation distinct variable (V) and joining (J) segments are rearranged from germ line DNA to form a functional TCR gen (genomic DNA). Due to modifications during the recombination, the N region occurs. Combinatorial (chosen V and J gene segment) and junctional (N region) diversity ensure uniqueness of the TCR gene sequence. B) TCR amplification. All TCR genes of a sample are amplified by PCR. In case of predominant rearrangement, multiple identical amplicons occur. C) Hetero-duplex temperature gradient gel electrophoresis. Amplicons are denatured and slowly re-natured. Predominant amplicons more often form homoduplices (right), whereas the remaining amplicons form heteroduplices (left). After electrophoresis in a temperature gradient, homoduplices appear as sharp bands (right), whereas heteroduplices form a broad smear (left). D) Fluorescence fragment analysis. Fluorescence labeled amplicons are separated on an automated sequencer. Fluorescence intensity is depicted dependent on fragment length. Predominant fragments form high peaks. In this approach, estimation of TCR clonality can be objectified by calculation of a peak-height-ratio[30].

Currently, PCR approaches represent the preferred technique for the estimation of TCR clonality. However, detection of TCR clonality does not demonstrate malignancy. Immune responses to human immunodeficiency virus or Epstein-Barr virus as well as to vaccines can result in T-cell clones representing up to 40% of all peripheral T-lymphocytes. Moreover, TCR clonality has been demonstrated in skin samples of various benign dermatoses (see table 3). Consequently, TCR clonality studies represent only a complementary tool in the diagnostics of CTCL. Since every single CTCL can be defined by the unique sequence of its TCR genes, the technique offers advantages in disease monitoring as staging procedures and therapy control. Moreover, it can be applied to identify the neoplastic cells in various issues of lymphoma research, as microanatomical studies or clone-

specific in-situ hybridization[31,32].

Entity	Detection of a T-cell clone		Technique
	n	%	
CTCL[33]	46/58	80	TCRγ PCR/ HD-TGGE
MF IA	9/12	75	
MF IB	17/24	71	
MF II-IV	3/4	(75)	
Sézary syndrome	3/4	(75)	
Pleomorph small/medium-sized CTCL	12/12	100	
CTCL, not otherwise specified	2/2	(100)	
MF[34]	47/64	74	TCRγ PCR/ FFA
MF IA	14/18	78	
MF IB	28/39	72	
MF II-IV	5/7	(71)	
Benign dermatoses[32]	11/100	11	TCRγ PCR/ HD-TGGE
Drug eruption	0/7	(0)	
Lupus erythematosus	5/23	22	
Morphea	4/27	15	
Lichen rubber	0/20	0	
Prurigo simplex	2/23	9	
Lichenoid dermatoses	56/144	39	
Pityriasis lichenoides et varioliformis acuta[35]	13/20	65	TCRγ PCR/HD-PAGE
Lichen sclerosus et atrophicus[36]	18/39	46	TCRγ PCR/ HD-TGGE
Lichen sclerosus et atrophicus[36]	16/29	55	TCRγ PCR/ FFA
Lichen ruber[37]	9/36	25	TCRγ PCR/ DGGE
Lichen ruber[32]	0/20	0	TCRγ PCR/ HD-TGGE
Chronic dermatitis vs. MF[38]			TCRγ PCR/ DGGE
MF	16/27	59	
Suggestive for MF, subsequently developing MF	11/22	50	
Suggestive for MF, subsequently MF excluded	6/32	19	
Chronic dermatitis	3/31	10	
Small plaque parapsoriasis (SPP)	3/25	12	
SPP[39]	2/3	(67)	TCRγ PCR/ DGGE
SPP[40]	1/8	(13)	TCRγ PCR/ DGGE
SPP[41]	0/14	0	TCRγ PCR/ HD-TGGE

Table 3. Detection of clonal T-cells by TCRγ PCR assays in different dermatoses and CTCL. HD, heteroduplex; PAGE, polyacrylamide gel electrophoresis; TGGE, temperature gradient gel electrophoresis; DGGE denaturing gradient gel electrophoresis; FFA, fluorescence fragment analysis.

1.4 Outline of the thesis

In the early 1990-ties, several groups applied SB techniques to evaluate an extracutaneous spread of malignant T-cells in MF and other CTCL. The majority of these studies detected circulating clonal T-cells only in Sézary syndrome and some cases of advanced MF or pleomorphic CTCL. Therefore, it was postulated that blood involvement is restricted to advanced cutaneous lymphoma and is associated with poorer prognosis, lymph node involvement as well as an enlarged total body tumor burden.[42,43,44,45] In contrast, frequent occurrence of multifocal or diffuse cutaneous MF lesions as well as the recirculational behavior of T-cells[46,47] support the hypothesis of an early occurrence of

malignant T-cells in the peripheral blood. Consequently, a few groups have supposed early hematogeneous involvement in MF[20,40,48,49].

The overall outline of the research described in this thesis was to obtain more insight into T-cell clonality in the peripheral blood of patients suffering from MF. Four main topics were addressed. The first concerned the occurrence of circulating clonal T-cells (chapter 2). Frequency of peripheral blood T-cell clonality with regard to MF stage was analyzed in detail, and the identity of the T-cell clones detected in skin and blood of a given patient was determined. Topics 2 and 3 focused on the significance of the peripheral blood T-cell clonality in CTCL. The prognostic relevance of simultaneous detection of clonal T-cells in peripheral blood and skin of MF patients was analyzed in extended studies with special emphasis for early stages (chapter 3). The linkage between clonal chromosomal aberrations indicating qualitative and functional alteration of the affected cells, and TCR clonality was addressed. In addition, the relation between genomic aberration and survival in CTCL was investigated (chapter 4). Main topic four concerned the confirmation of our TCR clonality data by a golden standard. After analyzing a large series of paraffin-embedded CTCL samples by our in-house PCR and the Biomed-2 protocol, the results of both approaches were compared with special regard to sensitivity, specificity and match of the outcomes (chapter 5). Finally, the main results of the studies presented in this thesis are summarized and the diagnostic and prognostic significance of T-cell receptor gene rearrangement analysis in CTCL is discussed (chapter 6).

References.

1 Groves FD, Linet MS, Travis LB, Devesa SS. Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. J Natl Cancer Inst. 92:1240-1251, 2000.

2 J.L.M.Alibert: Tableau du plan fonoide: Déscription des maladies de la peau observée a l'hospital St. Louis, et exposition des meilleuresméthods suivies pour leur traitement. Paris, Barrios l'Ainé et Files, 1806.

3 Willemze R, Kerl H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer (EORTC). Blood. 90:354-371, 1997.

4 Grange F, Hedelin G, Joly P, et al. Prognostic factors in primary cutaneous lymphomas other than mycosis fungoides and the Sezary syndrome: the French study group on cutaneous lymphomas. Blood. 93:3637-3642, 1999.

5 Fink-Puches R, Zenahlik P, Bäck B, Smolle J, Kerl H, Cerroni L. Primary cutaneous lymphomas: applicability of current classification schemes (EORTC, WHO) based on clinicopathologic features observed in a large group of patients. Blood. 99:800-805, 2002.

6 Jaffe ES, Harris NL, Stein H, Vardiman JW (Eds). World Health Organization Classification of Tumors: Pathology and Genetics of Tumours of Hematopoietic and Lymphoid Tissues. IARC Press, Lyon 2001.

7 Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, Grange F, Harris NL, Kempf W, Kerl H, Kurrer M, Knobler R, Pimpinelli N, Sander C, Santucci M, Sterry W, Vermeer MH, Wechsler J, Whittaker S, Meijer CJ. WHO-EORTC classification for cutaneous lymphomas. Blood. 105(10):3768-85, 2005.

8 Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (Eds.): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edition, IARC Press, Lyon 2008.

9 van Doorn R, van Haselen CW, van Voorst PC, et al. Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. Arch Dermatol. 136:504-510, 2000.

10 Zackheim H, Amin S, Kashani-Sabet M, McMillan A. Prognosis in cutaneous T-cell lymphoma by skin stage: long-term survival in 489 patients. J Am Acad Dermatol. 40:418-425, 1999.

11 Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol. 139:857-866, 2003.

12 Nickoloff BJ. Light-microscopic assessment of 100 patients with patch/plaque-stage mycosis fungoides. Am J Dermatopathol. 10:469- 477, 1988.

13 Ralfkiaer E. Controversies and discussion on early diagnosis of cutaneous T-cell lymphoma: phenotyping. Dermatol Clin. 12:329-334, 1994.

14 Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, Zackheim H, Duvic M, Estrach T, Lamberg S, Wood G, Dummer R, Ranki A, Burg G, Heald P, Pittelkow M, Bernengo MG, Sterry W, Laroche L, Trautinger F, Whittaker S; ISCL/EORTC. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood 110:1713-22 , 2007.

15 Kim YH, Willemze R, Pimpinelli N, Whittaker S, Olsen EA, Ranki A, Dummer R, Hoppe RT; ISCL and the EORTC. TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). Blood 110:479-84, 2007.

16 Bunn PA, Jr., Lamberg SI. Report of the Committee on Staging and Classification of Cutaneous T- Cell Lymphomas. Cancer Treat Rep 1979;63:725---28.

17 Meijer C, van der Loo E, van Vloten W, van der Velde E, Scheffler E, Cornelisse CJ: Early diagnosis of mycosis fungoides and Sézary syndrome by morphometric analysis of the lymph cells in the skin. Cancer 45: 2864-2871, 1980.

18 Willemze R, Cornelisse CJ, Hermans J et al: Quantitative electron microscopy in the early diagnosis of cutaneous T-cell lymphomas: A long-term follow-up study of 77 patients. American Journal of Pathology 123:166-173, 1986.

19 van Vloten WA, Schaberg A, van der Ploeg M: Cytophotometric studies on mycosis fungoides and other cutaneous reticuloses. Bulletins of Cancer 64: 249-258, 1977.

20 Kuzel TM, Roenigk HH, Rosen ST: Mycosis fungoides and the Sézary Syndrome: A Review of Pathogenesis, Diagnosis and Therapy. Journal of Clinical Oncology 9:1298-1313, 1991.

21 Smoller BR, Santucci M, Wood GS, Whittaker SJ. Histopathology and genetics of cutaneous T-cell lymphoma. Hematol Oncol Clin North Am. 17:1277-1311, 2003.

22 Karenko L, Hyytinen E, Sarna S, Ranki A. Chromosomal abnormalities in cutaneous T-cell lymphoma and its premalignant conditions as detected by G-banding and interphase cytogenetic methods. J Invest Dermatol. 108:22-29, 1997

23 Davis MM, Bjorkman PJ: T-cell antigen receptor genes and T-cell recognition. Nature 334: 395-402, 1988.

24 Wood GS, Bourguin A, Crooks CF, Sklar J: Quantitation of T-cell DNA in cutaneous lymphoid infiltrates. American Journal of Pathology 138:1503-1601, 1991.

25 Zehnbauer BA, Pardoll DM, Burke PJ, Graham ML, Vogelstein B: Immunoglobulin gene rearrangement in remission bone marrow specimens from patients with acute lymphoblastic leukemia. Blood 67: 835-841, 1986.

26 Mielke V, Staib G, Boehncke WH, Duller B, Sterry W: Clonal Disease in early cutaneous T-cel lymphoma. Dermatologic Clinics 2: 351-360, 1994.

27 Lukowsky A, Muche JM, Möbs M, Assaf C, Humme D, Hummel M, Sterry W, Steinhoff M. Evaluation of T-cell clonality in archival skin biopsy samples of cutaneous T-cell lymphomas using the Biomed-2 PCR protocol. Diagn Mol Pathol 2009 (accepted)

28 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 17:2257-2317, 2003.

29 Thurber SE, Zhang B, Kim YH, Schrijver I, Zehnder J, Kohler S. T-cell clonality analysis in biopsy specimens from two different skin sites shows high specificity in the diagnosis of patients with suggested mycosis fungoides. J Am Acad Dermatol. 57(5):782-90, 2007.

30 Lukowsky A, Richter S, Dijkstal K, Sterry W, Muche JM. A T-cell Receptor gamma Polymerase Chain Reaction Assay Using Capillary Electrophoresis for the Diagnosis of Cutaneous T-Cell Lymphomas. Diagn Mol Pathol 11:59-66, 2002.

31 Gellrich S, Lukowsky A, Schilling T, Rutz S, Muche JM, Jahn S, Audring H, Sterry W. Microanatomical compartments of clonal and reactive T cells in mycosis fungoides: molecular demonstration by single cell polymerase chain reaction of T cell receptor gene rearrangements. J Invest Dermatol. 115: 620-4, 2000.

32 Muche JM. Cutaneous Malignant Lymphoma. Value and Limits of the Detection of Clonal T-Cells. Akt Dermatol. 30: 559-565, 2004.

33 Muche JM, Lukowsky A, Asadullah K, Gellrich S, Sterry W. Demonstration of frequent occurrence of clonal T cells in the peripheral blood of patients with primary cutaneous T-cell lymphoma. Blood. 90: 1636 ± 1642, 1997.

34 Muche JM, Sterry W, Gellrich S, Rzany B, Audring H, Lukowsky A. Peripheral blood T-cell clonality in mycosis fungoides and nonlymphoma controls. Diagn Mol Pathol. 12:142-50, 2003.

35 Dereure O, Levi E, Kadin ME. T-Cell clonality in pityriasis lichenoides et varioliformis acuta: a heteroduplex analysis of 20 cases. Arch Dermatol. 136: 1483-86, 2000.

36 Lukowsky A, Muche JM, Sterry W, Audring H. Detection of expanded T cell clones in skin biopsy samples of patients with lichen sclerosus et atrophicus by T cell receptor-gamma polymerase chain reaction as- says. J Invest Dermatol. 115: 254-59, 2000.

37 Schiller PI, Flaig MJ, Puchta U, Kind P, Sander CA. Detection of clonal T cells in lichen planus. Arch Dermatol Res. 292: 568-569, 2000.

38 Ashton-Key M, Diss TC, Du MQ, Kirkham N, Wotherspoon A, Isaacson PG. The value of the polymerase chain reaction in the diagnosis of cu taneous T-cell infiltrates. Am J Surg Pathol. 21: 743-747, 1997.

39 Haeffner AC, Smoller BR, Zepter K, Wood GS. Differentiation and clonality of lesional lymphocytes in small plaque parapsoriasis. Arch Dermatol. 131: 321, 1995.

40 Theodorou I, Delfau-Larue MH, Bigorgne C, Lahet C, Cochet G, Bagot M, Wechsler J, Farcet JP. Cutaneous T-cell infiltrates: analysis of T-cell receptor gamma gene rearrangement by polymerase chain reaction and denaturing gradient gel electrophoresis. Blood 86: 305-310, 1995.

41 Muche JM, Lukowsky A, Heim J, Friedrich M, Audring H, Sterry W. Demonstration of frequent occurrence of clonal T cells in the peripheral blood but not in the skin of patients with small plaque parapsoriasis. Blood. 94: 1409-1417, 1999.

42 Weiss LM, Hu E, Wood GS, Moulds C, Cleary ML, Warnke R, Sklar J: Clonal Rearrangements of T-Cell Receptor Genes in Mycosis Fungoides and Dermatopathic Lymphadenopathy. N Engl J Med 313: 539, 1985.

43 Whittaker SJ, Smith NP, Jones RR, Luzzatto L: Analysis of Beta, Gamma, and Delta T-Cell Receptor Genes in Mycosis Fungoides and Sezary Syndrome. Cancer 68: 1572, 1991.

44 Bakels V, Van Oostveen JW, Gordijn RL, Walboomers JM, Meijer CJ, Willemze R: Frequency and Prognostic Significance of Clonal T-Cell Receptor β-Gene Rearrangemants in the Peripheral Blood of Patients with Mycosis Fungoides. Arch Dermatol 128: 1602, 1992.

45 Dommann SNW, Dommann-Scherrer, Dours-Zimmermann MT, Zimmermann DR, Kural-Serbes B, Burg G: Clonal disease in extracutaneous compartments in cutaneous T-cell lymphomas. A comparative study between cutaneous T-cell lymphomas and pseudo lymphomas. Arch Dermatol Res 288: 163, 1996 .

46 Edelson RL: Cutaneous T-Cell Lymphoma: Mycosis Fungoides, Sezary Syndrome, and other Variants. Am Acad Dermatol 2: 89, 1980.

47 Abel EA: Clinical features of cutaneous T-cell lymphoma. Dermatol Clinics 3: 647, 1985.

48 Bunn PA, Huberman MS, Whang-Peng J, Schechter GP, Guccion JG, Matthews MJ, Gazdar AF, Dunnick NR, Fischmann AB, Ihde DC, Cohen MH, Fossieck B, Minna JD: Prospective Staging Evaluation of Patients with Cutaneous T-Cell Lymphomas: Demonstration of a High Frequency of a Extracutaneous Dissemination. Ann Intern Med 93: 223, 1980.

49 Veelken H, Wood GS, Sklar J: Molecular Staging of Cutaneous T-Cell Lymphoma: Evidence for Systemic Involvement in Early Disease. J Invest Dermatol 104: 889, 1995.