

# ANALYSIS OF CLINICOPATHOLOGICAL CHARACTERISTICS OF CUTANEOUS LYMPHOMAS

Ph.D. thesis

**Nóra Erős, M.D.**

Semmelweis University  
School of Ph.D. Studies, Clinical Medicine



**Chairman: Zsolt Tulassay, M.D. Ph.D. D.Sc.**

**Program leader: Sarolta Kárpáti, M.D. Ph.D. D.Sc.**

**Supervisor: Márta Marschalkó, M.D. Ph.D.**

President of the Examination Committee:

Károly Rácz, M.D. Ph.D. D.Sc.

Members of the Examination Committee:

Katalin Gilde, M.D. Ph.D.

Péter Holló, M.D. Ph.D.

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## INTRODUCTION

Primary cutaneous lymphomas are malignant lymphoproliferative disorders within the group of non-Hodgkin's lymphomas. *Primary cutaneous lymphomas* are characterized by the presence of skin involvement and absence of extracutaneous manifestation at the time of diagnosis. The term of „cutaneous lymphoma” refers primary cutaneous lymphoma in this work.

Cutaneous lymphomas are classified by the WHO/EORTC consensus classification published in 2005, which divided the disorders into mature T-, NK-, and B-cell malignancies and precursor hematologic neoplasms. Cutaneous lymphomas are uncommon disorders with an estimated incidence of 1/100.000 inhabitants per year, however the incidence have increased worldwide and it is observed in our country too. T-cell lymphomas represent 65% of all cutaneous lymphoma cases, 25% are B-cell types, and rare or undefinied entities represent 10%.

The etiology and the pathogenesis of cutaneous lymphomas are unknown, however the development of the disease is thought to be a multifactorial, multistep process. The first event is supposed to be a chronic antigenic stimulation provoking an inflammatory reaction which initiates a malignant process subsequently. Several pathogenetic factors were suspected: environmental factors (insecticides, pesticides, chemical exposures in the petrochemical, textile, paint and metal industries), bacterial and viral agents, drugs, congenital or aquired immunodeficiencies and autoimmune disorders. Regarding the genetic backround many structural and numerical aberrations of chromosomes have been identified, which led to the conclusion that cutaneous lymphomas are not associated with a well defined genetic aberration, much rather various genetic alterations can induce

activation of oncogenes and/or inactivation of tumour suppressor genes.

### **AIMS OF THE STUDY**

1. To evaluate the presence of rare cutaneous lymphoma entities in our country, to analyze their clinicopathological features comparing to the published data.
2. To evaluate the diagnostic procedures currently used for the diagnosis of cutaneous lymphomas. The aim of the study was to investigate the diagnostic value of the simultaneously performed histological, immunohistochemical and molecular biologic analysis and to evaluate the percent of diagnostically uncertain cases.
3. To find a method for the improvement of the diagnostic procedure in cases with uncertain diagnosis.
4. To investigate the results of the clonal gene rearrangement in the peripheral blood and in the skin.

### **PATIENTS AND METHODS**

Investigations were performed in the Department of Dermatology, Semmelweis Hospital, Miskolc until 2005, followed by in the Department of Dermatology, Venerology and Dermatooncology, Semmelweis University, Budapest. Histopathological analysis was done in the Department of Pathology, Semmelweis Hospital, Miskolc then in the Histopathological Laboratory of Department of Dermatology, Venerology and Dermatooncology, and in the 1<sup>st</sup> Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest.

I analyzed the *clinical characteristics* of the patients: age, sex, duration and features of the cutaneous lesions,

localization, response to treatment and survival. I analyzed the results of the *staging procedure* including physical examination (skin, lymph nodes, liver, spleen), laboratory investigations (complete blood cell counts, ESR, CRP, urine analysis, hepatic and renal function tests, LDH,  $\beta$ 2 microglobulin, immunoelectrophoresis), imaging studies (chest X-ray, abdominal ultrasonography, computed tomography scans of the chest, abdomen and pelvis), lymph node biopsy in case of lymphadenopathy, as well as bone marrow biopsy.

Cutaneous B-cell lymphoma was found in 15 patients (7 marginal zone B-cell lymphomas, 4 follicle center lymphomas, 2 diffuse large B-cell lymphomas, leg type and 2 intravascular large B-cell lymphomas), non-mycosis fungoides/Sézary syndrome type cutaneous T-cell lymphoma in 7 patients (2 anaplastic large T-cell lymphomas, 5 CD4+ small/medium-sized pleomorphic T-cell lymphomas), CD4+/CD56+ hematodermic neoplasm in 2 patients and multiple malignant lymphoproliferative disorders in 4 patients. The value of the currently used diagnostic procedure was evaluated in 60 cases with suspected diagnosis of mycosis fungoides/Sézary syndrome.

The formalin fixed, paraffin embedded *skin biopsy specimens* were stained with hematoxylin-eosin and immunohistochemical analysis were performed with the following antibodies: CD2 (Novocastra), CD3 (DAKO), CD4 (Novocastra), CD5 (Novocastra), CD7 (Novocastra), CD8 (Novocastra), CD10 (Novocastra), CD20 (DAKO), CD21 (DAKO), CD23 (DAKO), CD30 (DAKO), CD34 (DAKO), CD45 (DAKO), CD45RO (DAKO), CD56 (Novocastra), CD68 (DAKO), CD79a (DAKO), CD138 (DAKO), kappa and lambda light chain (DAKO), bcl-2 (DAKO), bcl-6 (DAKO), Ki-67 (DAKO), p53 (DAKO), MUM-1 (DAKO),  $\beta$ F1 (Endogen), EMA (DAKO), ALK (DAKO), TIA-1, MPO (DAKO), TdT (DAKO), HLA-DR (DAKO), LMP-1 (DAKO),

granzyme-B (DAKO) and cytokeratin (DAKO). Molecular biologic analysis was performed on fresh frozen skin biopsy tissue with polymerase chain reaction (PCR) method.

The PCR amplification of the T-cell receptor (TCR)  $\gamma$  gene was performed by using a sense primer 5'-AGGGTTGTGTTGGAATCAGG-3' specific for V $\gamma$  gene and an antisense primer 5'-CGTCGACAACAAGTGTGTTCCAC-3' specific for J $\gamma$  gene. Linear amplification was performed with J $\gamma$  antisense primer for 25 cycles followed by further amplification with V $\gamma$  sense and J $\gamma$  antisense primers for 30 cycles. The PCR conditions were: denaturation at 94 °C for 30 seconds, annealing at 55 °C for 45 seconds and extension at 72 °C for 45 seconds.

The PCR analysis of the immunoglobulin (Ig) heavy chain was performed with amplification of the CDR3 gene region by using a IgV<sub>H</sub> specific 5'-ACACGGC(C/T)(G/C)TGTATTACTGT-3' sense and a J<sub>H</sub> specific 5'-ACCTGAGGAGACGGTCACC-3' antisense primer pairs. Amplification was performed for 30 cycles. The following PCR conditions were used: denaturation at 95 °C for 2 minutes, annealing at 57 °C for 1 minute and extension at 72 °C for 2 minutes.

The PCR products were evaluated by electrophoresis on a 2% agarose gel, stained with ethidium bromide. Positive controls were obtained from clonal skin samples previously analyzed with Southern blot technique, negative controls were obtained from peripheral blood DNA of healthy persons and distilled water. A PCR result considered positive (clonal) when a distinct band was detected, whereas smear-like pattern represented negative result (polyclonal population).

From the *peripheral blood* the following analyses were performed: serological tests for *Borrelia burgdorferi*, EBV and HIV infection, flow cytometry, molecular biologic and cytogenetic analysis.

The flow cytometric analysis was performed using an antibody panel labelled with FITC, phycoerthrin and phycoerythrin-Cy5 fluorescents then incubated at 4 °C for 15 minutes. Red blood cells were lysated with a hypotonic solution, then the cell debris was removed by centrifugation, and the pellet was suspended in PBS buffer. The following antibodies were used: CD3, CD4, CD8, CD10, CD19, CD45, CD26, TCR $\alpha$ - $\beta$ , TCR $\gamma$ - $\delta$ . The measurement was performed with a BD FACSCalibur flow cytometer.

The molecular biologic analysis was performed from EDTA containing blood samples with the above mentioned PCR method.

Cytogenetic analysis was performed with G-banding: the blood sample was stimulated with phytohemagglutinin for 72 hours, centrifuged in a Heparin containing tube followed by cell separation and culture in 10 ml culture medium at 37 °C for 72 hours, 0,06  $\mu$ g/ml colcemid was added, then hypotonisation with potassium chloride, tri-sodium citrate and distilled water containing hypotonic solution, and fixation was performed. The samples were dried at 37 °C for 3 days followed by digestion with trypsin, staining with Giemsa, finally karyotyping was performed.

*Lymph node biopsy* was performed in cases of palpable lymph nodes and histopathological, immunohistochemical, molecular biologic and flow cytometric analysis were done with the above mentioned methods.

*Bone marrow examination* was performed with crista biopsy technique which makes possible the simultaneous analysis of the formalin fixed bone as well as the bone marrow aspirate. Histopathological, cytological, immunohistochemical, molecular biologic, flow cytometric and cytogenetical analyses were performed as described above.

*Fluorescence in situ hybridization (FISH) analysis* was performed in anaplastic large T-cell lymphoma for the

demonstration of the t(2;5) translocation. After hypotonisation the sample was dried at room temperature overnight, then dehydrated with 70%, 85% and 100% ethanol at  $-20^{\circ}\text{C}$ . The DNA probes containing complementary sequences (Vysis LSI ALK) were denaturated at  $72^{\circ}\text{C}$  for 10 minutes, then hybridization was performed at  $37^{\circ}\text{C}$  for overnight in a wet chamber. After removing the non-hybridized DNA probes, the samples were covered with  $6\ \mu\text{l}$  of Bioview Blue Dapi DNA specific stain. Finally the analysis was performed with a fluorescent microscope, assaying of 200 interphase nuclei.

## **RESULTS**

### **I. CLINICOPATHOLOGICAL CHARACTERISTICS OF UNCOMMON ENTITIES**

#### **1. CUTANEOUS B-CELL LYMPHOMA**

##### **1.1. Marginal zone B-cell lymphoma**

Seven patients (5 males, 2 females) were investigated with marginal zone B-cell lymphoma, age ranged between 32-68 years, average age was 49.5 year. Violaceous-erythematous nodules were detected in all cases, 5 patients had multiple, 2 patients had solitary skin lesions, mainly located on the back. Histopathological analysis revealed dense nodular or diffuse lymphoid infiltrate with CD20+, CD79a+, CD3-, CD5-, CD10- and CD30 negative phenotype in the deep dermis and occasionally in the subcutis, without epidermal involvement. Presence of monoclonal immunoglobulin light chain was detected in 2 patients only (1 kappa and 1 lambda light chain restriction). *Borrelia burgdorferi* serology was positive in 3 out of 7 patients, 2 cases with IgM and 1 case with IgG positivity. Two patients received antibiotic treatment, however only 1 case showed regression, the other cases needed further

treatment. I observed indolent course of the disease in every cases, the skin lesions responded to the treatment, and extracutaneous manifestation was not detected.

### **1.2. Follicle center lymphoma**

Four patients were investigated (3 males, 1 female), age ranged between 28-61 years, average age was 43.7 year. Skin lesions manifested as violaceous-erythematous papules, nodules and larger tumours in most of cases, typically located on the trunk. Pruritic eczematiform lesions, erythematous gyrated plaques and B-symptoms (fever, weight loss, weakness, night sweats) were observed in cases with widespread skin symptoms. Histopathology was characterized by the presence of diffuse or nodular lymphoid infiltrate in the dermis, composed of CD20+, CD45+, CD10+, bcl-6+, bcl-2 negative centrocytes and centroblasts. The patients resonded to the treatment, however relapses were frequently observed. Two patients had bone marrow and one patient lymph node infiltration, however nodal follicular lymphoma with secondary skin involvement could be excluded by the medical history and the bcl-2 negativity of the skin biopsy specimen.

### **1.3. Diffuse large B-cell lymphoma, leg type**

Two elderly (68 and 66 years) female patients were observed with ulcerating erythematous-violaceous papules, nodules, and a larger solitary tumour on both and unilateral leg, respectively. The histopathological analysis revealed a CD20+, CD45+, p53+, bcl-2+, LMP-1 negative centroblastic and immunoblastic infiltrate in the dermis and subcutis, with a Ki-67 proliferation rate of 80-90%. Complete remission was observed in the first patient without any therapy, however the second case displayed relapse after the tratment. In the latter case 4 malignant tumours were presented in the medical history (gastric lymphoma, breast cancer, basocellular carcinoma and the diffuse large B-cell lymphoma, leg type).

The cutaneous lymphoma occurred 3 years after the chemotherapy and radiotherapy of the breast cancer, so an anticancer treatment induced secondary malignant tumour may be hypothesized.

#### **1.4. Intravascular large B-cell lymphoma**

Two elderly (73 and 64 years) female patients were observed. The first patient had had uncertain central nervous system symptoms, panniculitis-like nodules on the thighs, then induration of the skin occurred with oedematous and orange-like surface and multiple teleangiectasias. The second patient presented with brownish-red thick nodules on the anterior aspect of the thighs. Histopathology revealed atypical, centroblast-like CD20+, CD45+, CD79a+, CD3-, CD30-, CD34-, CD45RO-, CD68-, HLA-DR+, LMP-1 negative cells within the lumen of the widened dermal and subcutaneous blood vessels in both cases. After having the diagnosis we could consider the first patient's neurological signs as specific symptoms related to the central nervous system involvement. In the second case in addition to the skin symptoms pulmonary manifestation was suspected by the chest CT scan, and without histological verification we could consider them as a specific infiltration based on the disappearance after the R-CHOP therapy. Both cases responded to the treatment with a survival of 5 and 3 years, and the second patient is alive and symptom free.

## **2. CUTANEOUS T-CELL LYMPHOMA**

### **2.1. Anaplastic large T-cell lymphoma**

Two females (68 and 24 years) were observed. The first patient presented with rapidly enlarging ulcerated unilateral skin tumours on the trunk. Local X-ray radiotherapy resulted in complete response. The second young patient

presented with a rapidly growing nodule on the dorsal surface of the tongue. Histopathology revealed a deep lymphoid cell infiltrate between the muscle fibres, composed of atypical large cells, admixed with small lymphocytes, histiocytes, eosinophil and neutrophil granulocytes. The atypical cells larger than 30  $\mu\text{m}$  proved to be CD3+, CD30+, CD4-, CD8-, CD20-, ALK negative lymphoid cells. The tumour completely regressed after the biopsy procedure.

## **2.2. CD4+ small/medium-sized pleomorphic T-cell lymphoma**

Five patients were analyzed (3 males, 2 females), age ranged between 33-68 years, average age was 50.4 year. Short history of the skin lesions was observed in all cases with a median time of 2 months, which proved to be a strikingly short period compared to the 5-year-history observed in the cutaneous B-cell lymphomas. The reason for the short history could be the head and neck localization of the skin lesions in 4 cases. Solitary erythematous papules and nodules were observed in most cases, multifocal skin lesions occurred in 1 case. The histology revealed dense lymphoid infiltrate in the dermis in all cases, accompanied by involvement of the subcutis in 2 cases, and folliculotropism in 3 patients. The cells of the infiltrate proved to be mixed: several small-medium sized CD2+, CD3+, CD4+, CD5+, CD8-, CD20-, CD30-, TIA-1 negative tumour cells admixed with reactive eosinophils, histiocytes and B-cells. The Ki-67 proliferation rate was 10-30%. Clonal TCR  $\gamma$  gene rearrangement was found in 2 skin samples. Spontaneous regression was observed in 2 cases and 3 patients remained in complete remission after surgical excision without further therapy.

### **3. CD4+/CD56+ HEMATODERMIC NEOPLASM**

Two cases (75-year-old male and 69-year-old female) were analyzed with precursor hematologic neoplasm. A survival of 13 and 9 months was observed, respectively. The first patient presented with skin, lymph node, liver, spleen, peripheral blood and bone marrow involvement at onset of the disease. Polychemotherapy resulted in temporary remission, followed by central nervous system manifestation and fatal outcome. Autopsy revealed lymphoid infiltration in almost every organ including the central nervous system. The second patient had skin, lymph node, mesopharyngeal and bone marrow involvement at the presentation. In spite of the systemic and prophylactic intrathecal chemotherapy leukemic phase and central nervous system manifestation developed and fatal outcome was observed. Autopsy revealed generalized skin, lymph node, bone marrow and internal organ involvement, however the brain and the meninges proved to be tumour-free. Both cases were characterized by the development of central nervous system manifestation at 7 and 8 months after the diagnosis, respectively. The central nervous system involvement was demonstrated by cytological and flow cytometric analysis of the cerebrospinal fluid, and regarded as an additional marker of the aggressiveness of the disease.

### **4. MULTIPLE MALIGNANT LYMPHOPROLIFERATIVE DISORDERS**

#### **4.1. T-cell disease associated with T-cell lymphoma**

The first patient (15-year-old male) had simultaneously developed *lymphomatoid papulosis (LyP)* and *mycosis fungoides (MF)*. The spontaneously resolving lesions of LyP proved to be type A by histological examination, and the MF plaques showed folliculotropism and follicular mucinosis.

The second patient (48-year-old male) presented with longstanding symptoms of *LyP type A followed by an ALK negative nodal anaplastic large T-cell lymphoma (ALCL)*. Subsequently a secondary cutaneous ALCL occurred, then generalized skin tumours and lymph node involvement were observed, finally liver failure resulted in fatal outcome. The hepatic manifestation was not proved as autopsy was not performed. All biopsy samples (LyP, nodal ALCL, secondary cutaneous ALCL) showed the same histology and immunophenotype indicated the presence of the same tumour clone.

#### **4.2. B-cell disease associated with T-cell lymphoma**

The first patient (72-year-old male) presented with associated *B-cell chronic lymphoid leukemia (B-CLL) and MF*. The second patient (75-year-old male) had *B-CLL followed by cutaneous ALCL and MF*. In this patient one B- and two T-cell disorders, altogether 3 malignant lymphoproliferative diseases were associated. It was unique that the ALCL was followed by MF, reversed order is more commonly observed. The 2 cutaneous T-cell lymphomas proved to be independent by their different clinical, histological and immunophenotypic features as well as molecular biologic findings: clonal TCR  $\gamma$  gene rearrangement was detected only in the MF sample, and it was absent in the former cutaneous ALCL skin sample.

## **II. ASSESSMENT OF THE DIAGNOSTIC PROCEDURE OF CUTANEOUS LYMPHOMAS**

I investigated the diagnostic value of the simultaneously performed histopathological, immunophenotypic and molecular biologic analysis for the diagnosis of cutaneous lymphoma. A total of 60 patients with clinical suspicion of MF and Sézary syndrome, 43 males and

17 females, aged between 24 and 84 years (average age: 58.4 year) were investigated. This group consisted of 20 patients with clinical diagnosis of MF-like plaques, 12 patients with small plaque parapsoriasis, 9 patients with erythroderma, 9 patients with eczema, 5 patients with large plaque parapsoriasis, 2 patients with actinic reticuloid, and 1-1 patients with pityriasis lichenoides chronica, granuloma annulare and papuloerythroderma.

Clear distinction could be made between the benign dermatoses (31/60, 52%) and cutaneous lymphomas (17/60, 28%) by the results of the complex histological, immunophenotypic and molecular biologic analysis. In 20 % of the patients (12 out of 60) the results were not sufficient enough to establish the diagnosis of lymphoma with certainty, so these patients were followed for 4-65 months (average: 22 months) and subsequent skin biopsies were performed. Evolution of MF was detected in 6/12 patients, and the final results were proved as the follows: benign dermatosis: 31 out of 60 patients (52%) cutaneous lymphoma: 23 out of 60 patients (38%) and indeterminate cases: 6 out of 60 patients (10%).

The complex histopathological, immunophenotypic and molecular biologic analysis proved to be a useful method to verify the dignity of the diseases in 80% of cases at the first biopsy, and this proportion could be reached 90% by the second biopsy. The remaining 10% indeterminate cases needed further follow-up and repeated skin biopsies until the final diagnosis.

I investigated the diagnostic value of the molecular biologic analysis and the presence of clonal T-cell receptor  $\gamma$  gene rearrangement in the peripheral blood. Dominant T-cell clone was not found in the skin of benign and indeterminate cases, however it was detected in the skin of 9/23 (39%) lymphoma patients. The PCR analysis proved to be a reliable method as monoclonality was detected only in lymphoma

patients, and all positive results were accompanied by other lymphoma-related signs such as CD4 restriction or epidermotropism, and false positive results were not obtained. Interestingly T-cell clones were detected more commonly in the blood than in the skin samples: in 1/31 (3%) patients of the benign cases and in 12/23 (52%) of the lymphoma cases, mostly observed in the elderly patients, average age was 61 year. Circulating T-cell clones were regarded as true tumour cells if the same clone was detected simultaneously in the skin, according to the published data. Identical clone was detected in 6 patients, at the same base pair level.

## CONCLUSIONS

Based on my results it could be established that **uncommon primary cutaneous lymphoma entities could be found in our country.**

*Marginal zone B-cell lymphoma* proved to be the most common cutaneous B-cell lymphoma with the following characteristic features:

- skin lesions were located mainly on the back, in contrast to the localization on the extremities as described in the literature
- monoclonal Ig production was detected infrequently by immunohistochemistry, 1 kappa and 1 lambda light chain restriction was detected out of 7 cases
- serological results for *Borrelia burgdorferi* were positive in 1 patient with IgG positivity, and 2 patients with IgM positivity, so we could not verify the association between *B. burgdorferi* infection and the development of marginal zone B-cell lymphoma. Further studies are needed to detect *B. burgdorferi* DNA in the lesional skin samples.

- we found that antibiotic treatment is not successful in every cases with positive *B. burgdorferi* serology

In our patients the second most frequent cutaneous B-cell lymphoma was *follicle center lymphoma*, with the following characteristics:

- two patients were 28-year-old at the time of the diagnosis, and their skin symptoms have been persisted for 2 and 3 years. The occurrence of the disease should be expected in younger patients.
- B-symptoms were observed only in this group
- extracutaneous dissemination (lymph node-, bone marrow infiltration) was observed in untreated cases with longstanding symptoms
- the disease proved to be more aggressive than the marginal zone B-cell lymphoma

In cases with *diffuse large B-cell lymphoma, leg type* my observations were partly concordant with the literature with some new observations:

- skin lesions occurred on one or both legs of elderly female patients
- spontaneous regression should be expected even in cases with multiple skin lesions
- extracutaneous dissemination was not observed, however local recurrence developed in one patient
- the disease could be associated with other malignancies: in one of our patients had 3 other malignant tumours previously

My observations regarding *intravascular large B-cell lymphoma* were the following:

- the disease occurred in elderly female patients
- CHOP and R-CHOP treatment resulted in several years of disease-free survival

The following observations were made in cases with *anaplastic large T-cell lymphoma*:

- the disease responded to local radiotherapy even in rapidly progressive cases with severe ulcerative tumours
- we observed a young female patient with a unique localization of ALCL on the dorsal surface of the tongue, previously not described by the literature
- spontaneous regression could occur even in cases with unusual localization or rapid progression

The characteristics of *CD4+ small/medium-sized pleomorphic T-cell lymphoma* were as follows:

- short history of skin symptoms
- solitary lesion in the head-neck region was the most characteristic lesion
- complete regression was observed in all cases, relapse or systemic involvement did not occur
- based on these findings the disease could be established as an indolent disorder

Analyzing the cases with *CD4+/CD56+ hematodermic neoplasm* the following observations were made:

- the disease proved to be aggressive, therapy-resistant with tendency of dissemination
- meningeal involvement could be expected as it was proved by the cytological and flow cytometric analysis of the cerebrospinal fluid. In these cases the imaging studies (CT, MR) were not informative
- we recommend early cerebrospinal fluid analysis and prophylactic intrathecal chemotherapy similarly to the acute lymphoid leukemia cases

I observed *multiple malignant lymphoproliferative disorders*: 2 cases with multiple T-cell lymphomas and 2 cases

with B-cell lymphoproliferative disease associated with T-cell lymphoma. The clinicopathological characteristics of the patients were the following:

- LyP followed by MF is more usual, however we found a case of a 15-year-old male patient with simultaneously occurring LyP and MF
- a patient was observed with LyP and systemic ALCL, in whom the longstanding and untreated LyP preceded the development of a secondary high grade lymphoma
- ALK-negative systemic ALCL proved to be more aggressive than ALK-positive systemic ALCL or cutaneous ALCL
- histopathological investigation revealed type A LyP in both patients with LyP associated with second malignancy, in accordance with the literature
- association with B- and T-cell lymphoproliferations is rare, however we found a case in whom 3 malignant lymphoproliferative disorders developed, one B- and two T-cell lymphomas, which proved to be independent
- we observed a rare occurrence of cutaneous ALCL followed by MF

I investigated the diagnostic value of the **simultaneously performed histological, immunohistochemical and molecular biologic analysis** in the diagnosis of cutaneous T-cell lymphoma, and evaluated the frequency of cases with indeterminate diagnosis. I investigated the **possible method for improving the diagnostic procedure** in the diagnostically indefinite cases. My observations are the follows:

- the simultaneous histological, immunophenotypic and molecular biologic analysis of the skin biopsy specimen proved to be a useful method for the distinction between benign, reactive dermatoses and cutaneous lymphomas. we could establish definitive diagnosis in 80% of cases at first skin biopsy

- follow-up, repeated skin biopsy and complex histopathological analysis assured further diagnostic help in the unclassified, indeterminate cases, as definitive diagnosis was established in further 10% of cases
- in the remaining indeterminate cases further follow-up and repeated skin biopsies are needed

**I investigated the presence of clonal TCR  $\gamma$  gene rearrangement in the peripheral blood and in the skin.** The following results were found:

- T-cell clones were detected more commonly in the blood than in the skin of patients with benign dermatosis as well as in lymphoma patients
- peripheral blood monoclonality in a patient with benign dermatosis should not to be considered to be a tumour clone
- in the indeterminate cases clonal gene rearrangement was not detected in the skin and blood samples
- identical T-cell clones at the same base pair level in the skin and blood were detected in 6 lymphoma patients, regarded as true tumour cells
- T-cell clones in the blood were observed in the elderly patients, in accordance with the literature

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