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Cutaneous CD30-positive lymphoproliferations : clinical and molecular aspects and differential diagnosis

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Clinical and molecular aspects and differential diagnosis

Gineke Benner

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Cutaneous CD30-positive lymphoproliferations

Clinical and molecular aspects and differential diagnosis

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CONTENTS

Chapter 1	General introduction	7
Chapter 2	Applicability and prognostic value of the new TNM classification system in 135 patients with primary cutaneous anaplastic large cell lymphoma	23
Chapter 3	Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lymphoproliferative disorders	35
Chapter 4	Bone marrow examination has limited value in the staging of patients with an anaplastic large cell lymphoma first presenting in the skin. Retrospective analysis of 107 patients	49
Chapter 5	Primary cutaneous anaplastic large cell lymphoma shows a distinct miRNA expression profile and reveals differences from tumor stage mycosis fungoides	57
Chapter 6	Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases	71
Chapter 7	Summary and discussion	89
Chapter 8	Appendix	101
	Nederlandse samenvatting	102
	List of publications	106
	List of abbreviations	107
	Curriculum Vitae	108
	Nawoord	109

1 General introduction

PRIMARY CUTANEOUS T-CELL LYMPHOMAS

Primary cutaneous lymphomas represent a heterogeneous group of malignant non-Hodgkin lymphomas that clinically originate in the skin with no evidence of extracutaneous disease at the time of diagnosis. The incidence is being estimated on 1:100,000 individuals per year.[1] Primary cutaneous lymphomas often differ in clinical behavior, prognosis and biological features from their histologically similar nodal counterparts with or without secondary skin involvement, indicating a different pathogenesis and requiring a different treatment.[2] For these reasons, primary cutaneous lymphomas were included as distinct entities in recent classifications for malignant lymphomas (EORTC 1997; WHO-EORTC 2005; WHO 2008).[2-4] Two main categories can be distinguished: primary cutaneous T-cell lymphomas (CTCL), accounting for 75-80% of cases and primary cutaneous B-cell lymphomas (CBCL), accounting for 20-25% of cases. Within the group of CTCLs three subgroups can be distinguished: (1) the group of classical CTCLs, including mycosis fungoides (MF), variants of MF, and Sézary syndrome (SS); (2) the group of primary cutaneous CD30-positive lymphoproliferative disorders (CD30+ LPD); and (3) a group of rare and often aggressive cutaneous T/NK-cell lymphomas. Table 1 shows the WHO-EORTC classification and relative frequencies of most cutaneous lymphomas. The studies in this thesis focused on cutaneous CD30-positive lymphoproliferations, in particular primary cutaneous anaplastic large cell lymphoma (C-ALCL). As C-ALCL is part of the group of primary cutaneous

Table 1. WHO-EORTC classification for cutaneous lymphomas

	Frequency (%)*	**5-yr DSS (%)**
Cutaneous T-cell lymphoma		
Mycosis fungoides (variants)	48	88-100
Sézary syndrome	3	24
Primary cutaneous CD30+ lymphoproliferative disorders		
Primary cutaneous anaplastic large cell lymphoma	8	95
Lymphomatoid papulosis	12	100
Subcutaneous panniculitis-like T-cell lymphoma	1	82
Primary cutaneous NK/T-cell lymphoma, nasal type	<1	NR
Primary cutaneous CD4+ small/medium pleomorphic T-cell lymphoma	2	75
Primary cutaneous aggressive CD8+ T-cell lymphoma	<1	18
Primary cutaneous γ/δ T-cell lymphoma	<1	NR
Primary cutaneous peripheral T-cell lymphoma, not otherwise specified	2	16
Cutaneous B-cell lymphoma		
Primary cutaneous marginal zone B-cell lymphoma	7	99
Primary cutaneous follicle centre lymphoma	11	95
Primary cutaneous large B-cell lymphoma, leg type	4	55

*Data are based on 1905 patients with a primary cutaneous lymphoma registered by the Dutch and Austrian Cutaneous Lymphoma Groups[2]; NR: not reached; DSS: disease specific survival.

CD30+ LPD, this introductory chapter focuses on the clinical aspects, differential diagnosis and molecular aspects involved in the pathogenesis of this subgroup of CTCL.

CD30

In 1982, Schwab et al. described a new molecule that was initially termed Ki-1 and subsequently designated CD30.[5] CD30 is a 120-kDa transmembrane cytokine receptor of the tumor necrosis factor receptor family, located on chromosome 1p36.[6;7] Interaction between CD30 and its natural ligand CD30L (CD153, located on chromosome 9q33), which is expressed constitutively by granulocytes, activated T-cells and histiocytes, results in pleiotropic effects depending on cell type, state of differentiation or activation and the presence of other stimuli.[8] The CD30 antibody was first reported to react selectively with Hodgkin and Reed-Sternberg cells in Hodgkin's disease and with scattered activated blast cells in the perifollicular areas in reactive lymph nodes and tonsils.[9] Further studies showed that CD30 was also expressed by highly activated B- and T-cells and by a group of diffuse large cell lymphomas, which generally show a T- or null-cell phenotype and sometimes a B-cell phenotype.[10] Because of constant CD30 expression and frequent anaplastic features, these lymphomas were accepted as a distinct morphologic entity, initially called Ki-1 or CD30-positive (large cell) lymphoma and later anaplastic large cell lymphoma (ALCL).[11] In addition, a primary cutaneous variant was recognized (C-ALCL), and it was found that the large atypical cells in skin biopsies of lymphomatoid papulosis (LyP) also express the CD30 molecule.[12;13] Because of the overlapping clinical, histological and immunophenoptypical features, it was then suggested that C-ALCL and LyP are parts of a spectrum of primary cutaneous CD30+ LPD.[14]

THE GROUP OF PRIMARY CUTANEOUS CD30-POSITIVE LYMPHOPROLIFERATIVE DISORDERS

Primary cutaneous CD30+ LPD represent the second most common subgroup of CTCL, accounting for approximately 25% of all CTCL (Table 2). This group includes C-ALCL, LyP and borderline cases.[2] C-ALCL is composed of large cells with anaplastic, pleomorphic, or immunoblastic cytomorphology and expression of the CD30 molecule by more than 75% of the tumor cells and without clinical evidence or history of MF, or another type of CTCL. LyP is defined as a chronic, recurrent, self-healing papulonecrotic or papulonodular skin disease with histologic features suggestive of a (CD30+) malignant lymphoma. As both disorders show overlapping clinical, histological and immunophenotypical features the clinical appearance and course are used as decisive criteria for the definitive diagnosis and choice of treatment. The main clinical characteristics are summarized in Table 2. The term 'borderline cases' was initially used for cases with a discrepancy between the clinical features and the histological appearance.[14] Nowadays it refers to cases in which, despite careful clinicopathologic correlation, a definite distinction between C-ALCL and LyP cannot be made yet. Clinical examination during follow-up generally discloses whether the patient has C-ALCL or LyP.[2]

Table 2.

	C-ALCL	LyP
Extent skin lesions	Solitary/localized	Generalized
Spontaneous remission	20% complete 20% partial	100%
Staging	Yes	No
Therapy	Excision/RT	None (MTX/RT)
Risk to develop systemic disease at 10 years	16%	4%
Disease related 10-year-survival	>90%	100%

RT: Radiotherapy; MTX: methotrexate

Clinical features

Primary cutaneous anaplastic large cell lymphoma

The median age of C-ALCL patients is around 60 years with a male to female ratio of 2-3:1.[15;16] Most patients present with solitary or localized nodules or tumors, and sometimes papules, often showing ulceration (Figure 1). Multifocal lesions are seen in about 20% of the patients. In about 40% of the cases, the skin may show partial or complete spontaneous regression.[15] Skin relapses occur frequently, but extracutaneous dissemination is uncommon.[15;16] Prognosis is usually favorable with a 10-year disease-specific survival of approximately 90%.[15-17] Risk factors that predict an unfavorable course, occurring in few patients with C-ALCL, are largely unknown. However, several studies have suggested that age older than 60 years, absence of spontaneous remission, and presentation with multifocal skin lesions may correlate with reduced survival.[15;18-20] Moreover, extensive single limb involvement and localization on the head and neck have been associated with a less favorable prognosis.[16;21;22] In **chapter 2** we investigated the prognostic significance of a large number of parameters including sex, age (≤60 vs. >60 years), extent of disease, site of presentation, and complete spontaneous remission of initial skin lesions in a large group of patients (n=135) with C-ALCL. For extent of disease we used the recently described TNM classification system for primary cutaneous lymphomas other than MF and SS, as proposed by the International Society for Cutaneous Lymphomas (ISCL) and Cutaneous Lymphoma Task Force (CLTF) of the EORTC.[23] This classification system is meant to replace the use of the Ann Arbor system, which is the primary means for classifying the extent of disease in patients with non-Hodgkin lymphomas. This, because the Ann Arbor system has a number of shortcomings for lymphomas that arise primarily in extranodal sites such as the skin. For instance, in primary cutaneous lymphomas, the initial stage according to the Ann Arbor system would either be IE (if single skin site) or IVD + (if multiple skin sites), thereby disproportionally or inappropriately placing many patients in the highest stage, resulting in unnecessarily aggressive treatments.[23] A major goal of the study described in **chapter 2** was to investigate the applicability and prognostic value of this new TNM classification system.

Lymphomatoid papulosis

LyP generally occurs in adults, but may occur in children as well.[15;16] The median age is around 45 years with a male to female ratio of 1.5-2:1. Most patients present with generalized papular, papulonecrotic, and/or nodular skin lesions at different stages of development, predominantly on the trunk and limbs (Figure 1).[15;16;20] Individual skin lesions disappear within 3-12 weeks, and may leave behind superficial scars. The duration of the disease may vary from several months to more than 40 years.[2] LyP has an excellent prognosis. In up to 20% it may be preceded by, associated with, or followed by another type of malignant (cutaneous) lymphoma, generally MF, (C-)ALCL, or Hodgkin lymphoma.[15] Risk factors that identify patients most likely to develop another type of malignant (cutaneous) lymphoma or extracutaneous disease are not known.

Histologic features

Primary cutaneous anaplastic large cell lymphoma

C-ALCL show diffuse nonepidermotropic infiltrates with cohesive sheets of large CD30+ tumor cells (Figure 1).[11;14] In most cases, the tumor cells have the characteristic morphology of anaplastic cells, with round, oval, or irregularly-shaped nuclei, prominent (eosinophilic) nucleoli, and abundant cytoplasm. Less commonly (20-25%), they have a nonanaplastic (pleomorphic or immunoblastic) appearance. Reactive lymphocytes are often present at the periphery of the lesions. Ulcerating lesions may show a LyP-like (type A) histology with an abundant inflammatory infiltrate of reactive T-cells, histiocytes, eosinophils, neutrophils, and few CD30-positive cells.

Lymphomatoid papulosis

The histologic picture of LyP is extremely variable and in part correlates with the age of the biopsied skin lesion. Four histologic subtypes (types A, B, C, and D) have been described.[11;24;25] In LyP type A lesions, scattered or small clusters of large, sometimes multinucleated or Reed-Sternberg-like, CD30+ cells are intermingled with numerous inflammatory cells, such as histiocytes, small lymphocytes, neutrophils, and/or eosinophils (Figure 1). LyP type C lesions demonstrate a monotonous population or large clusters of large CD30+ T-cells with relatively few admixed inflammatory cells. LyP type B is uncommon (less than 10%) and is characterized by an epidermotropic infiltrate of small CD4+, CD30- atypical cells with cerebriform nuclei similar to that observed in MF. LyP type D consists of an epidermotropic infiltrate of small- to medium-sized CD8+ and CD30+ atypical lymphoid cells that histologically resembles primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma.[24] Recognition of the different histologic subtypes of LyP has contributed to a better definition and to a better understanding of the relationship between LyP and other types of CTCL. However, from a clinical point of view, differentiation between these different subtypes is not useful, as they do not differ clinically.

Immunophenotype

Except for LyP type B, which are generally CD30 negative, CD30+ LPD show CD30 staining on the cell membrane and in the Golgi region of most of the neoplastic cells.[18] In most cases these neoplastic cells have an activated CD4+ T-cell phenotype with variable loss of CD2, CD5 and/or CD3, and frequent expression of granzyme B, TIA-1 and perforin (cytotoxic

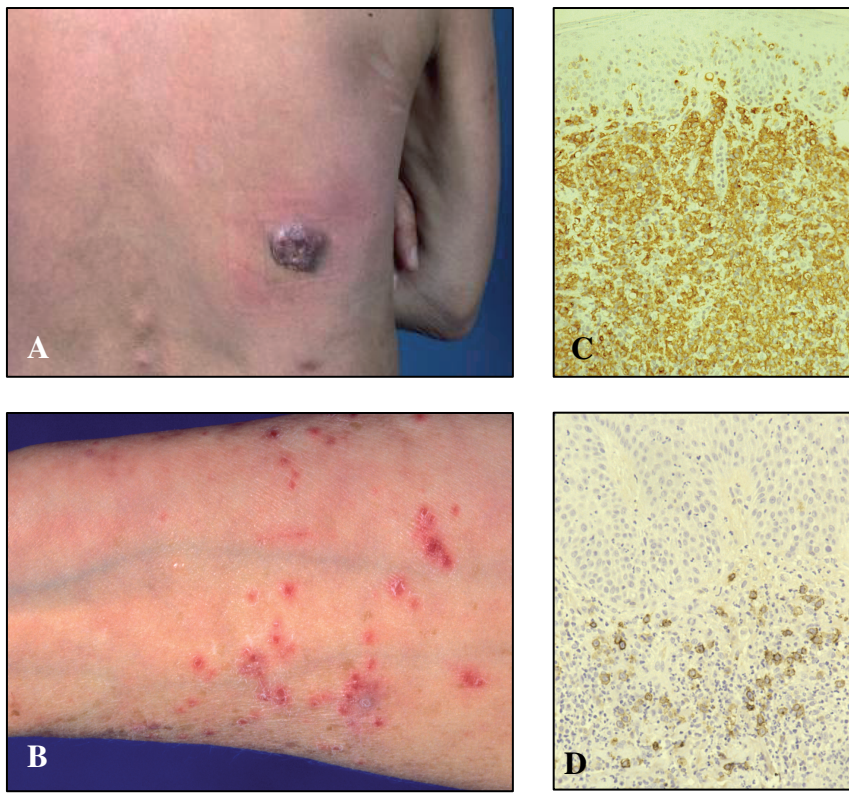

Figure 1. A: Clinical example of C-ALCL; B: clinical example of LyP; C: cohesive sheets of large CD30+ tumor cells, suggestive for a C-ALCL lesion; D: scattered CD30+ large tumor cells, suggesting LyP, type A; However, the histologic picture in panel C and D can be found both in C-ALCL and in LyP. The final diagnosis depends therefore on the clinical presentation.

proteins).[13;26] Some cases have a CD8+ T-cell phenotype. Most primary cutaneous CD30+ LPD express the cutaneous lymphocyte antigen (CLA), but do not express epithelial membrane antigen (EMA) and anaplastic lymphoma kinase (ALK), indicative of the 2;5 chromosomal translocation or its variants in systemic ALCL.[27;28] Recent studies have reported on the utility of immunohistochemical markers to differentiate between C-ALCL and LyP, such as BCL2, clusterin, TNF-receptor associated factor 1 (TRAF1) and IFN regulatory factor 4 (IRF4; also known as multiple myeloma antigen 1 or MUM1)[29-32]. The results of these studies are often unconfirmed or conflicting. **Chapter 3** evaluates the value of some of these phenotypical markers in a large group of cutaneous CD30+ lymphoproliferations.

Genetic features

Clonally rearranged T-cell receptor genes have been detected in nearly all C-ALCL and approximately 60%-70% of LyP lesions.[33;34] Identical rearrangements have been demonstrated in LyP lesions and associated lymphomas.[35;36]

The translocation t(2;5) (p23;q35) resulting in expression of NPM-ALK protein (p80), which is predominantly found in systemic ALCL in children, is not or rarely found in LyP and C-ALCL.[27] Recently, a new translocation involving the IRF4 gene locus has been discovered in 20-25% of C-ALCL cases.[24;37;38] In other CTCL this translocation has been rarely found.

The results of recent studies employing array-based comparative genomic hybridization (aCGH) and gene expression profiling are described in the following paragraph.

Etiology and pathogenesis

The mechanisms that are involved in the development of primary cutaneous CD30+ LPD are largely unknown.[11] So far, no causative agent has been identified. Because viral antigens may strongly induce CD30 expression in T-and B-cells, a viral etiology has been suggested. However, studies for an etiologic role of human T-lymphotrophic virus-1, Epstein-Barr virus (EBV) and other herpes viruses, including herpes simplex virus (HSV) type 1 and type 2 and human herpesvirus-6, 7, and 8, have been consistently negative.[39-42]

Spontaneous remission is one of the most characteristic features of these CD30-positive lymphomas and occurs, by definition, in all patients with LyP and in approximately 40% of cases of C-ALCL[15]. Several studies have investigated the expression of apoptosis related proteins that may contribute to the spontaneous disappearance of skin lesions. For instance, it was shown that the FAS receptor is consistently expressed by CD30+ LPD tumor cells, in contrast to aggressive types of CTCL.[43;44] In addition, lower levels of the antiapoptotic protein BCL2 were found in LyP compared with nonregressing lesions of C-ALCL suggesting that increased BCL2 expression in nonregressing lesions of C-ALCL protects tumour cells from apoptosis.[44] It was also suggested that interactions between CD30 and CD30L may contribute to apoptosis of the neoplastic T-cells.[45] However, the exact mechanisms responsible for the regression of skin lesions are still unknown. Regarding possible mechanisms for tumor progression, unresponsiveness to the growth inhibitory effects of transforming growth factor-beta (TGF-beta) by point mutations and deletions in the TGF-beta type I and type II receptors[46;47], as well as high levels of BCL2 expression by the CD30-positive tumor cells[44] have been suggested.

Recent studies have shed light on molecular (epi)genetic features of C-ALCL. Using aCGH analysis van Kester et al. identified several recurrent copy number alterations including gains on chromosome 7q and 17q and losses on 6q and 13q.[48] Nearly identical results were found in a recent study by Laharanne et al., using a different aCGH platform.[49] In both studies the 9p21 deletion (involving the CDKN2A-CDKN2B gene locus) was rare or absent in C-ALCL patients, which is in contrast to patients with transformed mycosis fungoides (MF-TR).[48-51] In the same study by van Kester et al. gene expression profiling showed increased expression of skin-homing chemokine receptors compared to cutaneous peripheral T-cell lymphoma, not otherwise specified (PTL-NOS), which may contribute to the lower tendency to disseminate to extracutaneous sites.[48] Additionally, based on the increased expression of CD30, TRAF1 and IRF4 Wozniak and Piris speculate that CD30-mediated NF-κB activation may play a role in the pathogenesis of C-ALCL.[48;52]

On the epigenetic level van Doorn et al. found that BCL7a was hypermethylated at a lower frequency in C-ALCL (14%) compared to aggressive types of CTCL (64%). They suggested that this gene functions as a tumor suppressor in lymphoid cells.[53] Little is known about histon modifications and miRNA expression in C-ALCL, which next to DNA methylation influence the epigenetic landscape.

Several studies have demonstrated specific miRNA expression profiles in different types of CTCL suggesting a role in the pathogenesis of these disorders. In **chapter 5** we investigated the miRNA expression profile of C-ALCL and compared the results with tumor stage MF.

Differential diagnosis

CD30 is not only expressed by the conditions included in the group of primary cutaneous CD30+ LPD, but also by systemic anaplastic large cell lymphoma involving the skin secondarily, MF-TR, certain other lymphomas and reactive skin conditions, which may mimic CD30+ LPD histologically (Table 3).

Secondary cutaneous anaplastic large cell lymphomas

Two types of systemic ALCL are included as separate entities in the WHO 2008 classification: ALK+ and ALK- ALCL.[4] ALK+ ALCL is associated with the t(2;5) translocation resulting in expression of the anaplastic large cell lymphoma kinase (ALK) protein. ALK+ ALCL occur most frequently in the first three decades of life and often involve both lymph nodes and extranodal sites such as skin.[54] It has a relatively good prognosis with a 5-year overall survival of approximately 80%.[55] Extranodal sites are less commonly involved in ALK- ALCL, but can include skin as well. Patients with ALK- ALCL have a higher median age than ALK+ ALCL and their survival is significantly worse with an overall 5-year survival rate of only 46%.[55]

In order to differentiate between a primary and secondary cutaneous ALCL adequate staging investigations have to be performed. According to guidelines from the Dutch Cutaneous Lymphoma Group (DCLG), this staging work-up should include physical examination, laboratory studies, imaging studies and a bone marrow biopsy. However, recent data from the registry of the DCLG show that in an increasing number of patients with an ALCL presenting in the skin, in particular those presenting with a solitary tumor that has resolved spontaneously

Table 3 .Differential diagnosis of cutaneous CD30+ lymphoproliferations

Primary cutaneous CD30+ lymphoproliferative disorders • Primary cutaneous anaplastic large cell lymphoma • Lymphomatoid papulosis • Borderline cases
Transformed mycosis fungoides
Systemic ALCL with secondary skin involvement
Other lymphomas • Other CTCL that sometimes express CD30: epidermotropic CD8+ CTCL, pagetoid reticulosis, patch or plaque stage MF • CD30+ cutaneous B-cell lymphomas: e.g. posttransplant (EBV+) LPD, MTX-associated lymphomas • Hodgkin lymphoma with secondary skin involvement
Reactive skin conditions • Atopic dermatitis • Drug reactions • Viral infections: Milker's nodule, HIV, HSV or VZV infections, molluscum contagiosum • Persistent arthropod bite reactions • Cutaneous lymphoid hyperplasia (pseudolymphoma)

or has been excised completely, staging is incomplete and particularly a bone marrow biopsy is not always performed. Moreover, the recently published classification system for non-MF/SS primary cutaneous lymphomas suggests that in cutaneous lymphomas with an indolent clinical behavior such as C-ALCL, bone marrow evaluation should be considered, but is not required, unless indicated by other staging assessments.[23] In **chapter 4** we investigated whether the current policy to advice bone marrow examination in all patients with C-ALCL should be maintained or whether it should be performed only in selected cases.

Transformed mycosis fungoides

MF is the most common type of CTCL (Table 1), clinically characterized by the slow progression from patches to plaques and in a proportion of patients to tumors and development of extracutaneous disease.[2] Histologically, the early stages of MF show superficial band-like or lichenoid infiltrates with atypical small- to medium-sized T-cells with highly indented (cerebriform) nuclei into the epidermis (epidermotropism). With progression to tumor stage, the dermal infiltrates become more diffuse, with an increase in the proportion of tumor cells as well as an increase in the number of blast cells, and epidermotropism may get lost. While patients with early patch or plaque stage MF generally run an indolent course with a 10-year DSS over 80%, patients developing skin tumors or extracutaneous disease have a reduced 10-year DSS of 42% and <20%, respectively.[56] Apart from clinical stage, large cell transformation (LCT) has been associated with an aggressive clinical course and a poor survival. LCT is defined by the presence of large T cells exceeding 25% of the total lymphoid infiltrate or forming microscopic nodules. These large T-cells may be CD30+ or CD30-. In view of the excellent prognosis of C-ALCL (CD30+), several studies have evaluated whether CD30 expression in MF-TR is associated with a good survival as well.[57-59] However, in none of the published studies a significant difference between CD30+ and CD30- cases was found, probably because of the small size of the study groups. Evaluation of studies to prognostic factors in MF-TR is further hampered by the inclusion of not only patients with MF but also variable numbers of patients with SS and by variable proportions of patients with LCT at extracutaneous sites. To find out whether CD30 expression and other prognostic factors are related to a better survival of patients with MF-TR, we have evaluated clinicopathologic and immunophenotypical data in a large group of patients. The results are presented in **chapter 6**.

Other lymphomas

Other types of cutaneous lymphoma that may express CD30 can be divided in three groups: (1) well-defined types of CTCL that sometimes express the CD30 antigen including CD8-positive epidermotropic CTCL[60], pagetoid reticulosis[61] and rare cases of patch or plaque stage MF; (2) skin localizations of CD30+ B-cell lymphomas, such as (EBV positive) posttransplant LPD or MTX associated lymphoma and (3) classical Hodgkin lymphoma with secondary skin involvement.[4]

Reactive skin conditions

Increasing numbers of benign skin conditions are being identified, in which the reactive inflammatory infiltrate may contain scattered or sometimes large clusters of large CD30+ T-cells, which mimic LyP or C-ALCL histologically. These include atopic dermatitis, lymphomatoid drug reactions, viral infections (e.g. Milker's nodule, human immunodeficiency virus (HIV), HSV or

varicella zoster virus (VZV) infections, molluscum contagiosum), persistent arthropod bite reactions and pseudolymphoma (Table 2).

Treatment

Treatment of CD30+ LPD should primarily be based on the size, the extent, and the clinical behavior of the skin lesions.[15] In LyP, one should take into account that a curative therapy is not available and that none of the available treatment modalities affects the natural course of the disease. For that reason, the short term benefits of active treatment should be balanced carefully against the potential side effects. For patients with few non-scarring lesions active treatment is not necessary. For patients with numerous, disseminated, or stigmatizing lesions, low-dose methotrexate (5-25 mg/week) and phototherapy, in particular PUVA, are preferred options.[62]

Solitary or localized C-ALCL are usually treated with surgical excision or radiotherapy.[15;62] The preferred type of treatment in patients with multifocal skin lesions has been subject of debate. For a long time, multiagent chemotherapy has been used as first-line therapy in patients with multifocal skin lesions. However, skin relapses are common. In recent EORTC, ISCL and United States Cutaneous Lymphoma Consortium consensus recommendations multiagent chemotherapy is therefore only advised in patients developing extracutaneous disease.[15;62] For multifocal skin lesions low-dose Methotrexate (5-25 mg/week), as in LyP, is suggested. Alternatively, retinoids or interferon can be considered.

AIM AND OUTLINE OF THE THESIS

The studies presented in this thesis have aimed to address questions regarding clinical aspects, differential diagnosis and molecular aspects involved in the pathogenesis of cutaneous CD30+ lymphoproliferations.

Chapter 2 evaluates the applicability and prognostic value of the new TNM classification system for primary cutaneous lymphomas other than MF and SS in patients with C-ALCL and investigates the prognostic significance of other clinical variables.

Chapter 3 analyses the diagnostic and prognostic value of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in several cutaneous CD30-positive lymphoproliferations including C-ALCL, LyP, CD30+ MF-TR and skin localizations of systemic ALCL.

Chapter 4 evaluates the current policy to advice bone marrow examination to patients with an ALCL presenting in the skin.

Chapter 5 provides an analysis of the miRNA expression profiles of skin biopsies from C-ALCL patients compared to skin biopsies from patients with benign inflammatory dermatoses and tumor stage MF.

Chapter 6 retrospectively analyses prognostic factors in patients with MF-TR and provides a prognostic index.

Chapter 7 summarizes and discusses the findings described in the preceding chapters.

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2

Applicability and prognostic value of the new TNM classification system in 135 patients with primary cutaneous anaplastic large cell lymphoma

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ABSTRACT

Objectives: To test the applicability and prognostic value of the new TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome in patients with primary cutaneous anaplastic large cell lymphoma (C-ALCL) and to evaluate the prognostic significance of other clinical parameters, in particular the site of presentation.

Design: Retrospective cohort analysis.

Setting: Dutch Cutaneous Lymphoma Group database.

Patients: One hundred thirty-five patients with C-ALCL.

Main Outcome Measures: Clinical variables, including T category and site of presentation.

Results: Eighty patients (59.3%) presented with T1 disease, 37 (27.4%) with T2 disease and 18 (13.3%) with T3 disease. Median follow-up was 56 months (range, 11-288 months). Five-year disease-specific survival (DSS) was 93% for T1 disease, 93% for T2 disease, and 77% for T3 disease (P=.19). Patients with skin lesions on a leg had a reduced 5-year DSS compared with lesion on other sites (82% for leg vs 95% for head and neck, 96% for trunk, and 95% for arm; p=0.23). Patients with leg involvement (n=32) had significantly worse 5-year DSS than did patients without leg involvement (n=103; 76% vs 96%; P=.03 after adjustment for T-category).

Conclusions: The new TNM system can be applied well to patients with C-ALCL and may provide prognostic information, in particular, P when combined with site of presentation. Patients with T2 or T3 disease with skin lesions on the leg may have a reduced survival and require close surveillance during follow-up.

INTRODUCTION

Primary cutaneous anaplastic large cell lymphoma (C-ALCL) is a non-Hodgkin lymphoma of T-cell origin that presents in the skin without evidence of extracutaneous disease at the time of diagnosis. It is characterized by large cells with an anaplastic, pleomorphic or immunoblastic cytomorphology and expression of the CD30 antigen by more than 75% of the tumor cells.[1] Patients with C-ALCL show overlapping clinical, histological and immunophenotypical features with lymphomatoid papulosis (LyP) that together form a spectrum of disease, collectively designed as primary cutaneous CD30-positive lymphoproliferative disorders.[2] Distinction between C-ALCL and LyP is based on a combination of clinical, histological and immunophenotypical criteria.[3] C-ALCL is regarded as an indolent type of cutaneous T-cell lymphoma (CTCL), as illustrated by several large studies[3-5] showing 10-year disease-specific survival (DSS) of approximately 90% and 10-year overall survival (OS) of approximately 75%. Risk factors that predict an unfavorable course, occurring in few patients with C-ALCL, are largely unknown. However, several studies[3,6-8] have suggested that ages older than 60 years, absence of spontaneous remission, and presentation with multifocal skin lesions may correlate with reduced survival. Moreover, extensive single-limb involvement and localization on the head and neck have been associated with a less favorable prognosis.[4,9]

Recently, a new TNM classification system has been developed for primary cutaneous lymphomas other than mycosis fungoides (MF) and Sézary syndrome (SS). This classification system is primarily meant to document extent of disease in a consistent manner, facilitating comparison of studies at different institutes (Table 1).[10] Recent studies[11-13] have started to evaluate the clinical usefulness of this TNM system. Studies of large groups of cutaneous B-cell lymphomas confirmed its applicability and suggested that this system has prognostic significance in primary cutaneous diffuse large B-cell lymphomas, leg type, but not in primary cutaneous follicle centre lymphomas and primary cutaneous marginal zone lymphomas. However, studies in CTCL other than MF and SS, have not been published thus far.

The aim of the present study was to test the applicability and prognostic value of this TNM classification system for C-ALCL. In addition, the prognostic significance of other clinical parameters, in particular the site of presentation, was evaluated.

METHODS

Patients

Between January 1, 1986, and December 31, 2007, 155 patients with C-ALCL had been included in the database of the Dutch Cutaneous Lymphoma Group, and follow-up data were collected yearly for each patient. All of the cases were reviewed by an expert panel of dermatologists and hematopathologists before entry in this database. All of the cases met the criteria of the World Health Organization-European Organization for Research and Treatment of Cancer classification, and none of them had evidence of extracutaneous disease at the time of diagnosis.[1] Patients with a follow-up of less than 12 months, unless they died of their lymphoma (n=17), and patients with human immunodeficiency virus-associated (n=2) or posttransplantation (n=1) ALCL were excluded. Six patients with skin lesions suggesting C-ALCL but who developed

characteristic skin lesions of MF during follow-up were excluded because a diagnosis of transformed MF was considered more likely. The final study group contained 135 patients. Seventy-nine of these 135 patients were included in a previous study[3] that aimed to define guidelines for diagnosis, management and treatment for this group of cutaneous lymphomas. This study was approved by the Medical Ethical Committee of the Leiden University Medical Center, Leiden, the Netherlands.

Assessment of clinical variables (including T category)

In all of the patients, the following clinical parameters were scored retrospectively: sex, age at diagnosis, site of presentation, extent of disease at presentation, spontaneous remission of initial skin lesions, type and result of initial therapy, occurrence and site of relapse, disease-free survival after complete remission (in months), duration of follow-up (in months) and current status. Extent of disease was scored using the proposed TNM classification system for primary cutaneous lymphomas other than MF and SS (Table 1). Because patients with C-ALCL have, by definition, no extracutaneous disease (lymph node or visceral) at the time of diagnosis, only T categories were scored.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc, Chicago, Illinois). Rates of DSS and OS were calculated from date of diagnosis until death from lymphoma and death from any

Table 1. Classification and description of the proposed TNM classification system for cutaneous lymphomas other than mycosis fungoides and Sézary syndrome[10]

Classification	Description
T1	Solitary skin involvement. T1a: Solitary lesion ≤ 5 cm in diameter T1b: Solitary lesion > 5 cm in diameter
T2	Regional involvement of skin: multiple lesions limited to 1 body region or 2 contiguous body regions. T2a: All-disease-encompassing in a ≤ 15 cm diameter circular area T2b: All-disease-encompassing in > 15 and ≤ 30 cm diameter circular area T2c: All-disease-encompassing in a > 30 cm diameter circular area
T3	Generalized skin involvement. T3a: Multiple lesions involving two noncontiguous body regions T3b: Multiple lesions involving ≥ 3 body regions
N0	No clinical or pathologic involvement of lymph node
N1	Involvement of one peripheral lymph node region that drains an area of current or prior skin involvement
N2	Involvement of ≥ 2 peripheral lymph node regions or involvement of any lymph node region that does not drain an area of current or prior skin involvement
N3	Involvement of central lymph nodes
M0	No evidence of extracutaneous non-lymph node disease
M1	Extracutaneous non-lymph node disease present

cause, respectively, or last follow-up without an event. Survival curves were estimated using the Kaplan-Meier technique, and comparison between curves was performed using the log-rank test. Prognostic factors were evaluated by means of univariate and multivariate analysis with OS and DSS as end points, and $P<0.05$ were considered significant. Clinical parameters included for univariate analysis were sex, age (≤ 60 vs. > 60 years), extent of disease (T category), site of presentation, and complete spontaneous remission of initial skin lesions. Multivariate analysis was performed using significant univariate variables from the Cox proportional hazards regression analysis.

RESULTS

Clinical characteristics at diagnosis, type of initial treatment and follow-up data are provided in Table 2. The study group included 97 males (71.9%) and 38 females (28.1%), with a median age at diagnosis of 61 years (range, 8-89 years). Using the TNM system, 80 patients initially had a solitary skin lesion (T1), 37 had regional skin lesions (T2), and only 18 had generalized skin lesions (T3). Representative examples are presented in Figure 1.

Figure 1. Examples of different T categories in patients with C-ALCL. Examples of different T categories in patients with C-ALCL: A, T1a; B, T1b; C, T2a; D T2b; E and F, T3b (skin lesions on trunk not shown).

Table 2. Clinical characteristics at diagnosis, type of initial treatment and follow-up data of 135 patients with C-ALCL

Variable		Value
Sex*	Male	97 (71.9)
	Female	38 (28.1)
Median age (range)		61 (8-89)
Extent of disease*	T1 (solitary skin lesion)	80 (59.3)
	T2 (regional skin lesions)	37 (27.4)
	T3 (generalized skin lesions)	18 (13.3)
Site of skin involvement*	Head/neck	42 (31.1)
	Trunk (incl. buttock)	32 (23.7)
	Arm	22 (16.3)
	Leg	21 (15.6)
	Generalized skin involvement	18 (13.3)
Spontaneous remission of initial skin lesions*		
	Absent	88 (65.2)
	Partial	23 (17.0)
	Complete	24 (17.8)
Initial therapy*	Radiotherapy	58 (43.0)
	Surgery	39 (28.9)
	None/topical steroids	24 (17.8)
	Multiagent chemotherapy	8 (5.9)
	Methotrexate	3 (2.2)
	PUVA	2 (1.5)
	Topical nitrogen mustard	1 (0.7)
Results initial therapy*	Complete remission	129 (95.5)
	Partial remission	2 (1.5)
	Progressive disease	4 (3.0)
Median disease free survival after initial therapy (range)		21 (1-182)
Occurrence and site of relapses*		
	None	62 (45.9)
	Skin only	53 (39.3)
	Extracutaneous disease	20 (14.8)
Median duration of follow-up (range)		56 (11-288)
Current status*	Alive without disease	95 (70.4)
	Alive with disease	9 (6.6)
	Death from lymphoma	12 (8.9)
	Death from other cause	19 (14.1)
Disease specific survival,%	5 y	91
	10 y	89
Overall survival,%	5 y	80
	10 y	71
Risk for extracutaneous disease,%		
	5 y	13
	10 y	24

PUVA: psoralen-UV-A; *Data are given as number (percentage)

Table 3. Distribution of different T categories in 135 patients with C-ALCL

T category	No. (%)	5-year DSS (%)	5-year OS (%)
T1	80 (59.3)	93	85
T1a	75 (55.6)	96	88
T1b	5 (3.7)	60	40
T2	37 (27.4)	93	81
T2a	24 (17.8)	96	86
T2b	8 (5.9)	100	86
T2c	5 (3.7)	80	60
T3	18 (13.3)	77	63
T3a	7 (5.2)	83	71
T3b	11 (8.1)	71	57

The distribution of the different T categories and the subgroups within these main T categories, and the corresponding 5-year DSS and OS rates are given in Table 3. Solitary or regional skin lesions at presentation (T1 and T2 disease) were localized on the head and neck in 42 patients (31.1%), on the trunk in 32 (23.7%), on a single arm in 22 (16.3%), and a single leg in 21 (15.6%). Eighteen patients had generalized skin lesions (T3 disease).

Initial therapy consisted of radiotherapy or excision in most patients (Table 2). Only 8 of 135 patients had been treated with multiagent systemic chemotherapy initially. Twenty-three of 24 patients with spontaneous remission had not received any treatment other than topical steroids in 6 of them because of complete spontaneous remission of the skin lesions. During follow-up, none of these 24 patients, including 4 cases initially presenting with multifocal skin lesions, showed the waxing and waning of skin lesions typical of LyP, thus confirming a diagnosis of C-ALCL.

During follow-up 53 of 135 patients (39.3%) developed 1 or multiple cutaneous relapses, while 20 of 135 (14.8%) patients developed extracutaneous disease, including 10 patients with involvement of only peripheral lymph nodes draining an area of current or previous skin involvement and 10 with more extensive nodal or visceral disease. The median duration for development of extracutaneous disease was 18 months (range 2 to 125 months). Development of extracutaneous disease in these 20 patients was not associated with progression to a higher T category. After a median follow-up of 56 months (range, 11-288 months), 95 patients were alive without disease, 9 were alive with disease, 12 patients died of lymphoma, and 19 patients died from unrelated causes. Ten-year DSS was 89% and 10-year OS was 71%.

Prognostic parameters

Univariate analysis showed that sex, age (≤ 60 vs. > 60 years), extent of disease (T category), site of presentation, and complete spontaneous remission of initial skin lesions were not significantly related to survival. Multivariate analysis was, therefore, not performed. Regarding extent of disease, 5-year DSS for patients with T1disease was 93%, with T2 disease was 93%, and with T3 disease was 77%, indicating that patients with T3 disease have a reduced, although

statistically nonsignificant, survival rate compared with patients with T1 or T2 (P=.19) (Table 3 and Figure 2). Analysis of survival in different subgroups of T categories showed a significantly reduced 5-year DSS for patients with T1b vs T1a disease (60% vs 96%, P<0.001), but the number of patients (n=5) with T1b disease does not allow firm conclusions to be drawn. Subgroups of T2 or T3 disease showed no significant differences in survival.

Analysis of site showed a trend toward reduced 5-year DSS in patients presenting with skin lesions on a leg (82% for leg vs 95% for head and neck, 96% for trunk, and 95% for arm; P=0.23) (Table 4). Thus, in contrast to previous studies,[9] skin lesions on the head or neck were not associated with a less favorable prognosis.

Further analysis showed that in patients with multifocal skin lesions (category T3), those with involvement of one (n=4) or both legs (n=7) had a 5-year DSS of 67% compared with 100% in

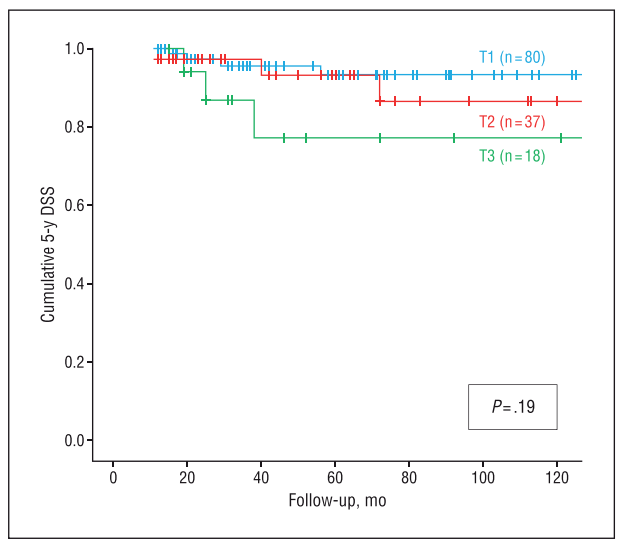

Figure 2. Five-year disease-specific survival (DSS) for the different T categories in patients with cutaneous anaplastic large cell lymphoma

Table 4. Different sites of skin involvement in 135 patients with C-ALCL

Site of skin involvement	No. (%)	5-yr DSS (%)	5-yr OS (%)
Head	42 (31.1)	95	87
Trunk	32 (23.7)	96	85
Arm	22 (16.3)	95	90
Leg	21 (15.6)	82	62
Generalized skin involvement	18 (13.3)	77	63
Legs involved	11 (8.1)	67	53
Legs not involved	7 (5.2)	100	86

patients without leg involvement (P=0.20) (Table 4). Moreover, in the total study group, 5-year DSS of patients with leg involvement (n=32) and patients without leg involvement (n=103) were 76% and 96%, respectively (P=0.007; after adjustment for T category, P=0.03 (Figure 3 and Table 5).

COMMENT

In the present study the clinical usefulness of the new TNM classification system for primary cutaneous lymphomas other than MF and SS was tested on a group of 135 patients with a C-ALCL. Although primarily meant to document extent of disease in a consistent manner, we also evaluated the prognostic value of this classification system for this group of C-ALCL. The results of this study show that this new TNM system can be applied well on this group of CTCL. Five-year DSS in patients with T1 disease was 93%, with T2 disease was 93%, and with T3 disease

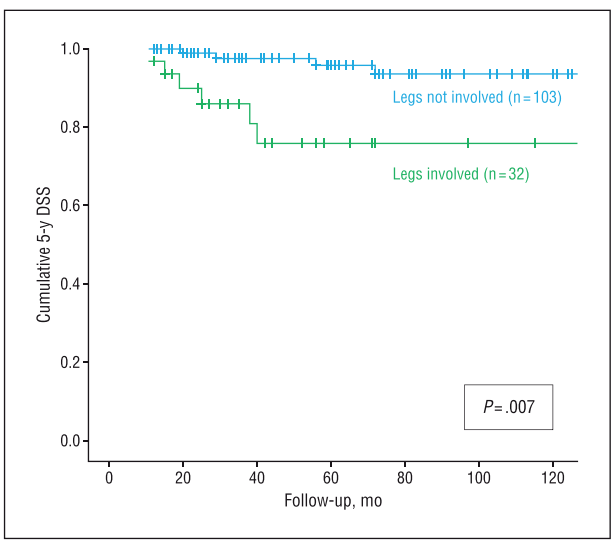

Figure 3. Five-year disease-specific survival (DSS) of patients with cutaneous anaplastic large cell lymphoma with and without leg involvement

Table 5. 5-year DSS in patients with and without leg involvement in different T categories

		Legs not involved (n=103)		Legs involved (n=32)		
T category	**No. (%)**	**D+ (%)**	**5-yr DSS, %**	**D+**	**5-yr DSS, %**	**p-value**
T1	80 (59.3)	4/70 (6)	94	1/10 (10)	90	0.39
T2	37 (27.4)	2/26 (8)	100	2/11 (18)	76	0.13
T3	18 (13.3)	0/7	100	3/11 (27)	67	0.20
Total group	135 (100)	6/103 (6)	96	6/32 (19)	76	0.03*

D+: patients who died from lymphoma/number of patients in each T category; *Adjusted for T category

was 77%, suggesting that patients with generalized skin lesions have a less favorable prognosis than do patients with solitary or localized skin lesions. Previous studies[3,4,6] have also suggested a correlation with reduced survival in patients with multifocal skin lesions. In the group of 18 patients with generalized skin lesions (T3 disease), 3 of 11 with involvement of the legs died of lymphoma (all were patients with involvement of both legs), compared with none of the 7 patients without leg involvement (5-year DSS 67% vs 100%). Also, in the patients with regional skin lesions (T2 disease), leg involvement was associated with reduced 5-year DSS (76% vs 100%4). In the total group of 135 patients, those with leg involvement had significantly worse survival than did those without leg involvement. These observations are in agreement with those of a previous study, which suggested that patients with extensive limb involvement are at risk for a poor prognosis.[4] The fact that in univariate analysis site of presentation was not statistically significantly related to survival can be explained by small sample sizes (Table 4). The same holds true when analyzing the association with survival for leg involvement within the 3 T categories separately (Table 5).

Apart from localization on the leg, presentation on the head and neck has also been associated with a less favorable prognosis.[9] In the retrospective cohort analysis of 157 patients with solitary or localized C-ALCL retrieved from the Surveillance, Epidemiology, and End Results (SEER) database, patients with skin lesions on the head and neck showed a significantly increased risk of death. In contrast, in the present study, patients presenting with skin lesions on the head and neck had a 5-year DSS of 95%. Only one of 42 patients (2.4%) with a solitary skin lesion on the head andneck region died of lymphoma (56 months after diagnosis). These different results are difficult to explain. However, because diagnoses in the SEER database are not verified independently, it cannot be excluded that the SEER cohort contains several patients with folliculotropic MF. Such patients preferentially present at the head and neck region, commonly contain many CD30-positive blast cells, and have a worse prognosis than C-ALCL.[14,15]

In conclusion, these results show that the new TNM system can be applied well to patients with C-ALCL and may provide prognostic information, in particular when combined with site of presentation. Patients with T2 and T3 disease with skin lesions on the leg were found to have a worse prognosis compared with patients without leg involvement. However, we do not believe that there is enough reason to adapt the current guidelines for the initial treatment of C-ALCL in these patients. These guidelines indicate that patients with solitary or localized skin lesions can best be treated with excision or radiotherapy, whereas in patients with multifocal skin lesions low-dose oral methotrexate or, in the case of few scattered skin lesions, radiotherapy is preferred.[3] However, patients with regional of generalized skin lesions that involve the leg should be controlled very closely and may require systemic chemotherapy in an earlier phase of disease progression.

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3

Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lymphoproliferative disorders

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SUMMARY

Background: CD30 is expressed on various types of cutaneous lymphomas, including lymphomatoid papulosis (LyP), primary cutaneous anaplastic large cell lymphoma (C-ALCL), some cases of mycosis fungoides showing large cell transformation (MF-TR) and skin localizations of systemic anaplastic lymphoma kinase (ALK)-positive or ALK-negative ALCL. Differentiation between these entities is often not possible on the basis of histology alone, but several markers, including TRAF1, MUM1 and BCL2, have been reported to provide additional diagnostic information.

Objective: To evaluate the diagnostic and prognostic significance of these markers in a large group of cutaneous CD30-positive lymphoproliferations.

Methods: An immunohistochemical study on the expression of TRAF1, MUM1, BCL2 and CD15 was performed on skin biopsies of 28 patients with C-ALCL, 39 patients with LyP, 11 patients with CD30-positive MF-TR, two with ALK-positive ALCL and six with ALK-negative ALCL. In addition, the prognostic significance of these markers was evaluated.

Results: TRAF1 was expressed in roughly 70-80%, and MUM1 expression in 70-100% of all groups of cutaneous CD30-positive lymphoproliferations. Highest levels of BCL2 were expressed in MF-TR (73%), in contrast to 21% in C-ALCL and 36% in LyP. Highest levels of CD15 were expressed in C-ALCL (44%), compared with 18% in LyP and 9% in MF-TR. A relation with survival was not clear.

Conclusions: The results of the present study suggest that expression of TRAF1, MUM1, BCL2 and CD15 can not be considered as useful diagnostic or prognostic makers in cutaneous CD30-positive lymphoproliferations. Differentiation between these different conditions should be based on a combination of clinical, histological and immunophenotypical criteria.

INTRODUCTION

Primary cutaneous CD30-positive lymphoproliferative disorders are the second most common group of cutaneous T-cell lymphoma (CTCL), accounting for 25-30% of all CTCL.[1] This group includes primary cutaneous anaplastic large cell lymphoma (C-ALCL), lymphomatoid papulosis (LyP) and borderline cases. These conditions show overlapping clinical, histological and immunophenotypical features and are considered as a spectrum of disease. Consequently, differentiation between LyP and C-ALCL is often not possible on the basis of histological criteria alone.[2] In addition, some cases of mycosis fungoides showing large cell transformation (MF-TR) and systemic ALCL involving the skin secondarily may show histological features indistinguishable from C-ALCL.[1;3] Distinction between these different types of cutaneous CD30-positive lymphoproliferations is however important, as they have a different clinical behaviour and require a different clinical approach. LyP is characterized by the presence of a chronic, recurrent, self-healing papulonodular skin eruption, rarely disseminates to extracutaneous sites and has an excellent prognosis with a 10-year disease-specific survival of 100%. Therefore, staging is generally not required and most patients do not require any specific treatment. Most patients with C-ALCL present with solitary or localized skin lesions and have an excellent prognosis as well. However, a small proportion of C-ALCL may disseminate to extracutaneous sites and require systemic chemotherapy.[2;4] In contrast to LyP patients, patients with C-ALCL should therefore be adequately staged. Systemic ALCL involving the skin secondarily includes cases associated with the t(2;5) translocation, resulting in strong expression of anaplastic lymphoma kinase (ALK) protein (ALK-positive ALCL), as well as cases which are ALK-negative.[3;5] Both groups should be treated with systemic chemotherapy and the prognosis is often poor. Also patients with MF showing histological transformation to a large cell lymphoma, which may be CD30-positive or CD30-negative, generally have an unfavourable clinical course.

In recent years several phenotypical markers, including TRAF1 [tumour necrosis factor (TNF) receptor-associated factor], MUM1 (multiple myeloma oncogene 1) and BCL2, have been reported as valuable diagnostic adjuncts that facilitate differentiation between the different groups of cutaneous CD30-positive lymphoproliferations.[6-9] However, the results of these studies are often unconfirmed or conflicting. Therefore, in this study we evaluated the diagnostic significance of these markers in a large group of CD30-positive lymphoproliferative disorders involving the skin. In addition, the prognostic significance of these markers, as well as CD15, was evaluated.

MATERIALS AND METHODS

Patients

Paraffin-embedded skin biopsies of 27 patients with C-ALCL, 39 patients with LyP, 11 patients with CD30-positive MF-TR and eight patients with secondary cutaneous ALCL were available for this study. Skin biopsies from seven patients with a primary cutaneous peripheral T-cell lymphoma, not otherwise specified (PTL-NOS) and nine patients with plaque-stage MF, were included as a control group for TRAF1 and/or MUM1 staining.

Information on all the patients was retrieved from the database of the Dutch Cutaneous Lymphoma Group (DCLG). The information had been reviewed by an expert panel of

dermatologists and hematopathologists before entry in this database, using criteria of the World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification for primary cutaneous lymphomas and the WHO classification for systemic lymphomas involving the skin secondarily.[1;10] In all patients with C-ALCL routine staging procedures including physical examination, full and differential blood cell counts and serum biochemistry, computed tomography scan of the neck, chest and abdomen and a bone marrow biopsy had been negative, except for one case. This patient presenting with a solitary tumor on the head and involvement of only one draining lymph node was considered to be a primary cutaneous lymphoma and included in the C-ALCL group. Patients with LyP had not been staged, with a few exceptions. The group of secondary cutaneous ALCL included two patients with ALK-positive ALCL who developed skin lesions during follow-up, and six patients with ALK-negative ALCL who either presented with cutaneous and extensive extracutaneous localizations (n=4) or developing skin lesions during follow-up (n=2). A summary of the clinical and follow-up data of the different groups is presented in Table 1.

Immunohistochemistry

Four-micrometre sections of formalin-fixed, paraffin-embedded tissues were put on APES (3-aminopropyltriethoxysilane) slides (Sigma A-3648; Sigma, St Louis, MO, U.S.A.) and dried overnight (37°C). Sections were then dewaxed and rehydrated. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in methanol. After antigen retrieval by boiling for 10 min in 10 mmol L^{-1} Citrate buffer (pH 6.0) for TRAF1, CD30, and BCL2 and in 1.0 mmol L^{-1} ethylenediaminetetraacetic acid (pH 9.0) for CD15 and MUM1, tissue sections were incubated overnight with antibodies against TRAF1 (1:20; Ber-TRAF1A antibody was kindly provided by Dr. Horst Dürkop, Dept. of Pathology, Charité, Berlin, Germany), CD30 (1:800), bcl-2 (1:400), CD15 (1:100) and MUM1 (1:100) (DAKO, Glostrup, Denmark). Sections were then incubated with biotin-

Table 1. Clinical characteristics of patients with cutaneous CD30-positive lymphoproliferations.

				Sec cutaneous ALCL	
	C-ALCL	**LyP**	**MF-TR**	**ALK+**	**ALK-**
Male	19	25	8	1	0
Female	8	14	3	1	6
Median age *(range)*	63 (41-86)	51 (7-84)	65 (37-87)	16 (11-24)	63 (19-87)
Median duration of follow-up, months *(range)*	63 (1-288)	65 (1-337)	78 (22-277)	70* (8-131)	49* (1-216)
Status at last follow-up:					
- Alive without disease	13	8	3	1	1
- Alive with disease	6	29	3	0	1
- Died of lymphoma	6	0	4	1	3
- Died of other cause	2	2	1	0	1

*Median duration of follow-up from first skin lesions is 64 months (1 and 126 months, respectively); Sec., secondary

labelled rabbit antimouse antibodies (1:200 E-0354), followed by incubation with peroxidase labeled streptavidin-biotin-complex (sABC-HRP; 1:100; K-0377 Dako). All secondary and tertiary antibodies were diluted in phosphate-buffered saline containing 1% bovine serum albumin for 30 min at room temperature. Immunoreactivity was detected using diamino-benzidine (DAB)-reagents and counterstaining was performed with Mayer's haematoxylin (Klinipath, Duiven, the Netherlands). For confirmation of TRAF1 staining ten selected cases were also stained in the Dept. of Pathology, Centro Nacional de Investigaciones Oncológicas, Madrid, with the commercially available TRAF1 monoclonal antibody H3 (Santa Cruz, Heidelberg, Germany).

Immunostaining for TRAF1, Mum1, BCL2 and CD15 were semi-quantitatively scored as follows: - = no or less than 10% of tumor cells stained; ± = 10-50% of tumor cells stained; + = 50-75% of tumor cells stained; ++ = more than 75% of tumor cells stained.

Statistical evaluation

Statistical calculations were performed using SPSS 14.0 (SPSS Inc, Chicago, IL). Comparison of TRAF1, MUM1, BCL2 and CD15 expression between C-ALCL, LyP and MF-TR was performed using the Fisher's exact test. All p-values were two-tailed. A p-value less than 0.5 was considered statistically significant. Comparison of prognosis between positive and negative cases of C-ALCL and MF-TR, respectively, was done using the Kaplan-Meier technique and log-rank testing. Disease-specific survival (DSS) was calculated from date of diagnosis until death from lymphoma or last follow-up without an event. For these purposes, patients with expression in more than 50% of tumor cells were considered as one group and compared with lymphomas containing less positive tumor cells or without expression in tumor cells.

RESULTS

TRAF1

No significant differences were found in expression of TRAF1 between cases with C-ALCL, LyP and MF-TR (Table 2). Clear cytoplasmic staining for TRAF1 in more than 50% of neoplastic cells was found in 20 of 23 (87%) cases with C-ALCL, 31 of 37 (84%) cases with LyP and eight of 11 (73%) cases with MF-TR (Figure. 1). In only three of 23 (13%) C-ALCL cases, and three of 37 (8%) LyP cases, the neoplastic cells were completely negative for TRAF1, while scattered dendritic cells were TRAF1 positive, serving as an internal control. Three cases of C-ALCL and two cases of LyP had been excluded, since both tumor cells and dendritic cells were completely negative for TRAF1. In one of the two ALK-positive ALCL ca. 40% of the neoplastic T-cells was TRAF1 positive, whereas four of six (67%) ALK-negative ALCL showed expression of TRAF1 in more than 50% of the neoplastic cells. The tumor cells of the PTL-NOS and plaque stage MF cases were consistently negative apart from few scattered positive cells, which correlated with CD30-positive cells in serial sections, and dendritic cells serving as an internal control (Figure. 2). Because of the discrepant results between the present study and previous studies[6] a selection of ten biopsies of our series, including seven unequivocal positive or negative cases and three cases with in our hands questionable or at most very weak TRAF1 staining results were also stained in the Dept. of Pathology, Centro Nacional de Investigaciones Oncológicas, Madrid, with the commercially

Table 2. Overall positivity (%) and level of expression of TRAF1, MUM1, BCL2 and CD15 in four different groups of cutaneous CD30-positive lymphoproliferations.

				Sec cutaneous ALCL	
	C-ALCL	**LyP**	**MF-TR**	**ALK+**	**ALK-**
TRAF1					
-	3	3	2	1	2
±	0	3	1	1	0
+	6	18	3	0	0
++	14	13	5	0	4
Overall positivity*	87% (20/23)	84% (31/37)	73% (8/11)	0% (0/2)	67% (4/6)
MUM1					
-	0	0	0	0	0
±	0	3	0	0	0
+	1	5	3	1	2
++	17	9	6	0	3
Overall positivity*	100% (18/18)	82% (14/17)	100% (9/9)	100% (1/1)	100% (5/5)
BCL2					
-	17	21	3	2	3
±	4	4	0	0	0
+	2	4	2	0	0
++	4	10	6	0	3
Overall positivity*	22% (6/27)	36% (14/39)	73% (8/11)	0% (0/2)	50% (3/6)
CD15					
-	15	31	9	2	5
±	0	1	1	0	0
+	7	2	1	0	0
++	5	5	0	0	1
Overall positivity*	44% (12/27)	18% (7/39)	9% (1/11)	0% (0/2)	17% (1/6)

- = complete negative or less than 10% of tumor cells stained; ± = 10-50% of tumor cells stained; + = 50-75% of tumor cells stained; ++ = more than 75% of tumor cells stained;
Overall positivity: more than 50% of tumor cells stained.

available TRAF1 monoclonal antibody H3 (Santa Cruz, Heidelberg, Germany). Identical results were obtained in the seven unequivocal cases, while the three questionable cases of C-ALCL also showed TRAF1 expression in more than 80% of the CD30-positive tumor cells.

MUM1

There were no significant differences in the expression of MUM1 between C-ALCL, LyP and MF-TR (Table 2). Nuclear staining for MUM1 was found in more than 50% of tumor cells in all (100%) cases with C-ALCL, MF-TR and skin localizations of ALK-positive or ALK-negative ALCL.

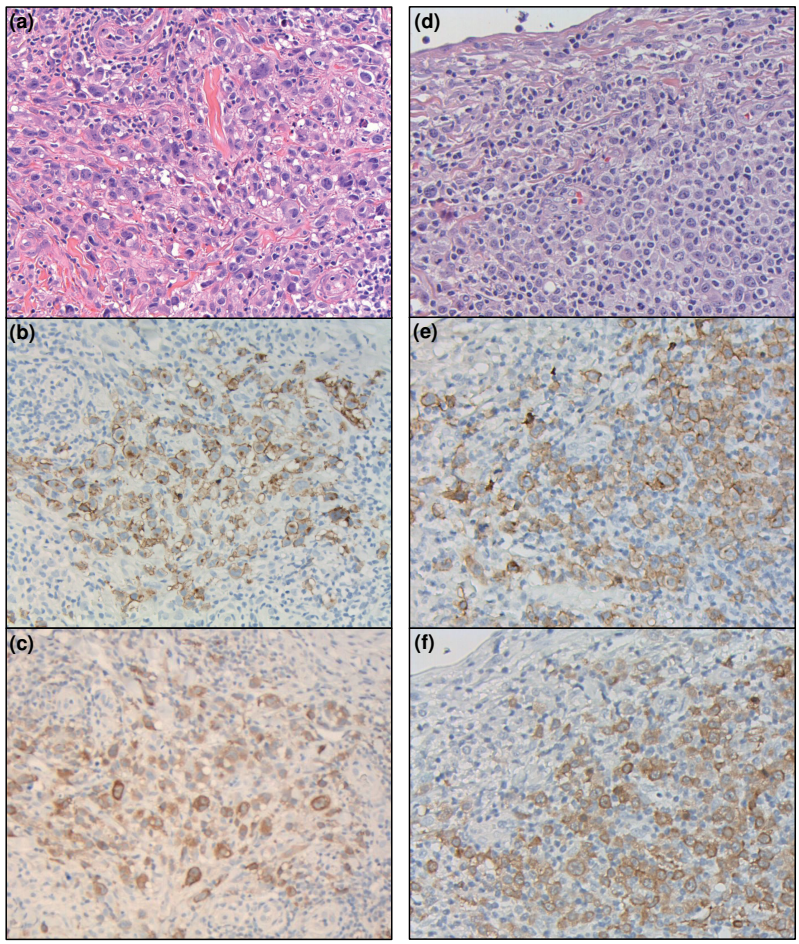

Figure 1. Serial sections of a lymphomatoid papulosis skin biopsy (a-c) and a primary cutaneous anaplastic large cell lymphoma skin biopsy (d-f) showing haematoxylin and eosin staining (a;d) and positive staining for CD30 (b;e) and TRAF1 (c;f).

In LyP, MUM1 was expressed by more than 50% of the CD30-positive T-cells in 14 of 17 (82%), while in the other three cases approximately 40% of the neoplastic cells were MUM1 positive. None of the tumor cells in the PTL-NOS cases showed expression of MUM1 except for few scattered positive cells, which largely correlated with CD30-positive cells in serial sections.

BCL2

Cytoplasmic staining for BCL2 in more than 50% of tumor cells was observed in six of 27 (22%) cases with C-ALCL, in 14 of 39 (36%) cases with LyP and in eight of 11 (73%) cases with MF-TR (Table 2). In the group of secondary cutaneous ALCL, the tumor cells in the two ALK-positive ALCL cases were completely negative for BCL2, while BCL2 positivity was observed in three of

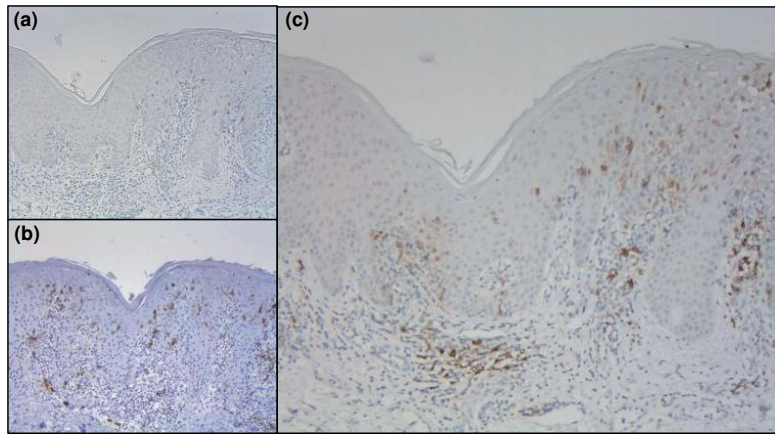

Figure 2. Plaque-stage mycosis, with few CD30 positive tumor cells (a) and scattered CD1a-positive Langerhans cells (b). TRAF1 is expressed by (epi)dermal dendritic cells (internal control), but not by the neoplastic T-cells (c).

six (50%) ALK-negative ALCL cases. In all cases strong expression of BCL2 in reactive T-cells was observed. While there were no significant differences in BCL2 expression between C-ALCL and LyP, BCL2 expression was significantly higher in the group of MF-TR as compared to C-ALCL (p=0.008) and LyP (p=0.042).

CD15

Cytoplasmic and/or membranous staining for CD15 in more than 50% of tumor cells was observed in 12 of 27 (44%) cases with C-ALCL, seven of 39 (18%) cases with LyP and only one of 11 (9%) cases with MF-TR (Table 2). In all cases strong cytoplasmic expression of CD15 in granulocytes was observed. In the group of secondary cutaneous ALCL, the two ALK-positive ALCL were completely negative for CD15, while CD15 was expressed by more than 50% of the tumor cells in one of six ALK-negative cases.

Prognostic significance

In the group of C-ALCL and the group of MF-TR, expression of BCL2 or CD15 by more than 50% of the neoplastic cells was not related to survival (data not shown). In C-ALCL, two of three (67%) TRAF1 negative cases died of lymphoma, in contrast to four of 20 (20%) TRAF1 positive cases (5-year DSS 0% vs. 93%; p=0.014).

DISCUSSION

In the present study we evaluated the diagnostic and prognostic significance of TRAF1, MUM1, BCL2 and CD15 expression in four groups of cutaneous CD30-positive lymphoproliferations. None of these markers were found to be a reliable diagnostic adjunct facilitating differentiation between LyP, C-ALCL, CD30-positive MF-TR and secondary cutaneous ALCL.

TRAF1 is an adaptor protein which is involved in intracellular signal transduction of several TNF receptor family members, including CD30.[11] The exact function of TRAF1 is not clear. Previous studies showed that TRAF1 is strongly expressed in the neoplastic cells of classical Hodgkin lymphoma, but not by the CD30-positive tumor cells of systemic ALCL.[11;12] In a recent study, Assaf et al. reported high TRAF1 expression in more than 80% of LyP cases, including cases showing cohesive sheets of CD30-positive large anaplastic cells (LyP, type C), while TRAF1 was expressed by only a small minority (7%) of primary and secondary cutaneous ALCL.[6] They suggested that TRAF1 expression might be a valuable diagnostic marker for LyP, and speculated that activation of TRAF1 might play a role in the mechanism of spontaneous remission in this condition.

In the present study, using the same Ber-TRAF1A antibody, TRAF1 expression by more than 50% of the neoplastic cells was found in roughly 70-80% of cases in all groups and no significant differences between LyP and C-ALCL were found. In contrast, cases of primary cutaneous PTL, NOS and plaque stage MF were consistently negative apart from few scattered positive cells, which correlated with CD30-positive cells in serial sections, and dendritic cells serving as an internal control.[13] Because of the discrepant results between the present study and the study of Assaf et al,[6] a selection of cases was also stained in a separate lab with the commercially available antibody H3 (Santa Cruz, Heidelberg, Germany), but staining with Ber-TRAF1A and H3 gave similar results. It should be noted that Assaf et al. used a cut-off point of more than 80% for a positive staining, while in the present study 50% was taken as a cut-off point. Taking staining of >75% as a cut-off point, the percentages of TRAF1 positive C-ALCL, LyP and MF-TR in our study were 61%, 35% and 45%, respectively (Table 2), leaving the discrepant results between both studies unexplained.

In the group of C-ALCL two of three TRAF1 negative cases died of lymphoma, in contrast to four of 20 (20%) TRAF1 positive cases. Although statistically significant, the small number of TRAF1 negative cases does not allow firm conclusions and further studies are required.

MUM1, also known as interferon regulatory factor-4 (IRF4) is a member of the interferon regulatory factor family of transcription factors. It was first identified in multiple myeloma, but is also expressed by plasma cells, activated T-cells, Hodgkin's and Reed-Sternberg cells in classical Hodgkin lymphoma, and by the CD30-positive cells in systemic ALCL.[14-17] Studies on MUM1 expression in primary cutaneous CD30-positive lymphoproliferations appeared only recently.[7;9;18] Kempf et al. found MUM1 expression by neoplastic cells in 87% (13/15) of LyP cases (varying from 10-90%) and in only 20% (2/10) of C-ALCL cases (10% and 30%, respectively) and suggested that MUM1 expression may be a valuable tool for the distinction between LyP and C-ALCL. In contrast to this study, Wasco et al. found Mum1 expression by more than 50% of the cells in 63% (12/19) of LyP cases, in 80% (4/5) of C-ALCL cases, in 80% (4/5) of secondary cutaneous ALCL cases and in 100% (9/9) of MF-TR cases. Consistently, Feldman et al. reported MUM1 expression in 93% (13/14) of C-ALCL cases.[18] In addition, these authors described the presence of a recurrent translocation involving the IRF4 gene in 57% (8/14) of C-ALCL cases. Also in our study expression of MUM1 by more than 50% of the neoplastic cells was found in 70-100% of all four groups of cutaneous CD30-positive lymphoproliferations (Table 2), while staining in PTL, NOS was restricted to few scattered positive cells which largely correlated with CD30-positive and/or TRAF1-positive cells in serial sections. Taken together, these observations

suggest that both TRAF1 and MUM1 expression are closely associated with CD30 expression and are no useful diagnostic markers in the differentiation of cutaneous CD30-positive lymphoproliferations. This is further supported by the expression of TRAF1 by CD30-positive blast cells in patients with skin localizations of Hodgkin lymphoma, EBV-positive diffuse large B-cell lymphomas and a patient with Orf (data not shown). Moreover, recent gene expression profiling studies from our group showed increased levels of TRAF1 mRNA in skin biopsies from patients with C-ALCL and CD30-positive MF-TR as compared to skin biopsies of patients with PTL-NOS and CD30-negative MF-TR (van Kester, submitted).

In 1998 Paulli et al. reported lower levels of the anti-apoptotic protein BCL2 in LyP compared to nonregressing lesions of C-ALCL, and suggested that increased BCL2 expression in nonregressing lesions of C-ALCL protects tumor cells from apoptosis. In our study, expression of BCL2 by more than 50% of the tumor cells was found in 36% of LyP cases and 22% of C-ALCL cases, which is consistent with percentages reported in other studies.[19;20]

Highest levels of BCL2 were expressed in MF-TR. It is tempting to speculate that high expression of this antiapoptotic protein in MF-TR contributes to treatment resistance and poor prognosis, but functional studies supporting such an assumption have not been performed.

CD15, also known as Lewis X, is a carbohydrate adhesion molecule, which is expressed on myeloid cells and mediates neutrophil adhesion and phagocytosis. In hematopathology, CD15 has been described as a useful marker to differentiate between ALCL (CD15 negative) and classical Hodgkin lymphoma (CD15 positive).[21] However, the presence of CD15 in a varying number of tumor cells in ALCL is reported in small numbers of patients as well.[3;21-23] Expression of Pax-5 (pan-B-cell marker) is a more reliable marker to differentiate between both conditions, since it is consistently negative in ALCL.[24] During preparations for a slide seminar for cutaneous lymphomas we noticed two cases of C-ALCL which strongly expressed CD15. Since both of them had a fatal outcome, which is uncommon in C-ALCL, we wondered if CD15 expression was predictive of a poor survival in this group and decided to include CD15 in the present study. Cytoplasmic and/or membranous CD15 staining in more than 50% of the neoplastic cells, was found in 18% of LyP cases and even in 44% of C-ALCL cases, but was not associated with a more unfavorable prognosis in the latter group. In contrast, CD15 was expressed in only one of eleven MF-TR cases. The results in C-ALCL and LyP are very similar to those of a French study, which reported CD15 positivity in a varying number of tumor cells in 2 of 16 LyP cases and 6 of 25 C-ALCL cases.[25]

In conclusion, the results of the present study suggest that expression of TRAF1, MUM1, BCL2 and CD15 can not be considered as useful diagnostic or prognostic markers in cutaneous CD30-positive lymphoproliferations. Differentiation between these different conditions in this group should always be based on a combination of clinical, histological and immunophenotypical criteria.

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4

Bone marrow examination has limited value in the staging of patients with an anaplastic large cell lymphoma first presenting in the skin. Retrospective analysis of 107 patients

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SUMMARY

Background: According to criteria of the World Health Organization-European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas a diagnosis of primary cutaneous CD30-positive anaplastic large cell lymphoma (C-ALCL) should be made only when systemic localizations have been excluded by adequate staging procedures, including a bone marrow biopsy. It has recently been questioned whether or not bone marrow examination should be performed routinely in indolent cutaneous lymphomas such as C-ALCL. Studies addressing this issue have never been performed.

Objectives: To determine the incidence of bone marrow involvement in patients with an ALCL first presenting in the skin to find out if the current policy to advice bone marrow examination should be maintained or whether a bone marrow biopsy should be performed only in selected cases.

Methods: All patients presenting with skin lesions with histological and immunophenotypical features of an ALCL were retrieved from the database of the Dutch Cutaneous Lymphoma Group. Patients with a history of systemic ALCL and patients without bone marrow examination were excluded from the study. The final study group included 107 patients with an ALCL first presenting in the skin, who had been staged completely.

Results: Staging procedures showed the presence of extracutaneous disease in 20 patients, but bone marrow involvement was not detected in any of the 107 patients. Moreover, only one patient developed bone marrow involvement during follow-up (median follow-up period: 69 months)

Conclusions: Bone marrow examination has limited value in the staging of patients with an ALCL first presenting in the skin, and should be performed only in selected cases.

INTRODUCTION

Primary cutaneous CD30-positive anaplastic large cell lymphoma (C-ALCL) is defined as a malignant lymphoma composed of large cells with an anaplastic, pleomorphic or immunoblastic cytomorphology and expression of the CD30 antigen by more than 75% of tumour cells.[1] Clinically, C-ALCL most often present with solitary or localized skin lesions; they may regress spontaneously, often relapse in the skin, but uncommonly disseminate to extracutaneous sites and have an excellent prognosis with a 10-year disease-specific survival (DSS) of approximately 90%.[2;3] Radiotherapy or, in cases of a small solitary tumour surgical excision are the preferred modes of treatment, while systemic chemotherapy is only required in exceptional cases.[2]

C-ALCL must be differentiated from systemic ALCL involving the skin secondarily. These systemic ALCLs consist of lymphomas which are associated with the t(2;5) translocation resulting in expression of the anaplastic large cell lymphoma kinase (ALK) protein (ALK-positive ALCLs) and of lymphomas which are not (ALK-negative ALCLs).[4;5] Importantly, C-ALCLs are not associated with the t(2;5) translocation and do not express ALK protein. Both ALK-positive and ALK-negative ALCL with secondary cutaneous involvement have a worse prognosis and require a completely different therapeutic approach as compared to C-ALCL.

According to the criteria of the World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification for cutaneous lymphomas a diagnosis of C-ALCL can only be made after adequate staging procedures have been conducted, including a bone marrow biopsy.[1] Consistently, current guidelines of the Dutch Cutaneous Lymphoma Group (DCLG) require complete staging including a bone marrow biopsy.[2] However, recent data from the registry of the DCLG show that in an increasing number of patients with an ALCL presenting in the skin, in particular those presenting with a solitary tumour that has resolved spontaneously or has been excised completely, staging is incomplete and particularly a bone marrow biopsy is not always performed. Moreover, in a recently published consensus paper from the International Society for Cutaneous Lymphomas (ISCL) and the European Organization of Research and Treatment of Cancer (EORTC) Cutaneous Lymphoma Group, it was suggested that in indolent cutaneous lymphomas such as C-ALCL, bone marrow examination is recommended, but not required, unless indicated by other staging investigations.[6]

Studies on the incidence of extracutaneous manifestations and, in particular, a positive bone marrow biopsy, in patients with an ALCL first presenting in the skin have never been published. In the present study, we therefore evaluated retrospectively the results of staging in a large group of such patients. The main purpose of this study was to determine the incidence of bone marrow involvement in these patients in order to find out if our current policy to advice bone marrow examination in all patients with an ALCL first presenting in the skin should be maintained or that a bone marrow biopsy should be performed only in selected cases.

PATIENTS AND METHODS

All patients presenting with skin lesions with the histological and immunophenotypical features of an ALCL between 1986 and 2007 were retrieved from the database of the DCLG (n = 157). All cases in the Dutch registry have been reviewed by an expert panel of dermatologists and

hematopathologists before entry in this database. Moreover, for each patient in this database follow-up is collected yearly. These procedures guarantee that this initial study group did not contain patients with lymphomatoid papulosis or transformed mycosis fungoides, both of which may show histological features similar to a (C-)ALCL. From the initial group of 157 patients, patients with a history of systemic ALCL who developed specific skin lesions during follow-up (n = 4) and patients without bone marrow examination (n = 46) were excluded from the study. The final study group consisted of 107 patients with an ALCL first presenting in the skin. Staging investigations had consisted of physical examination, full and differential blood cell count and serum biochemistry, computed tomographic scanning of chest and abdomen and other imaging studies if required, and a bone marrow biopsy.

Statistical calculations were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, U.S.A.). DSS and overall survival (OS) were calculated from date of diagnosis until death from lymphoma and death from any cause respectively, or last follow-up without an event. Survival curves were estimated using the Kaplan-Meier technique and comparison between curves was done by log-rank testing.

RESULTS

The median age at diagnosis of our final study group was 57 years (Table 1). In total 72 patients were male (67%) and 35 patients were female (33%). Median follow-up was 69 months (range 1-308 months). Staging investigations showed no evidence of extracutaneous disease in 87 of 107 cases (81%), which were therefore classified as C-ALCL. In the remaining 20 patients staging was positive. In 11 of these 20 cases there was only involvement of peripheral lymph nodes draining a skin area containing ALCL lesions, suggesting that these were C-ALCLs with secondary lymph node involvement. The other nine patients showed more widespread disease with involvement of central lymph nodes and/or other extracutaneous localizations such as lung (three patients), central nervous system (two patients), bone (two patients), liver and spleen (one patient) and nasopharynx (one patient). Bone marrow involvement by ALCL was not observed in any of the 107 cases investigated. However, bone marrow biopsies showed B-cell chronic lymphocyic leukemia (B-CLL) in four cases (4%) and myelodysplastic syndrome in one case (1%). Patients with negative staging results and patients with positive staging results had a 5-year DSS of 90% and 51%, respectively ($p = 0.001$; Figure 1), whereas the 5-year OS was 80% and 51%, respectively ($p = 0.144$).

DISCUSSION

The results of the present study indicate that bone marrow examination has limited clinical value in normal staging work-up of patients with an ALCL first presenting in the skin. Bone marrow involvement was not detected at the time of diagnosis in any of the 107 patients investigated, including 20 patients with extracutaneous disease at other sites. Moreover, in the database of the DCLG, which collects follow-up information for every included patient every year, bone marrow involvement during follow-up had only once been recorded. In this particular patient all staging investigations at diagnosis were negative, but six months after diagnosis involvement of multiple

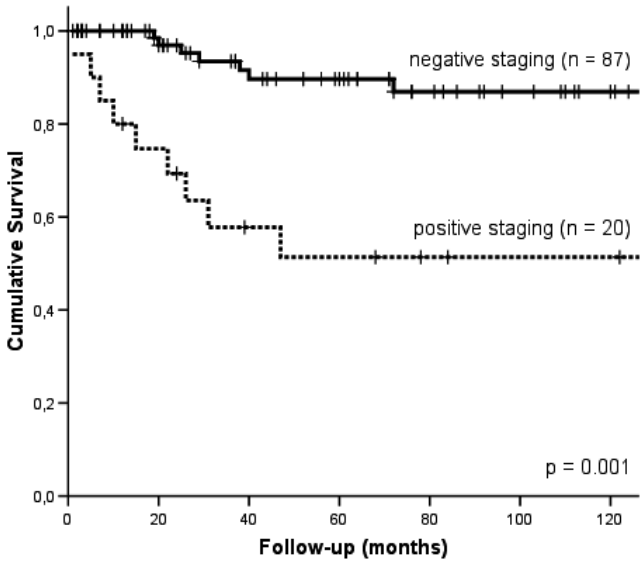

Figure 1. Survival curves of patients with an anaplastic large cell lymphoma first presenting in the skin with negative (n=87) and positive (n=20) staging results.

peripheral lymph nodes, spleen, adenoid and bone marrow was observed. In addition, none of the 46 patients in whom bone marrow examination had not been performed initially, and who were therefore not included in the present study, had developed bone marrow involvement during follow-up. Taken together, these observations indicate that bone marrow involvement is not or is rarely found in ALCL first presenting in the skin, and argue against bone marrow examination as an essential part of initial staging procedures.

An unexpected finding in our study was the presence of a B-CLL in bone marrow biopsies of 4 of 107 patients. The coexistence of C-ALCL and B-CLL is uncommon with only few cases published.[7;8] Several hypotheses exist for the coexistence of both T- and B-cell malignancies, including an origin from a common stem cell progenitor, exposure to carcinogens or viruses affecting oncogenes or tumour suppressor genes of both T- and B-cell precursors, and coincidental occurrence of two unrelated neoplasms.[9]

A limitation of the present study is that the results of bone marrow examinations were obtained from a database, and not by review of the original bone marrow specimens. Current guidelines for bone marrow evaluation request a biopsy specimen with a length of at least 2 cm and with sufficient marrow fields, and in the case of doubtful infiltration additional immunohistochemistry.[10] Consistently, in a study of ALK-positive ALCL, the incidence of bone marrow involvement increased from approximately 10% when only haematoxylin and eosin sections were analyzed, to 30%, when immunohistochemical stainings for CD30, epithelial membrane antigen and/or ALK were used.[11] Considering the fact that our patients were collected during a period of 20 years, it cannot be excluded that our series contain a number of false-negative marrows, as some biopsies from the early years might not meet current standards

and immunohistochemistry was not widely used at that time. However, no differences were observed between bone marrows included in the database before and after 2000, and we therefore believe that the number of false-negative bone marrows will be minimal, and will not significantly influence our conclusions. Moreover, even if we assume a small number of false-negative bone marrows, the clinical consequences of not performing a bone marrow biopsy are expected to be minimal. It is of interest that the 5-year DSS and OS for the 46 patients in

Table 1. Staging results and survival data of anaplastic large cell lymphomas first presenting in the skin, from the database of the DCLG.

	Number	5-year DSS (OS)
Total number of patients with ALCL	157	-
History of non-cutaneous ALCL	4	-
Bone marrow not investigated	46	95% (72%)
Final study group	107	81% (74%)
Male:female	72:35	-
Median age , years (range)	57 (7-88)	-
Median duration of follow-up, months (range)	69 (1-308)	-
Staging negative	87	90% (80%)
Staging positive*	20	51% (51%)
Involvement of bone marrow	0	-

*Bone marrow involvement by B-CLL is not included in this category

whom a bone marrow biopsy had initially not been performed were 95% and 72%, respectively, which is similar to that of the group of C-ALCL (Table 1).

In conclusion, the results of the present study indicate that bone marrow examination has limited value in the staging of patients with an ALCL first presenting in the skin, and should be performed only in selected cases, such as patients with other positive staging assessments (e.g. involved lymph nodes) or rare patients requiring multiagent chemotherapy.

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5

Primary cutaneous anaplastic large cell lymphoma shows a distinct miRNA expression profile and reveals differences from tumor stage mycosis fungoides

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNA molecules that repress gene expression post-transcriptionally. They may have oncogenic or tumor suppressing properties depending on their target genes. Although several studies have suggested that miRNAs are involved in the pathogenesis of cutaneous T-cell lymphoma (CTCL), the miRNA expression profile of primary cutaneous anaplastic large cell lymphoma (C-ALCL), an indolent type of CTCL, is not investigated.

In this study we analyzed the miRNA expression profiles of skin biopsies from 14 C-ALCL patients and 12 patients with benign inflammatory dermatoses (BID) by miRNA microarrays. We identified 13 miRNAs that are differentially expressed between C-ALCL and BID using ANOVA analysis ($P<0.05$, Benjamini-Hochberg corrected). Of these miRNAs the up-regulation of miR-155, miR-27b, miR-30c and miR-29b in C-ALCL was validated by miRNA-Q-PCR on independent study groups (C-ALCL and BID).

In a second line of experiments the miRNA expression profiles of C-ALCL were compared with those of tumor stage mycosis fungoides (MF), a CTCL with less favorable prognosis. Although miRNA microarray analysis did not show statistically significant differences, miRNA-Q-PCR demonstrated statistically significantly differential expression of miR-155, miR-27b, miR-93, miR-29b and miR-92a between tumor stage MF and C-ALCL.

This study, the first describing the miRNA expression profile of C-ALCL, reveals differences with tumor stage MF, suggesting a different contribution to the pathogenesis of these lymphomas.

INTRODUCTION

Primary cutaneous anaplastic large cell lymphoma (C-ALCL) belongs to the group of CD30+ lymphoproliferative disorders, which is the second most common group of cutaneous T-cell lymphoma (CTCL). It is characterized by large cells with an anaplastic, pleomorphic, or immunoblastic cytomorphology and by expression of the CD30 antigen by more than 75% of the tumor cells [1]. Although its histology indicates an aggressive lymphoma, C-ALCL often shows an indolent clinical behavior. It usually presents with solitary or localized skin lesions, which may regress spontaneously. Relapses in the skin occur frequently but extracutaneous disease is uncommon. C-ALCL patients have an excellent prognosis with a 10-year disease-specific survival (DSS) of approximately 90% [2-4]. The molecular mechanisms involved in the development of this disorder are largely unknown.

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression post-transcriptionally. They are involved in crucial biological processes such as development, immune function, proliferation, apoptosis and the stress response [5-7]. There is increasing evidence that miRNAs are involved in the development of several types of cancers, including haematological malignancies [8,9]. Several studies have demonstrated specific miRNA expression profiles in different types of CTCL suggesting a role in the pathogenesis of these disorders [10-14]. Only one study focusing on the miRNA expression profiling of CTCL in general has included C-ALCL biopsies [12]. Hence the miRNA expression profile of C-ALCL is as yet unknown.

To gain better insight in the molecular pathogenesis of C-ALCL, we determined the miRNA expression profile of C-ALCL compared to benign inflammatory dermatoses (BID) by miRNA microarray analysis and validated our results by miRNA-Q-PCR.

In a second line of experiments we compared the miRNA expression profiles of C-ALCL with those of tumor stage mycosis fungoides (MF), a CTCL with a 10-year DSS of 42% [15,16]. The differences in miRNA expression described in this study suggest a different role in the pathogenesis of these lymphomas.

METHODS

Material selection

Formalin fixed paraffin embedded (FFPE) skin biopsies containing more than 75% of CD30+ large cells were selected from 21 C-ALCL patients, present in the database of the Dutch Cutaneous Lymphoma Group. In all cases the diagnosis had been confirmed by an expert panel of dermatologists and pathologists before entry in this database, using criteria of the WHO-EORTC classification [1]. The study group included 15 males and 6 females with a median age at the time of diagnosis of 62 years (range 33-83). Median follow-up period after diagnosis was 22 months (range 2-279). At last dates of follow-up 12 patients were alive with complete remission, four patients were alive with disease, two patients died of other causes and three patients died of disease. To assess the percentage of (tumor) cells in all biopsies, hematoxylin and eosin and CD3 stainings were reviewed, cut directly before and after slides sectioned for isolation. In total 14 biopsies were selected for array analysis and 7 additional biopsies were selected as a test group for validation by miRNA-Q-PCR. As a control group FFPE biopsies of

BID containing T-cell rich infiltrates were selected: 5 eczema and 7 lichen planus cases for array analysis and 5 additional eczema and 6 lichen planus cases for miRNA-Q-PCR.

Results of miRNA microarray analysis of C-ALCL were compared with those recently published on 19 tumor stage MF[10], that were generated using the same platform[11]. Both studies were performed in accordance with the Dutch code and Leiden University Medical Center guidelines on leftover material.

miRNA isolation

Total RNA was isolated from 6x20 µm sections of C-ALCL biopsies and 8x20 µm sections of BID biopsies using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Warrington UK) according to the manufacturers' protocol.

miRNA array analysis

The complete miRNA profile of samples (3 µg total RNA) were elucidated using µRNA microarrays (containing complete human miRnome (miRBase 10.1)) as previously described[10,17], using a synthetic human miRNA universal reference pool containing 454 miRNAs as a common reference. Image analysis was carried out using Bluefuse software (BlueGnome, Cambridge, UK). Raw fold ratio data were global loess-normalized within arrays and quantile normalized between arrays using the LIMMA package[18]. The normalized log ratios (average of four replicates per probe) were used for subsequent analysis in Genespring 7.2 (Agilent Technologies, CA, US). MiRNAs that had a median intensity >300 fluorescence units background in more than 50% of the arrays were removed prior to ANOVA analysis. ANOVA analysis was performed to identify miRNAs differentially expressed between sample types and multiple testing correction was done using the Benjamini-Hochberg method.

MiRNA-Q-PCR

MiRNA cDNA synthesis and miRNA-Q-PCR was performed as described before[10], using 300 ng RNA as input material for reverse transcription with the miRNA reverse transcription kit and the stem-loop Megaplex primer pool A v. 2.1 (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). MiRNA-Q-PCR was performed using Taqman miRNA assays and 2x Universal PCR mastermix (Applied Biosystems). All reactions were run on the LightCycler480 (Roche, Almere, the Netherlands), according to manufacturer's protocol (Applied Biosystems). MiRNA expression levels were analyzed using the ΔCt method expressed relative to U6. Statistical analyses were performed using the Mann-Whitney U-test in SPSS version 17.0 (SPSS Inc, Chicago, Illinois).

RESULTS

We first determined the miRNA profiles from FFPE biopsies of 14 C-ALCL patients and 12 BID (eczema n = 5 and lichen planus n = 7) using miRNA microarrays (miRBase v.10.1). In total, 13 miRNAs were statistically significantly differentially expressed between C-ALCL and BID with an adjusted p-value <0.05 (Table 1). Twelve of these miRNAs showed higher expression in C-ALCL and 1 miRNA showed lower expression in C-ALCL. Of the 12 higher expressed miRNAs, 5 had a fold-change (FC) higher than 2 (highest FC: 5.95). The only miRNA lower expressed in C-ALCL

Table 1. Differentially expressed miRNAs with chromosomal location

miRNA	Fold change	Adj. P-value	Chromosomal location
miR-197	-1,52	0,0443	1: 110141515-110141589 [+]
miR-15b	1,50	0,0443	3: 160122376-160122473 [+]
miR-20a	1,54	0,0463	13: 92003319-92003389 [+]
miR-342-3p	1,63	0,0443	14: 100575992-100576090 [+]
miR-146b	1,66	0,0443	10: 104196269-104196341 [+]
miR-26b	1,83	0,0443	2: 219267369-219267445 [+]
miR-17-5p	1,95	0,0413	13: 92002859-92002942 [+]
miR-27b*	1,95	0,0443	9: 97847727-97847823 [+]
miR-92b	2,09	0,0443	1: 155164968-155165063 [+]
miR-30c*	2,15	0,0413	1: 41222956-41223044 [+]
			6: 72086663-72086734 [-]
miR-29b*	3,00	0,0443	7: 130562218-130562298 [-]
miR-155*	5,54	0,0035	21: 26946292-26946356 [+]
miR-425-5p	5,95	0,0443	3: 49057581-49057667 [-]

Positive fold changes are up regulated in C-ALCL samples and negative value is down-regulated compared to controls. Adj. P-value is adjusted P-value after Benjamini-Hochberg multiple testing correction. *validated

was miR-197 (FC: -1.52). To validate these results, miRNA-Q-PCR was performed for miR-155, miR-29b, miR-30c and miR-27b in an additional group of C-ALCL (n = 7) and BID (eczema n = 5 and lichen planus n = 6). All miRNA-Q-PCR results are consistent with the microarray data, showing significant differential expression between C-ALCL and BID ($P < 0.05$; Figure 1A).

Comparison of miRNA microarray results from C-ALCL with those from tumor stage MF, showed differential expression of several miRNAs, but none remained statistically significant after multiple testing correction. In line with these results the unsupervised cluster analysis, also including BID, was unable to classify the different disease entities into separate groups, although there was a tendency to cluster separately from BID (Figure 2).

Although miRNA microarray analysis did not show statistically significant differences between C-ALCL and tumor stage MF, we noticed that the set of miRNAs discriminating C-ALCL from benign controls (this study) is different from the set discerning tumor stage MF from the same controls [10]. We therefore extended the analysis of C-ALCL and used miRNA-Q-PCR to determine the expression of miR-93, miR-92a, miR-30b, miR-16 and miR-383, which were differentially expressed between tumor stage MF and BID. Except for miR-383, all miRNAs showed significant up-regulation in C-ALCL vs. BID (Figure 1B). In analogy, we also measured miR-29b and miR27b in tumor stage MF, using the validation set as described by van Kester et al.[10], resulting in the up-regulation of miR-29b in tumor stage MF vs. BID ($p < 0.01$), while miR-27b is not differentially expressed (data not shown). Finally, we determined whether any of the miRNAs assayed by Q-PCR is differentially expressed between C-ALCL and MF. As depicted in Figure 3, this analysis identified differential expression of miR-155, miR-27b, miR-93, miR-92a and miR-29b.

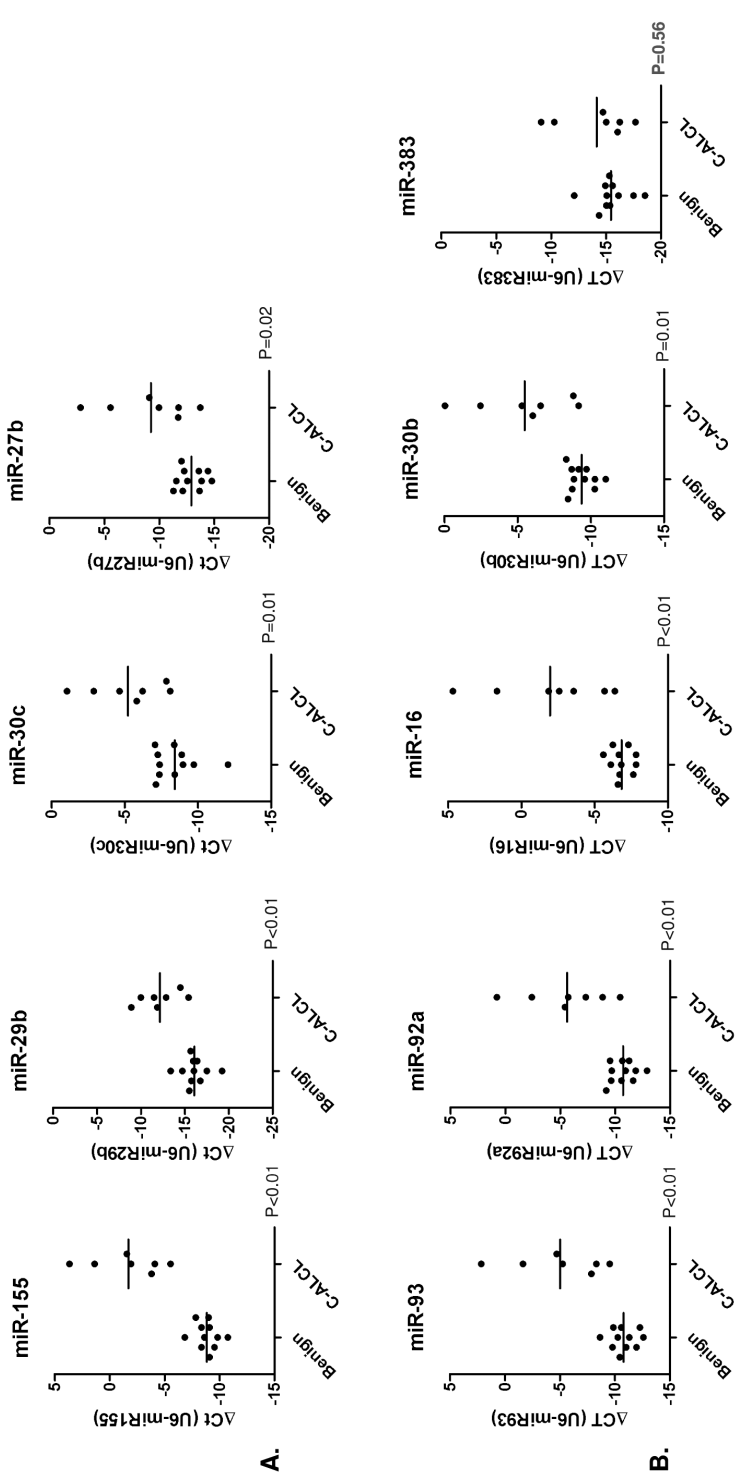

Figure 1. Expression levels of selected miRNAs as measured by miRNA-Q-PCR in an additional test group. Expression levels of miR-155, miR-29b, miR-93, miR-92a, miR-16, miR-30b, miR-27b, miR-30c and miR-383 in C-ALCL (n=7) and benign controls (Eczema, n=5 and lichen planus, n=6), calculated by Delta Ct method. Horizontal bars represent the mean. A: confirmation of four miRNAs selected for validation. B: four of five miRNAs additionally tested are significant differentially expressed between C-ALCL and benign controls (p-values measured by Mann-Whitney U-test).

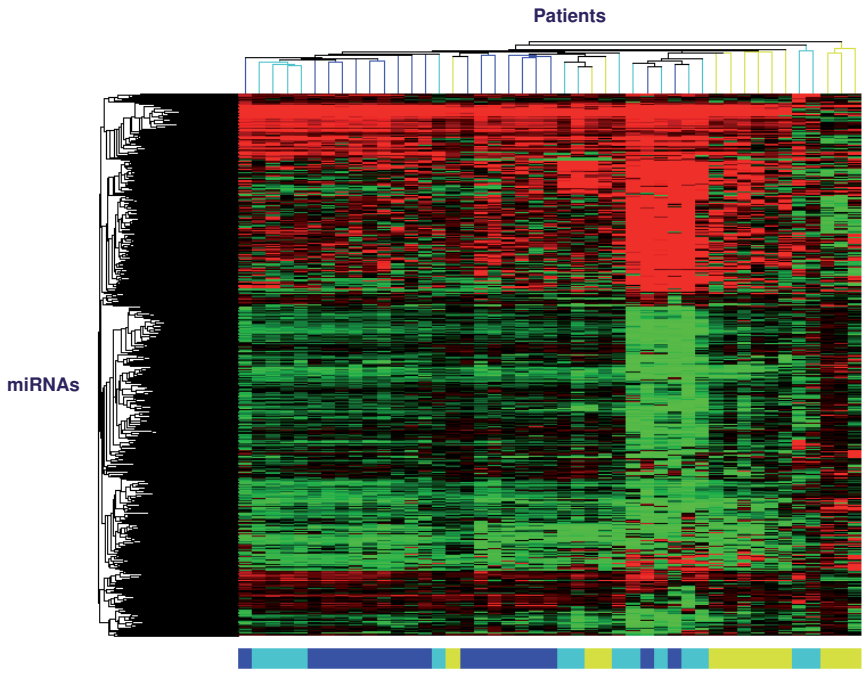

Figure 2. Unsupervised cluster analysis C-ALCL, tumor stage MF and benign controles. Heatmap representing miRNA array expression data of C-ALCL samples (n=14, depicted in light blue), tumor stage MF samples (n=19, depicted in dark blue) and benign controls (n=12, depicted in yellow).

DISCUSSION

In this study we evaluated the miRNA expression profile of C-ALCL with miRNA microarrays and miRNA-Q-PCR, by comparing C-ALCL with BID and tumor stage MF. Using miRNA microarrays, we found thirteen miRNAs differentially expressed between C-ALCL and BID, twelve up-regulated and one down-regulated. We validated and confirmed expression of miRNA-155, miR-27b, miR-30c and miR-29b with miRNA-Q-PCR in two independent groups of C-ALCL and BID. Since miRNA deregulation in cancer is often correlated with DNA copy number alterations[5,19], we subsequently compared the genomic location of differentially expressed miRNAs in C-ALCL with previous described copy number alterations. However, a correlation to genomic regions of gain could only be established for up regulation of miR-29b[20-23]. This suggests that other mechanisms such as transcriptional deregulation or epigenetic alterations probably are more important in deregulation of miRNA expression in C-ALCL[19].

Since these array-based analyses of miRNA profiles of C-ALCL were performed using the same platform as the recently published experiments on miRNA profiling of tumor stage MF, a proper comparison (including normalisation and statistical analysis) was feasible[10,11]. Under stringent statistical conditions no differences between C-ALCL and MF were identified. However, the miRNA-Q-PCR based analysis of an additional selection of individual miRNAs (chosen as

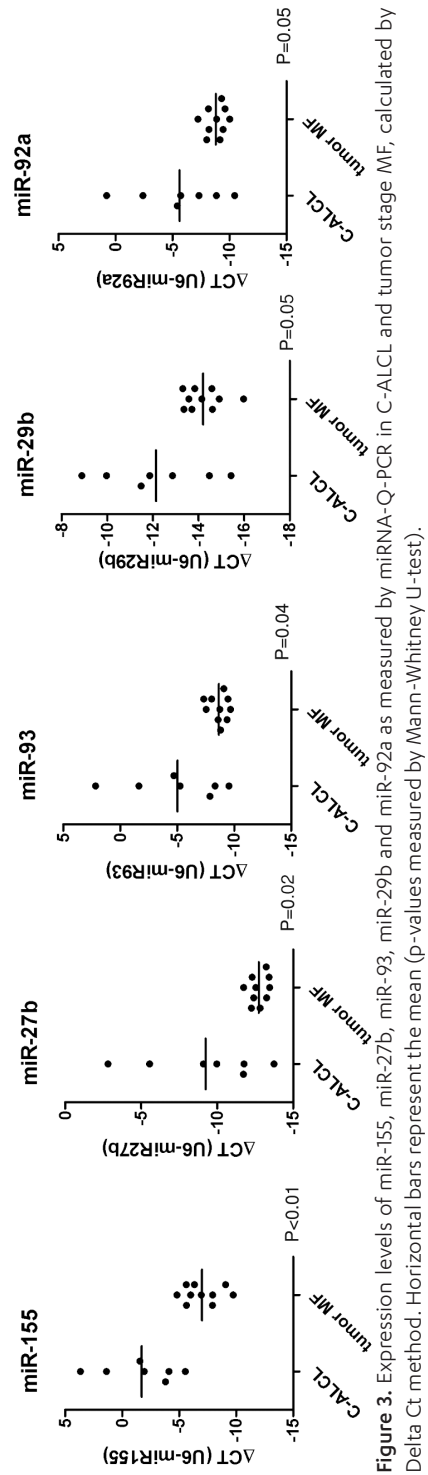

Figure 3. Expression levels of miR-155, miR-27b, miR-93, miR-29b and miR-92a as measured by miRNA-Q-PCR in C-ALCL and tumor stage MF, calculated by Delta Ct method. Horizontal bars represent the mean (p-values measured by Mann-Whitney U-test).

being differentially expressed between tumor stage MF or C-ALCL and BID) revealed not only further differences between the two disease entities (miR-155, miR-27b, miR-93, miR-92a and miR-29b) but also additional differences between C-ALCL and BID (miR-93, miR-92a, miR-27b, miR-30b and miR-16). We concluded that miRNA arrays are well suited for initial genome-wide screening, but apparently are less sensitive for detection of more subtle changes in miRNA expression. A similar observation was recently made by Ralfkiaer et al. in the course of their miRNA profiling studies for diagnostic markers of CTCL supporting the notion that PCR assays are more sensitive than miRNA microarrays[12].

Several of the aberrantly expressed miRNAs were previously also identified in other malignancies including lymphoma. For instance, miR-155 is known to play a role in immune responses and required for T-cell function[7,24]. It is also a well-known oncogene involved in several hematological malignancies such as systemic ALCL and CTCL (12,25). In some B-cell lymphoproliferative disorders it appears that higher expression of miR-155 correlates with disease severity and prognosis[26]. In contrast, we find higher expression of miR-155 in C-ALCL, having a far better prognosis than tumor stage MF. Differential expression of miR-155 between CTCL with a good and a bad prognosis was also observed in another study from our group, showing differential expression of the miR-155 precursor (BIC) being high in C-ALCL and low in PTL-NOS which have a worse prognosis[23].

Altered expression of miR-27b has been found in several malignancies such as breast cancer and neuroblastoma, but has not been described in relation to lymphoma so far[27,28]. Peroxisome proliferator-activated receptor γ (PPARγ) is one of the validated targets of miR-27b in cardiomyocytes and human macrophages[29,30]. Previously this receptor has been implicated in tumorigenesis[31], although it remains debatable whether this receptor is stimulatory or inhibitory. Yang et al. found that PPARγ is highly expressed in systemic ALCL cases and contributes to malignant T-cell survival[32]. It is tempting to speculate that in C-ALCL miR-27b by targeting of PPARγ might contribute to the relative favorable prognosis compared to systemic ALCL, although validation experiments for this hypothesis are warranted.

MiR-30c and miR-29b have been reported to be down regulated in ALK- systemic ALCL and ALK+ ALCL, respectively[25]. In contrast to this, we demonstrate up regulation for both miRNAs in C-ALCL, possibly relating to the different pathogenesis of these lymphomas. MiR-30c is considered a tumor suppressive miRNA, predicting sensitivity to treatment in different tumour types[33]. MiR-29b is part of the tumor suppressive miR-29a/b1 cluster located within the common fragile site FRA7H on chromosome 7q32.3. Feldman et al. showed that the $t^{6;7}$ (p25.3;q32.3) in systemic ALK-negative ALCL was associated with up-regulation of miR-29b1, illustrating that this location is indeed susceptible for chromosomal changes[34]. MiR-29b can regulate the expression of the oncogenes MCL1, TCL1 and CDK6 and serves as a prognostic marker in mantle cell lymphoma[35,36]. As miR-29b functions as a tumour suppressive miRNA its higher expression in C-ALCL compared to tumor stage MF might play a role in its indolent clinical behaviour and is in agreement with the described low expression of MCL1 in C-ALCL[37].

MiR-93 is previously described as an oncomir preventing apoptosis and promoting tumor growth[38,39] and higher expression correlates with a poor prognosis in ovarian cancer[40].On the other hand, miR-93 over expression is reported in ALK+ ALCL cell lines when compared to ALK-

systemic ALCL cell lines[25], correlating to the better prognosis of ALK+ vs ALK- systemic ALCL. MiR-92a is part of the oncogenic miR-17-92 cluster, involved in many malignancies including lymphoma[41]. It was previously established that higher expression of miR-92a correlates with poor prognosis in several malignancies including human esophageal squamous cell carcinoma[42] and in small cell lung cancer[43].

In summary, we have determined the miRNA expression profile of C-ALCL compared to BID using miRNA microarrays and observed that more miRNAs appeared differentially expressed using miRNA-Q-PCR. The same was true for the comparison of C-ALCL with tumor stage MF. We therefore suspect that the real number of differentially expressed miRNAs of C-ALCL compared to BID or tumor stage MF might be higher then observed in this study, probably due to cross-hybridization problems or a restricted dynamic range of miRNA microarrays. Moreover, miRNA microarrays are only capable of measuring the relative expression levels of known (annotated) miRNAs and hitherto unknown miRNAs might be differentially expressed between the different disease entities as well. These restrictions might be overcome in the future using techniques like next generation deep sequencing, though the limited availability of suitable (frozen) material with a high tumor cell content will remain a challenge. The current miRNA expression profile of C-ALCL and the differences compared to tumor stage MF provide a framework for further (functional) studies and may help to reveal the molecular pathogenesis of these lymphomas.

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6

Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases

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ABSTRACT

Large cell transformation (LCT) in mycosis fungoides (MF) is generally associated with an aggressive clinical course and poor survival, requiring aggressive therapeutic approach. However, a proportion of cases may follow an indolent clinical course. To identify prognostic factors we analyzed the prognostic relevance of clinical, histologic, and immunophenotypical features in a large cohort of transformed MF patients, including, 75 patients with only skin lesions, 19 patients with LCT in skin and lymph nodes and 6 patients with LCT in lymph nodes only. Multivariate analysis of the total group showed that CD30 negativity, folliculotropic MF, extent of skin lesions and extracutaneous transformation were associated with reduced disease-specific survival (DSS) and, except for CD30 negativity and folliculotropic MF, also overall survival. In a multivariate analysis of 75 patients with only skin lesions at the time of LCT, CD30 negativity, folliculotropic MF and extent of skin lesions were independent parameters for both DSS and overall survival. Using the most discriminating parameters as a prognostic index, in both study groups differences in DSS between patients with 0-1 unfavorable prognostic factor(s) and ≥2 unfavorable prognostic factors were statistically significant ($p<0.001$). This prognostic index may be helpful in predicting prognosis and selecting the most appropriate treatment in patients with transformed MF.

INTRODUCTION

Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma, clinically characterized by the slow progression from patches to plaques and in a proportion of patients to tumors. In a minority of patients dissemination to nodal sites, other extracutaneous sites, or both may occur. Although patients with early patch/plaque stage MF generally run an indolent course with a 10-year disease-specific survival (DSS) over 80%, patients developing skin tumors or extracutaneous disease have a reduced 10-year DSS of 42% and less than 20%, respectively.[1,2] Apart from clinical stage, large cell transformation (LCT) in MF has been associated with an aggressive clinical course and a poor survival.[3-10] Most studies report a median survival between 2 and 36 months (Table 1). Data on prognostic factors within these studies are however inconsistent and often conflicting and are probably related to the small size of the groups studied thus far (12-45 patients; median, 22 patients). Evaluation of these previous studies is further hampered by the inclusion of not only patients with MF, but also variable numbers of patients with Sézary syndrome (SS) in 5 of 7 studies, and by the variable proportions (17%-92%) of patients with transformation at extracutaneous sites.

Remarkably, in a recent study of 1502 patients with MF/SS, including 70 patients with transformed MF at the time of first diagnosis, the median survival was 8.3 years and the 5-year overall and DSSs were 63% and 65%, respectively.[1] Although not described in much detail, these survival data suggests a much better prognosis of transformed MF than reported in all previous studies (Table 1).

To better define the prognosis and prognostic factors in patients with transformed MF, we evaluated the clinical, histologic, and immunophenotypical features of a large cohort of 100 transformed MF patients. This study reveals independent prognostic factors and provides a prognostic index that may be helpful in the clinical management of patients with transformed MF.

METHODS

Patient selection

One hundred and thirty patients with a diagnosis of transformed MF or a histologic diagnosis of (early) blastic transformation were retrieved from the cutaneous lymphoma database of the Leiden University Medical Center (LUMC). For each case clinical records, skin biopsies (2 to 6 biopsies/case) and lymph node biopsies, if applicable, were reviewed. Hundred cases were included because they had clinical and histological features consistent with MF, and at least one biopsy showing LCT according to the criteria described previously: the presence of large T-cells exceeding 25% of the total lymphoid infiltrate or forming microscopic nodules.[4] This study group did not contain cases with coexisting lymphomatoid papulosis (LyP) or primary cutaneous anaplastic large cell lymphoma (C-ALCL).

In a previous study no difference in survival was found between patients presenting with tumor stage MF (stage IIB) with or without LCT.[11] For that reason 25 patients with stage IIB MF with LCT at first presentation, were compared with 27 patients presenting with stage IIB MF without LCT at first presentation, including 15 patients who developed LCT during follow-up and 12 patients who did not meet the criteria of LCT and were therefore excluded from the main study.

Table 1. Studies on patients with transformed CTCL (Mycosis fungoides/Sézary syndrome).

	MF patients with LCT	LCT at first diagnosis	Stage at time LCT			Median survival (months)
			IA-IIA	IIB	IV	
Dmitrovsky[8]	12	0/12 (0%)	1/12 (8%)		11/12 (92%)	2
Salhany[4]	17	7/17 (41%)	6/17 (35%)		11/17 (65%)	12
Greer[3]	22	9/22 (41%)	9/22 (41%)		13/22 (59%)	12
Diamandidou[7]	26	9/26 (35%)	7/26 (27%)	10/26 (38%)	7/26 (27%)	19
Vergier[9]*	45	8/45 (18%)	2/45 (4%)	24/45 (53%)	20/45 (44%)	36
Barberio[6]*	17	2/17 (12%)	7/17 (41%)	7/17 (41%)	3/17 (18%)	27
Arulogun[5]	22	7/22 (32%)	3/22 (14%)	11/22 (50%)	7/22 (32%)	27
Agar[1]	70	70/70 (100%)	NR	NR	NR	100
Present study*	100	42/100 (42%)	10 (10%)	65 (65%)	25 (25%)	24

MF: mycosis fungoides; LCT: large cell transformation ; NR: not reported. *Studies that included only patients with mycosis fungoides

Clinicopathologic evaluation

All patients were classified according to the new criteria proposed by the International Society for Cutaneous Lymphomas and European Organization for Research and Treatment of Cancer.[12] Staging included complete physical examination, blood cell count and chemistry; in most cases CT scan of abdomen, chest, neck, and head (in cases of head lesions or head and neck lesions); and 1 or multiple skin biopsies. In all patients with clinically significant adenopathy a lymph node biopsy had been performed.

For each patient the following clinical data were recorded: sex, age at diagnosis MF and age at diagnosis of LCT, duration of skin lesions before MF, clinical stage at diagnosis MF, time interval between MF and LCT, clinical stage at LCT, extent of skin tumors at the time of LCT, first treatment after LCT, result of treatment, duration of follow-up and survival status. For the first skin biopsy showing transformation folliculotropic MF and the percentage of large T-cells (nodules of large cells but < 25%, 25-75% or >75%) were recorded. Immunohistochemical stainings against T-cell antigens (CD2, CD3, CD4, CD5, CD8), B-cell antigens (CD20, CD79a), histiocytes (CD68) and CD30 were studied to determine the phenotype of the atypical cells, to differentiate large T-cells from admixed histiocytes, and to determine the percentage of large T-cells expressing CD30 (0-25%, 26-50%, 51-75% or >75%). In the MF stage IIB group without transformation only age, sex, folliculotropic MF, extent of skin lesions, duration of follow-up and survival status were recorded.

Prognostic factors

The following parameters were analyzed for their prognostic significance in transformed MF: sex, age at diagnosis of LCT (≤60 yrs *vs* >60 yrs), time interval between MF and LCT (≤24 months after diagnosis MF *vs* >24 months after diagnosis MF), clinical stage at the time of LCT, folliculotropic MF, CD30 expression by > than 50% of the neoplastic T-cells and the extent of skin tumors. Extent

of skin lesions tumors was scored as solitary, regional (multiple skin lesions within 1 anatomic region) or generalized (multiple lesions in > one anatomic region). In a separate analysis of the 75 patients who presented with only skin lesions at the time of transformation, the percentage of large T-cells (< 25% but clusters, 25-75% or >75%) was included in the analysis as well.

Statistical analysis

All statistical calculations were performed using SPSS version 17.0 (SPSS Inc). DSS was calculated from the date of first biopsy showing LCT until death as result of lymphoma or date of last follow-up. Overall survival (OS) was calculated from the date of first biopsy showing transformation until the patient's death or date of last follow-up. Survival curves were estimated by the method of Kaplan and Meier and comparison between curves was done by log-rank testing. Univariate analysis of parameters with possible prognostic significance for DSS and OS was performed using Cox proportional hazards regression analysis. Factors significant at the 0.25 level in univariate analysis were included in a multivariate analysis model. In this model *P* values below 0.05 were considered significant and all parameters included were categoric.

RESULTS

Clinical characteristics and follow-up data

The main clinical and histological characteristics and follow-up data of the 100 patients with transformed MF are summarized in Table 2. The study group included 64 males and 36 females (male-female ratio, 1.8:1), with a median age at transformation of 68 years (range, 33-90 years). The median time between the initial biopsy-proven diagnosis of MF and transformation was 10 months (range, 0-222 months). Forty-two patients had transformation at first presentation. The other 58 patients developed LCT 1 to 222 months (median, 44 months) after the diagnosis MF had been made. At the time of LCT, 75 patients had transformation only in skin lesions; 19 patients had transformation in both skin and lymph nodes (n=19), in 2 of them also in bone marrow or tongue; and 6 patients had transformation only in lymph nodes, but not in concurrent skin lesions (stage IVA). Altogether, 10 patients had MF stage IB, 65 stage IIB, 24 stage IVA and 1 patient stage IVB. Initial treatment after transformation, listed in Table 2, resulted in a complete remission in only 18 of 100 patients; the remission was however generally short-lived. Median survival after transformation was 24 months (range, 1-235 months). DSS after 2, 5 and 10 years was 62%, 38% and 36%, respectively, and OS was 57%, 33% and 24%, respectively.

Histopathologic and immunophenotypical features

Biopsies from tumorous lesions generally showed a dense and diffuse infiltrate throughout the entire dermis, whereas the infiltrates in biopsies from patients with plaques stage (stage IB) disease were generally confined to the upper dermis. In this latter group, 3 patients showed a predominantly intraepidermal accumulation of large CD30+ (1 case) or CD30- (2 cases) T-cells simulating pagetoid reticulosis. However, the clinical presentation and the presence of part of these large blast cells in the superficial dermal compartment ruled out this diagnosis. In the majority of cases, the large cell population consisted of a mixture of large T-cells with

Table 2: Clinical and histological characteristics of 100 patients with transformed mycosis fungoides.

Males : Females	64 : 36
Median duration skin lesions before diagnosis MF (months; range)	33 (1-480)
Median interval between diagnosis MF and LCT (months; range)	10 (0-222)
Median age at diagnosis MF (range)	64 (29-90)
Median age at diagnosis MF with LCT (range)	68 (33-90)
Site of LCT	
Only skin	75
Only lymph node	6
Skin + lymph node	19
Stage at MF with LCT	
IB	10
IIB	65
IV	25
Folliculotropic MF	
Absent	69
Present	31
Percentage of blast cells	
<25% (clusters)	9 (10%)
25-75%	39 (41%)
>75%	46 (49%)
CD30 expression(1)	
0-25%	53
26-50%	0
51-75%	8
76-100%	39
First therapy after LCT	
Local radiotherapy (2)	44
Total skin electron beam therapy	12
Polychemotherapy	28
Other (local steroids, excision, photochemotherapy, etc.)	16
Median duration follow-up after:	
start skin lesions (months; range)	100 (15-640)
diagnosis MF (months; range)	54 (5-318)
transformation (months; range)	24 (1-235)
Current status	
Alive without disease	6
Alive with disease	25

Continued on the next page

Died of lymphoma	55
Died of other cause	14
Survival	
2-yr DSS/OS	62%/57%
5-yr DSS/OS	38%/33%
10-yr DSS/OS	36%/24%

MF: mycosis fungoides; LCT: large cell transformation; FU: follow-up; DSS: disease specific survival; OS: overall survival; (1) CD30 expression by more than 50% of the cells was taken as prognostic factor for statistical analysis; (2) often combined with other skin directed therapies such as local steroids or photochemotherapy.

cerebriform nuclei, blast cells with prominent nucleoli (T-immunoblasts), intermediate forms, and variable numbers of large anaplastic cells. Cases with a monotonous population of either large cerebriform cells without prominent nucleoli (n=3) or T-immunoblasts (n=4) or large anaplastic cells (n=8) comprising > 75% of the total lymphoid infiltrate were less commonly observed. In 31 cases the histologic (and clinical) features were consistent with the diagnosis of folliculotropic MF. The percentages of large T-cells of the total lymphoid population in the skin biopsies showing transformation are presented in Table 2. Immunophenotypic analysis showed that 70 cases had a $CD3^+CD4^+CD8^-$ T-cell phenotype, 7 cases a $CD3^+CD4^-CD8^+$ T-cell phenotype, and 19 cases a $CD3^+CD4^-CD8^-$ T-cell phenotype. In 4 cases the phenotype could not be assessed. In many cases there was (partial) loss of 1 or more pan-T-cell antigens, whereas 4 cases showed aberrant expression of CD20, CD79a, or both. CD30 was expressed by > 75% of the large T-cells in 39 cases, by 50 to 75% in 8 cases, and between 5% and 20% in 8 cases. In the other 45 cases, CD30 staining was either completely negative or expressed by only very few (<5%) large T-cells.

Prognostic factors

In the total group of patients (n=100), both univariate and multivariate analysis established that extent of skin lesions, extracutaneous transformation, negative staining for CD30 and folliculotropic MF were associated with a reduced DSS (Table 3). Further analysis showed that the difference in survival between stage IB versus IIB was not significant, but that the differences between stage IV versus IIB and stage IV versus combined stage IB and IIB were significant for DSS (P=0.048 and P=0.018, respectively). Multivariate analysis for OS showed that extent of skin lesions and extracutaneous transformation, but not folliculotropic MF and CD30 negativity, were independent parameters for reduced OS (Table 3).

In the group with only transformation in skin lesions (stage IB-IIB; n=75) multivariate analysis showed that CD30 negativity, folliculotropic MF and extent of skin tumors were independent parameters for reduced DSS and OS (Table 4). Further analysis showed no significant differences between solitary and regional skin tumors or between regional and generalized skin tumors. However, patients with generalized skin tumors had a significantly worse DSS (P=0.005) and OS (P=0.002) compared with the combined group of solitary and regional skin tumors. DSS curves of all independent parameters in the total group of patients (n=100) and in the patients with only skin lesions and LCT (n=75) are shown in Figure 1.

Table 3: Univariate and multivariate analysis of prognostic factors in 100 patients with transformed mycosis fungoides.

Characteristics	No.	Median survival (months)	DSS (%)			Univariate analysis DSS	
			2-yr	5-yr	10-yr	HR (95% CI)	P
Sex							0.418
Male	64	25 (1-235)	65	38	38	1	
Female	36	20 (2-160)	56	38	32	1.3 (0.7-2.2)	
Age							0.395
<60 years	34	42.5 (3-215)	70	40	40	1	
≥60 years	66	19 (1-235)	57	38	33	1.3 (0.7-2.2)	
Time interval between MF and LCT							0.089
≤24 months	63	30 (2-215)	71	44	39	1	
>24 months	37	17 (1-235)	46	29	29	1.6 (0.9-2.7)	
Clinical stage at LCT							0.021
Skin disease (stage IB+stage IIB)	75	26 (1-235)	69	44	44	1	
Extracutaneous disease (stage IV)	25	20 (3-215)	42	23	17	1.9 (1.1-3.3)	
CD30 expression							0.003
Positive	47	38 (1-215)	78	55	50	1	
Negative	53	20 (2-235)	48	24	24	2.3 (1.3-4.1)	
Folliculotropic MF							0.060
Absent	69	25 (1-235)	67	45	42	1	
Present	31	22 (2-134)	52	25	25	1.7 (1.0-3.0)	
Extent of skin lesions							0.005
Solitary	22	43 (5-164)	89	68	59	1	
Regional	27	23 (1-235)	74	39	39	1.9 (0.8-4.9)	
Generalized	45	20 (2-100)	46	23	.	3.6 (1.6-8.5)	

MF: mycosis fungoides; LCT: large-cell transformation; DSS: disease specific survival; OS: overall survival; HR: Hazard ratio; Missing data: extent of skin lesions: 6.

Prognostic index

Because patients may have a combination of favorable and unfavorable prognostic factors, we developed a prognostic index, that may better predict prognosis and be an useful tool in selecting appropriate treatment. For that purpose the most discriminating independent prognostic factors for DSS were selected. For the total group of patients with transformed MF these were the presence of generalized skin lesions, extracutaneous transformation, CD30 negativity, and folliculotropic MF. Patients with 0 (16 cases), 1 (36 cases), 2 (31 cases), 3 (14 cases) or 4 (3 cases) unfavorable prognostic factors had a 2-year DSS of 83%, 85%, 52%, 14% and 33%, respectively. Sub analysis showed that the difference in DSS between the 52 patients with 0-1 unfavorable prognostic factor and the 48 patients with 2 to 4 unfavorable prognostic factors

Multivariate analysis DSS		OS (%)			Univariate analysis OS		Multivariate analysis OS	
HR (95% CI)	P	2-yr	5-yr	10-yr	HR (95% CI)	P	HR (95% CI)	P
						0.980		
		59	31	21	1			
		54	37	30	1 (0.6-1.6)			
						0.043		0.071
		70	40	36	1		1	
		47	29	16	1.7 (1.0-2.9)		1.9 (0.9-4.0)	
	0.322					0.246		0.475
1		66	36	25	1		1	
1.4 (0.7-2.5)		42	26	23	1.3 (0.8-2.2)		1.3 (0.7-2.3)	
	0.046					0.224		0.002
1		59	36	26	1		1	
1.9 (1.0-3.7)		42	23	17	1.4 (0.8-2.3)		3.2 (1.5-6.6)	
	0.039					0.007		0.068
1		70	49	33	1		1	
2.0 (1.0-3.7)		46	19	16	2.0 (1.2-3.2)		1.8 (1.0-3.3)	
	0.048					0.318		
1		57	37	25	1			
1.9 (1.0-3.4)		50	24	24	1.3 (0.8-2.2)			
	0.034					0.002		0.023
1		81	61	54	1		1	
1.7 (0.7-4.4)		62	29	29	2.0 (0.9-4.5)		1.7 (0.6-4.4)	
3.0 (1.2-7.2)		44	20	.	3.5 (1.7-7.4)		3.2 (1.3-7.7)	

was statistically significant (*P*<0.001; Figure 2A). For the group of patients with only transformed skin lesions CD30 negativity, folliculotropic MF and the presence of generalized skin lesions were selected. Patients with 0 (14 cases), 1 (33 cases), 2 (21 cases) or 3 (7 cases) unfavorable prognostic factors had a 5-year DSS of 73%, 61%, 19% and 0%, respectively. The difference in DSS between the 47 patients with 0 to 1 unfavorable prognostic factor and the 28 patients with 2 to 3 unfavorable prognostic factors was statistically significant (*P*<0.001; Figure 2B).

Comparison between patients with tumor stage MF (stage IIB) with or without transformation at first presentation.

Although transformed MF is generally associated with a poor prognosis, in a previous study no difference in survival was found between patients with stage IIB with or without LCT at the

Table 4: Univariate and multivariate analysis of prognostic factors in 75 transformed mycosis fungoides patients presenting with only skin lesions.

Characteristic	No.	Median survival (months)	DSS (%)			Univariate analysis DSS	
			2-yr	5-yr	10-yr	HR (95% CI)	P
Sex							0.390
Male	51	27 (1-235)	73	46	46	1	
Female	24	22.5 (2-160)	60	39	39	1.4 (0.7-2.7)	
Age							0.515
<60 years	19	45 (4-164)	48	48	48	1	
≥60 years	56	21 (1-235)	65	42	42	1.3 (0.6-2.8)	
Time interval between MF and LCT							0.050
≤24 months	48	31 (2-164)	80	51	51	1	
>24 months	27	20 (1-235)	51	31	31	2.0 (1.0-3.8)	
Clinical stage at LCT							0.205
IB	10	48 (11-160)	77	64	64	1	
IIB	65	25 (1-235)	68	40	40	2.2 (0.7-7.0)	
CD30 expression							0.008
Positive	36	38 (1-160)	90	57	57	1	
Negative	39	21 (2-235)	54	33	33	2.6 (1.3-5.6)	
Folliculotropic MF							0.014
Absent	53	27 (1-235)	73	55	55	1	
Present	22	23.5 (2-134)	61	22	22	2.3 (1.2-4.6)	
% large cells in infiltrate							0.784
<25 (nodules)	7	26 (11-44)	100	.	.	1	
25-75	32	27 (2-235)	71	49	49	0.67 (0.2-2.1)	
>75	36	24.5 (1-164)	62	46	46	0.75 (0.2-2.2)	
Extent of skin tumors							0.024
Solitary	19	30 (5-164)	87	69	69	1	
Localized	22	29 (1-235)	84	49	49	1.6 (0.5-5.0)	
Generalized	34	20.5 (2-100)	52	30	.	3.4 (1.3-9.5)	

MF: mycosis fungoides; LCT: large-cell transformation; DSS: disease specific survival; OS: overall survival; HR: Hazard ratio

Multivariate analysis DSS		OS (%)			Univariate analysis OS		Multivariate analysis OS	
HR (95% CI)	**P**	**2-yr**	**5-yr**	**10-yr**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
						0.962		
		60	36	22	1			
		56	37	37	1 (0.6-1.9)			
						0.084		0.012
		42	42	42	1		1	
		53	32	20	1.9 (0.9-3.7)		2.8 (1.8-6.1)	
	0.830					0.180		0.537
1		67	49	21	1		1	
1.1 (0.5-2.4)		45	27	22	1.5 (0.8-2.6)		1.2 (0.6-2.5)	
	0.220					0.207		0.127
1		77	64	38	1		1	
2.2 (0.6-6.4)		57	32	25	1.8 (0.7-4.6)		2.1(0.8-5.6)	
	0.006					0.026		0.027
1		76	50	33	1		1	
3.2 (1.4-7.1)		45	24	21	1.9 (1.0-3.5)		2.1 (1.1-3.9)	
	<0.001					0.178		0.001
1		60	43	29	1		1	
4.0 (1.9-8.2)		58	21	21	1.5 (0.8-2.8)		3.4 (1.7-7.0)	
						0.789		
		80	.	.	1			
		60	41	29	0.74 (0.3-2.0)			
		55	38	28	0.71 (0.3-1.9)			
	0.024					0.009		0.002
1		78	62	62	1		1	
1.2 (0.4-4.0)		69	35	35	1.7 (0.7-4.4)		0.9 (0.3-2.6)	
3.4 (1.2-9.7)		44	25	.	3.4 (1.5-7.8)		3.3 (1.3-8.0)	

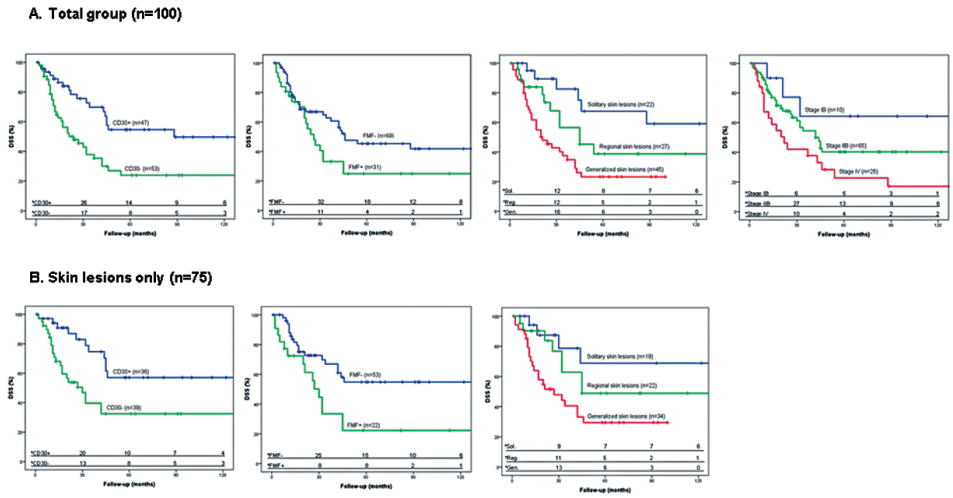

Figure 1. DSS curves of independent parameters in patients with transformed MF. (A) Curves for total group of patients with transformed MF (n=100), indicating CD30 expression, folliculotropic MF (FMF), extent of skin lesions, and clinical stage, respectively. (B) Curves for subanalysis of patients with only skin lesions and LCT (n=75), indicating CD30 expression, FMF and extent of skin lesions. *indicates number of persons at risk at the specified times after diagnosis of transformed MF.

time of first presentation.[11] In our study, 65 patients had stage IIB at time of transformation. Within this group, 25 patients had LCT at first presentation. These 25 patients were compared 27 patients with stage IIB MF without LCT at first presentation. Differences in DSS and OS between both groups were not statistically significant (Table 5). Further analysis between those patients who presented with stage IIB without LCT, but who developed LCT during follow-up (n=15) and those patients who did never develop LCT (n=12), also showed no statistically significant differences in survival (data not shown).

DISCUSSION

Previous studies demonstrated that LCT in MF is associated with an aggressive clinical course and a poor survival.[3-10] Notwithstanding, in the past 20 years we have regularly seen MF patients with transformation in skin and even lymph node biopsies, who after treatment followed an indolent course for many years. This is reflected in the present study, in which 23 of 100 patients, including 19 with LCT in the skin and 4 with LCT in lymph nodes, had an indolent course for 5 to almost 20 years (median, 99 months; range, 60-235 months) after LCT. Only 4 of these 23 long-survivors died of lymphoma, 88 to 215 months after transformation. These observations underscore the need to define parameters that may help to predict which patients will have an aggressive and which a more indolent clinical course. Data on prognostic factors from previous studies are however conflicting, perhaps largely because of the small and heterogeneous groups studied. For that purpose we analyzed the clinical, histological, and immunophenotypical features of the largest cohort of transformed MF patients studied thus far.

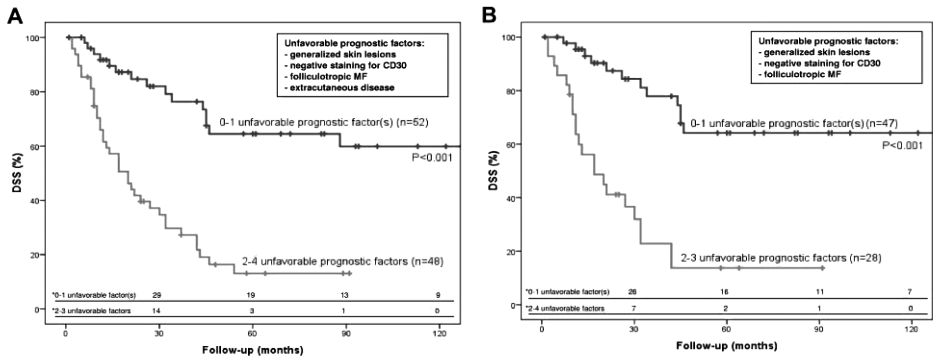

Figure 2. Prognostic index indicating differences in DSS. Prognostic index indicating differences in DSS in total group of patients with transformed MF (n=100; A) and in patients with only skin lesions and LCT (n=75;B). *indicates number of persons at risk at the specified times after diagnosis of transformed MF.

The median survival of the 100 patients was 24 months (range, 1-235 months) and the 5-year DSS and OS 38% and 33%, respectively. These results are similar to those of previous studies, (median survival, 12-36 months; 5-yr OS, 11%-32%), but contrast to those of Agar et al, who reported a median survival of 100 months and a 5-yr DSS and OS of 65% and 63%, respectively, in a group of 70 patients with LCT at first diagnosis.[1] In this latter study, survival data and prognostic factors, including LCT, were analyzed on a group of 1502 patients with MF or SS.[1] Because further details on the group of 70 patients with LCT are not provided, the difference in survival between this recent study and all other studies in transformed MF cannot be explained.

The most important prognostic factors in patients with transformed MF from the present and previous studies include advanced stage at transformation, CD30 expression, folliculotropic MF and increased extent of skin lesions.

Advanced stage at transformation

Several studies reported that patients with extracutaneous transformation have a shorter median survival than patients with transformation limited to the skin.[3,4] In contrast, in the study by Diamandidou et al, no difference in survival was found between patients with cutaneous and extracutaneous transformation.[7] In fact, patients with transformed stage IIB (tumor stage) did as poorly as or even worse than patients with extracutaneous transformation (stage IV). Patients with transformed stage I-IIA (plaques) did significantly better than the combined transformed IIB-IV groups.[7] In the present study, more advanced stage was significantly associated with reduced survival (Table 3). Subanalysis showed that extracutaneous transformation (stage IV) had a significantly poorer survival than cutaneous disease transformation (combined IB-IIB vs IV; *P*=0.018). No significant difference in survival was found between stage IB and stage IIB, indicating that extracutaneous transformation is the main factor responsible for shorter survival.

CD30 expression

Several studies have suggested that CD30 expression in transformed MF may be associated with a better prognosis.[5,6,9] However, in none of the published studies a significant difference between

Table 5. Summary of clinical characteristics and survival data of patients with tumor stage mycosis fungoides (stage IIB) with or without large cell transformation at presentation.

	MF stage IIB with LCT at presentation (n=25)	**MF stage IIB without LCT at presentation (n=27)**
Male/Female	16 / 9	23 / 4
Median age (range)	72 years (42-90)	63 years (40-82)
CD30 expression - positive (>50% of blast cells) - negative (<50% of blast cells)	 13/25 (52%) 12/25 (48%)	 See footnote*
Folliculotropic MF	10/25 (40%)	13/27 (59%)
Extent of skin lesions - solitary - regional / localized - generalized	 8/25 (32%) 9/25 (36%) 8/25 (32%)	 8/27 (30%) 8/27 (30%) 11/27 (40%)
Median survival (months)	42 (5-153)	53 (12-262)
2-yr DSS/OS	95% / 75%	88% / 84%
5-yr DSS/OS	56% / 40%	54% / 48%
10-yr DSS/OS	56% / 30%	29% / 16%
P-value DSS/OS	0.644 / 0.721	

MF: mycosis fungoides; LCT: large-cell transformation; *in most cases scattered CD30+ blast cells (<1% to 5% of total infiltrate)

CD30+ and CD30- cases was found, probably because of the small size of the study groups. In the present study, CD30 expression proved a strong and independent predictor of improved survival, both in the total group of patients and in patients with transformation limited to the skin. The favourable prognosis of the patients with CD30+ transformed MF raises the question whether part of them might not have a primary cutaneous CD30+ lymphoproliferative disorder (LyP of C-ALCL) coexisting with MF. Indeed differentiation between transformed MF and these primary cutaneous CD30+ lymphoproliferative disorder may be difficult, in particular in case the infiltrates contains >75% CD30+ blast cells, as observed in 39 of 100 cases (Table 2). However, none of our patients had a chronic recurrent self-healing papulonodular eruption consistent with LyP. In all CD30+ cases, a diagnosis of C-ALCL was unlikely, because 1) tumors developed in areas with pre-existent patches or plaques; 2) tumors showed an admixture with small- to medium-sized atypical T-cells with cerebriform nuclei (characteristic of MF); or 3) tumors showed significant folliculotropism, a condition that is rarely seen in cases of C-ALCL. Although it cannot be excluded, we do not believe our study contained patients with combined MF and C-ALCL.

Folliculotropic MF

The significance of folliculotropic MF in the prognosis of transformed MF has not been studied in previous studies. Although included as a distinct variant of MF in recent classifications for cutaneous lymphomas, cases of folliculotropic MF were included in the present study to allow comparison with previously published series. Both in the total group and in patients with

transformation in only skin lesions, folliculotropic MF was a strong and independent predictor of reduced survival. Similarly, in other studies on large cohorts of MF patients folliculotropic MF was associated with a poorer survival.[1,2]

Extent of skin tumors

In the group with transformation only in skin lesions we investigated the prognostic significance of the extent of transformed skin lesions. It must be emphasized that transformation was generally demonstrated in only one lesion, assuming that other, clinically similar lesions, showed the same histology. The presence of generalized skin tumors was strongly associated with reduced DSS and OS, when compared with the combined group of solitary and localized skin tumors. However, no significant differences were found between solitary and localized skin tumors or between localized and generalized skin tumors.

Percentage of blast cells

Progression from plaque- to tumor-stage MF is accompanied by a gradual increase of blast cells and a decrease of atypical T-cells with cerebriform nuclei and of inflammatory cells, and associated with a reduced prognosis. One might therefore expect that high percentages of blast cells (>75%), reflecting further tumor progression, are associated with a worse prognosis. However, no association between the percentage of blast cells and survival was found, consistent with the results of a previous study.[9] Also, when stratified for CD30 expression, an association was not found (data not shown).

In conclusion, previous studies of transformed MF emphasized above all the aggressive clinical behavior and poor prognosis of these patients. As a result, recent National Comprehensive Cancer Network guidelines suggest that a more aggressive therapeutic approach should be considered in patients with transformed MF compared to patients with the same stage of MF without LCT.[14] However, some caution is warranted. First, consistent with previous studies we did not find a difference in survival between patients with stage IIB with or without LCT at first diagnosis.[11,13]There were no major differences in age, percentage of folliculotropic MF, and extent of skin lesions between both groups (Table 5). In fact, prognostic unfavourable factors (eg, folliculotropic MF, generalized skin lesions) were even slightly more frequent in the MF stage IIB group without LCT, strengtening our conclusions that MF stage IIB with LCT at presentation do not necessarily worse than MF stage IIB without LCT at presentation. This observation is important for clinical management, and suggests that patients with stage IIB with LCT should not be treated more aggressively than patients presenting with stage IIB without LCT, as suggested by recent National Comprehensive Cancer Network guidelines.[14] Second, although the median and 5-year survival in the present study were similar to that of previous studies, the results of this study clearly demonstrate that not all patients with transformed MF run an aggressive clinical course. The prognostic index described herein may be a valuable tool to predict which patients will have an aggressive and which a more indolent clinical course, and they may aid to select the most appropriate treatment in patients with transformed MF. However, the applicability and usefulness of this new tool needs to be validated by future studies.

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7

Summary and discussion

The studies presented in this thesis focused on cutaneous CD30-positive lymphoproliferations, particularly on primary cutaneous anaplastic large cell lymphoma (C-ALCL). In recent classifications for malignant lymphomas C-ALCL is recognized as a distinct type of cutaneous T-cell lymphoma (CTCL). Characteristic clinical features of C-ALCL include presentation with solitary or localized skin lesions, tendency to spontaneous remission and in most patients an indolent course and excellent prognosis.

Although fairly well defined as a distinct type of CTCL, a number of controversies and outstanding questions remained, that needed to be addressed.

The main issues concerned:

1. the utility of recently described phenotypic markers in the differentiation between the different types of CD30-positive lymphoproliferations.
2. the lack of prognostic markers predictive of an unfavorable clinical course in a minority of patients with C-ALCL
3. the extent of staging procedures necessary to exclude systemic disease
4. molecular mechanisms underlying the favorable prognosis of C-ALCL
5. the prognostic significance of CD30 expression in transformed mycosis fungoides (MF).

In this final chapter, these issues are discussed on the basis of studies described in this thesis and data from literature.

Diagnostic value of phenotypical markers in cutaneous CD30-positive lymphoproliferations

Distinction between the different types of cutaneous CD30+ lymphoproliferations is important, as they have a different clinical behavior and require a different clinical approach. Since the histological and immunophenotypical features of these conditions show considerable overlap, clinicopathologic correlation is often required to differentiate between these disorders. However, this does not provide a definite diagnosis in all patients. For instance, in patients presenting with ALCL in the skin and regional lymph nodes, it may be difficult to differentiate between C-ALCL with secondary regional lymph node involvement and a secondary cutaneous ALCL. Differentiation may be facilitated by the use of phenotypical markers. Unlike systemic ALCL, most C-ALCL express the cutaneous lymphocyte antigen (CLA), but do not express epithelial membrane antigen (EMA) and anaplastic lymphoma kinase, which is indicative of the t(2;5) chromosomal translocation or variant translocations.[1;2] Despite careful clinicopathologic correlation in some patients presenting with multifocal skin lesions, differentiation between LyP and C-ALCL may be impossible at first presentation. In such patients, designated as borderline cases, careful follow-up will normally disclose within weeks or months whether they have a waxing and waning eruption and can be labeled as LyP.[3] Recent studies suggested that immunophenotypical markers like TRAF1, IRF4 (also known as MUM1) and BCL2 may be valuable diagnostic adjuncts in the differentiation between LyP and C-ALCL.[4-7] Assaf et al. reported high TRAF1 expression in more than 80% of LyP cases, but in only 7% of cases of primary or secondary cutaneous ALCL.[6] In another study, Kempf et al. described IRF4 (MUM1) expression by the neoplastic T-cells in 13 of 15 (87%) cases of LyP, and in only 2 of 10 (20%) cases of C-ALCL.[4] Wasco et al. did however not find a difference in IRF4 (MUM1) expression between these disorders.[5] Paulli et al. reported lower levels

of the antiapoptotic protein BCL2 in LyP compared with nonregressing lesions of C-ALCL, and suggested that increased BCL2 expression in nonregressing lesions of C-ALCL protects tumor cells from apoptosis.[7] Since the results of these studies are either unconfirmed or conflicting, in **chapter 3** we evaluated the diagnostic value of these markers in a large group of cutaneous CD30+ lymphoproliferations, including these from 28 patients with C-ALCL, 39 patients with LyP, 11 patients with CD30+ MF-TR, two with ALK+ ALCL and six with ALK- ALCL.

Both TRAF1 and IRF4 (MUM1) were found to be expressed by the CD30+ neoplastic T-cells in 70-80% of LyP, C-ALCL and CD30+ MF-TR, which suggests that they are not useful to differentiate between these disorders. Expression of BCL2 by > 50% of the tumor cells was found in 36% of LyP cases and 22% of C-ALCL cases, which also argues against diagnostic value in the differentiation between these disorders. Interestingly, highest levels of BCL2 were expressed in MF-TR. It is tempting to speculate that high expression of this antiapoptotic protein in MF-TR contributes to treatment resistance and poor prognosis, although functional studies supporting such an assumption have not been performed. In summary, TRAF1, MUM1 and BCL2 do not aid in the differentiation between different types of cutaneous CD30+ lymphoproliferations.

Prognostic parameters in C-ALCL

While most C-ALCL patients have an excellent prognosis, risk factors that predict an unfavorable course, occurring in few patients with C-ALCL, are largely unknown. Several studies have suggested that age older than 60 years, absence of spontaneous remission, and presentation with multifocal skin lesions may correlate with reduced survival.[8-11] Moreover, in recent studies extensive limb involvement and localization on the head and neck have been associated with a less favorable prognosis as well.[12-14] In **chapter 2** the potential prognostic significance of sex, age (≤60 vs. >60 years), extent of disease, site of presentation, and complete spontaneous remission of initial skin lesions was analyzed on a large group of 135 patients with C-ALCL. Extent of disease was scored using the new TNM classification system for primary cutaneous lymphomas other than MF and Sézary syndrome, as proposed by the ISCL/EORTC.[12] Because these patients by definition have no extracutaneous disease (N and M categories) at the time of diagnosis, only T categories were scored, as either T1 (solitary skin lesion), T2 (regional skin lesions) or T3 (generalized skin lesions).

Analysis of extent of skin lesions showed a 5-year disease-specific survival (DSS) of 93% for patients with T1 disease 93% for patients with T2 disease and 77% for patients with T3 disease, indicating that patients with T3 disease have a reduced, although statistically nonsignificant, survival rate compared with patients with T1 or T2 disease (P=.19). In addition, analysis of site showed a trend toward reduced 5-year DSS in patients with skin lesions on a leg, compared to localizations on other sites (82% for leg vs. 95% for head and neck, 96% for trunk, and 95% for arm; P=.23). Moreover, the 5-year DSS of patients with leg involvement (n=32) and patients without leg involvement (n=103) were 76% and 96%, respectively (P=.007; after adjustment for T category, P=.03). Sub analysis within the group of 18 patients with generalized skin lesions (T3 disease) showed that those patients with involvement of one or both legs had a 5-year DSS of 67% compared with 100% in patients without leg involvement (P=.20). Also, in the patients with regional skin lesions (T2 disease), leg involvement was associated with a reduced 5-yr DSS (76% vs. 100%; P=.13). Taking together, extent of disease may correlate with survival, in particular in

patients with leg involvement. This is in line with other studies reporting on the poor prognosis of C-ALCL with extensive limb disease or leg involvement.[12;14] Interestingly, also in other types of cutaneous lymphomas leg involvement has been associated with an aggressive clinical behavior. For instance, Poligone et al. described three patients with a CTCL localized to the lower leg and (locally) aggressive behavior, including two patients with tumor stage MF and one patient with peripheral T-cell lymphoma, not otherwise specified (PTL-NOS).[15] Moreover, cutaneous lymphomas with the histological features of a diffuse large B-cell lymphoma located on the leg have a worse prognosis compared to primary cutaneous follicle centre lymphoma with a diffuse population of large B-cells located on the trunk or head, and have therefore been included as a separate entity (primary cutaneous large B-cell lymphoma of the leg or leg-type) in recent classifications for cutaneous lymphomas. The biological mechanism responsible for this poor prognosis is unclear. Cluster analysis of gene expression profiling data from C-ALCL patients showed that patients with extensive limb disease (including 5 samples from 3 patients) clustered separately from C-ALCL patients without extensive limb disease (including 9 samples from 7 patients).[14] Among the upregulated genes in patients with extensive limb disease were STAT5 and IL2RA. The authors speculate that aberrant activation of the IL2R/STAT5 pathway may contribute to the more aggressive behavior of this subgroup of patients. A limitation of this study is the fact that validation experiments have not been performed. Moreover, as the group with extensive limb involvement included biopsies of only three patients, further studies are warranted to confirm these findings.

Apart from leg-involvement, other parameters investigated in this study were not significantly related to survival. For instance, the results of a previous study indicating that patients with skin lesions on the head or neck had a significant poor survival could not be confirmed.[13] In that particular study, C-ALCL patients were retrieved from the Surveillance, Epidemiology, and End Results (SEER) database, which includes cases without independent histological review. It can therefore not be excluded that the SEER cohort contains several patients with folliculotropic MF, which preferentially present at the head and neck region, commonly contain many CD30-positive blast cells and have a worse prognosis than patients with C-ALCL, as shown in **chapter 6**.

In addition to the clinical parameters investigated in **chapter 2**, we also investigated the prognostic significance of immunophenotypical markers described in **chapter 3**, including TRAF1, BCL2 and CD15, in a group of 28 patients with C-ALCL. In C-ALCL, two of three TRAF1-negative patients died of lymphoma, in contrast to four of 20 (20%) TRAF1-positive patients (5-year DSS 0% vs. 93%; P=.014). However, no difference in expression of BCL2 and CD15 was found between C-ALCL patients with a favorable prognosis and those who died of disease.

Extent of staging procedures in C-ALCL

According to criteria of the WHO-EORTC classification for cutaneous lymphomas a diagnosis of C-ALCL should be made only when systemic localizations have been excluded by adequate staging procedures, including a bone marrow biopsy. Review of the database of the Dutch Cutaneous Lymphoma Group showed that bone marrow examination is often not performed, in particular in patients with a solitary tumor that has resolved spontaneously or has been excised completely. Moreover, the recently published staging classification system for non-MF/SS

primary cutaneous lymphomas suggests that in cutaneous lymphomas with an indolent clinical behavior, such as C-ALCL, bone marrow evaluation should be considered, but is not required unless indicated by other staging investigations.[16]

Since the incidence of bone marrow involvement in patients with an ALCL first presenting in the skin was not known, the study described in **chapter 4** presents the staging results of 107 patients with an ALCL first presenting in the skin. In 20 patients extracutaneous disease was found. Eleven of these patients had involvement of peripheral lymph nodes, draining a skin area containing ALCL lesions, suggesting that these were C-ALCLs with secondary lymph node involvement. The other nine patients had more widespread disease with involvement of central lymph nodes and/or other extracutaneous localizations, suggesting systemic ALCL with secondary skin involvement. Bone marrow involvement by ALCL was never observed at the time of diagnosis and only one patient who had originally negative staging results, developed widespread systemic disease, including bone marrow involvement six months after diagnosis.

In conclusion, the results indicate that bone marrow examination has limited value in the staging of patients with an ALCL first presenting in the skin, and should be performed only in selected cases. Obviously, in ALK+ ALCL cases presenting in the skin bone marrow examination should be included in the initial staging work-up, as ALK expression is indicative of a systemic (ALK+) ALCL which has (often subtle) bone marrow involvement in 10-30% of the cases.[17]

Molecular mechanisms underlying the favorable prognosis of C-ALCL

Although its histology indicates an aggressive lymphoma, C-ALCL usually shows an indolent clinical behavior. The molecular mechanisms responsible for this favorable prognosis are largely unknown. It has been suggested that interaction between CD30 and CD30L may contribute to apoptosis and the subsequent regression as observed in approximately 40% of the cases.[18] In addition, it has been speculated that increased expression of skin-homing chemokine receptors and CD30-mediated NF-kB activation may contribute to the pathogenesis of C-ALCL.[19] Interestingly, two recent studies, using array-CGH analysis, showed that the 9p21 deletion (involving the CDKN2A-CDKN2B gene locus), which was often found in MF-TR, was not or rarely observed in C-ALCL.[20;21] Apart from genetic also epigenetic factors may play a role in the pathogenesis of C-ALCL. For instance, van Doorn et al. found that BCL7a was hypermethylated at a lower frequency in C-ALCL (14%) compared to aggressive (64%) CTCL entities and suggested that this gene functions as a tumor suppressor in lymphoid cells.[22] However little is known on histon modifications and miRNA expression in C-ALCL, which influence the epigenetic landscape as well. In **chapter 5** we investigated the miRNA expression profile of C-ALCL. MicroRNAs (miRNAs) are small RNA species that regulate gene expression post-transcriptionally and are aberrantly expressed in many malignancies including lymphoma.

Using a genome wide approach (μRNA™ microarrays), we found 13 miRNAs differentially expressed between C-ALCL and benign inflammatory dermatoses (BID). Of these miRNAs the up-regulation of miR-155, miR-27b, miR-30c and miR-29b in C-ALCL was validated by miRNA-Q-PCR on independent study groups of C-ALCL and BID. We also compared the miRNA expression profiles of C-ALCL with those of tumor stage MF.[23] Although miRNA microarray analysis did not show statistically significant differences, miRNA-Q-PCR identified statistically significant higher expression of miR-155, miR-27b, miR-93, miR-29b and miR-92a in C-ALCL compared to tumor

stage MF. This observation is in line with the current insights that arrays should preferably be used as screening tools and are unable to detect more subtle differences.

In conclusion, the differences in miRNA expression of C-ALCL and tumor stage MF described in this study might reflect a different role in the pathogenesis of these lymphomas.

This study illustrates two key limitations in the research on C-ALCL and CTCL in general. First of all, the limited availability of patient material with more than 70% of tumor cells, in order to avoid admixture of reactive lymphocytes. As a consequence the sample sizes for refined comparisons (e.g. C-ALCL with and without leg-involvement, or good versus poor prognosis) are too small for proper statiscal analysis. The same was true for additional comparisons between C-ALCL and tumor stage MF with transformation and CD30 expression (**chapter 6**). The second limitation relates to the fact that our study is purely descriptive with no (functional) data on any relevant miRNA target gene expression or dysregulation. Functional studies so far are hampered by the lack of suitable tools, e.g. animal models, which are available for other types of CTCL.[24]

The prognostic significance of CD30 expression in MF-TR

MF-TR is associated with an aggressive clinical course and a poor survival. Notwithstanding, in the past 20 years we have regularly seen MF-TR patients with transformation in skin and even lymph node biopsies who after treatment followed an indolent course for many years. This illustrates the need for parameters that help to predict which patients will have an aggressive and which a more indolent course and may help to select the most appropriate management for these patients. Given the excellent prognosis of C-ALCL (CD30+) compared to CTCL with a diffuse population of large CD30 negative neoplastic T-cells, one wonders whether also CD30+ MF-TR has a better prognosis than CD30 negative MF+TR.

In **chapter 6** we therefore investigated the prognostic significance of CD30 expression and other clinical and histological parameters in a group of 100 MF-TR patients. Multivariate analysis of the total group showed that CD30 expression, folliculotropic MF, extent of skin lesions and extracutaneous disease were independent prognostic parameters for disease specific survival. In a multivariate analysis of 75 patients with only skin lesions at the time of transformation, CD30 expression, folliculotropic MF and extent of skin lesions were independent prognostic parameters for both DSS and OS. Using the most discriminating parameters as a prognostic index, in both study groups differences in DSS between patients with 0-1 unfavorable prognostic factor(s) and ≥2 unfavorable prognostic factors were statistically significant (P<.001).

In summary, our analysis shows that CD30+ MF-TR has indeed a better prognosis. In particular, patients with CD30+ MF-TR presenting with a solitary tumor had a favorable prognosis (5-year DSS 68%). There is discussion whether such cases are MF-TR or MF with co-existent C-ALCL.[14;15] In favor of CD30+ MF-TR above concurrent MF and C-ALCL are tumors developing in areas with pre-existent patches or plaques, showing an admixture with small to medium sized atypical T-cells with cerebriform nuclei (characteristic of MF) and/or showing significant folliculotropism, which is rarely seen in cases of C-ALCL. Notwithstanding, the differential diagnosis is difficult as illustrated by four patients in our study, that were first misclassified as C-ALCL. Retrospectively, one patient did present with concurrent patches and plaques which were not biopsied at the time of diagnosis, but the other three patients presented with solitary skin lesions and developed typical patches and plaques during follow-up. Whether the same

molecular mechanisms contribute to the favorable prognosis of CD30+ MF-TR and C-ALCL remains to be elucidated. It is tempting to speculate that CD30 itself, or more likely CD30-CD30L interactions, is responsible for reduction of proliferation and even cell death, but CD30 activation can also result in opposite effects. For instance, in Hodgkin lymphoma cell lines CD30-CD30L interactions may contribute to autocrine growth regulation, while in C-ALCL the same interactions may contribute to apoptosis.[18] So far, CD30-CD30L interactions have not been studied in mycosis fungoides yet. Interestingly, the CDKN2A-CDKN2B deletion, which was often found in tumor stage MF, was associated with a lower survival rate.[21,22] Additional analysis of the patients included in the study by van Doorn et al. on tumor stage MF showed that loss of 9p was never observed in the CD30+ cases and in 8 of the 14 (57%) CD30- cases. Similarly, loss of 9p21 is not observed in C-ALCL, compared to 50% of primary cutaneous PTL-NOS patients[22]. One could therefore speculate that the tumor suppressors within the CDKN2A-CDKN2B gene locus may contribute to the favorable prognosis of both C-ALCL and CD30+ MF-TR.

CONCLUSIONS AND PERSPECTIVES

C-ALCL is a peculiar type of CTCL, as it combines a histology indicating an aggressive lymphoma with an excellent prognosis. Characteristic for its favorable clinical course is its tendency to spontaneous remission. The molecular mechanisms contributing to this spontaneous remission are largely unknown. Identifying these mechanisms is important as it might identify therapeutical targets, which can be used in the development of treatments not only for C-ALCL that do not show spontaneous regression, but potentially also for other types of aggressive CTCL.

Because its expression in normal cells is limited, CD30 itself is an attractive molecule for targeted therapies. Recent studies using the SGN-30 monoclonal antibody have shown high response rates in patients with cutaneous CD30+ LPD.[26] The results of a new anti-CD30 monoclonal antibody coupled to the anti-tubulin agent monomethyl auristatin E (SGN-35; brentuximab vedotin) are even more impressive.[27-30] In a phase II study of SGN-35 in patients with relapsed or refractory systemic ALCL 15 patients had skin lesions at the time of enrollment, while complete remission of skin lesions was found in 14 of them.[29;30] Future studies should reveal whether if SGN-35 is equally effective in patients with C-ALCL and patients with CD30+ MF-TR.

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8

Appendix

NEDERLANDSE SAMENVATTING

Non-Hodgkin lymfomen vormen een heterogene groep van aandoeningen die bestaan uit kwaadaardige woekeringen van witte bloedcellen. Ze ontstaan meestal in de lymfeklieren (nodaal), maar kunnen zich ook in andere organen presenteren (extranodaal). Primair cutane lymfomen (PCL) zijn non-Hodgkin lymfomen die zich in de huid (cutaan) presenteren, zonder dat er op het moment van diagnose aanwijzingen zijn voor lokalisaties elders in het lichaam. Er zijn twee hoofdcategorieën: 75-80% zijn primair cutane T-cel lymfomen (CTCL) en 20-25% primair cutane B-cel lymfomen (CBCL). Binnen de CTCL kunnen drie subgroepen worden onderscheiden: (1) de groep van klassieke CTCLs, waaronder mycosis fungoides (MF), varianten van MF en het Sézary syndroom (SS); (2) de groep van primair cutane CD30-positieve lymfoproliferatieve aandoeningen (CD30+ LPD); en (3) een groep van zeldzame en vaak agressieve cutane T/NK-cel lymfomen.

Een centraal onderwerp in dit proefschrift is het primair cutaan grootcellig anaplastisch T-cel lymfoom (C-ALCL). Dit lymfoom ziet er bij histopathologisch onderzoek onder de microscoop vaak agressief uit met velden grote ronde tumorcellen waarvan meer dan 75% het CD30 eiwit op zijn celmembraan tot expressie brengt. Patiënten met een C-ALCL presenteren zich meestal met solitaire of regionale huidafwijkingen, die in 20-40% van de gevallen de neiging hebben spontaan te verdwijnen (spontane remissie). Het beloop is doorgaans gunstig. De behandeling van persisterende afwijkingen bestaat meestal uit excisie of lokaal radiotherapie. Hoewel er frequent nieuwe afwijkingen in de huid kunnen ontstaan, vindt er maar zelden uitbreiding naar lokalisaties buiten de huid plaats. De ziektespecifieke 10-jaars overleving is dan ook ruim 90%. Het C-ALCL maakt samen met lymfomatoïde papulose (LyP) deel uit van de primair cutane CD30+ LPD. LyP wordt gekenmerkt door het komen en gaan van huidafwijkingen. Huidbiopten van beide aandoeningen vertonen bij histopathologisch onderzoek een aantal gemeenschappelijke kenmerken, waaronder expressie van het CD30 eiwit.

Naast primair cutane CD30+ LPD zijn er ook andere aandoeningen, die CD30 tot expressie brengen. Voorbeelden zijn de systemische variant van het ALCL dat zich bij aanvang in de lymfklieren presenteert, maar zich op termijn (ook) kan uitbreiden naar de huid, een bepaald stadium van MF (getransformeerde MF) en verschillende reactieve (goedaardige) huidaandoeningen. Met histopathologisch onderzoek is het onderscheid tussen deze aandoeningen niet altijd goed te maken, terwijl dit wel relevant is, omdat de aandoeningen een ander klinisch beloop en een andere prognose hebben en vaak ook een andere behandeling vereisen.

Ondanks dat in recente classificatie systemen voor cutane lymfomen het C-ALCL redelijk goed gedefinieerd is, bestonden er bij aanvang van dit promotietraject nog een aantal onduidelijkheden, zoals: 1) het nut van een aantal in de literatuur beschreven eiwitmarkers om het onderscheid te maken tussen verschillende soorten cutane CD30+ lymfoproliferaties; 2) het gebrek aan prognostische markers die voorspellend zijn voor het ongunstig beloop dat een klein deel van de patiënten met C-ALCL treft; 3) de uitgebreidheid van stageringsonderzoek, dat nodig is om lokalisaties buiten de huid vast te stellen; 4) de moleculaire mechanismen die verantwoordelijk zijn voor het gunstige klinisch beloop van de meeste patiënten met C-ALCL; en 5) de prognostische betekenis van CD30 expressie bij patiënten met getransformeerde MF.

Diagnostische waarde van bepaalde eiwitmarkers bij cutane CD30+ lymfoproliferaties

Recente studies hebben gesuggereerd dat de eiwitmarkers TRAF1, MUM1 (IRF4) en BCL2 verschillend tot expressie worden gebracht op bepaalde cutane CD30+ lymfoproliferaties, en bij histopathologisch onderzoek van nut kunnen zijn om de verschillende typen te kunnen onderscheiden. Omdat de resultaten van deze studies nog niet bevestigd zijn door andere studies of elkaar tegenspreken, hebben we in **hoofdstuk 3** de diagnostische waarde van deze markers in een grote groep van cutane CD30+ lymfoproliferaties onderzocht, waaronder 28 patiënten met C-ALCL, 39 patiënten met LyP, 11 patiënten met CD30+ getransformeerde MF en 8 patiënten met huidlokalisaties van de systemische variant van het ALCL. De resultaten van deze studie toonden aan dat TRAF1 en IRF4 niet nuttig waren om het onderscheid tussen de verschillende CD30+ lymfoproliferaties te maken omdat ze beide tot expressie werden gebracht in 70-80% van de gevallen van C-ALCL, LyP en CD30+ getransformeerde MF. Expressie van BCL2 in meer dan 50% van de tumorcellen, werd gevonden in 36% van de LyP gevallen en in 22% van de C-ALCL gevallen, wat eveneens pleit tegen het nut ervan als diagnostische marker. Het bleek dat in CD30+ getransformeerde MF 73% van de gevallen BCL2 in meer dan 50% van de tumorcellen tot expressie bracht. Omdat dit eiwit celdood tegengaat, zou de hoge expressie ervan mogelijk een rol kunnen spelen bij de slechtere prognose van patiënten met een getransformeerde MF.

Prognostische factoren in C-ALCL

In **hoofdstuk 2** hebben we in 135 C-ALCL patiënten de prognostische betekenis van een groot aantal factoren onderzocht, waaronder leeftijd, geslacht, uitgebreidheid van de huidafwijkingen, locatie en spontane remissie. Om de uitgebreidheid van de huidafwijkingen te scoren werd gebruik gemaakt van het Tumor, Node, Metastasis (TNM-) systeem, een systeem dat in gemodificeerde vorm al langer bestond voor MF en SS, maar pas recent is ontwikkeld voor andere PCL. Omdat deze patiënten op het moment van diagnose per definitie geen lokalisaties elders in het lichaam hebben, werd alleen het T-stadium gescoord, namelijk als T1 (solitaire huidafwijkingen), T2 (regionale huidafwijkingen) of T3 (gegeneraliseerde huidafwijkingen). Het bleek dat dit TNM-systeem goed toepasbaar was op patiënten met een C-ALCL. De analyse toonde verder aan dat patiënten met lokalisaties op één of beide benen, in tegenstelling tot lokalisaties elders op het lichaam, een significant slechter beloop hadden (ziektespecifieke 5-jaars overleving van 76% versus 96%). Vervolgens werd er in de groep van patiënten met lokalisaties op één of beide benen een trend gezien dat patiënten met een T2 of T3 score een slechter beloop hadden dan patiënten met een T1 score (ziektespecifieke 5-jaarsoverleving voor T1 90%, versus 76% en 67%, respectievelijk voor T2 en T3). Het lijkt daarom verstandig deze patiënten goed te controleren tijdens de follow-up.

Uitgebreidheid van stagering bij patiënten met een ALCL in de huid

De diagnose van een PCL kan pas worden gesteld als lokalisaties buiten de huid zijn uitgesloten met behulp van stageringsonderzoek. Dit bestaat doorgaans uit een grondig lichamelijk onderzoek, bloedonderzoek, beeldvormend onderzoek (CT scan) en een beenmergbiopt. Gegevens uit de database van de Werkgroep Cutane Lymfomen lieten echter zien dat bij C-ALCL

patiënten met een solitaire huidafwijking die spontaan verdwenen of volledig geëxcideerd is, in toenemende mate wordt afgezien van een beenmergbiopt. Omdat getallen over het voorkomen van beenmergbetrokkenheid bij patiënten met een ALCL in de huid echter niet bekend zijn, hebben we in **hoofdstuk 4** de resultaten van beenmergonderzoek in 107 patiënten die volledig gestageerd waren, retrospectief geanalyseerd. Deze resultaten werden gecorreleerd met uitkomsten van overige stageringsonderzoeken (laboratorium- en beeldvormend onderzoek) en overlevingsdata. Bij 20 patiënten werden op het moment van diagnose ook lymfoomlokalisaties elders in het lichaam gevonden, echter het beenmerg bleek nooit betrokken. Bovendien ontwikkelde slechts één patiënt beenmergbetrokkenheid gedurende de follow-up. Deze patiënt had behalve beenmergbetrokkenheid ook lokalisaties van het lymfoom in verschillende lymfeklieren, de milt en de neusamandel. Deze studie toont aan dat het beenmergbiopt slechts een beperkte waarde heeft bij de stagering van patiënten die zich met een ALCL in de huid presenteren en dat een beenmergbiopt alleen overwogen hoeft te worden wanneer andere stageringsonderzoeken aanwijzingen geven voor lokalisaties van het lymfoom buiten de huid.

De moleculaire mechanismen verantwoordelijk voor het gunstige beloop van C-ALCL

Illustratief voor het klinisch gunstige beloop van C-ALCL is de neiging tot spontane remissie, dat bij 20-40% van de patiënten optreedt. Wanneer we de mechanismen kennen die hiervoor verantwoordelijk zijn, kunnen deze wellicht gebruikt worden als startpunt voor de ontwikkeling van geneesmiddelen voor patiënten zonder spontane remissie van de huidafwijkingen, zowel bij C-ALCL als andere agressieve CTCL. Recent is meer aandacht gekomen voor de rol van miRNAs bij de ontwikkeling van lymfomen. MiRNAs zijn kleine stukjes niet-coderend RNA die de expressie van genen kunnen reguleren. In **hoofdstuk 5** hebben we daarom de miRNA expressie profielen in biopten van 14 C-ALCL patiënten onderzocht en vergeleken met 12 biopten van goedaardige ontstekingsziekten van de huid (benigne controles). We vonden significante verschillen in expressie van 13 miRNAs tussen C-ALCL en de benigne controles door gebruik te maken van miRNA microarrays, een platform waarmee in één keer heel veel (bekende) miRNAs tegelijkertijd onderzocht kunnen worden. Van deze miRNAs hebben we de verhoogde expressie van miR-155, miR-27b, miR-30c en miR-29b in C-ALCL ten opzichte van de benigne controles bevestigd op een onafhankelijke groep van 7 C-ALCL patiënten en 11 benigne controles met een onafhankelijke techniek: miRNA-Q-PCR. We hebben de miRNA expressie profielen van C-ALCL ook vergeleken met die van 19 patiënten met tumor stadium MF, die uit een eerdere studie reeds bekend waren. Hoewel er tussen beide CTCLs met miRNA microarrays geen significante verschillen werden gevonden, werd er met miRNA-Q-PCR een significant hogere expressie van miR-155, miR-27b, miR-93, miR-29b en miR-92a gevonden in C-ALCL ten opzichte van tumor stadium MF. Dit toont aan dat microarrays goed gebruikt kunnen worden als een screenende techniek, maar voor het identificeren van subtiele verschillen in expressie tussen bepaalde lymfomen tekort kunnen schieten. In het algemeen zouden de verschillen in miRNA expressie tussen C-ALCL en tumor stadium MF op een verschillende rol in de pathogenese van deze CTCLs kunnen wijzen en gebruikt kunnen worden als uitgangspunt voor functionele studies die het gunstige klinische beloop van C-ALCL kunnen verklaren.

De prognostische betekenis van CD30 expressie bij patiënten met getransformeerde MF

Het merendeel van de patiënten met MF heeft een klinisch beloop dat gekenmerkt wordt door de langzame ontwikkeling van oppervlakkige eczemateuze huidafwijkingen (patches) die langzaam steeds dikker worden (plaques). Een klein deel van de patiënten ontwikkelt op termijn ook tumoren en lokalisaties buiten de huid. Er kunnen zich gedurende deze ziekte ook op cellulair niveau veranderingen voordoen zoals blastaire transformatie van de kwaadaardige witte bloedcellen. In de literatuur spreekt men van blastaire transformatie bij MF indien bij histologisch onderzoek het infiltraat meer dan 25% blasten bevat. Blastaire transformatie bij MF is geassocieerd met een agressief klinisch beloop en een slechte prognose. De blastaire tumorcellen kunnen al dan niet CD30 tot expressie brengen. Vanwege de gunstige prognose van C-ALCL, waarbij CD30 expressie het belangrijkste kenmerk is, hebben wij in **hoofdstuk 6** retrospectief de prognostische betekenis van CD30 expressie bij 100 patiënten met getransformeerde MF onderzocht. Daarnaast hebben wij de prognostische waarde van andere factoren onderzocht die in de literatuur gesuggereerd worden, maar vaak niet hard gemaakt konden worden vanwege te kleine patiëntengroepen. Patiënten met getransformeerde MF bleken inderdaad een ongunstig klinisch beloop te hebben met een ziektespecifieke 5-jaars overleving van 38%. In de 75 patiënten met alleen huidafwijkingen op het moment van transformatie bleken CD30 negativiteit, folliculotropie en uitgebreide(re) huidafwijkingen significant gecorreleerd met een slechtere prognose. Voor de volledige studiegroep van 100 patiënten betrof dit dezelfde factoren en tevens lokalisaties met transformatie buiten de huid. Het bleek dat patiënten met 0-1 prognostisch ongunstige factor(en) een significant betere overleving lieten zien dan patiënten met 2 of meer prognostisch ongunstige factoren. Dit gegeven kan gebruikt worden om een prognose van patiënten met getransformeerde MF te bepalen en de meest geschikte behandeling voor deze patiënten te selecteren.

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LIST OF ABBREVIATIONS

aCGH	Array-based comparative genomic hybridization
ALCL	Systemic anaplastic large cell lymphoma
ALK	Anaplastic lymphoma kinase
BCL2	B-cell CLL/lymphoma 2
BCL7a	B-cell CLL/lymphoma 7A
BID	Benign inflammatory dermatoses
C-ALCL	Primary cutaneous anaplastic large cell lymphoma
CBCL	Primary cutaneous B-cell lymphoma
CD30+ LPD	CD30+ lymphoproliferative disorders
CLA	Cutaneous lymphocyte antigen
CTCL	Primary cutaneous T-cell lymphoma
DCLG	Dutch Cutaneous Lymphoma Group
DSS	Disease-specific survival
EBV	Epstein-Barr virus
EMA	Epithelial membrane antigen
EORTC	European Organization for Research and Treatment of Cancer
FC	Fold-change
FFPE	Formalin fixed paraffin embedded
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
IFN	Interferon
IRF4	IFN regulatory factor 4
ISCL	International Society for Cutaneous Lymphomas
LCT	Large cell transformation
LyP	Lymphomatoid papulosis
MF	Mycosis fungoides
MF-TR	Transformed mycosis fungoides
miRNA	MicroRNA
MTX	Methotrexate
MUM1	Multiple myeloma antigen 1
NPM	Nucleophosmin
PPARγ	Peroxisome proliferator-activated receptor γ
PTL-NOS	Peripheral T-cell lymphoma, not otherwise specified
PUVA	Psoralen Ultra-Violet A
RT	Radiotherapy
SS	Sézary syndrome
TGF-beta	Transforming growth factor-beta
TNM	Tumor, Node, Metastasis
TRAF1	Tumor necrosis factor receptor associated factor 1
VZV	Varicella-Zoster virus
WHO	World Health Organization

CURRICULUM VITAE

Gineke Benner is geboren op 10 oktober 1979 te Barendrecht. Na het behalen van het Gymnasium diploma aan de Christelijke Scholengemeenschap Johannes Calvijn te Rotterdam in 1998, begon zij in datzelfde jaar met de studie Rechtsgeleerdheid aan de Universiteit Leiden. In 2000 startte zij met de studie Geneeskunde aan dezelfde universiteit. Gedurende haar wetenschapsstage verrichte zij onderzoek naar fenotypen van dystrofinopathieën in relatie tot duplicaties in het dystrofine-gen (dr. H.B. Ginjaar en dr. A.T.J.M. Helderman-van den Enden). Ook behaalde zij in 2005 het bachelordiploma Rechtsgeleerdheid.

Gedurende haar co-schappen liep zij een extra wetenschapsstage bij de afdeling Dermatologie van het Leids Universitair Medisch Centrum, waar zij na het behalen van haar artsexamen in juni 2008 werd aangesteld als AIOSKO (assistent in opleiding tot specialist en klinisch onderzoeker). Dit leidde tot het in dit proefschrift beschreven onderzoek onder begeleiding van prof. dr. R. Willemze. In november 2010 is zij gestart met de opleiding tot dermatoloog (opleider prof. dr. R. Willemze).

NAWOORD

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