Cutaneous B-cell lymphoma : classification, prognostic factors and management recommendations

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C'est le temps que tu as perdu pour ta rose
qui fait ta rose si importante.

Uit: "Le petit prince"
Antoine de Saint-Exupéry (1900-1944)

Voor Philippe

Contents

List of abbreviations

ABC	Activated B-cell
aCGH	Array-based comparative genomic hybridization
Bcl-2	B-cell CLL/lymphoma 2
BMB	Bone marrow biopsy
CBCL	Primary cutaneous B-cell lymphoma
cFCL	Cutaneous follicle centre lymphoma
CTCL	Primary cutaneous T-cell lymphoma
DCLG	Dutch Cutaneous Lymphoma Group
DLBCL	Diffuse large B-cell lymphoma
DSS	Disease-specific survival
EMZL	Extranodal marginal zone lymphoma
EORTC(-CLG)	European Organization for Research and Treatment of Cancer (–Cutaneous Lymphoma Group)
FL	Follicular lymphoma
FOXP1	Forkhead box P1
GCB	Germinal centre B-cell
ISCL	International Society for Cutaneous Lymphomas
MF	Mycosis fungoides
MLPA	Multiplex Ligation-dependent Probe Amplification
MUM-1	Multiple myeloma oncogene 1
NHL	Non-Hodgkin lymphoma
OS	Overall survival
PCFCCL	Primary cutaneous follicle centre cell lymphoma
PCFCL	Primary cutaneous follicle centre lymphoma
PCI	Primary cutaneous immunocytoma
PCLBCL-leg	Primary cutaneous large B-cell lymphoma of the leg
PCLBCL, LT	Primary cutaneous diffuse large B-cell lymphoma, leg type
PCLBCL, other	Primary cutaneous diffuse large B-cell lymphoma, other
PCMZL	Primary cutaneous marginal zone B-cell lymphoma
RFS	Relapse-free survival
RT	Radiotherapy
SS	Sézary syndrome
WHO	World Health Organization

Chapter 1.

General Introduction

Malignant lymphomas can involve the skin either primarily or secondarily. The term "primary cutaneous lymphoma" refers to various T-cell and B-cell malignancies that present in the skin without evidence of extracutaneous disease at the time of diagnosis. It is important to differentiate these primary cutaneous T-cell lymphomas (CTCL) and primary cutaneous B-cell lymphomas (CBCL) from their nodal counterparts, that can involve the skin secondarily, since they have a completely different clinical behaviour and prognosis and most often require different therapeutic approaches. Primary cutaneous lymphomas are the second most common group of extranodal lymphomas after the lymphomas of the gastrointestinal tract, with an estimated annual incidence of 1:100.000. While in systemic non-Hodgkin lymphomas the large majority is derived from a population of atypical B-cells, in the skin only 20-25% are CBCL; the remainder mainly consist of CTCL.[1] In the United States the reported incidence of CBCL is even lower, being approximately 4.5%.[2]

Until recently, there has been much controversy regarding the terminology and classification of CBCL, which may have come to an end with the publication of the WHO-EORTC consensus classification for cutaneous lymphomas.[3] In this classification three main types of CBCL are described: primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle centre lymphoma (PCFCL) and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT). In addition, a category of primary cutaneous diffuse large B-cell lymphoma, other (PCLBCL, other) was included.

This thesis includes a number of clinical, clinicopathologic and molecular studies in different groups of CBCL. The aims of these studies were (1) to validate the clinical and prognostic significance of the WHO-EORTC classification, (2) to identify and validate (new) diagnostic and prognostic markers, (3) to evaluate the clinical significance of current staging procedures and of a new clinical staging system in CBCL, (4) to evaluate the efficacy of radiotherapy in the treatment of different types of CBCL, and (5) to formulate consensus recommendations for the management of these diseases.

In this introductory chapter a brief historical overview of the classification of CBCL and a short description of the different types of CBCL as currently recognized in the WHO-EORTC classification, will be presented first. In addition, the background of the issues investigated in this thesis will be discussed.

Historical overview of the classification of CBCL

Until the early 1980's CBCL were not recognized as distinct disease entities. Malignant B-cell proliferations in the skin were considered invariably as manifestations of systemic disease and classified by systems used by hematopathologists for systemic lymphomas. Patients with a B-cell proliferation in the skin who showed a good clinical response to local treatment and had a favourable prognosis, were regarded as having a benign condition (so called pseudolymphoma).[4-7]

In the late seventies, the introduction of immunohistochemistry had a major impact on the diagnosis and classification of B-cell proliferations in the skin. By accepting monotypic immunoglobulin light chain expression on tissue sections of skin biopsies as golden standard for the diagnosis of malignant B-cell lymphoma, it became possible to distinguish between malignant B-cell lymphomas and reactive B-cell proliferations. Moreover, it became clear that B-cell lymphomas can be confined to the skin without any systemic disease being present. This simple distinction between primary and secondary cutaneous B-cell lymphomas proved extremely important. According to the criteria of the **Kiel classification**, that was in use in these days, most CBCL were classified as immunocytoma, centroblastic/centrocytic lymphoma, centroblastic lymphoma or immunoblastic lymphoma. Studies on well-defined groups of patients showed that these different types of CBCL had a highly distinctive clinical presentation and often another clinical behaviour and prognosis as compared to their systemic counterparts. These studies led to the delineation of distinct types of CBCL, which were however not recognized separately in the existing classification schemes used for nodal lymphomas, and were therefore often treated inappropriately. In 1994, Willemze and co-workers proposed a separate classification scheme for primary cutaneous lymphomas, thereby using the terminology of the updated Kiel classification to define distinct disease entities (see Table 1).[8] This proposal was extensively discussed within the EORTC Cutaneous Lymphoma Group (EORTC-CLG) and was formalised in 1997 with the publication of the **EORTC classification for primary cutaneous lymphomas**.[1] In this classification three main types of CBCL were distinguished: primary cutaneous immunocytoma, later called primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle centre cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg). The distinction between the latter two groups was based on the observation that patients with tumours on the leg(s) had a much more aggressive clinical behaviour as compared to patients presenting with tumours on the head or trunk.[9]

In 2001 the **WHO classification for tumours of hematopoietic and lymphoid tissues** was published as a successor of the REAL classification.[10;11] This classification did not recognize CBCL as distinct disease entities. PCMZL was included in the broad category of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. PCFCCL with a (partially) follicular growth pattern, were classified as cutaneous

follicle centre lymphoma, which was considered as a variant of follicular lymphoma (FL), while PCFCCL with a diffuse growth pattern were classified as diffuse large B-cell lymphoma (DLBCL). The group of PCLBCL-leg was neither acknowledged as a distinct disease entity and was also classified as DLBCL. The classification of CBCL according to the different classification schemes is presented in Table 1.

The differences in terminology and definition of the three main types of CBCL between the EORTC and WHO classifications resulted in much debate and confusion.[12-15] The main point of discussion was formed by the group of PCFCCL. While this term resembles that of FL in the WHO classification, it is defined in a different way. FL denotes a neoplasm of follicle centre B-cells (a mixture of centrocytes and centroblasts), which has at least a partially follicular growth pattern.[11] FL is graded by the proportion of centroblasts, using a 3-grade system. The histological grade correlates with prognosis, with grades 1 and 2 showing an indolent clinical course, and grade 3 being more aggressive. The majority of FL is characterized by the presence of the characteristic chromosomal translocation t(14;18), resulting in overexpression of the anti-apoptotic protein Bcl-2.[16-19] In contrast, the term PCFCCL was introduced as an encompassing term for cutaneous lymphomas composed of cells with the morphology of follicle centre B-cells (centrocytes and centroblasts), regardless of their growth pattern.[20-23] Moreover, PCFCCL had an indolent clinical behaviour, irrespective of the proportion of centroblasts and thus, histological grading had no prognostic significance. In addition, PCFCCL generally showed no expression of Bcl-2 and the t(14;18) could only be identified in a small minority of cases.[24-26] However, because of the rarity of the disease, (hemato)pathologists were generally not familiar with this entity, and PCFCCL with a diffuse growth pattern were generally classified as DLBCL, leading to unnecessary aggressive treatment with multiagent chemotherapy.

To put an end to this ongoing controversy, in 2003 and 2004 consensus meetings were organized, in which representatives of the EORTC and the WHO systems succeeded to combine the best of the two systems and to reach agreement on a consensus classification, which was termed the **WHO-EORTC classification for cutaneous lymphomas.** In this classification four categories of CBCL were recognized, which will be discussed below and are summarized in Table 2.

CBCL in the WHO-EORTC classification for cutaneous lymphomas

Primary Cutaneous Marginal Zone B-cell Lymphoma (PCMZL), is an indolent lymphoma composed of small B cells including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells and plasma cells. The marginal zone cells express CD20, CD79a and Bcl-2, but are negative for CD5, CD10 and Bcl-6.[27;28] The plasma cells show monotypic cytoplasmic immunoglobulin light chain expression (kappa or lambda) on paraffin sections. This category includes cases previously classified as primary cutaneous

immunocytoma[29], cases of cutaneous follicular lymphoid hyperplasia with monotypic plasma cells[29;30] and rare cases of primary cutaneous plasmacytoma.[31] Clinically, PCMZL are characterized by red to violaceous papules, plaques or nodules preferentially localized on the trunk or extremities. They have a tendency to recur in the skin, but extracutaneous dissemination is exceedingly rare, which is reflected in the excellent 5-year survival rate of close to 100%.[29;32-35]

Primary Cutaneous Follicle Centre Lymphoma (PCFCL) is defined as a tumour of neoplastic follicle centre cells, usually a mixture of centrocytes (small and large cleaved follicle centre cells) and variable numbers of centroblasts (large noncleaved follicle centre cells with prominent nucleoli). It includes cases with a follicular, a follicular and diffuse and a diffuse growth pattern. The neoplastic follicle centre cells express CD20, CD79a and Bcl-6. CD10 is particularly observed in cases with a (partly) follicular growth pattern. Recent gene expression studies revealed that these PCFCL have the gene expression profile of germinal center B-cell (GCB)- like DLBCL.[36] Consistently, the neoplastic B-cells generally do not express Bcl-2, MUM-1 and FOXP1.[24;27;28;36-40] Clinically, PCFCL generally present with solitary or grouped plaques and tumours, preferentially located on the head or trunk.[20;22;23;41] Extracutaneous dissemination is uncommon and their prognosis is excellent, irrespective of the growth pattern or the number of blast cells (5-year survival of more than 95%).[20;22;23;38;40-42]

Primary Cutaneous diffuse Large B-cell Lymphoma, Leg Type (PCLBCL, LT) are diffuse large B-cell lymphomas with a predominance or confluent sheets of centroblasts and immunoblasts. These neoplastic cells express CD20, CD79a and, since PCLBCL, LT have the gene expression profile of activated B-cell (ABC)-like DLBCL, the great majority of cases strongly express Bcl-2, MUM-1 and FOXP1.[36] Bcl-6 is expressed in most cases, while CD10 is generally absent.[28;36;39;43-45] Characteristically, they present with tumorous skin lesions on the (lower) leg(s), but uncommonly can arise at other sites as well. The disease affects predominantly elderly patients, and females are more affected than males. These lymphomas often disseminate to extracutaneous sites and have an intermediate prognosis with a 5-year survival of approximately 50%.[9;43;46;47]

Primary Cutaneous diffuse Large B-cell Lymphoma, other (PCLBCL, other) is used for rare cases of primary cutaneous diffuse large B-cell lymphomas that do not belong to the group of PCFCL or PCLBCL, LT. These include morphologic variants, such as rare cases of anaplastic or plasmablastic lymphoma and intravascular large B-cell lymphomas presenting primarily in the skin. These latter entities are exceedingly rare and will not further be discussed in this thesis.

Table 1.

Classification of primary cutaneous B-cell lymphomas according to the Kiel classification, the EORTC classification, the WHO classification and the WHO-EORTC classification

Kiel classification	EORTC classification	WHO classification	WHO-EORTC classification
Immunocytoma	Primary cutaneous immunocytoma/ marginal zone B-cell lymphoma	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	Primary Cutaneous Marginal Zone B-cell Lymphoma (PCMZL)
Centroblastic/ centrocytic or centroblastic lymphoma	Primary cutaneous follicle centre cell lymphoma	Cutaneous follicle centre lymphoma* Diffuse large B-cell lymphoma#	Primary Cutaneous Follicle Centre Lymphoma (PCFCL)
Centroblastic lymphoma Immunoblastic lymphoma	Primary cutaneous large B-cell lymphoma of the leg	Diffuse large B-cell lymphoma	Primary Cutaneous diffuse Large B-cell Lymphoma, Leg Type (PCLBCL, LT)

* Cases with a (partly) follicular growth pattern

Cases with a diffuse growth pattern

Outstanding issues

At the time the WHO-EORTC classification was published, there were still several outstanding issues requiring additional study.

First, the definitions of PCFCL and PCLBCL, LT in the WHO-EORTC classification imply that differentiation between PCFCL and PCLBCL, LT is determined above all by morphological criteria, namely the absence or presence of confluent sheets of centroblasts and/or immunoblasts (designated previously as cleaved cell versus round cell morphology[46]) and no longer by site (non-leg versus leg) as in the EORTC classification.[1;9;48] This implies that a proportion of patients classified as PCFCCL or PCLBCL-leg in the EORTC classification will be assigned to another prognostic category, which may have important therapeutic consequences. Recent studies suggest that this concerns 10-15% of PCFCCL and PCLBCL-leg, but exact data are not available.[49;50]

Second, recent studies also illustrated that the interpretation of the group of PCLBCL, other is defined in different ways. Some authors exclude rare Bcl-2 negative cases from the group of PCLBCL, LT and include them in the heterogeneous group of PCLBCL, other[49], whereas others include all cases with characteristic round-cell morphological features within the group of PCLBCL, LT, irrespective of Bcl-2 expression.[50]

Third, previous studies have suggested several clinicopathologic and immunophenotypical parameters with prognostic significance in patients with CBCL.[44;46;51-54] The most interesting findings were that strong expression of Bcl-2 is associated with an unfavorable prognosis in PCFCL cases with a diffuse growth pattern[44] and that inactivation of CDKN2A is a negative prognostic predictor in PCLBCL, LT patients.[55] However, most of these studies were based on cases classified according to older classifications systems, on mixed groups of patients or, as in the latter study, on only a small number of patients.

Therefore, in the study described in **Chapter 2** we reviewed clinical data and histologic sections of 300 CBCL patients included in the database of the Dutch Cutaneous Lymphoma Group (DCLG) who were originally classified by the EORTC classification, and reclassified all cases according to the criteria of the WHO-EORTC classification as well as those of the WHO classification. The aims were to (1) assess the clinical significance of this new classification; (2) determine the percentage of CBCL patients assigned to a different prognostic category when compared with the EORTC and WHO schemes; (3) define more precisely the clinicopathologic features and prognostic factor of the newly defined categories; and (4) establish more clearly which cases should be assigned to the group of PCLBCL, other.

In addition, in **Chapter 3** we aimed to confirm inactivation of CDKN2A as a prognostic marker for the group of PCLBCL, LT on a larger patient group. This study describes the results of an European multicenter study, conducted within the framework of the EORTC-CLG.

Staging of cutaneous B-cell lymphomas

The TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary Syndrome

Staging of cancer is important in the appropriate and effective care of patients. The goals of a staging system for cutaneous lymphoma are to offer a clinically reasonable basis for appropriate management, to predict prognosis, and to facilitate comparison of results among different therapies and different institutions. The most widely used staging system for lymphoma is the Ann Arbor system, which was first introduced as a staging system for Hodgkin lymphoma in 1971.[56;57] Although its utility for the staging of other lymphomas has been challenged, it is still the primary means for classifying patients with non-Hodgkin lymphomas (NHL).[58] The Ann Arbor system is primarily an anatomical assessment of disease with modifications by presence or absence of systemic symptoms. However, it does not distinguish consistently between patients with different prognoses. Thus, in 1993, the International Prognostic Index was established to supplement the Ann Arbor staging to aid in the treatment decision or stratification of patients in clinical trials for patients with lymphoma.[59] However, the Ann Arbor system has a number of shortcomings, especially when staging lymphomas that arise primarily in extranodal sites such as the skin. 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), thus disproportionately or inappropriately placing high number of patients in the highest stage, resulting in unnecessarily aggressive treatments.

The TNM classification system is the most widely used means for classifying the extent of nonlymphoid malignant disease. An adjusted TNM + B (blood) system has been in use for nearly 30 years to stage patients with mycosis fungoides (MF) and Sézary

syndrome (SS), both forms of CTCL[60], and this system has recently been revised and updated.[61] However, this system is not appropriate for staging of other forms of primary cutaneous lymphomas. Therefore, representatives of the International Society for Cutaneous Lymphomas (ISCL) and EORTC-CLG recently published a consensus proposal for a TNM classification system applicable to all types of primary cutaneous lymphomas other than MF/SS.[62] This proposed TNM system is primarily meant to document extent of disease in a consistent matter, thereby facilitating comparison of studies at different institutes. Studies investigating the applicability of this TNM system on a group of CBCL have not been performed. Moreover, the prognostic significance of this proposed system for CBCL was unknown. In the study described in **Chapter 4** we therefore tested its clinical applicability on the large group of CBCL which were reclassified according to the WHO-EORTC classification as described in **Chapter 2**, and evaluated its prognostic relevance for the group of CBCL. In addition, these results were compared with the scoring of disease extent as used by the Dutch Cutaneous Lymphoma Group (DCLG) since many years.

Bone marrow examination in the staging of cutaneous B-cell lymphomas

Ever since the first introduction of a separate classification for primary cutaneous lymphomas, it was recognized that a diagnosis of true primary cutaneous lymphoma can only be made after adequate staging investigations have been performed. These include a thorough physical examination, laboratory studies, imaging techniques and a bone marrow biopsy (BMB). However, in the paper introducing the TNM staging system for primary cutaneous lymphomas other than MF and SS, described previously[62], it was for the first time suggested that bone marrow examination was recommended, but not required in indolent types of primary cutaneous lymphoma, including PCMZL and PCFCL. However, in most clinical practice guidelines for NHL, unilateral trephine BMB are part of the routine work-up in all patients with (a suspicion of) NHL. Moreover, the recommendation that a BMB is not required in indolent types of CBCL is confusing, as studies describing these PCMZL and PCFCL as indolent lymphomas, required negative staging procedures, including a negative BMB.[3]

Studies on the frequency of bone marrow involvement in this specific group of patients have never been performed. In the study described in **Chapter 5** we evaluated the frequency of bone marrow involvement in a large group of patients with histological skin features consistent with a marginal zone lymphoma (MZL) or follicle centre lymphoma (FCL), in order to determine whether our current policy to perform a BMB in all patients with a histological diagnosis of cutaneous MZL or FCL should be maintained.

Treatment of primary cutaneous B-cell lymphomas

It is generally recognized that PCMZL and PCFCL are indolent types of CBCL, which should not be treated primarily with systemic chemotherapy, and that PCLBCL, LT is characterized by a more unfavourable prognosis, justifying more aggressive therapeutic approaches. In recent years various new therapies for the treatment of CBCL, like rituximab and interferon alpha, have become available, supplementing traditional therapies like, surgical excision, local radiotherapy (RT), systemic multiagent chemotherapy or combinations of the latter two. However, the most appropriate therapy for each of the newly defined entities remains to be determined.

RT is the most widely applied and best known treatment in CBCL. Already in 1951 Crosti described seven cases of "reticulohistiocytoma of the dorsum" that were markedly sensitive to radiation therapy.[63] This entity is nowadays classified as PCFCL. Previous studies have shown that RT is a suitable treatment for both PCFCL as well as PCMZL, and perhaps also for PCLBCL, LT presenting with solitary or localized skin lesions.[23;41;42;64;65] However, data from the literature regarding efficacy and relapse rate show a wide variation.[41;42;64;66-69] Moreover, these studies are all based on cases classified according to the EORTC or WHO schemes, and it might be expected that a number of cases would be assigned to a different prognostic category, using the WHO-EORTC classification. These observations prompted us to evaluate the results of RT as initial treatment in a large group of CBCL classified according to the WHO-EORTC classification. The aim of this study, described in **Chapter 6**, was to define remission, relapse and survival rates of these newly defined groups after treatment with RT, and to establish for which patients this is a safe and effective treatment.

In summary, with the advent of the WHO-EORTC consensus classification for cutaneous lymphomas in 2005, uniform terminology and classification for this rare group of neoplasms was introduced. However, staging procedures and treatment strategies still varied between different cutaneous lymphoma centres, which may be due to the fact that consensus recommendations for the management of CBCL have never been published. Following the results of **Chapters 4-6** and based on an extensive literature review and discussions among experts in the field of cutaneous lymphoma, collaborating in the EORTC-CLG and the ISCL, we formulated consensus recommendations for the management of the three main groups of CBCL, which are described in **Chapter 7**.

Aims and outline of the thesis

The studies presented in this thesis have aimed to address outstanding questions regarding the classification, prognostication, staging and treatment of CBCL.

Chapter 2 investigates the clinical applicability of the WHO-EORTC classification on a group of 300 patients with CBCL and determined the percentage of patients that was assigned to a different prognostic category using this classification as compared to the previously used EORTC and WHO classifications. Furthermore, clinicopathologic features and prognostic markers for the new WHO-EORTC categories and more precise criteria for the group of PCLBCL, other are defined.

Chapter 3 investigates if previously reported findings of inactivation of the CDKN2A gene as a prognostic marker can be confirmed on a large group of PCLBCL, LT and aims to further fine-map the aberrations found in this region by using MLPA.

Chapter 4 evaluates the clinical applicability and prognostic significance of the newly proposed TNM classification system for primary cutaneous lymphomas other than MF and SS on a group of 300 CBCL patients classified according to the new WHO-EORTC criteria and in addition, compares this classification system with the system used by the Dutch Cutaneous Lymphoma Group.

Chapter 5 investigates the frequency of bone marrow involvement in patients presenting with skin lesions that show histological features suggesting an indolent type of cutaneous B-cell lymphoma and evaluates whether the current practice to perform bone marrow biopsies in all these patients should be maintained.

Chapter 6 retrospectively analyzes the results of radiotherapy in a large group of CBCL classified according to the WHO-EORTC classification and establishes for which groups of patients this is a safe and effective treatment.

Chapter 7 provides a review concerning consensus recommendations for the management of CBCL, which were based on an extensive literature study and discussions among a broad panel of dermatologists, haematologists and (radiation) oncologists involved in the treatment of CBCL patients.

Chapter 8 summarizes and discusses the findings described in the preceding chapters.

Table 2.

Clinicopathologic features of the three main groups of primary cutaneous B-cell lymphomas recognized in the WHO-EORTC classification.

	Primary cutaneous marginal zone B-cell lymphoma	Primary cutaneous follicle centre lymphoma	Primary cutaneous diffuse large B-cell lymphoma, leg type
Clinical features	• solitary or multiple papules, plaques or nodules preferentially localized on the trunk or extremities • frequent cutaneous relapses • rarely extracutaneous dissemination	• solitary or grouped tumors presenting on the head or on the trunk • cutaneous relapses in 20% • extracutaneous dissemination in 5-10%	• solitary or multiple tumors presenting mainly on the leg(s) and rarely at other sites • frequent relapses and extracutaneous dissemination
Histopathology	patchy or diffuse infiltrates composed of small B-cells, including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells and plasma cells	follicular, follicular and diffuse or diffuse infiltrates composed of neoplastic follicle center cells , usually a mixture of centrocytes and variable numbers of centroblasts	diffuse infiltrates with a predominance or confluent sheets of of centroblasts and immunoblasts
Gene expression profile	Not applicable	Germinal centre B-cell (GCB)-like	Activated B-cell (ABC)-like
Immunophenotype	monotypic cIg, CD79a+, Bcl-2+, CD5-, cyclin D1-, Bcl-6-, CD10-, MUM-1+ (on plasma cells)	monotypic sIg or absence of sIg, CD20+, CD79a+, Bcl-6+, CD10+/-, Bcl-2-, MUM-1-, FOXP1- /(+/-)	monotypic sIg and/or cIg, CD20+, CD79a+, Bcl-6 +/(-), CD10-, Bcl-2+, MUM-1+, FOXP1+
Prognosis	5-year survival: 99%	5-year survival: 95%	5-year survival: 55%

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Chapter 2.

Reclassification of 300 primary cutaneous B-cell lymphomas according to the new WHO-EORTC classification for cutaneous lymphomas: comparison with previous classifications and identification of prognostic markers

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Abstract

PURPOSE: In the new WHO-EORTC classification for cutaneous lymphomas three major groups of primary cutaneous B-cell lymphoma (CBCL) are distinguished: primary cutaneous marginal zone B-cell lymphoma (PCMZL) and primary cutaneous follicle center lymphoma (PCFCL) with a good prognosis, and primary cutaneous large B-cell lymphoma, leg type (PCLBCL, LT) with an intermediate prognosis. This study aimed to assess the clinical significance of the new classification as compared to previous classification schemes (EORTC 1997; WHO 2001) and to define prognostic factors within the newly defined categories.

PATIENTS AND METHODS: In the present study clinical data and histological sections of 300 CBCL formerly classified according to the EORTC classification were reviewed and reclassified according to the WHO and the new WHO-EORTC classification schemes.

RESULTS: After reclassification the study comprised 71 PCMZL, 171 PCFCL and 58 PCLBCL, LT showing 5-year disease specific survivals of 98%, 95% and 50%, respectively. As compared with the EORTC and WHO schemes 5.3% and 36.3% of CBCL were reclassified into another prognostic category. Multivariate analysis of PCFCL revealed localization on the leg and expression of FOXP1 as independent parameters associated with a poor prognosis. Expression of Bcl-2 or MUM-1 had no significant effect on survival in this group. In PCLBCL, LT no independent prognostic parameters were found.

CONCLUSIONS: These results emphasize the clinical significance of the WHO-EORTC classification, but suggest that within the group of PCFCL distinction should be made between cases presenting on the legs and cases presenting at other sites.

Introduction

In recent years, there has been considerable debate regarding the classification and terminology of the group of primary cutaneous B-cell lymphomas (CBCL).[1-4] This controversy, which resulted from differences between the EORTC and WHO schemes in the classification of CBCL, may have come to an end with the recent introduction of the new WHO-EORTC classification for cutaneous lymphomas.[5-8] In this new classification, three main types of CBCL are recognized: primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous large B-cell lymphoma, leg type (PCLBCL, LT). In addition, it contains a category called primary cutaneous large B-cell lymphoma, other (PCLBCL, other) for diffuse large B-cell lymphomas, which do not fit in the group of PCLBCL, LT or the group of PCFCL with a diffuse infiltration of large centrocytes.

PCFCL is defined as a tumor of neoplastic follicle center cells, which may show a follicular, a follicular and diffuse or diffuse growth pattern, and which is predominantly composed of generally large centrocytes (large cleaved cells). In most cases the tumor cells do not express Bcl-2 and MUM-1. PCLBCL, LT is defined by a predominance or confluent sheets of medium-sized to large B-cells with round nuclei resembling centroblasts and/or immunoblasts, which generally show strong expression of Bcl-2 and MUM-1. These definitions imply that differentiation between PCFCL and PCLBCL, LT is determined above all by morphological criteria, namely the absence or presence of confluent sheets of centroblasts and/or immunoblasts (designated previously as cleaved cell versus round cell morphology[9]) and no longer by site (non-leg versus leg) as in the EORTC classification.[7;10;11] This implies that a proportion of patients classified as primary cutaneous follicle center cell lymphoma (PCFCCL) or primary cutaneous large B-cell lymphoma of the leg (PCLBCL, leg) in the EORTC classification will be assigned to another prognostic category, which may have important therapeutic consequences. Recent studies suggest that this concerns 10-15% of PCFCCL and PCLBCL, leg, but exact data are not available. [12;13]These studies also illustrate that the definition of the group of PCLBCL, other leaves room for different interpretations.

In the present study clinical data and histologic sections of 300 CBCL included in the registry of the Dutch Cutaneous Lymphoma Group and originally classified by the EORTC classification, were reviewed and reclassified by the new WHO-EORTC classification as well as by the WHO classification. The aims of this study were: 1. to assess the clinical significance of this new classification; 2. to determine the percentage of CBCL patients assigned to a different prognostic category as compared to the EORTC and WHO schemes; 3. to define more precisely the clinicopathologic features and prognostic factors of the newly defined WHO-EORTC categories; 4. to establish whether PCLBCL, LT not expressing Bcl-2 should be assigned to the group of PCLBCL, other, as suggested previously.[12]

Patients and Methods

Patient selection

Between 1985 and 2005, 320 CBCL, including 72 PCMZL/ PCI (primary cutaneous immunocytomas), 185 PCFCCL and 63 PCLBCL, leg, had been included in the registry of the Dutch Cutaneous Lymphoma Group. All cases had been classified by an expert panel of dermatologists and pathologists at the time of diagnosis using the criteria of the EORTC classification.

In all cases, the presence of extracutaneous disease at the time of diagnosis had been excluded by standard staging procedures including computerized tomography of thorax and abdomen, and bone marrow cytology and histology. Follow-up data had been collected yearly for each patient.

Histological review

For each case, hematoxylin-eosin stained sections, routine immunostainings and paraffin blocks were collected from the archives of the participating or referring departments. If paraffin blocks were available, additional stainings for Bcl-2, Bcl-6, Ki-67 (Dako, Glostrup, Denmark), CD10 and CD35 (Novocastra, Newcastle upon Tyne, United Kingdom) and in cases of PCFCCL and PCLBCL, leg also for MUM-1 (Dako, Glostrup, Denmark) and FOXP1 (kindly provided by Dr. A. Banham, Nuffield Department of Clinical and Laboratory Sciences, Oxford, United Kingdom) was performed, using standard techniques described previously.[14;15]

Bcl-2, Bcl-6 and CD10 staining was considered positive if more than 50% of the neoplastic B-cells showed an unequivocal positive staining.[12;16;17] In case of CD10, only a membranous staining pattern on the tumor cells was considered positive. For expression of MUM-1, nuclear staining of more than 30% of the neoplastic B-cells was considered positive.[18;19] Consistent with prior studies[12;20] FOXP1 was scored into 3 groups: positive (> 90% strongly positive cells), weak (20-90% weakly-moderately positive cells) and negative (< 20% weakly stained cells).

From the initial group of 320 patients 10 patients were excluded because of incomplete clinical details or lack of follow-up. Another 10 cases were excluded because histological sections were either not available or of insufficient quality to allow proper reclassification. The final study group comprised 300 CBCL including 71 PCMZL, 169 PCFCCL and 60 PCLBCL, leg (see Table 1). These 300 cases were reclassified as PCMZL, PCFCL or PCLBCL, LT following the WHO-EORTC classification, and as extranodal marginal zone B-cell lymphoma (EMZL), cutaneous follicle center lymphoma (cFCL) or diffuse large B-cell lymphoma (DLBCL) using the WHO classification.

Statistical analysis

All statistical calculations were performed using SPSS 12.0.1 (SPSS Inc, Chicago, IL). Overall survival (OS) was calculated from the date of diagnosis until the patient's death or date of last follow-up. Disease specific survival (DSS) was calculated from the date of diagnosis until death due to lymphoma or date of last follow-up. Survival curves were estimated by the method of Kaplan and Meier and statistical comparison between curves was done by log-rank testing. Prognostic factors within the different entities were evaluated by univariate and multivariate analysis with OS and DSS as endpoints and *p* values below 0.05 were considered significant. Parameters included for univariate analysis were: age (≤ 70 vs. > 70 years), gender, localization of skin lesions (leg vs. non-leg), extent (solitary vs. localized vs. multifocal), growth pattern (only in PCFCL: follicular vs. follicular-diffuse vs. diffuse), Bcl-2 (positive vs. negative), MUM-1 (positive vs. negative), FOXP1 (positive vs. weak vs. negative). Multivariate analysis was performed with the use of a Cox proportional hazards regression model with a stepwise selection of the significant variables. Multivariate analysis was stratified for age. All other parameters included in the univariate analysis were introduced in the multivariate analysis and all parameters were categorical.

Results

Comparison between the EORTC, the WHO and the WHO-EORTC classification

This is the first study which allows direct comparison of the clinical significance of the EORTC[7], the WHO[5;7] and the WHO-EORTC[6;8] classification for the group of CBCL. The number of cases reclassified into another prognostic category are presented in Table 1 (EORTC versus WHO-EORTC) and Table 2 (WHO versus WHO-EORTC).

All 71 PCMZL/PCI in the EORTC classification were classified as EMZL in the WHO classification and as PCMZL using the criteria of the WHO-EORTC classification (Tables 1 and 2).

Of the 169 cases classified as PCFCCL in the EORTC classification, 62 cases showing a follicular (four cases; 2.4%) or a follicular and diffuse (58 cases; 34.3%) growth pattern, were classified as cFCL, while 107 cases (63.3%) showing a diffuse population of large centrocytes were classified as DLBCL using the WHO classification (Table 2). Following the WHO-EORTC classification the large majority of PCFCCL (162 of 169 cases: 95.9%) was reclassified as PCFCL. The remaining seven PCFCCL showing a predominance or confluent sheets of cells with a centroblast- or immunoblasts-like morphology were reclassified as PCLBCL, LT.

All 60 cases classified as PCLBCL, leg by the EORTC scheme were classified as DLBCL in the WHO classification. Fifty-one of 60 cases showed diffuse infiltrates of centroblasts and/or immunoblasts and were therefore classified as PCLBCL, LT following the WHO-

EORTC classification (Table 1). Nine of 60 cases (15.0%) showed diffuse infiltrates predominantly composed of large cleaved cells, in the absence of sheets or clusters of centroblasts and/or immunoblasts, and were therefore classified as PCFCL.

The 5-year OS for the three main types of CBCL according to the EORTC, WHO and WHO-EORTC classifications are presented in Figure 1.

Table 1.
Classification of 300 CBCL: comparison between the EORTC classification and the WHO-EORTC classification.

EORTC	*No*	*WHO-EORTC*	*No*
Primary cutaneous marginal zone B-cell lymphoma	71	Primary cutaneous marginal zone B-cell lymphoma	71
Primary cutaneous follicle center cell lymphoma	169	Primary cutaneous follicle center lymphoma	162
		Primary cutaneous large B-cell lymphoma, leg type	**7**
Primary cutaneous large B-cell lymphoma, leg	60	**Primary cutaneous follicle center lymphoma**	**9**
		Primary cutaneous large B-cell lymphoma, leg type	51

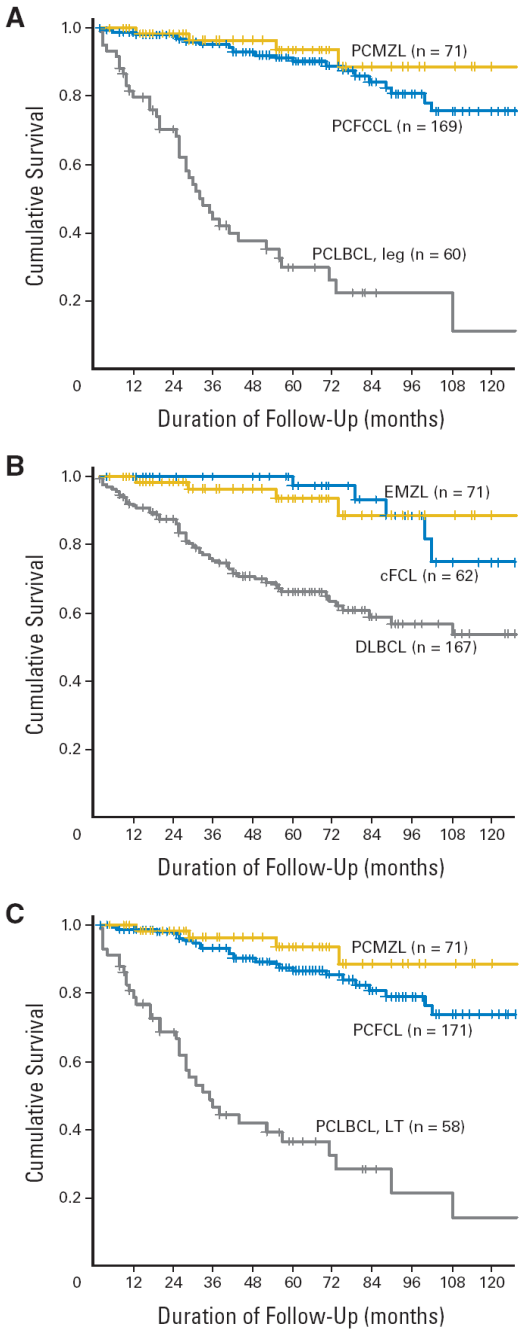
16 of 300 cases (5,3%) were reclassified into another prognostic category (indicated in bold).

Table 2.
Classification of 300 CBCL: comparison between the WHO classification and the WHO-EORTC classification.

WHO	*No*	*WHO-EORTC*	*No*
Extranodal marginal zone B-cell lymphoma	71	Primary cutaneous marginal zone B-cell lymphoma	71
Cutaneous follicle center lymphoma	62	Primary cutaneous follicle center lymphoma	62
Diffuse large B-cell lymphoma	167	**Primary cutaneous follicle center lymphoma**	**109**
		Primary cutaneous large B-cell lymphoma, leg type	58

109 of 300 cases (36,3%) were reclassified into another prognostic category (indicated in bold).

Figure 1. Overall survival of 300 CBCL classified according to the criteria of the EORTC classification (A), the WHO classification (B) and the WHO-EORTC classification (C).



Clinical features and prognostic parameters of the WHO-EORTC groups
After reclassification, the study group contained 71 PCMZL, 171 PCFCL and 58 PCLBCL, LT. The main clinical characteristics and follow-up data are presented in Table 3. In the following section relevant clinicopathologic and immunophenotypical data and their prognostic significance are presented.

PCMZL
Follow-up data of the 71 PCMZL showed development of extracutaneous disease in six of 71 (8.5%) cases, and overall and disease specific 5-year-survivals of 94% and 98%, respectively (Table 3). The clinicopathologic details of 50 of these 71 patients have been reported recently, and will therefore no further be discussed.[21]

PCFCL
This group contained 171 cases, including nine cases classified previously as PCLBCL, leg. Characteristically, most patients presented with localized lesions on the head (37.4%) or trunk (39.2%); 11 cases (5.8%) presented with skin lesions on the leg(s).
Histologically, a follicular, a follicular and diffuse or diffuse growth pattern was observed in 2.3% (4/171), 33.9% (58/171), and 63.7% (109/171) of cases.
Expression of Bcl-2 by more than 50% of the neoplastic B-cells was observed in 16 of 148 (10.8%) cases and expression of MUM-1 by more than 30% of the neoplastic B-cells in 13 of 128 (10.2%) cases (Table 4). Strong nuclear expression of FOXP1 was observed in only four of 104 (3.8%) cases. Bcl-6 and CD10 were expressed by 122 of 132 (92.4%) and 9 of 141 (6.0%) cases. CD10 expression was found in seven of 48 (14.6%) PCFCL with a follicular or follicular and diffuse growth pattern, and in only two of 93 (2.2%) PCFCL with a diffuse growth pattern. After a median follow-up period of 62 months (range, 2 – 336 month), 18 of 171 developed extracutaneous disease, and nine patients died of lymphoma, four of whom due to central nervous system involvement. The overall and disease specific 5-year survivals were 87% and 95%, respectively.

Prognostic factors
Univariate analysis of OS on the total group of PCFCL (n = 171) showed that the following variables were related to a shorter survival: age ($p < 0.0001$), female gender ($p = 0.028$), localization on the leg(s) ($p < 0.0001$), extent of skin lesions ($p = 0.045$) and expression of FOXP1 ($p = 0.002$). Growth pattern, expression of Bcl-2 or MUM-1 had no significant effect on OS. In multivariate analysis, only localization on the leg(s) ($p = 0.001$) and expression of FOXP1 ($p = 0.033$) remained significant independent factors associated with a poor prognosis. Both weak and positive expression of FOXP1 was associated with inferior prognosis as compared to the negative cases. PCFCL presenting with skin lesions on the leg(s) developed extracutaneous disease in five of 11 cases (46%) and had a 5-year OS and DSS of 22% and 41%, respectively, whereas PCFCL presenting at other sites than

the leg developed extracutaneous disease in 13 of 160 cases (8%) and showed 5-year OS and DSS of 92% and 98%, respectively (Figure 2). Multivariate analysis of OS in only those PCFCL with a diffuse infiltrate of large centrocytes (n = 109) revealed localization on the leg as the only independent parameter significantly related with a poor survival (p = 0.006).

PCLBCL, leg type

This group contained 58 cases, including seven cases classified previously as PCFCCL using the EORTC classification. Consistently, most cases presented with skin lesions on one (40 cases) or both legs (six cases) or with lesions on the leg(s) and other skin site(s) (five cases); seven patients had presented with skin lesions restricted to sites other than the leg.

Characteristically, the neoplastic B-cells strongly expressed Bcl-2 (44 of 49 cases; 89.8%), MUM-1 (36 of 40 cases; 90.0%) and FOXP1 (26 of 32 cases; 81.3%). Bcl-6 and CD10 were expressed in 30 of 44 (68.2%) and zero of 47 (0%) cases, respectively (Table 4).

After a median follow-up of 26 months (range 2 – 276 months), 27 of 58 cases had developed extracutaneous disease and 26 of 58 cases had died of lymphoma. The overall and disease specific 5-year-survivals were 37% and 50%, respectively.

Prognostic factors

In univariate analysis of OS only age (p = 0.041) and extent of skin lesions (p = 0.023) were associated with a poor prognosis. The 5-year OS for patients presenting with a solitary tumor was 70% as compared to 27% and 0% for patients presenting with localized or multifocal disease respectively. Gender, localization on the leg, expression of Bcl-2, MUM-1 and FOXP1 had no prognostic significance in this group. In multivariate analysis, no independent prognostic factors were found. Importantly, no differences in survival were found between PCLBCL, LT presenting on the leg(s) and PCLBCL, LT presenting at sites other than the leg (Figure 2).

Table 3. Clinical and treatment characetristics of 300 Dutch patients with CBCL.

	All patients		PCMZL		PCFCL		PCLBCL, leg type	
	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
Total no. of patients	300		71	23.7	171	57.0	58	19.3
Age, Years								
Median	62		53		58		78	
Range	21-92		23-87		21-89		42-92	
Gender								
Male	177	59.0	48	67.6	109	63.7	20	34.5
Female	123	41.0	23	32.4	62	36.3	38	65.5
Ratio M/F	1.4		2.1		1.8		0.5	
Site of cutaneous involvement								
Head/ neck	90	30.0	10	14.1	75	43.9	5	8.6
Trunk	139	46.3	39	54.9	93	54.4	7	12.1
Upper extremities	33	11.0	26	36.6	4	2.3	3	5.2
Lower extremities	81	27.0	19	26.8	11	6.4	51	87.9
Other	2	0.7	0	0	2	1.2	0	0
Extent of cutaneous involvement								
Single lesion	103	34.3	17	23.9	70	40.9	16	27.6
Regional	121	40.3	17	23.9	73	42.7	31	53.4
Multifocal	76	25.3	37	52.1	28	16.4	11	19.0
Treatment								
No therapy	19	6.3	10	14.1	7	4.1	2	3.5
Radiotherapy	174	58.0	31	43.7	111	64.9	32	55.2
Chemotherapy	42	14.0	5	7.0	23	13.5	14	24.1
Chemotherapy & radiotherapy	18	6.0	0	0	12	7.0	6	10.3
Surgery	21	7.0	9	12.7	11	6.4	1	1.7
Other	26	8.7	16	22.5	7	4.1	3	5.2
Clinical course after treatment								
Complete remission	276	92.0	58	81.7	169	98.8	49	84.5
Relapse rate*	118	42.8	33	56.9	51	30.2	34	69.4
Extracutaneous progression	51	17.0	6	8.5	18	10.5	27	46.6
Status at last follow-up								
Alive and well	202	67.3	49	59.0	136	79.5	17	29.3
Alive with disease	30	10.0	17	23.9	7	4.1	6	10.3
Died of lymphoma	36	12.0	1	1.4	9	5.3	26	44.8
Died of other cause	32	10.7	4	5.6	19	11.1	9	15.5
Survival								
5-yr overall survival		78.5		93.7		86.7		36.6
5-yr disease specific survival		87.2		98.0		94.5		50.2

* Relapses were only considered in patients who achieved a complete remission (n = 276).

Table 4. Expression patterns in PCFCL, PCLBCL, LT and subgroups

	PCFCL (total)	PCFCL (non-leg)	PCFCL (leg)	PCLBCL, LT (total)	PCLBCL, LT (leg)	PCLBCL, LT (non-leg)
Bcl-2						
Positive (%)	16/148 (11%)	13/140 (9%)	3/8 (38%)	44/49 (93%)	40/43 (93%)	4/6 (67%)
MUM-1						
Positive (%)	13/128 (10%)	9/121 (7%)	4/7 (57%)	36/40 (90%)	33/36 (92%)	3/4 (75%)
FOXP1						
Positive (%)	4/104 (4%)	3/99 (3%)	1/5 (20%)	26/32 (81%)	23/29 (79%)	3/3 (100%)
Weak (%)	25/104 (24%)	22/99 (22%)	3/5 (60%)	5/32 (16%)	5/29 (17%)	0/3 (0%)
Negative (%)	75/104 (72%)	74/99 (75%)	1/5 (20%)	1/32 (3%)	1/29 (3%)	0/3 (0%)
Bcl-6						
Positive (%)	122/132 (92%)	118/126 (94%)	4/6 (67%)	30/44 (68%)	26/39 (67%)	4/5 (80%)
CD10						
Positive (%)	9/141 (6%)	9/133 (7%)	0/8 (0%)	0/47 (0%)	0/42 (0%)	0/5 (0%)

Discussion

In this study histological sections of 300 Dutch CBCL patients, formerly classified according to the EORTC classification[7], were reviewed and classified according to the WHO[5] and the new WHO-EORTC classification.[6;8] Using the criteria of the WHO-EORTC classification PCMZL and PCFCL showed 5-year DSS of 98% and 95%, respectively, whereas PCLBCL, LT had a 5-year DSS of 50%. These results are in agreement with the results of recent studies and confirm that the new WHO-EORTC classification adequately distinguishes between CBCL with an indolent and CBCL with a more aggressive clinical behavior.[3;12;13]

Comparison between the three classification schemes showed no other than semantic differences in the classification of PCMZL and PCFCL with a follicular or follicular and diffuse growth pattern. However, with respect to CBCL with a diffuse population of large neoplastic B-cells 16 of 167 (9.6%) and 109 of 167 (65.3%) cases, classified according to the EORTC and WHO classification, respectively, were reclassified in another prognostic category. Seven cases originally classified as PCFCCL in the EORTC classification, were reclassified as PCLBCL, LT, because of the presence of confluent sheets of cells with a centroblast- or immunoblast-like morphology. Conversely, nine cases originally classified as PCLBCL, leg, showed diffuse infiltrates of large cleaved cells and were therefore reclassified as PCFCL. As compared to the WHO classification, 109 of 167 cases (65%) classified as DLBCL were reclassified as PCFCL, which implies that these patients should be treated primarily with radiotherapy rather than with multi-agent chemotherapy.[22-27] Taken together, these results illustrate that the WHO-EORTC classification is a major step forward, and may contribute to more appropriate treatment in patients with CBCL.
A second goal of the present study was to define prognostic parameters within the redefined categories PCFCL and PCLBCL, LT. Both in the total group of PCFCL and in the group of PCFCL with a diffuse infiltrate of large cleaved cells, site of clinical presentation appeared

the most important independent prognostic factor. Consistent with previous studies, PCFCL presenting on the leg had a much worse prognosis than PCFCL presenting at other sites, but similar to PCLBCL, LT[9;12] (see Figure 2).We therefore suggest that within the group of PCFCL further distinction should be made on the basis of site to prevent undertreatment of patients presenting with skin lesions on the leg. Expression of Bcl-2 and MUM-1 were no independent prognostic parameters, neither in the total group of PCFCL, nor in PCFCL with a diffuse infiltrate of large cleaved cells.
In the group of PCLBCL, LT the extent of skin lesions at the time of diagnosis and age were the only prognostic parameters. Comparison of Bcl-2 positive and Bcl-2 negative PCLBCL, LT showed no significant difference in 5-year DSS (47% versus 60%, p = 0.512) or 5-year OS (38 % versus 40%, p = 0.579), which is consistent with the results of Kodama et al.[12] Similarly, in both studies no significant differences in 5-year OS and DSS were found between PCLBCL, LT with or without expression of MUM-1 or FOXP1. These data indicate that distinction between cases with or without expression of Bcl-2, MUM-1 or FOXP1 is not useful, and that categorization of Bcl-2 negative cases as PCLBCL, other is not justified.[12] We therefore propose that this term should only be used for exceptional cases of CBCL that do not fit the criteria of PCFCL or PCLBCL, LT, such as rare cases of intravascular large B-cell lymphoma, T-cell/histiocyte-rich B-cell lymphoma or plasmablastic lymphoma with only skin lesions at presentation.

In the WHO-EORTC classification, differentiation between PCFCL with a diffuse large cell infiltrate and PCLBCL, LT has become more difficult, since it is based primarily on cell morphology (cleaved versus round) and no longer on the basis of site (non-leg versus leg), as in the EORTC classification. Classification on the basis of morphology may be difficult and is associated with a considerable inter-observer variation.[9] In such difficult cases, the presence of a considerable proportion of admixed T-cells, the presence of a stromal reaction as well as demonstration of (remnants of) follicular dendritic cells networks by staining with appropriate antibodies (CD35 or CD21) may serve as useful additional criteria suggesting a diagnosis of PCFCL. Moreover, since PCLBCL, LT, unlike PCFCL, characteristically shows strong expression of Bcl-2, MUM-1 and FOXP1, this phenotypic profile might also be a useful adjunct, supporting a diagnosis of PCLBCL, LT. However, since Bcl-2, MUM-1 and to a lesser extent FOXP1 are also expressed by a small minority of PCFCL, these markers can not be used as a golden standard to differentiate between both conditions (Table 4).

In conclusion, the results of this study emphasize the clinical significance of the new WHO-EORTC classification and suggest that it may contribute to better diagnosis and treatment. Reclassification of PCFCCL (EORTC) with a predominant round cell morphology as PCLBCL, LT proved clinically relevant, which implies that such cases should be treated primarily with multi-agent chemotherapy. However, reclassification of PCLBCL, leg

(EORTC) with a predominant cleaved cell morphology into the group of indolent PCFCL proved less fortunate, since these cases have a much worse prognosis than PCFCL not presenting on the leg and should not be treated routinely with radiotherapy. Consistent with the results of a previous European multicenter study[9], we suggest that in addition to the WHO-EORTC classification also site of presentation is taken into account to select the most appropriate treatment in patients with PCFCL.

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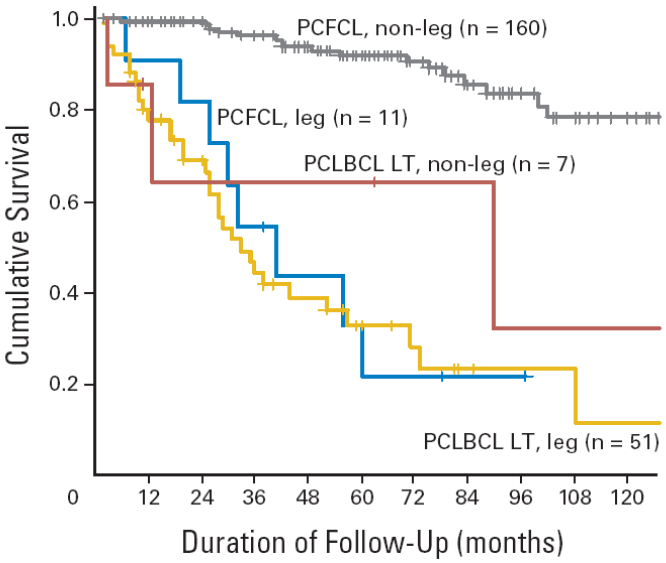
Figure 2. Overall survival of PCFCL and PCLBCL, LT further subdivided by site (leg versus non-leg).



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Chapter 3.

Fine-mapping chromosomal loss at 9p21: correlation with prognosis in primary cutaneous diffuse large B-cell lymphoma, leg type

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Abstract
Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT) is the most aggressive type of primary cutaneous B-cell lymphoma. In a recent study on 12 patients it was found that inactivation of CDKN2A by either deletion of 9p21.3 or promoter hypermethylation is correlated with a worse prognosis.
In the present EORTC multi-centre study, skin biopsies of 64 PCLBCL, LT patients were analyzed by Multiplex Ligation-dependent Probe Amplification to validate these previous results and to fine-map the losses in this region. Although no minimal common region of loss could be identified, most homozygous loss was observed in the CDKN2A gene (43/64; 67%) encoding p16 and p14ARF. Promoter hypermethylation of p16 and p14/ARF was found in six and zero cases respectively. Survival was markedly different between patients with versus without aberrations in the CDKN2A gene (5-year disease-specific survival 43% versus 70%; $p = 0.06$). In conclusion, our results confirm that deletion of chromosome 9p21.3 is found in a considerable proportion of PCLBCL, LT patients and that inactivation of the CDKN2A gene is associated with an unfavourable prognosis. In most patients the deletion involves a large area of at least several kilobasepairs instead of a small minimal common region.

Introduction

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT) is the most aggressive type of primary cutaneous B-cell lymphoma (CBCL). It is generally characterized by rapidly growing tumours that present on the leg(s), but in a minority of patients skin lesions can also arise at other sites. Histologically it is defined as a tumour with a predominance or confluent sheets of large, atypical B-cells (resembling centroblasts and immunoblasts), which generally express Bcl-2 and MUM-1. The disease has an intermediate prognosis with a 5-year survival rate of only 50%.[1] Since it is recognized that this disease represents a distinct type of CBCL, it will be included as a separate entity in the forthcoming WHO 2008 classification.

In a recent study by our group, using array-based comparative genomic hybridization (aCGH), it was found that inactivation of the CDKN2A region, encoding for the tumour suppressor genes p16 and p14ARF, by either deletion of chromosome 9p21.3 or promoter hypermethylation, is associated with a worse prognosis. However, these results were based on only 12 cases.[2]

Multiplex Ligation-dependent Probe Amplification (MLPA) has recently been described as a new method for relative quantification of multiple different DNA sequences in a single reaction, requiring only small amounts of DNA.[3] Moreover, the application of this technique on DNA isolated from formalin-fixed, paraffin-embedded (FFPE) material has previously been reported to be reliable and less sensitive to DNA degradation.[4-6] Targeted MLPA probe panels are commercially available including a set of probes targeting the chromosomal region of 9p21 containing several known genes (CDKN2A, coding for p16 and p14ARF, CDKN2B, coding for p15 and MTAP).

In the present study, MLPA was used to confirm that inactivation of CDKN2A is an unfavourable prognostic marker in a large patient group and to further fine-map the 9p21.3 region in order to determine a possible minimal common region.

Results

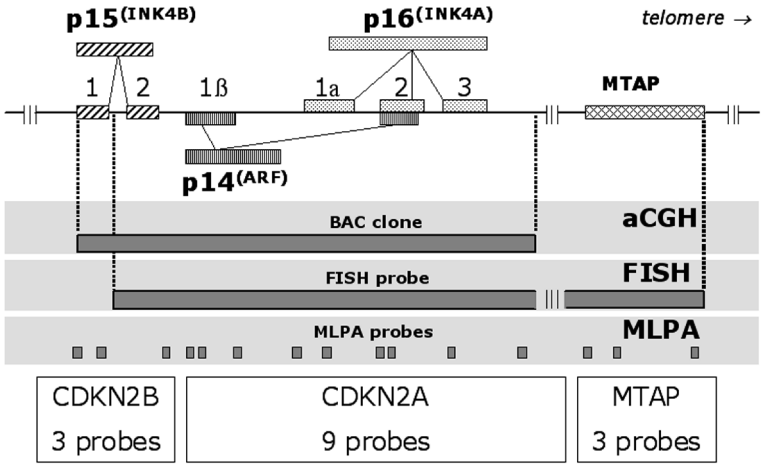
Testing the reliability of the MLPA technique

We first investigated whether results generated by MLPA were in accordance with our previous aCGH results.[2] In addition, we aimed to obtain more information on the precise location of deletions as the MLPA technique has a much higher spatial resolution than the aCGH technique applied previously (see Figure 1).[2] To that end, we subjected available isolated DNA from 12 patients (9 PCLBCL, LT and 3 primary cutaneous follicle centre lymphomas (PCFCL) previously investigated by aCGH to MLPA. We were indeed able to confirm chromosomal aberrations in 9p21.3 as detected by aCGH in all patients, with homozygous loss in four of 12 patients and hemizygous loss in two of 12 patients using MLPA (See Figure 2). Moreover, in three of the six patients that did not show loss in the aCGH analysis (three PCLBCL, LT and three PCFCL), MLPA allowed detection of hemizygous loss (and in one patient even homozygous loss) of individual probes within the

complete set (two PCLBCL, LT and one PCFCL) (see Figure 2). This demonstrates the higher sensitivity of the MLPA technique as compared to the aCGH platform previously used. However, we could not detect a minimal common region of deletion within the CDKN2A gene region.

Since a considerable part of our samples was derived from FFPE material, it was felt important to test the claim that MLPA can be applied as reliably to DNA isolated from FFPE material as to DNA isolated from frozen material.[6] To accomplish this, we analysed DNA from fresh-frozen samples and FFPE material taken simultaneously from the same tumour. This was done in two patients from whom at three different time points biopsy material was collected (primary tumour, first skin relapse and second skin relapse). We observed full concordance of the results as depicted for one patient in Figure 3, thereby demonstrating the applicability of this technique on partly degraded DNA. In addition, it was noted that the genetic lesions in the 9p21.3 region showed a stable pattern over time and did not alter with disease progression and treatment.

Figure 1. Schematical representation of 9p21.3 showing the spatial resolution of different techniques.



MLPA can be used to fine-map the chromosomal aberrations as found by aCGH (BAC clone RP11-149I2 corresponding to 21899259-22000413 (according to Ensembl) on chr. 9) and FISH (e.g. LSI p16 probe from Vysis which, according to the manufacturer, at least includes the region from 21792942 (D9S1749) - to 21995210 (D9S1752) on chromosome 9). Exact genomic positions of the MLPA probes can be requested at MRC Holland (info@mlpa.com).

Figure 2. Array CGH vs. MLPA results for PCLBCL, LT (n = 9) and PCFCL patients (n = 3).

MLPA results	PCLBCL, LT (n = 9)									PCFCL (n = 3)		
	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	# 11	# 12
p15, promotor+exon 1	0.17	0.13	0.41	0.5	1.02	0.99	1.03	0.86	1.13	0.93	1.09	0.81
p15, end exon 1	0.13	0.13	0.41	0.45	1.04	0.88	1.05	0.98	1.02	0.99	1.09	0.95
Intron 1 p15/p14ARF	0.17	0.14	0.23	0.42	0.74	0.65	1.01	0.54	0.24	0.95	1.03	0.93
p14ARF, promotor	0.18	0.09	0.5	0.42	1.14	0.91	1.09	0.77	0.67	0.99	1.4	1.17
p14ARF, promotor near TSS	0.13	0.11	0.42	0.52	1.18	0.97	1.07	0.83	1.33	0.97	1.23	0.99
p14ARF, exon 1	0.16	0.08	0.25	0.32	0.93	0.73	0.9	0.73	0.63	1.04	1.17	1.01
Intron 1 p14ARF/p16	0.13	0.09	0.28	0.43	0.4	0.61	0.92	0.62	1	0.91	0.93	0.86
Intron 1 p14ARF/p16	0.12	0.06	0.37	0.42	0.41	0.74	0.86	0.67	1.05	0.73	0.81	0.67
p16, exon 1	0.19	0.09	0.57	0.48	0.59	1.02	1.32	0.82	1.15	1.19	2	1.45
p16, exon 1	0.17	0.07	0.5	0.41	0.57	0.91	1.13	0.82	1.35	1.04	1.23	0.97
p16, exon 2	0.13	0.09	0.27	0.46	0.45	0.76	0.99	0.58	1.36	1.09	0.95	0.68
p16, exon 3	0.13	0.07	0.43	0.38	0.47	0.8	0.92	0.77	1.13	0.97	1.03	0.93
MTAP, End	0.13	0.07	0.31	0.34	0.81	0.69	0.85	0.89	0.78	0.95	1.01	0.95
MTAP	0.19	0.1	0.4	0.38	0.82	0.76	1.06	0.97	0.7	1.02	1.13	1.02
MTAP, Start	0.19	0.09	1.16	1.09	1	1.08	1.03	1.09	0.66	1.21	1.41	1.05
aCGH results (Log2 ratio)	-0.92	-0.94	-0.48	-0.50	-0.26	-0.24	-0.05	-0.15	-0.06	-0.04	-0.02	-0.07
SD	0.12	0	0.05	0.04	0.05	0.03	0.03	0.03	0.03	0.07	0.03	0.01
Follow-up	D+ 12	D+ 26	D+ 21	D+ 11	A- 85	A- 60	A- 82	D+ 54	D- 18	A- 264	A- 62	A- 60

D+ = died of lymphoma, D- = died of other cause, A- = alive in complete remission. Black = homozygous loss, grey = hemizygous loss, white = no loss.

Allelic loss at the 9p21.3 locus

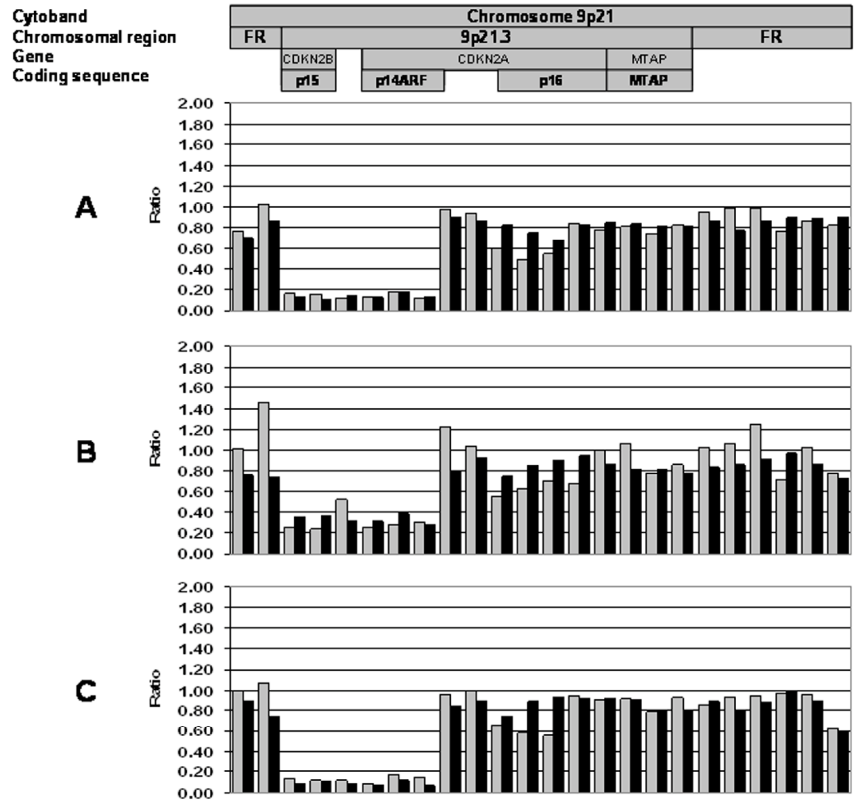
Having confirmed that MLPA can reliably detect genetic lesions in frozen as well as in FFPE material, we subjected the whole study group to MLPA. In total, tumour biopsies from 64 patients with a diagnosis of PCLBCL, LT were included in the final study group. This included the nine patients previously analyzed by aCGH and in addition 55 new patients. The overall MLPA results for these patients are depicted in Figure 4. It was found that 45 patients (70%) showed homozygous loss of one or multiple probes within the 9p21.3 region. Hemizygous loss was found in 14 patients and five patients did not show any detectable loss in this region.

Most chromosomal aberrations were localized in the CDKN2A gene. Homozygous loss within this region was found in 43/64 cases (67%). Homozygous losses in the coding regions for p16 (exon 1α, 2 and 3) and p14ARF (exon 1β and 2), as well as both promoter regions, were found in 40/64 (63%) and 37/64 (58%) cases respectively. Specific probes, most often lost were located in exon 1α coding for p16 and exon 2 coding for both p16 and p14ARF (both probes were lost in 31/64 cases; 48%). In most patients however, the deletion covered a large part of chromosome 9p21.3 instead of a smaller minimal common region (see Figure 4).

Analysis of p16 and p14ARF promoter methylation status

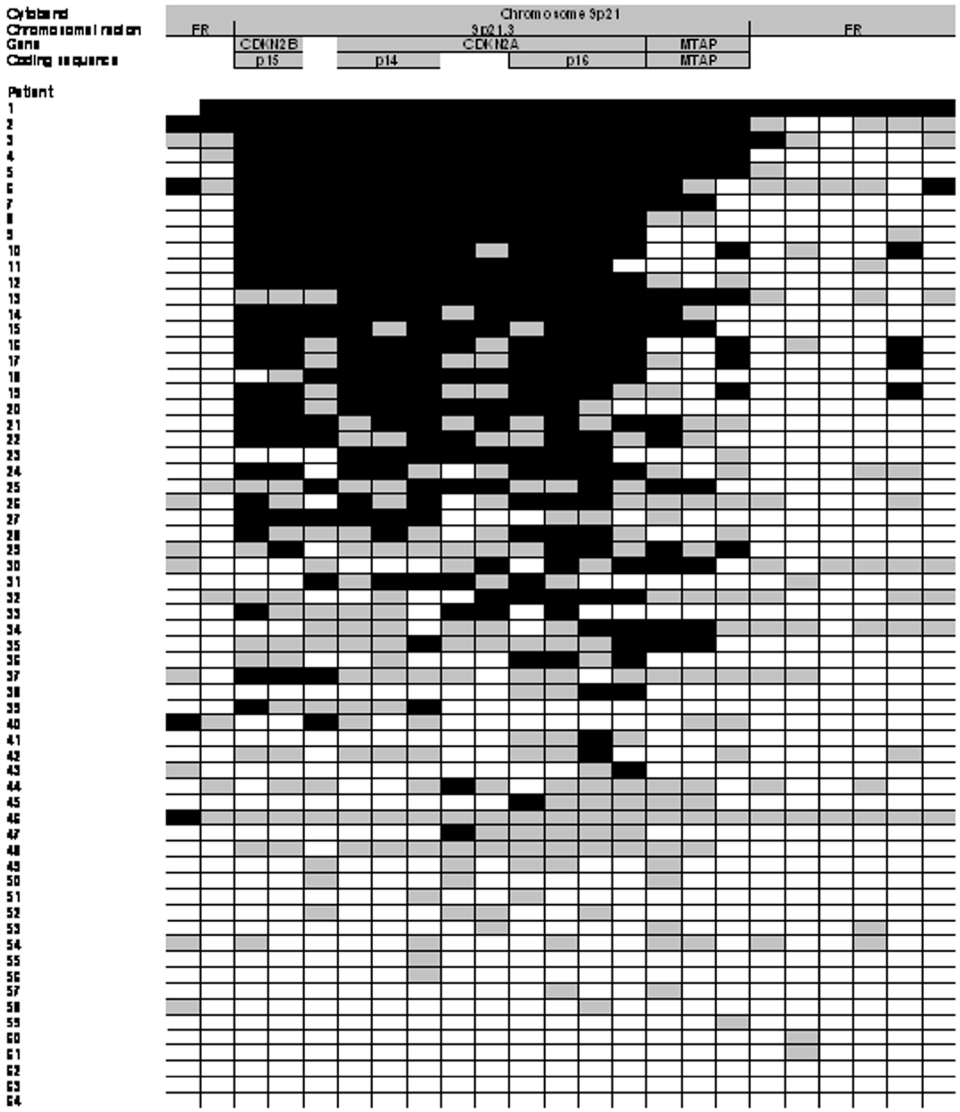
From 20/21 patients without homozygous loss in CDKN2A, sufficient DNA was available to determine the promoter methylation status of p16 and p14ARF. In none of the samples methylation of the p14ARF promoter was found. However, methylation of the p16 promoter was detected in 6/20 samples. Five of these samples showed hemizygous loss within the p16 coding and promoter region in MLPA analysis and one sample had no loss within this region.

Figure 3. MLPA results for DNA from fresh frozen (black bars) versus FFPE material (grey bars) from one patient.



Skin biopsies obtained at diagnosis (A), at first skin relapse after 32 months (B) and at second skin relapse after 39 months (C). FR: flanking region

Figure 4. Total MLPA results for 64 PCLBCL, LT patients.

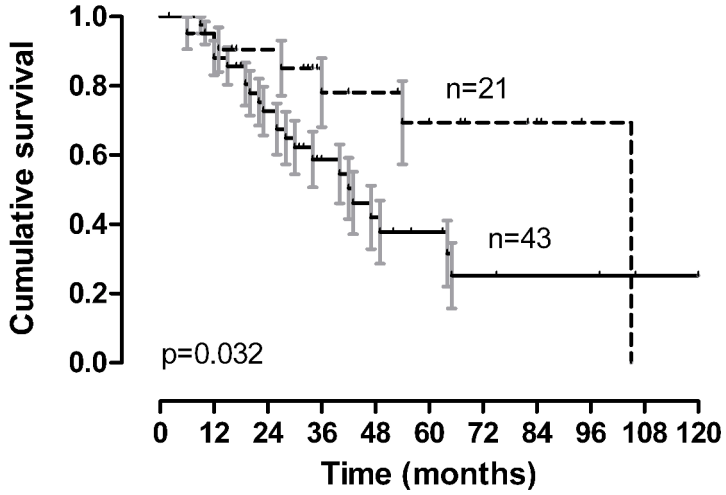


Black = homozygous loss (ratio < 0.4), grey = hemizygous loss (ratio between 0.4 and 0.7), white = no loss (ratio > 0.7). FR: flanking region

Correlation with survival

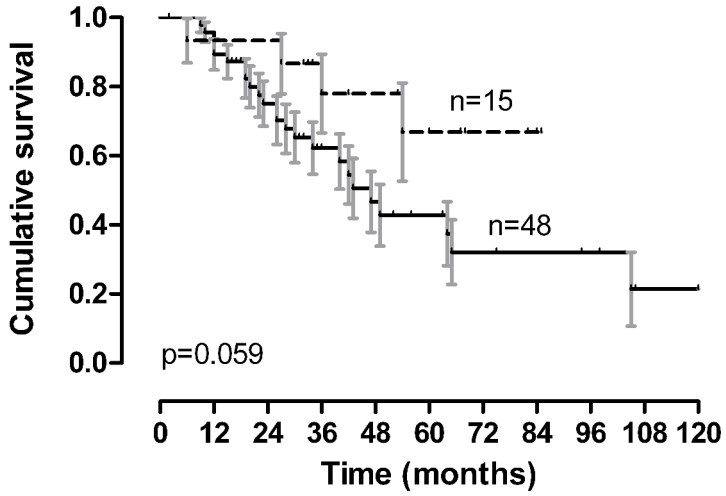
Survival analysis revealed a clear correlation between homozygous loss in chromosome 9p21.3 and reduced survival. Patients without homozygous loss in this region had an actuarial 5-year disease-specific survival (DSS) of 68%, while patients with loss of at least one probe in this region had a 5-year DSS of 39% (p = 0.06). Although statistically not significant, these results are in line with the findings described by Dijkman et al.[9] Since most loss occurred in the CDKN2A region, we also performed survival analysis for losses specific to this region and in addition also for loss in areas coding specifically for p14ARF and p16 separately. Loss in the CDKN2A region was most strongly correlated with prognosis. Five-year DSS for patients with or without loss in this region was 38% versus 69% (p = 0.03) (See Figure 5). Differences in 5-year DSS between patients with or without losses in the regions coding p14ARF or p16 were not or borderline significant, respectively. Finally, we tested whether inclusion of the methylation data affected the survival analysis. In the complete study group a total of 48 patients can be considered to have an inactivated CDKN2A gene (43 patients with homozygous loss in the CDKN2A gene and 5 patients with a methylated p16 promoter combined with hemizygous loss in CDKN2A). Five-year DSS for patients with and those without inactivation of CDKN2A are 70% and 43% respectively (p = 0,059)(See Figure 6).

Figure 5. Disease-specific survival of 64 PCLBCL, LT patients according to chromosomal aberrations within CDKN2A.



Solid line: patients with homozygous loss of one or multiple probes in CDKN2A (n = 43), dashed line: patients with no or hemizygous loss in CDKN2A (n = 21). Error bars indicate Standard Error.

Figure 6. Disease-specific survival of 63 PCLBCL, LT patients according to CDKN2A status.



Solid line: patients with inactivation of CDKN2A (n = 48), dashed line: patients without inactivation of CDKN2A (n = 15). In one patient no DNA was available for determining methylation status, this patient is not included in this analysis. Error bars indicate Standard Error.

Discussion

In this study we aimed to confirm recently reported data describing that loss of 9p21.3 and more specifically, inactivation of CDKN2A is commonly found and associated with inferior prognosis in PCLBCL, LT.[2] By using MLPA we were able to confirm loss of 9p21.3, including the CDKN2A gene, on a large group of patients with PCLBCL, LT. We observed full concordance with the results as obtained by aCGH, and, in addition, detected small areas of loss in three patients. So comparison between aCGH and MLPA confirms the higher sensitivity of the latter technique and its ability to fine-map larger areas of loss as found by genome-wide analyses such as aCGH using BAC clones as in the previous study. It was found that in most patients the deletion covers a substantial part (up to several tens of thousands of basepairs) of this chromosomal region. Although no minimal common region of loss could be detected, most chromosomal aberrations converged on the CDKN2A gene. An additional advantage of the MLPA technique is that it can be applied reliably on FFPE material of CBCL patients. Comparison between DNA derived from fresh-frozen and FFPE sections, obtained from the same tumour in two patients, showed identical results. Moreover, comparison of skin biopsy specimens obtained from consecutive tumours in these two patients, demonstrated identical chromosomal aberrations, indicating that these losses can display a stable pattern over time.

Loss or inactivation of the CDKN2A gene either by deletion or promoter hypermethylation has been extensively reported in haematological malignancies, including B-cell non-Hodgkin lymphomas.[7-10] CDKN2A codes for p16 and p14ARF, both of which are tumour suppressor genes and are negative regulators of cell cycle progression. In our study group, inactivation of CDKN2A was mostly due to (homozygous) deletion. Promoter hypermethylation of p16 was found in a minority of cases, which is in accordance with the results of previous studies in CBCL.[11;12] Promoter hypermethylation of p14 was never detected.

Besides confirming the loss in this chromosomal region we further wanted to validate the prognostic significance of the findings as reported previously. Although less striking than the results reported by Dijkman et al[2], loss or inactivation of CDKN2A, was still associated with reduced survival (See Figures 5 and 6), which is also consistent with previous reports of others.[11-13] Even though the results described herein show a clear, and borderline significant, correlation with reduced survival, loss of CDKN2A can not be used as the sole tool to optimize management in individual patients.

In our study group there are several patients with deletions in 9p21.3, that have a favourable clinical course thus far. More importantly, five of 21 patients without inactivation of CDKN2A died of lymphoma 6-54 months (median 27 months) after diagnosis. Especially this latter group runs the risk of being undertreated when management would be solely based on CDKN2A status.

In conclusion, in a large part of PCLBCL, LT patients chromosomal loss is seen in 9p21.3. In most patients these losses are concentrated on the CDKN2A gene coding for p16 and p14ARF. Inactivation of this gene is caused by homozygous deletion or, less commonly, by promoter hypermethylation and is associated with a worse prognosis. However, caution is warranted before these results are incorporated into clinical decision making.

Material and Methods

Sample collection

Cases were collected from centres collaborating in the EORTC Cutaneous Lymphoma Group. Tumour DNA from pre-treatment skin biopsies of 80 patients were initially submitted for the study. Patients with incomplete staging investigations (minimum requirements being routine laboratory screening, CT scans of chest and abdomen and bone marrow biopsy) or follow-up of less than 12 months (unless caused by death due to lymphoma) were excluded from further analysis (n = 6). In addition, of all submitted cases H&E sections were reviewed for morphological reference and estimation of percentage tumour- and admixed reactive cells. In case of doubt about the percentage of tumour cells, we reviewed stainings for CD3 and CD20. If these were not available, the case was excluded. Combined with information on the expression of Bcl-2, MUM-1 and FOXP1, a diagnosis of PCLBCL, LT was confirmed or discarded. Cases in which a diagnosis of PCLBCL, LT could not be confirmed and cases with more than 30% admixed reactive T-

cells were excluded (n = 5). Finally, five cases could not be analyzed due to poor quality DNA. The final study group consisted of 64 patients with a diagnosis of PCLBCL, LT. The study group contained 25 males and 39 females (male-female ratio: 0,6), with a median age at diagnosis of 78 years (range 47-92 years) and a median duration of follow-up of 34 months (range 2-158 months). Clinical characteristics and treatment data are presented in Table 1. In addition, 3 patients with a diagnosis of primary cutaneous follicle centre lymphoma (PCFCL) were included in the experiment validating the MLPA technique. Twelve of the above described patients (9 PCLBCL, LT and 3 PCFCL) were formerly analyzed with aCGH.[2] Genomic DNA was extracted from either fresh frozen material, or FFPE sections, using local protocols.

Table 1. Clinical and treatment characteristics of 64 patients with PCLBCL, LT.

Total number of patients	**64**
Age, years	
Median (range)	78 (47-92)
Sex	
Male	25
Female	39
male:female ratio	0.6
Site of skin lesions (%)	
Head/ neck	2 (3%)
Trunk	7 (11%)
Arm(s)	2 (3%)
Leg(s)	59 (92%)
Extent of skin lesions (%)	
Solitary	26 (41%)
Regional	30 (47%)
Multifocal	8 (13%)
Treatment (%)	
Radiotherapy	32 (50%)
Chemotherapy	15 (23%)
Chemotherapy and radiotherapy	9 (14%)
Surgery	3 (5%)
Surgery and radiotherapy	2 (3%)
Rituximab	1 (2%)
Other	2 (3%)
Result of treatment (%)	
Complete remission	54 (84%)
Partial remission	6 (9%)
No response	3 (5%)
Progressive disease	1 (2%)
Status at last follow-up (%)	
Alive and well	23 (36%)
Alive with disease	6 (9%)
Died of lymphoma	30 (47%)
Died of other cause	5 (8%)

Fine-mapping chromosomal loss at 9p21.3 using MLPA

A commercially available MLPA Kit (SALSA MLPA Kit P024B; MRC-Holland, Amsterdam, the Netherlands) targeting the 9p21 region was used according to the manufacturer's protocol. The P024B kit contains 23 probes of which 9 probes are specific for the CDKN2A region, 3 probes for the CDKN2B region and 3 probes for the MTAP gene, while 8 probes hybridize to regions flanking these genes. For the experiments we used 60-80 ng of genomic DNA and normal control DNA (a DNA mix of 15 healthy donors) was always included in the same reaction. The principles of the MLPA technique are concisely described by Vorstman et al.[14], while detailed methodology can be found in the paper by Schouten et al.[3]

Briefly, genomic DNA diluted in 5 ul of Tris-EDTA 10mM, was denatured at 95°C for 5 min, mixed with the probe set and the MLPA buffer, and incubated for 16 hr at 60°C. After probe hybridisation, products were ligated for 15 min at 54°C. The ligase enzyme was then inactivated by incubation for 5 min at 98°C. The ligation products were subsequently amplified by PCR using universal FAM-labelled primers. All these reactions were carried out in a PTC-200 Thermal cycler with heated lid (MJ Research, Waltham, Massachusetts). The resulting products were separated according to size on an ABI Prism 3730 DNA analyzer (Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands) by the inclusion of GeneScan ROX 500 as internal size standard (Applied Biosystems). Resulting fragment analysis chromatograms were sized to standard fragment lengths by GeneMapper v3.7 (Applied Biosystems).

Promoter hypermethylation analysis

Since promoter hypermethylation can be involved in gene inactivation, we also evaluated the methylation status of the CpG islands, located in the promoter regions of p16 and p14ARF, in patients without homozygous loss of (parts of) the CDKN2A gene. Promoter methylation status was determined by performing melting curve analysis of bisulfite converted and PCR amplified tumour DNA, as described previously.[15] Tumour DNA was modified with sodium bisulfite by using the EZ Methylation Kit (Zymo Research Corporation, Orange, CA). PCR primers were designed to anneal to bisulfite-converted DNA as template which amplified a region of the p16 and p14ARF gene promoter CpG islands (see Table 2). PCR amplification of bisulfite-treated DNA and subsequent melting curve analysis in the presence of SYBRGReen (MyiQ Real-time PCR Detection System; Bio-Rad Laboratories BV, Veenendaal, the Netherlands) allowed detection of methylation present in the sample DNA, by generating a peak with a higher melting temperature as compared to unmethylated DNA. Ratios for methylated versus unmethylated DNA in each sample were determined by dividing the total area under the melting temperature curve(s) by the area under the methylation specific peak. All samples showing a ratio above 0.3 were considered to contain methylated tumour DNA.

Table 2. PCR primer sequences, designed to anneal to bisulfite-converted DNA as template, for p16 and p14ARF gene promoter CpG islands.

Gene	Primer sequence (5' - 3')	CpGs in Amplicon	Position of Amplicon Relative to Transcription Start Site	Amplicon Size (bp)
p16	GATTTAATTTGGTAGTTAGGAAGGTTGT	10	-299, -159	140
	GGTTGGGAGTAGGGAGGTCG			
p14ARF	GAGGGGAGTTAGGAATAAAATAAGG	10	-413, -268	145
	CTAAAACGCAACTCCAAACAACT			

Data analysis and statistical methods

Analysis of MLPA results was carried out upon the transfer of GeneMapper results to Coffalyser software, a data analysis tool which was designed by MRC-Holland for normalization of MLPA fragment data files. With this programme, DNA copy number ratios of test samples can be computed, by comparison and normalization to a control sample (for full description see: http://www.mlpa.com/coffalyser/). Since sample DNA is compared against a normal control sample, a ratio of 0.5 would ideally indicate hemizygous loss and zero would indicate homozygous loss. However, considering the fact that our samples contained a maximum of 30% admixed reactive cells, ratios between 0.4 and 0.7 were considered as hemizygous loss, while ratios below 0.4 were considered as homozygous loss.

For analysis of clinical data and performing survival analyses, SPSS 14.0 (SPSS Inc., Chicago, IL, U.S.A.) was used. Disease-specific survival (DSS) was calculated from the date of diagnosis until death from lymphoma (including therapy-related death) or last follow-up without event. Survival curves were estimated using the method of Kaplan and Meier and statistical comparison between curves was done by log-rank testing.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

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Chapter 4.

The applicability and prognostic value of the new TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome: results on a large cohort of primary cutaneous B-cell lymphomas and comparison with the system used by the Dutch Cutaneous Lymphoma Group

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Summary
Background: Recently, a consensus proposal was published for a TNM classification system for all primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome, meant to document extent of disease in a consistent manner. The applicability and the prognostic significance of this system have not been investigated thus far.
Objectives: To test the applicability and prognostic relevance of the proposed TNM classification system on a cohort of primary cutaneous B-cell lymphomas (CBCL).
Patients/ methods: The study group included 71 primary cutaneous marginal zone lymphomas (PCMZL), 171 primary cutaneous follicle centre lymphomas (PCFCL) and 58 primary cutaneous diffuse large B-cell lymphomas, leg type (PCLBCL, LT). Since only patients with primary cutaneous lymphoma were included (T1-3, N0M0), only T-rating was scored. The results were compared with the scoring as applied by the Dutch Cutaneous Lymphoma Group.
Results: The system was easily applicable to all cases. In PCMZL and PCFCL no correlation was found between T-score and survival (5-year disease-specific survival: T1: 100% and 98%, T2: 94% and 93%, T3: 100% and 88% respectively). In PCLBCL, LT there was an obvious, although statistically not significant, association between increasing T-score and reduced survival (5-year disease-specific survival: T1: 75%, T2: 49%, T3: 0%; p = 0.077). Comparing the TNM system with the Dutch Cutaneous Lymphoma Group system, there was a discrepancy in the classification of 20 cases.
Conclusions: The new TNM- system is a useful tool to document disease extent in patients with CBCL and provides prognostic information in the group of PCLBCL, LT patients.
Keywords: B cell, cutaneous lymphoma, disease extent, prognosis, TNM staging

Introduction

Recently, representatives of the International Society for Cutaneous Lymphomas and the Cutaneous Lymphoma Group of the European Organization for Research and Treatment of Cancer (EORTC) published a consensus proposal for a TNM classification system applicable to all primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome.[1] This proposed TNM system is primarily meant to document extent of disease in a consistent manner, thereby facilitating comparison of studies at different institutes. In the proposed system the T-classification reflects the extent/ distribution of primary cutaneous involvement, the N-classification describes lymph node involvement and the M-classification is used to describe if extracutaneous non-lymph node disease is present. Since, by definition, in all primary cutaneous lymphomas, extracutaneous disease (lymph node or visceral) is absent at the time of diagnosis, the N- and M-scores are only used to document disease extent at the time of relapse or disease progression. A detailed description of the different categories is given in Table 1.

Studies investigating the applicability of this TNM system have not been performed thus far. Moreover, whether this TNM system provides prognostically relevant information for the different types of primary cutaneous lymphoma and thus may serve as an additional guide in the appropriate management of these lymphomas remains to be elucidated.

In the present study we applied the proposed TNM system on a large cohort of 300 primary cutaneous B-cell lymphomas (CBCL), recently reclassified according to the new WHO-EORTC classification.[2;3] This classification distinguishes 3 main types of CBCL: primary cutaneous marginal zone lymphoma (PCMZL) and primary cutaneous follicle centre lymphoma (PCFCL), both indolent types of CBCL and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT), which represents a more aggressive type of CBCL. Since these groups have been redefined as compared to the formerly used classifications[4;5], we have summarized the main features of these 3 groups, as found in a recent study[2], in Table 2.

The aims of this retrospective cohort analysis were to test the clinical applicability of the proposed TNM system and to evaluate its prognostic relevance for the group of CBCL. In addition, these results were compared to the scoring of disease extent as used by the Dutch Cutaneous Lymphoma Group.

Methods

Patient selection

The study group is a retrospective cohort of 300 CBCL patients reclassified according to the criteria of the WHO-EORTC classification.[3] In all cases, gender, age at diagnosis, site, size and extent of skin lesions (see below), status at last follow-up and duration of follow-up were recorded as part of a recent study.[2] In addition, in approximately 50% of cases clinical photographs were available for evaluation.

This group included 71 primary cutaneous marginal zone lymphomas (PCMZL), 171 primary cutaneous follicle centre lymphomas (PCFCL) and 58 primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT).

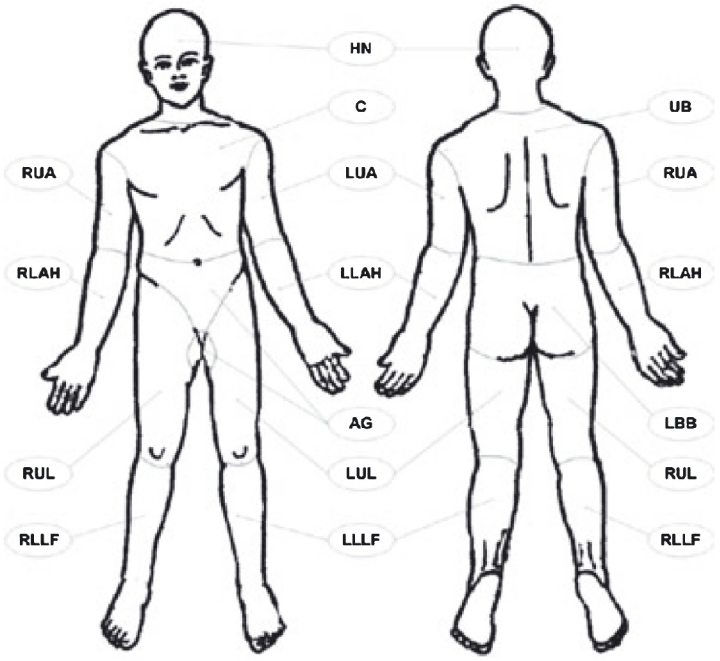
Table 1. Description of different scres in the TNM classification.

Classification		Description
T	**T1**	Solitary skin involvement categories: • **T1a** a solitary lesion $\leq$5 cm diameter • **T1b** a solitary lesion >5 cm diameter
	T2	Regional skin involvement categories: Multiple lesions limited to one body region or two contiguous body regions • **T2a** all disease encompassing in a $\leq$ 15 cm diameter circular area • **T2b** all disease encompassing in a > 15 and $\leq$ 30 cm diameter circular area • **T2c** all disease encompassing in a > 30 cm diameter circular area
	T3	Generalized skin involvement: • **T3a** multiple lesions involving 2 non-contiguous body regions • **T3b** multiple lesions involving $\geq$ 3 body regions
N	**N0**	No clinical or pathologic lymph node involvement
	N1	Involvement of one peripheral lymph node region that drains an area of current or prior skin involvement
	N2	Involvement of two or more 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
M	**M0**	No evidence of extracutaneous non-lymph node disease
	M1	Extracutaneous non-lymph node disease present

Assessment of disease extent

In all cases extent of skin lesions at the time of diagnosis was scored using the proposed TNM system.[1] Since only patients with primary cutaneous lymphoma were included (T1-3, N0M0), only T-rating was scored (see Table 1 and Figure 1). In case no measurements were recorded in the database of the Dutch Cutaneous Lymphoma Group and clinical photographs were not available, measurements of skin lesions were obtained from patients charts or, in case the patient had undergone radiotherapy, from the files of the radiotherapy department. The results were compared with the scoring of the Dutch Cutaneous Lymphoma Group, used in our previous study.[2] In this Dutch Cutaneous Lymphoma Group system, extent of involved skin is defined as solitary when it concerns a single tumour, as localized when the lesion consists of multiple skin lesions that can be irradiated within one radiation field, and as multifocal for separate lesions in adjacent body regions that can not be irradiated within one radiation field or for lesions involving multiple nonadjacent body regions. In contrast to the TNM system, no further subdivision is made on the basis of the size of the skin lesions or the affected areas.

Figure 1. Body regions as defined in the proposed TNM system for the designation of T-classification. Left and right extremities are assessed as separate body regions.



HN	Head & neck
C	Chest
LUA	Left upper arm
LLAH	Left lower arm & hand
AG	Abdominal & genital
LUL	Left upper leg
LLLF	Left lower leg & feet
RUA	Right upper arm
RLAH	Right lower arm & hand
RUL	Right upper leg
RLLF	Right lower leg & feet
UB	Upper back
LBB	Lower back & buttock

Statistical analysis

Statistical calculations were performed using SPSS 12.0.1 (SPSS Inc, Chicago, IL). Disease-specific survival was calculated from the date of diagnosis until death of lymphoma or last follow-up without event. Survival curves were estimated using the method of Kaplan and Meier and statistical comparison between curves was done by log-rank testing.

Results

The median follow-up for the whole group was 56 months (range 2-336 months).
The results of scoring extent of disease by T-score and by the Dutch Cutaneous Lymphoma Group system, as well as the corresponding 5-year disease-specific survival rates, are summarized in Table 3. Because of the clear descriptions of the main T-score groups and the different subgroups (see Table 1), combined with the strictly defined body regions (see Figure 1), this system could be easily applied to all cases.

The study included 71 PCMZL patients (48 males, 23 females) with a median age of 53 years (range 23-87). Using the TNM system most patients were classified as T3 (n = 28; 39%), followed by T2 (n = 25; 35%) and T1 (n = 18; 25%). According to the Dutch Cutaneous Lymphoma Group system 36 patients (51%) had multifocal disease, 17 patients (24%) had localized disease, while 18 patients (25%) had presented with a solitary lesion. Eight cases with multiple separate lesions at adjacent body regions were classified as multifocal according to the Dutch Cutaneous Lymphoma Group system, but as T2 using the TNM system. Representative examples of the different stages are presented in Figure 2. The five-year disease-specific survival for T1, T2 and T3 were 100%, 94% and 100% respectively, and 100%, 92% and 100% for solitary, localized and multifocal disease according to the Dutch Cutaneous Lymphoma Group system. These results clearly show that extent of disease, either by TNM or Dutch Cutaneous Lymphoma Group system has no prognostic significance in this group.

Table 2. Clinical characteristics of the 3 main groups of primary cutaneous B-cell lymphoma.

	PCMZL	PCFCL	PCLBCL, LT
Median age, years (range)	53 (23-87)	58 (21-89)	78 (42-92)
Male: female ratio	2.1	1.8	0.5
Clinical presentation	solitary or multiple papules, plaques or nodules preferentially on the trunk (55%) or extremities (67%)	solitary or grouped tumours or plaques preferentially on the head (44%) or the trunk (54%) lesions on the leg(s) uncommon (6%)	solitary or multiple nodules and/or tumours most often on the leg(s) (88%) lesions at sites other than the leg(s) uncommon
Relapse rate	57%	30%	69%
Extracutaneous progression	8.5%	10.5%	47%
Prognosis: **5-year DSS** **5-year OS**	 98% 94%	 95% 87%	 50% 37%
Preferred treatment	non-aggressive local treatment or radiotherapy	radiotherapy	multi-agent chemotherapy

Patients with a PCFCL (n = 171; 109 males and 62 females) had a median age of 58 years (range 21-89). Using the TNM system, most patients presented with stage T1 (n = 68; 40%) or T2 (n = 88; 51%). Only 15 patients (9%) were classified as T3 at the time of diagnosis (see Table 3). According to the Dutch Cutaneous Lymphoma Group system 68 patients (40%) had presented with a solitary skin lesion, 76 patients (44%) with localized skin lesions and 27 patients (16%) with multifocal skin lesions. Twelve cases with multiple separate lesions at adjacent body regions were classified as multifocal according to the Dutch Cutaneous Lymphoma Group system, but as T2 using the TNM system. Representative examples of the different stages are presented in Figure 3.
The 5-year disease-specific survival for T1, T2 and T3 was 98%, 93% and 88% and 99%, 95% and 85% for solitary, localized and multifocal according to the Dutch Cutaneous Lymphoma Group system respectively. Although there was a tendency toward reduced survival with increasing T-score, these differences were not significant (p = 0.560). Moreover, no significant differences were found between subgroups within the different T-categories (T1a vs. T1b; T2a vs. T2b vs. T2c; T3a vs. T3b; see Table 3).

Patients with PCLBCL, LT (n = 58; 20 males, 38 females) had a median age of 78 years (range 42-92). In this group the numbers according to the T-score corresponded entirely with the numbers in the extent categories as used by the Dutch Cutaneous Lymphoma Group: T1/ solitary 24% (n = 14), T2/ localized 57% (n = 33) and T3/ multifocal 19% (n = 11) (see Figure 4). Although statistically not significant (p = 0.077) an obvious correlation was seen between extent and survival (5-year disease-specific survival of 75%, 49% and 0% respectively). Moreover, the classification into subgroups within the main T-scores provided additional prognostic value. For instance, patients in the T2 group showed a decreased survival with increasing size of the affected areas (5-year disease-specific survival for involved area of ≤ 15 cm, 15-30 cm or > 30 cm is 67%, 48% and 0% respectively; see Table 3).

Discussion

This study investigates the applicability and prognostic significance of the newly proposed TNM system and describes for the first time how this system is applied to a large group of primary cutaneous lymphomas.
Our results show that the TNM system can easily be applied to the three commonest groups of CBCL. In both indolent entities, PCMZL and PCFCL, the system does not appear to have prognostic significance, but in PCLBCL, LT, the group with an intermediate prognosis, increasing T-score seems to be associated with worse survival. In addition, subdivision based on the size of the skin lesion or the affected body area, or in case of T3 classification, the amount of involved body regions, provided additional prognostic information in this group of PCLBCL, LT.

Table 3. Scoring of disease extent in 300 CBCL according to the TNM-system and the Dutch Cutaneous Lymphoma Group -system.

	PCMZL (n = 71)				PCFCL (n = 171)				PCLBCL, LT (n = 58)			
	TNM no. of patients (%)	**5-yr DSS**	**DCLG** no. of patients (%)	**5-yr DSS**	**TNM** no. of patients (%)	**5-yr DSS**	**DCLG** no. of patients (%)	**5-yr DSS**	**TNM** no. of patients (%)	**5-yr DSS**	**DCLG** no. of patients (%)	**5-yr DSS**
T1	**18 (25%)**	**100%**	**Solitary**		**68 (40%)**	**98%**	**Solitary**		**14 (24%)**	**75%**	**Solitary**	
T1a	15 (21%)	100%	18 (25%)	100%	53 (31%)	98%	68 (40%)	99%	8 (14%)	86%	14 (24%)	75%
T1b	3 (4%)	100%			15 (9%)	100%			6 (10%)	56%		
T2	**25 (35%)**	**94%**	**Localized**		**88 (51%)**	**93%**	**Localized**		**33 (57%)**	**49%**	**Localized**	
T2a	7 (10%)	75%	17 (24%)	92%	44 (26%)	91%	76 (44%)	95%	18 (31%)	67%	33 (57%)	47%
T2b	7 (10%)	100%			27 (16%)	96%			9 (16%)	48%		
T2c	11 (15%)	100%			17 (10%)	92%			6 (10%)	0%		
T3	**28 (39%)**	**100%**	**Multifocal**		**15 (9%)**	**88%**	**Multifocal**		**11 (19%)**	**0%**	**Multifocal**	
T3a	3 (4%)	100%	36 (51%)	100%	4 (2%)	100%	27 (16%)	85%	5 (9%)	20%	11 (19%)	0%
T3b	25 (35%)	100%			11 (6%)	83%			6 (10%)	0%		

Consistently, in two recent studies on PCLBCL, LT, patients presenting with a solitary tumour had a better prognosis than patients presenting with multiple tumours on one or both legs.[6;7] However, in a study of 40 PCLBCL, LT by Kodama et al[8] no difference in survival was found between patients with solitary or multiple tumours. Zinzani et al reported a significantly higher overall survival for PCMZL/ PCFCL patients with a single skin lesion compared to those with regional/ disseminated disease.[7] Since the authors do not describe this relation for PCMZL and PCFCL separately, comparison with the results of the present study is impossible.

Comparing the TNM-system with the Dutch Cutaneous Lymphoma Group system, there was a discrepancy in the classification of 20 cases (8 PCMZL and 12 PCFCL) with multiple separate lesions at two contiguous body regions (see Figure 2B). Since there is no anatomical or biological relation between such separate lesions, in the Dutch Cutaneous Lymphoma Group such lesions are classified as multifocal or generalized disease, and not as regional disease. However, since the T3 category in the TNM system is defined as involvement of 2 non-contiguous body regions or 3 or more body regions, such cases are not classified as T3, but as T2, which denotes regional skin involvement. Classification of such cases as T2 is of minor clinical importance in PCMZL and PCFCL, since both groups represent indolent types of CBCL, which can be treated with non-aggressive therapies, irrespective of the extent of skin lesions. However, in other types of lymphoma, classifying separate lesions at adjacent body sites as T2 might have important therapeutic consequences.

In conclusion, our results show that the proposed TNM system provides the clinician with a useful tool to document the disease extent in patients with CBCL in a consistent manner. In cases of PCLBCL, LT it also provides prognostic information. The applicability of the definitions for T2c and T3a/b should be evaluated in cohorts of other types of primary cutaneous lymphomas, because this might have undesirable therapeutic consequences.

Figure 2. Examples of different T-scores in primary cutaneous marginal zone lymphoma. A: T2a; Dutch Cutaneous Lymphoma Group: localized, B: T2c; Dutch Cutaneous Lymphoma Group: multifocal.

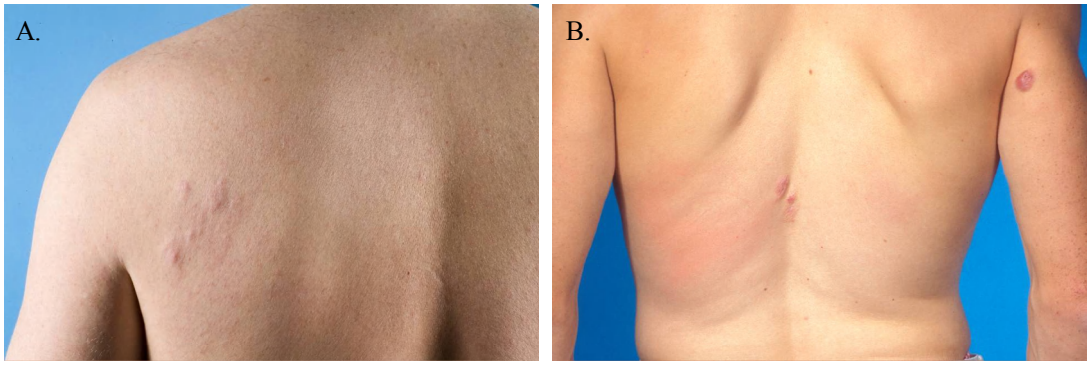


Figure 3. Examples of different T-scores in primary cutaneous follicle centre lymphoma. A: T1b; Dutch Cutaneous Lymphoma Group: solitary, B: T2b; Dutch Cutaneous Lymphoma Group : localized, C and D: T3b; Dutch Cutaneous Lymphoma Group : multifocal.

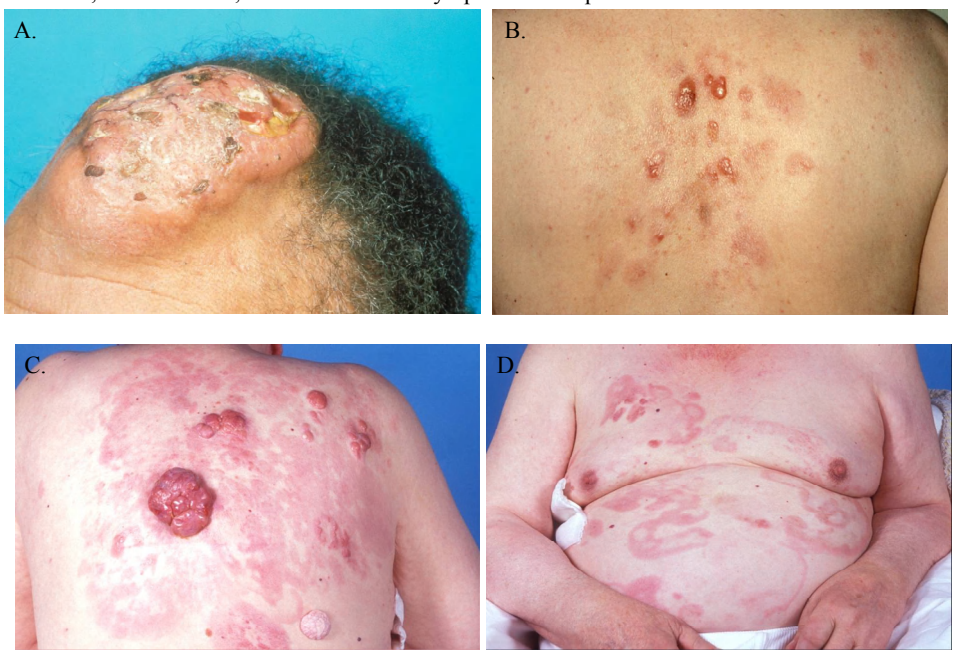
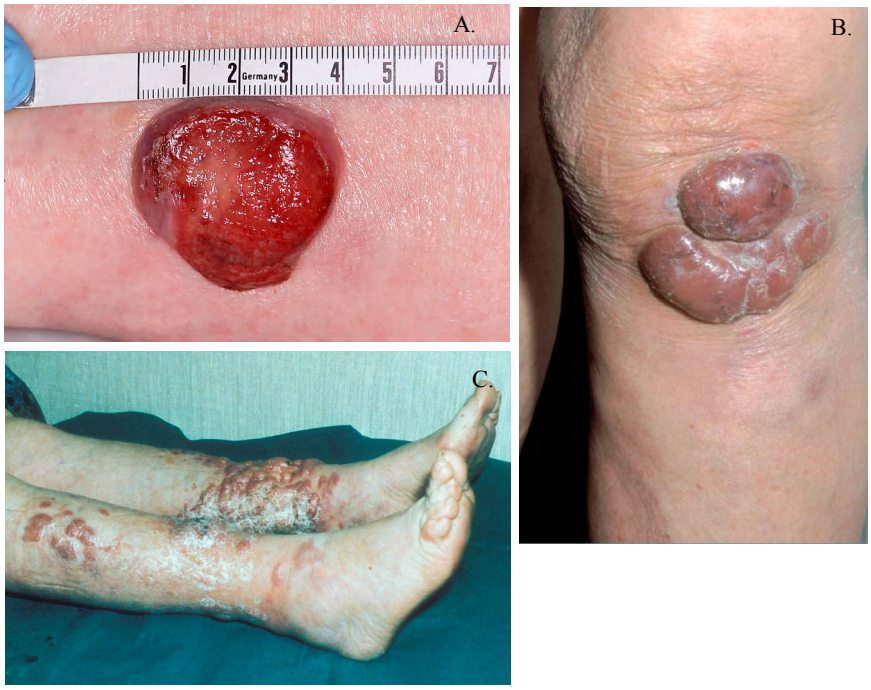


Figure 4. Examples of different T-scores in primary cutaneous diffuse large B-cell lymphoma, leg type. A: T1a; Dutch Cutaneous Lymphoma Group : solitary, B: T2a; Dutch Cutaneous Lymphoma Group: localized, C: T3a; Dutch Cutaneous Lymphoma Group: multifocal



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Chapter 5.

Results of bone marrow examination in 275 patients with histological features that suggest an indolent type of cutaneous B-cell lymphoma

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Summary
Recently, discussions have started whether or not bone marrow biopsies should be performed routinely in patients with skin lesions that show histologic features consistent with an indolent B-cell lymphoma (marginal zone lymphoma (MZL) or follicle centre lymphoma (FCL). As no studies have addressed this question for this group of lymphomas, we evaluated the results of bone marrow biopsy examination in 275 patients with histologic features consistent with MZL (n = 82) or FCL (n = 193) first presenting in the skin. In the MZL group, two of 82 patients (2%) showed bone marrow involvement and in one of these patients this was the only extracutaneous localization. In the group of FCL, 22 of 193 patients (11%) had bone marrow involvement. In nine of these this was the only extracutaneous localization. FCL patients with skin lesions and a positive bone marrow had a significantly worse prognosis as compared to patients with only skin lesions (5-year disease-specific survival 63% versus 95%; p = 0.001).
These results indicate that bone marrow investigation is an essential part in staging patients with a FCL first presenting in the skin. Bone marrow examination appears to have limited value in patients with MZL presenting in the skin.

Introduction

Primary cutaneous lymphomas (PCL) are defined as a group of non-Hodgkin lymphomas of either T-cell or B-cell origin, that present in the skin without evidence of extracutaneous disease at the time of diagnosis. Distinction between PCL and systemic lymphomas involving the skin secondarily is important since they often have a completely different clinical behaviour and prognosis, and may therefore require different types of treatment.[1]

A diagnosis of PCL can only be made after adequate staging investigations have been conducted. Adequate staging procedures include, besides a detailed history and physical examination, routine laboratory tests (complete and differential blood cell count and serum biochemistry), computed tomography (CT) scan of neck, chest and abdomen and a bone marrow biopsy. At recent consensus meetings of the European Organization for Research and Treatment of Cancer (EORTC) and the International Society of Cutaneous Lymphoma (ISCL), aimed to develop staging systems and guidelines for diagnosis and treatment of PCL other than mycosis fungoïdes (MF) and Sézary syndrome (SS), the necessity to perform a bone marrow biopsy in indolent PCL was questioned. In the subsequent consensus paper, it was suggested that bone marrow examination was recommended, but not required in indolent PCL, including primary cutaneous anaplastic large cell lymphoma, primary cutaneous marginal zone lymphoma (PCMZL) and primary cutaneous follicle centre lymphoma (PCFCL), unless indicated by other staging assessments.[2] Regarding the two types of indolent cutaneous B-cell lymphoma (CBCL), this is in accordance with the National Comprehensive Cancer Network (NCCN) clinical practice guidelines [v.1.2007: available from: http://www.nccn.org/professionals/physician_gls/PDF/nhl.pdf. Accessed 31 July; 2007]. In these guidelines bone marrow biopsies are not part of the essential workup of follicular lymphoma and marginal zone lymphoma and are only advised in selected cases. However, in most other guidelines, including those of the Dutch-Belgian Hemato-Oncology Group (HOVON) [available from: http://www.hovon.nl/hovon/stream.asp?hovnhl.pdf. Accessed 31 July; 2007], unilateral trephine bone marrow biopsies are part of routine work-up in all patients with (a suspicion of) non-Hodgkin lymphoma.[3;4]

The recommendation that a bone marrow biopsy is not required in indolent types of CBCL (PCMZL and PCFCL) is confusing, since studies describing these PCMZL and PCFCL as indolent lymphomas required negative staging procedures, including a negative bone marrow biopsy.[1] Henceforth, this recommendation means that in patients who present with skin lesions that are clinically and histologically highly suggestive or consistent with a diagnosis of PCMZL or PCFCL a bone marrow is not required, if other staging procedures are negative. In case of marginal zone lymphoma the consequences of not performing a bone marrow biopsy are expected to be limited. In case staging (including a bone marrow biopsy) is negative, it concerns a PCL, which, - according to the WHO-EORTC classification for cutaneous lymphomas -, is classified as PCMZL. If, however, a bone marrow biopsy and/or other staging procedures are positive, it does not concern a PCL, and

the WHO-EORTC classification should not be applied. Such cases should be classified according to the WHO classification as an extranodal marginal zone lymphoma with skin localizations. In both instances it concerns an indolent type of B-cell non-Hodgkin lymphoma, and the therapeutic consequences will be minimal. In case of PCFCL the situation is more complex. PCFCL are defined as tumors of neoplastic follicle center cells, usually a mixture of centrocytes and variable numbers of centroblasts, which may show a follicular (5%), follicular and diffuse (30%) or diffuse growth pattern (65%).[5] These PCFCL are preferably treated with radiotherapy and have an excellent prognosis with a 5-year-survival of 95%, irrespective of the growth pattern (follicular and/or diffuse) or the number of blast cells.[1] However, if in a patient with clinicopathologic features suggesting a PCFCL, a bone marrow biopsy and/or other staging procedure prove to be positive, a diagnosis of PCFCL can not be made. Such cases concern a secondary cutaneous lymphoma and should be classified according to the WHO classification as either follicular lymphoma or, in most cases, as a diffuse large B-cell lymphoma, which will have major therapeutic consequences. One would therefore assume that not performing a bone marrow biopsy in such cases may result in inappropriate treatment.
Studies on the frequency of extracutaneous manifestations and in particular a positive bone marrow biopsy in patients presenting with skin lesions otherwise consistent with a PCMZL or PCFCL have never been performed. In the present study we therefore evaluated retrospectively staging results of a large group of patients presenting with skin lesions that showed histological features consistent with a diagnosis of marginal zone lymphomas (MZL) or follicle centre lymphomas (FCL). The aim was to evaluate the frequency of bone marrow involvement in these patients in order to find out whether our current policy to perform a bone marrow biopsy in all patients with a histological diagnosis of cutaneous MZL or FCL should be maintained or that it should only be performed in selected cases as suggested recently.[2]

Patients and Methods

All patients who presented with skin lesions showing histologic and immunophenotypical features consistent with a diagnosis of MZL (n = 107) or FCL (n = 250) between 1985 and 2006 were retrieved from the database of the Dutch Cutaneous Lymphoma Group. The term FCL is herein used for tumors of neoplastic follicle centre cells with either a follicular, a follicular and diffuse or a diffuse growth pattern, which should be classified as PCFCL, in case staging procedures were negative. All cases had been reviewed by a panel of hematopathologists and dermato(patho)logists and classified by consensus prior to entry in the database. For each patient in this registry follow-up is collected yearly. Patients with a history of a prior systemic B-cell lymphoma (n = 16; 2 MZL and 14 FCL) and patients in whom a bone marrow biopsy was not performed (n = 66; 23 MZL and 43 FCL) were excluded from the study. The final study group consisted of 82 MZL and 193 FCL (Table 1). Median follow-up for the whole group was 43 months (range 1 – 443 months). Staging

procedures had included physical examination, complete and differential blood cell counts and serum biochemistry, CT-scan of neck, chest and abdomen and a bone marrow biopsy. The results of the staging investigations were extracted from the patient's files.
Statistical calculations were performed using SPSS 14.0 (SPSS Inc, Chicago, IL). Overall survival (OS) was calculated from the date of diagnosis until the patient's death or date of last follow-up. Disease-specific survival (DSS) was calculated from the date of diagnosis until death from lymphoma (including therapy-related death) or censoring. Patients who were alive at last follow-up or patients who died of other causes were considered censored. Survival curves were estimated using the method of Kaplan and Meier and statistical comparison between curves was done by log-rank testing.

Table 1. Selection of cases and results of bone marrow biopsy and disease-specific survival (DSS) in marginal zone lymphomas and follicle center lymphomas first presenting in the skin.

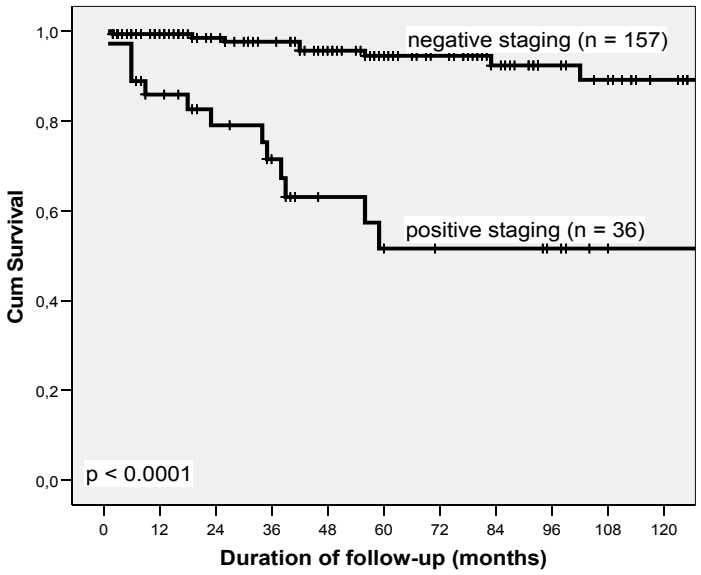
Histology	Marginal zone lymphoma	Follicle centre lymphoma
Total number present in database DCLG (*)	107	250
History of non-cutaneous lymphoma	2	14
Bone marrow biopsy not performed (5-year DSS)	23 (100%)	43 (86%)
Final study group (5-year DSS)	**82 (92%)**	**193 (86%)**
Male: female	52:30	118:75
Median age (range)	57 (23-90)	59 (21-90)
Median duration of follow-up, months (range)	31 (1-334)	51 (1-443)
Staging negative (5-yr DSS)	76 (98%)	157 (95%)
Staging positive (5-yr DSS)	6 (50%)	36 (51%)
Bone marrow involvement (5-yr DSS)	2 (50%)	22 (51%)
Only bone marrow involvement (5-yr DSS)	1 (100%)	9 (63%)

Results

Marginal zone lymphoma

In the group of 82 MZL patients, 76 patients showed no signs of extracutaneous involvement during staging investigations and were diagnosed as PCMZL (see Table 1).[1] In six cases (7%) extracutaneous localizations were found, including five patients with enlarged lymph nodes on the CT-scan. Bone marrow involvement was found in two of these six patients, in one of them as part of widespread disease with involvement of central lymph nodes and peripheral blood as well, and in the other patient as the only evidence of extracutaneous disease. The first patient was treated with chemotherapy, but died from secondary acute myeloid leukaemia three years after diagnosis. The second patient is in complete remission for over five years following an autologous bone marrow transplantation and radiotherapy for a skin relapse during follow-up. Patients with and without extracutaneous involvement had a 5-year OS of 33% and 91%, respectively and a 5-year DSS of 50% and 98% respectively (Table 1).

Figure 1: Disease-specific survival of follicle center lymphomas first presenting in the skin; cases with positive staging (n = 36) versus cases with negative staging (i.e. true PCFCL; n = 157).

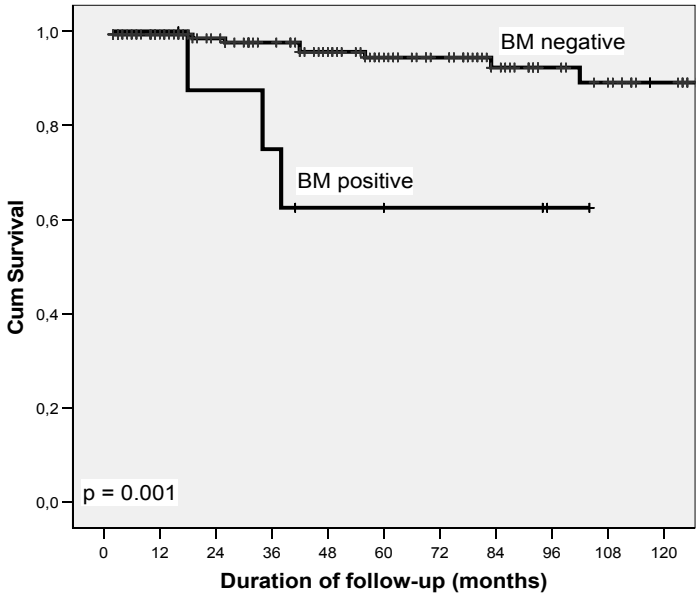


Follicle centre lymphoma

Of 193 FCL patients with complete staging work-up 157 patients showed no evidence of extracutaneous disease and were classified as PCFCL.[1] Extracutaneous disease was detected at the time of diagnosis in 36 cases (19%). Nine of 36 cases showed the characteristics of a follicular lymphoma, while the other 28 cases lacked a follicular component and were classified as diffuse large B-cell lymphoma. Taken together, in 22 of these 36 cases the bone marrow biopsy was positive or contained lymphoid aggregates highly suspect for lymphoma involvement (Table 1). In 13 patients, bone marrow involvement was accompanied by more extensive involvement of other organs or lymph nodes as assessed by CT-scans and/or lymph node histology. In the remaining nine cases the bone marrow was the only extracutaneous localization. The 14 patients with extracutaneous disease, but no bone marrow involvement showed nodal disease as assessed by CT-scan and/or lymph node biopsy (n = 13), while one patient showed bone involvement.

The 5-year OS for the 36 patients with and the 157 patients without extracutaneous involvement was 42% and 84%, respectively, whereas the 5-year DSS was 52% and 95% respectively ($p < 0.001$; Figure 1). Patients with bone marrow involvement with or without extracutaneous localizations at other sites had a 5-year OS of 30% and 44% and a 5-year DSS of 30% and 63%, respectively (Table 1).

Figure 2: Disease-specific survival of follicle center lymphomas first presenting in the skin; cases with only bone marrow in addition to skin lesions (n = 9) versus true PCFCL (n = 157).



Discussion

Recently, discussions have started whether or not bone marrow biopsies should be routinely performed in patients with a MZL or FCL first presenting in the skin.[2] Since there are, to the best of our knowledge, no studies that have addressed this question for this particular group of lymphomas, we evaluated the results of bone marrow biopsy examinations in 275 patients with a MZL or FCL first presenting in the skin. Considering the whole group of 275 patients, 24 patients (9%) had bone marrow involvement at initial diagnosis, while in 10 patients (4%) this was the only evidence of extracutaneous disease.

In the group of MZL, bone marrow was involved in only two of 82 (2%) patients, and in one of these two patients it was the only evidence of extracutaneous disease. Thus, if bone marrow biopsies would not have been performed in this group, only one of 82 cases would have been misdiagnosed as PCMZL.

Recent studies on large cohorts of nongastric extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), report bone marrow involvement at initial diagnosis in 14% to 28% of patients.[6-8] Cutaneous MZL included in these studies showed bone marrow involvement in zero of seven[7] and two of 22 (9%)[8] cases, respectively. Since MALT lymphomas present with disseminated disease in a considerable proportion of cases, these studies emphasize the need of extensive staging procedures, including a bone marrow biopsy. In addition, in these studies bone marrow involvement was associated with an inferior prognosis. In contrast, a recent Austrian study found bone marrow involvement in only three of 140 (2%) MALT lymphomas, including one of 79 (1%) nongastric MALT lymphomas, thus arguing against the necessity of a routine bone marrow biopsy. [9]The different frequencies of bone marrow involvement in these studies are as yet unexplained.

The results of the present study and data from the literature indicate that bone marrow involvement is uncommon in MZL first presenting in the skin, and that it is rarely the only manifestation of extracutaneous disease. Moreover, treatment of MZL with skin lesions and isolated bone marrow involvement will not be different from that of MZL presenting with only skin lesions (PCMZL). In both cases, radiotherapy will be the primary option, although the (known) presence of bone marrow involvement will ask for a closer follow-up of such a patient compared to the patient with only skin involvement. It is also interesting to note that the 23 patients with cutaneous MZL in whom no bone marrow biopsy was performed, and who were excluded from the present study, had a 5-yr DSS of 100% (see Table 1). Thus, from a clinical point of view, there does not seem to be a good reason to consider a bone marrow biopsy mandatory in these patients. However, if bone marrow biopsies are no longer performed, a definite diagnosis of PCMZL can no longer be made with certainty. Given the major pathogenetic differences in terms of translocations and antigenic stimuli involved in the development of MALT lymphomas at different sites[10-12],

not performing a bone marrow biopsy may obscure distinction between primary and secondary cutaneous cases and hamper elucidation of the pathogenesis of genuine PCMZL.

In the group of FCL first presenting in the skin, bone marrow was involved in 22 of 193 cases (11%), whereas bone marrow involvement was the only evidence of extracutaneous disease in nine of them. Thus, if bone marrow biopsies had not been performed in this group, nine cases would have been incorrectly classified as PCFCL. The finding of bone marrow involvement in these nine patients appears clinically relevant, since they had a significantly worse survival as compared to the 157 PCFCL patients (5-year OS 44% versus 84%; p = 0.001; 5-year DSS 63% versus 95%; p = 0.001; Figure 2). In addition, although the clinical presentation (site and extent of skin lesions) in these nine patients did not differ from patients with a PCFCL, the presence of bone marrow involvement may also have therapeutic consequences.
In three of nine cases the skin infiltrates showed a partial follicular growth pattern and were classified as follicular lymphoma with skin and bone marrow involvement. In the other six cases the skin biopsies showed a diffuse population of large cleaved cells without a follicular component and were classified as diffuse large B-cell lymphoma according to the WHO classification. Taken together, these data indicate that a bone marrow biopsy is mandatory in patients with a FCL first presenting in the skin.

Due to the fact that these patients were collected during a 20-year period, all data were retrieved from a database or patient's files, and some of these could not be reviewed. The quality of CT scans has very much improved, and PET scans have recently contributed to the upgrading of staging procedures. As far as bone marrow biopsies are concerned, much has changed during the last decade. The Cheson guidelines[3] request a well-defined quality, regarding length of the specimen (>2 cm) with sufficient marrow fields, and in the case of doubtful infiltration additional immunohistochemistry, which obviously was not available in the early years of this study. Although some *false-positive* marrows might have been present, one would rather expect *false-negative* bone marrows. We therefore believe that there are two reasons why the percentage of positive bone marrow biopsies might be even higher than found in our study. First, in the absence of a histologic review of the bone marrow biopsies, it can not be excluded that some designated negative, were in fact of insufficient quality to allow a definite conclusion. Exclusion of such 'false-negative' bone marrow biopsies would result in a higher percentage of positive bone marrow examinations. Second, one of the main goals of the Dutch Cutaneous Lymphoma Group is to assist referring dermatologists and pathologists in making a correct diagnosis of patients with (suspicion of) a *cutaneous* lymphoma. Although the database contains both primary and secondary cutaneous lymphomas, patients presenting with skin lesions and extensive extracutaneous disease, and who were primarily referred to a haematologist, may not have been included in the Dutch Cutaneous Lymphoma Group database. If such patients would

have been included, the percentage of positive bone marrow biopsies would definitely have been higher. However, we believe that this will apply to only a few patients as compared to the large number of cases included in this study, and therefore will not significantly influence our conclusions.

In conclusion, the results of our study indicate that bone marrow investigation is an essential part of staging procedures in patients with a FCL first presenting in the skin. From a clinical point of view, bone marrow examination appears to have limited value in patients with MZL presenting in the skin, and may be considered only in selected cases, as previously suggested.[2]

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Chapter 6.

Results of radiotherapy in 153 primary cutaneous B-cell lymphomas classified according to the WHO-EORTC classification

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Abstract

Objective: To evaluate the results of radiotherapy in patients with primary cutaneous B-cell lymphoma (CBCL) classified according to the criteria of the WHO-EORTC classification.

Design: A multicenter, 20-year, retrospective, cohort analysis.

Setting: Eight dermatology departments collaborating in the Dutch Cutaneous Lymphoma Group.

Patients: From 1985 until 2005 153 CBCL patients were initially treated with radiotherapy with curative intent. These cases were classified according to the WHO-EORTC classification and consisted of 25 primary cutaneous marginal zone lymphoma (PCMZL), 101 primary cutaneous follicle centre lymphoma (PCFCL) and 27 primary cutaneous large B-cell lymphoma, leg type (PCLBCL, LT).

Interventions: Local radiotherapy with a median dose of 40 Gy (range 20-46 Gy) applied to all visible skin lesions.

Main Outcome Measures: Complete remission rate, relapse rate, 5-year relapse-free survival, 5-year overall survival and 5-year disease-specific survival (DSS).

Results: Complete remission was reached in 151 of 153 (99%) patients. Relapse rates for PCMZL, PCFCL and PCLBCL, LT were 60%, 29% and 64% and the 5-year DSS was 95%, 97% and 59% respectively. PCFCL presenting on the leg(s) had a higher relapse rate (63%) and a much lower 5-year DSS (44%) as compared to PCFCL presenting at other sites (relapse rate: 25% and 5-year DSS: 99%).

Conclusions: Radiotherapy is a suitable treatment for a large group of CBCL patients. However, patients with PCFCL presenting with lesions on the leg(s) and patients with PCLBCL, LT display a more unfavourable clinical course and should therefore be treated with more aggressive treatment modalities.

Introduction

The term primary cutaneous B-cell lymphomas (CBCL) refers to a heterogeneous group of B-cell non-Hodgkin lymphomas, that present in the skin without evidence of extracutaneous disease at the time of diagnosis. In the past years there has been much debate regarding the terminology and classification of these lymphomas. Recently, representatives of the European Organization for Research and Treatment of Cancer (EORTC) classification and the World Health Organisation (WHO) classification have reached consensus regarding a new classification for primary cutaneous lymphomas. In this WHO-EORTC classification three main types of CBCL are recognized: primary cutaneous marginal zone lymphoma (PCMZL) and primary cutaneous follicle centre lymphoma (PCFCL), both indolent types of CBCL with a 5-year disease-specific survival over 95%, and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT), which represents a more aggressive type of CBCL with a 5-year-disease-specific survival of approximately 50%.[1]

Previous studies have shown that radiotherapy (RT) is a suitable treatment in both types of indolent CBCL, and perhaps also in PCLBCL, LT presenting with solitary or localized skin lesions.[2-6] However, data from the literature regarding efficacy and relapse rate show a wide variation.[2;3;5;7-10] Moreover, these studies are all based on cases classified according to the EORTC[11] or WHO[12] classifications. In a recent study we showed that approximately 5% and 36% of cases formerly classified according to the EORTC and WHO classifications respectively, were assigned to another prognostic category, when the WHO-EORTC classification was used.[13] These observations prompted us to evaluate the results of RT as initial treatment in CBCL reclassified according to the criteria of the new WHO-EORTC classification.

The purpose of this retrospective study was to define complete remission (CR) rates, relapse rates, relapse-free survival (RFS), 5-year overall survival (OS) and 5-year disease-specific survival (DSS) following RT for these newly defined groups of CBCL, and to establish for which patients RT is a safe and effective mode of treatment.

Patients and Methods

Selection of patients

Between 1985 and 2005 320 patients with CBCL were included in the registry of the Dutch Cutaneous Lymphoma Working Group. Follow-up data had been collected each year from patients' medical charts or referring physicians. In a recent study clinical data and histological sections of 300 CBCL were reviewed and reclassified according to the criteria of the new WHO-EORTC classification.[13] This group included 174 patients, who had received RT as initial treatment. Patients in which RT was administered in palliative dosages with no intention to be curative were excluded (n = 7), as were patients with a short follow-up of less than 12 months (n = 14). However, patients that died within 12 months after diagnosis due to their lymphoma were not excluded from the study. The final study

group consisted of 153 CBCL. According to the WHO-EORTC classification this group included 25 PCMZL, 101 PCFCL and 27 PCLBCL, LT (see Figure 1).
For all patients the following data were recorded: age at diagnosis, gender, involved skin site, extent of involved skin, result of initial therapy, occurrence and site of relapse, relapse treatment, result of relapse treatment, duration of follow-up and status at last follow-up. Extent of involved skin was defined as solitary when it concerned a single tumour, localized when the lesion consisted of multiple plaques and/or tumours that could be irradiated within one radiation field and multifocal if multiple nonadjacent body sites were involved or when the lesion(s) could not be irradiated within one radiation field.

Treatment

Most patients had been treated with electron beam irradiation (4 to 10 MeV), while nine patients received 6 to 10 MV photon beams. The radiation dose varied between 20 and 46 Gy, with a median dose of 40 Gy. In all patients, a margin of at least 2 cm of healthy skin was included in the radiation field. In PCFCL localized on the trunk, which often present with tumors surrounded by annular erythemas, the erythematous areas were included in the radiation field because they represent early manifestations of the neoplastic process.[14-16] Treatment response was evaluated 4-6 weeks after the end of radiotherapy by clinical examination and classified as CR, partial remission (PR), no response (NR) and progressive disease (PD); CR was defined as the disappearance of all visible skin lesions, PR was defined as a 50% or more remission of clinical lesions, NR as less than 50% remission and PD as development of new skin lesions during treatment. Outcome measures for results of RT were complete remission (CR) rate, relapse rate, 5-year relapse-free survival (5-yr RFS), 5-year overall survival (5-yr OS) and 5-year disease-specific survival (5-yr DSS).

Statistical analysis

Overall survival was calculated from the date of diagnosis until the patient's death or last follow-up without event. Disease-specific survival was calculated from the date of diagnosis until death of lymphoma or last follow-up without event. Relapse-free survival was calculated from the date complete remission was reached until first relapse or last follow-up without event. Survival curves were estimated using the method of Kaplan and Meier and statistical comparison between curves was done by log-rank testing. The χ^2 test was used to analyze differences between subgroups. All statistical analyses were done with Statistical Product and Services Solutions software, version 12.0.1 (SPSS Inc, Chicago, Ill).

Results

The total group consisted of 87 men and 66 women, with a median age of 63 years (range 23-92 years). Median follow-up for the whole group was 62 months (range 3-336 months). The clinical characteristics, results of RT and follow-up data of the total group of CBCL are presented in Table 1. Clinical characteristics, treatment results and follow-up data of the

three subgroups of CBCL are also summarized in Table 1 and will be described in more detail below.

Primary cutaneous marginal zone B-cell lymphoma
The group of PCMZL consisted of 18 men and seven women, with a median age of 49 years (range 23-79 years). Nine patients had a solitary lesion, five patients had localized skin lesions, while 11 patients presented with multifocal disease. Most patients presented with lesions on the trunk or arms (see Table 1).
RT resulted in a complete remission in all 25 patients. Fifteen patients (60%) experienced a relapse after a median relapse-free interval of 16 months (range 3-144 months). Twelve of these patients showed relapses confined to the skin, one patient had a cutaneous and an extracutaneous relapse and two patients experienced an extracutaneous relapse without concurrent skin lesions. Cutaneous relapses always occurred at unirradiated sites. Patients with a PCMZL had an excellent prognosis with a 5-yr OS and DSS of 90% and 95%, respectively (see Figure 2).

Primary cutaneous follicle center lymphoma
The PCFCL group consisted of 61 men and 40 women, with a median age of 58 years (range 27-85 years). Most patients (93/101; 92%) presented with solitary lesions (n = 48) or localized skin lesions (n = 45). Only eight of 101 patients had multifocal disease at the time of presentation. The large majority presented with the typical lesions on the head (n = 41) or trunk (n = 55). Eight patients had lesions on one (n = 7) or both (n = 1) leg(s). Six of these eight patients were previously classified as PCLBCL, leg following the EORTC classification. RT resulted in a complete remission in all 101 patients. A relapse was noted in 29 of 101 cases, two to 62 months (median 12 months) after initial treatment. In 21 patients the relapse was confined to the skin, three patients had a relapse in the skin and an extracutaneous localization, while 5 patients had a relapse at an extracutaneous site without concurrent skin lesions. All cutaneous relapses occurred outside the irradiated area. Skin relapses were generally treated with an additional course of RT, which resulted in another CR in all cases. Ultimately, 10 of 101 patients developed extracutaneous disease and four of 101 patients died of lymphoma. The five-year OS and 5-yr DSS survival was 90% and 97% respectively (see Figure 2).

Comparison between PCFCL with (solitary or localized) lesions confined to the head (n = 38) or trunk (n = 48) showed no difference in relapse rate (26% vs. 21%) or 5-yr DSS (95% vs. 98%). However, PCFCL presenting with lesions on the leg(s) (n = 8) had a higher relapse rate (63% versus 25%; $p = 0.028$), developed more often extracutaneous disease (38% versus 8%; $p = 0.006$), and had a considerably lower 5-yr DSS (44% versus 99%; $p < 0.001$), compared to the patients without lesions on the leg(s).

Our study group contains only five PCFCL presenting with multifocal skin lesions without involvement of the leg. Three of these five patients had a relapse in the skin; one of these three patients eventually developed intracerebral lesions and died of the lymphoma 26 months after the initial diagnosis.
Comparison between PCFCL with a follicular or follicular and diffuse growth pattern (32 cases; classified as cutaneous follicle center lymphoma in the WHO classification) and PCFCL with a diffuse growth pattern (69 cases; classified as diffuse large B-cell lymphoma (DLBCL) in the WHO classification) showed no significant difference in relapse rate (13/32; 41% vs. 16/69; 23% resp; $p = 0.072$) and no difference in DSS (100% vs. 95% resp; $p = 0.712$) between the 2 groups. This illustrates the fact that the growth pattern of the malignant infiltrate has no prognostic significance and does not justify more aggressive treatment.

Primary cutaneous large B-cell lymphoma, leg type
The PCLBCL, LT group contained eight men and 19 women. The age at diagnosis in this group (median 78 years, range 50-92 years) was considerably higher as compared to the other two groups. Twenty-five of 27 patients presented with skin lesions on the leg(s), while one patient presented with a solitary tumour on the scalp and another patient had lesions localized to the left forearm. These two patients were formerly classified as primary cutaneous follicle center cell lymphoma (PCFCCL) in the EORTC classification.
According to the WHO classification all 27 patients would have been classified as DLBCL.
Twelve patients had a solitary lesion at initial diagnosis and 15 patients presented with localized disease. This group did not contain patients presenting with multifocal skin lesions.
RT resulted in a complete remission in 25 of 27 patients (93%). In two patients, including the patient presenting with a solitary tumor on the scalp, new skin lesions developed outside the irradiated areas during initial radiotherapy, and both patients died of lymphoma three and nine months after diagnosis. The patient previously classified as PCFCCL (EORTC classification) and presenting with skin lesions on the left forearm reached a sustained complete remission now for more than five years.
Of the 25 patients that did reach a complete remission, seven patients relapsed only in the skin, four in the skin as well as in an extracutaneous site, while five patients showed only extracutaneous relapses. Two of the 11 skin relapses were located within the irradiated area, although the cumulative dose in these patients (36 and 40 Gy) was comparable to that of the other patients.
Ultimately, 10 patients showed extracutaneous dissemination and 11 patients died due to lymphoma. For the total group of PCLBCL, LT the 5-yr OS was 40% and the 5-yr DSS was 59% (see Figure 2). Comparison between patients presenting with a solitary lesion and patients presenting with multiple localized skin lesions did not show significant differences in 5-yr RFS (40% vs 32%; $p = 0.510$) or 5-yr DSS (61% versus 58%; $p = 0.727$).

Table 1. Patient characteristics, therapy results and follow-up data.

	Total group		PCMZL		PCFCL		PCLBCL, LT	
Total no. of patients	n = 153	%	n = 25	%	n = 101	%	n = 27	%
Age, years								
Median (range)	62 (23-92)		49 (23-79)		58 (27-85)		78 (50-92)	
Gender								
Male	87	57%	18	72%	61	60%	8	30%
Female	66	43%	7	28%	40	40%	19	70%
Ratio M/F	1.3		2.6		1.5		0.4	
Skin site								
Head	46	30%	4	16%	41	41%	1	4%
Trunk	68	44%	13	52%	55	54%	0	0%
Upper extremities	12	8%	9	36%	2	2%	1	4%
Lower extremities	38	25%	5	20%	8	8%	25	93%
Other	1	0.7%	0	0%	1	1%	0	0%
Extent								
Solitary	69	45%	9	36%	48	48%	12	44%
Localized	65	43%	5	20%	45	45%	15	56%
Multifocal	19	12%	11	44%	8	8%	0	0%
Status after RT								
CR	151	99%	25	100%	101	100%	25	93%
PR	0	0%	0	0%	0	0%	0	0%
NR	0	0%	0	0%	0	0%	0	0%
PD	2	1%	0	0%	0	0%	2	7%
First relapse*								
None	91	59%	10	40%	72	71%	9	36%
Skin	40	26%	12	48%	21	21%	7	28%
Skin & extracutaneous	8	5%	1	4%	3	3%	4	16%
Extracutaneous	12	8%	2	8%	5	5%	5	20%
Time to first relapse, months								
Median (range)	16 (1-144)		16 (3-144)		12 (2-62)		17 (1-61)	
Extracutaenous dissemination	24	16%	3	12%	11	11%	10	37%
Status at last follow-up								
Alive and well	117	76%	23	92%	84	83%	10	37%
Alive with disease	3	2%	0	0%	2	2%	1	4%
Died of lymphoma	16	10%	1	4%	4	4%	11	41%
Died of other cause	17	11%	1	4%	11	11%	5	19%
5-yr RFS		60%		45%		70%		36%
5-yr DSS		90%		95%		97%		59%

* Relapses were only considered in patients who achieved a complete remission (n = 151).

Discussion

In the present study we evaluated the results of RT in 153 patients with a CBCL, reclassified according to the criteria of the WHO-EORTC classification.

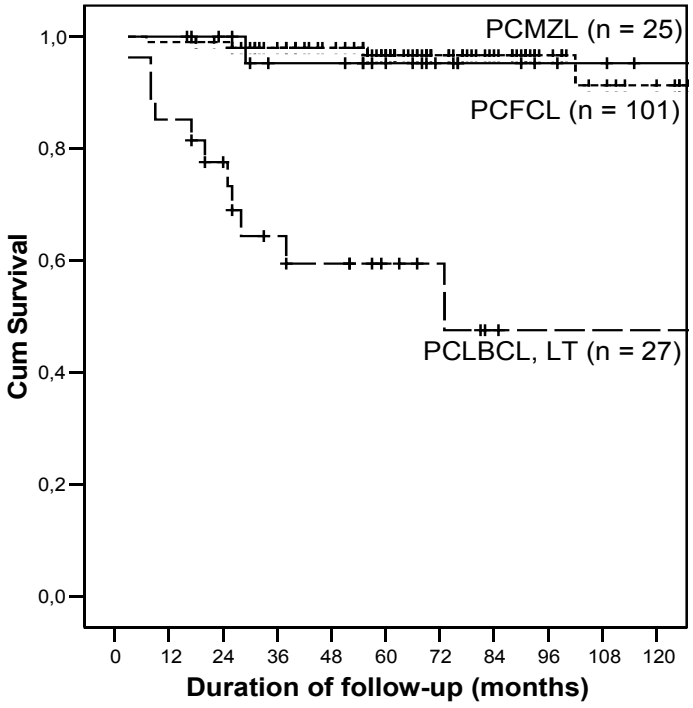
PCFCL formed the largest group (101 of 153 patients; 66%). In the WHO-EORTC classification these PCFCL are defined as a tumor of neoplastic follicle center cells, with a predominance of (large) centrocytes and variable numbers of centroblasts, which may have a follicular, a follicular and diffuse or a diffuse growth pattern, and which generally present on the head or trunk, and uncommonly on the legs.[1] In the EORTC classification these rare cases presenting on the legs were included in the category PCLBCL, leg.[11] The results of our study showed CR following initial RT in all 101 cases. The 5-yr RFS was 70% (see Figure 3). Twenty-one patients showed one or multiple relapses confined to the skin, while eight patients relapsed at extracutaneous sites with or without concurrent skin lesions. No infield recurrences were observed. In most patients skin relapses were successfully treated again with RT. The 5-yr DSS and OS of the total group were 97% and 90%, respectively. These observations are consistent with the results of most previous studies in patients with PCFCCL as defined by the criteria of the EORTC classification,[3;5;7;10] but differ considerably from the results reported by Piccino and coworkers.[8;9] These authors performed a retrospective study of 102 PCFCCL and found a relapse rate of 75% and a 5-yr RFS of only 23%. Moreover, infield relapses were noted in 18 of 102 cases. These differences might be explained by the use of orthovoltage techniques and the narrow margins (0.5-1.0 cm.) of clinically uninvolved skin included in the irradiation field, as previously postulated.[7] The reported survival data and the low proportion of patients developing extracutaneous disease however, are comparable in all these studies, which illustrates the favorable biologic behavior of this lymphoma.

Subgroup analysis showed that patients presenting with skin lesions on the leg more often relapsed, more often developed extracutaneous disease, and had a much more unfavorable prognosis than patients presenting with skin lesions on the head or trunk. These observations confirm the results of recent studies and suggest that presentation on the leg is an unfavorable risk factor in PCFCL.[13;17] It also suggests that such cases should not be treated routinely with radiotherapy.

Patients presenting with multifocal skin lesions represent another subgroup subjected to much debate.[2;3;9;18] In a previous study of our group it was found that PCFCL presenting with multifocal skin lesions have the same clinical behavior and prognosis as PCFCL presenting with solitary or localized skin lesions.[2] RT of all visible skin lesions proved equally effective as multi-agent chemotherapy. In fact, (skin) relapses were observed in three of nine patients treated with multi-agent chemotherapy and not in any of the five patients treated with RT. The present study group contained only five PCFCL presenting with multifocal skin lesions, but not located on the leg. Three of these five patients relapsed in the skin, and one of them died of CNS involvement.

Recent analysis of 13 PCFCL with multifocal skin lesions (excluding localization on the leg) treated with multi-agent chemotherapy showed (skin) relapses in 4/13 patients, while one of them developed extracutaneous disease during follow-up (unpublished data). Taken together, these small series do not allow firm conclusions to be drawn. Prospective collaborative studies on larger numbers of patients are required to evaluate if PCFCL presenting with multiple skin lesions can indeed be treated safely and effectively with RT.

Figure 2. Disease-specific survival after radiotherapy according to diagnostic category.



PCLBCL, LT are defined as tumors with a predominance or confluent sheets of centroblasts and immunoblasts, characteristically presenting with skin lesions on the (lower) legs. Cases with a similar morphology and phenotype (strong Bcl-2 and MUM-1 expression) arising at sites other than the leg are included in this group. In the EORTC classification such cases were included in the group of PCFCCL. In recent studies it was found that cases presenting on the leg and cases presenting at other sites have a similar clinical behavior and prognosis, indicating that reclassification of such cases as PCLBCL, LT is an improvement.[13;17] In the present study 25 of 27 patients presented with skin lesion on the legs, while two patients presented with skin lesions at other sites. In general, patients with PCLBCL, LT should be treated as other systemic DLBCL, for instance, with a regiment of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) in combination with Rituximab (anti-CD20 antibody). However, in a previous European multicenter study including 48 PCLBCL, leg, patients presenting with a solitary tumor on one leg had a significantly better prognosis than patients presenting with multiple tumors on one or both legs.[13;17;19] It was therefore suggested that patients with a solitary tumor could be treated with radiotherapy, while all other patients in this group should be treated with systemic chemotherapy. The results of the present study do not support this suggestion. No difference in relapse rate and OS and DSS were observed between patients with a solitary lesion and patients presenting with multiple localized lesions.
Moreover, two patients developed new skin lesions outside the irradiated areas still during initial treatment, and 16 of the other 25 patients showed relapses after initial therapy. In addition, this was the only group in which skin relapses developed within a previously irradiated area (2 patients). Only 9 of 27 patients showed a sustained CR after initial RT. Taken together, these results indicate that RT should not be used as first line treatment in these PCLBCL, LT, irrespective of the number of skin lesions. These patients should be routinely treated as other DLBCL following current protocols. Only in patients, who do not tolerate systemic chemotherapy because of a poor clinical condition or patients who refuse this type of treatment, RT may be considered as an alternative option.

As described recently[20], RT is also a highly effective treatment in PCMZL, either presenting with a solitary or few scattered lesions. However, the relapse rate in PCMZL proved much higher than in PCFCL (5-year RFS 36% versus 70%, respectively, see Figure 3). In patients presenting with many scattered skin lesions, RT is no longer first choice of treatment. In such patients beneficial effects have been reported of chlorambucil[20] or intralesional treatment with interferon alpha or rituximab.[21-23] In patients developing chronically relapsing disease, treatment is aimed at palliation and no longer at cure, and therefore the benefits of treatment should be weighed carefully against their potential side effects. In such patient, an expectant policy should be considered. The results of a recent pilot study suggest that low dose RT with 2 x 2 Gy is a useful alternative for such patients experiencing multiple skin relapses. (Neelis K.J. et al, manuscript in preparation)

Figure 1. Clinical examples of the studied disease entities.
A. Primary cutaneous marginal zone lymphoma. B. Primary cutaneous follicle centre lymphoma.
C. Primary cutaneous large B-cell lymphoma, leg type.

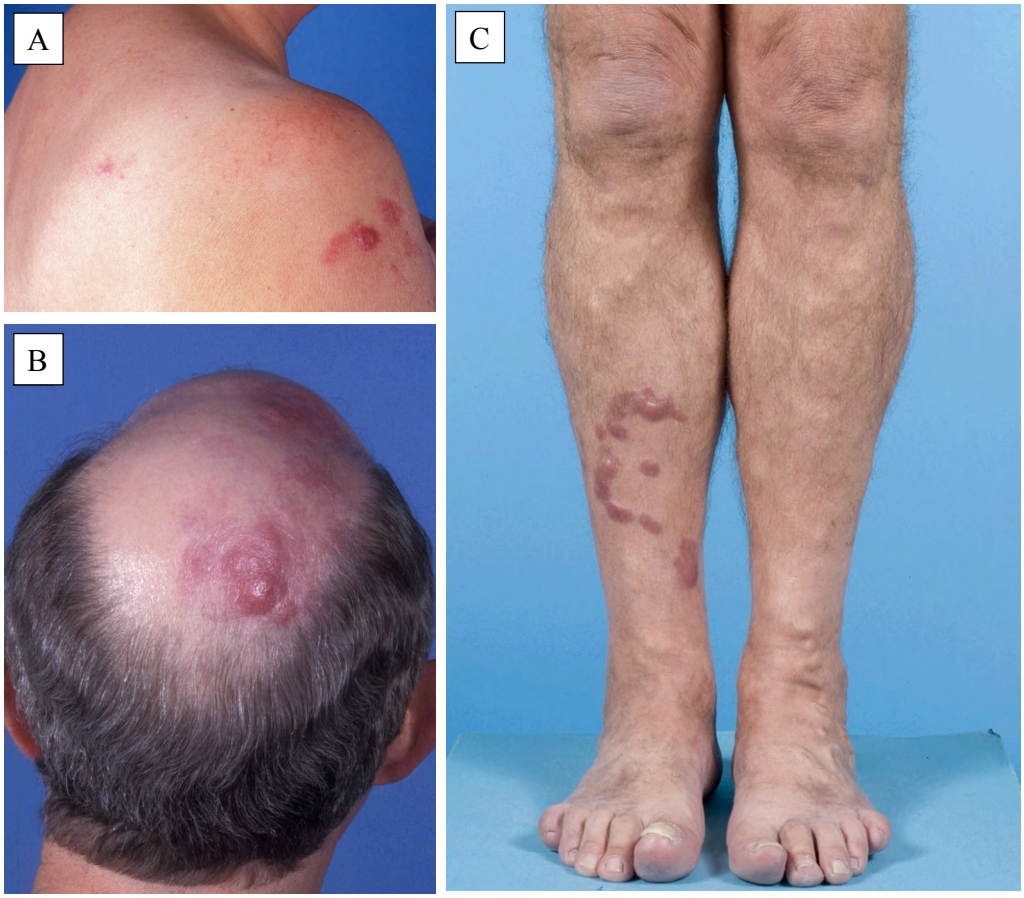
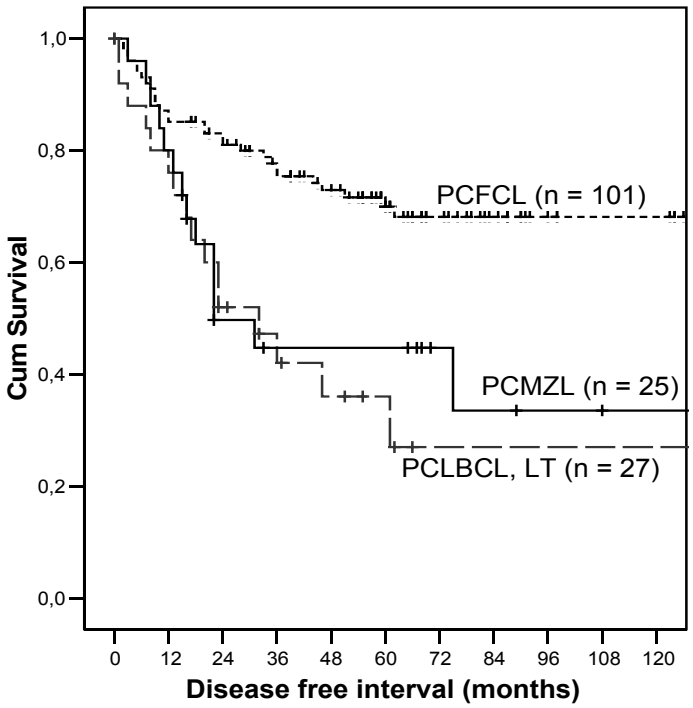


Figure 3. Relapse-free survival after radiotherapy according to diagnostic category.



In conclusion, the results of our study indicate that RT is a safe and effective treatment in many patients with CBCL. In PCFCL and PCMZL, RT is the first line of treatment, not only in patients presenting with solitary or localized skin lesions, but probably also in patients presenting with few scattered skin lesions. However, prospective, collaborative studies are required to confirm this latter conclusion. PCLBCL, LT and rare patients with a PCFCL presenting with skin lesions on the leg appear to have a more aggressive clinical course, and RT should not be considered as first choice of treatment in these patients.

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Chapter 7.

European Organization for Research and Treatment of Cancer (EORTC) and International Society for Cutaneous Lymphoma (ISCL) consensus recommendations for the management of cutaneous B-cell lymphomas

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Abstract
Primary cutaneous B-cell lymphomas (CBCL) represent approximately 20-25% of all primary cutaneous lymphomas. With the advent of the World Health Organization-European Organization for Research and Treatment of Cancer (EORTC) consensus classification for cutaneous lymphomas in 2005, uniform terminology and classification for this rare group of neoplasms was introduced. However, staging procedures and treatment strategies still vary between different cutaneous lymphoma centres, which may be due to the fact that consensus recommendations for the management of CBCL have never been published.
Based on an extensive literature search and discussions within the EORTC Cutaneous Lymphoma Group and the International Society for Cutaneous Lymphomas, the present report aims to provide uniform recommendations for the management of the three main groups of CBCL. Since no systematic reviews or (randomized) controlled trials were available, these recommendations are mainly based on retrospective studies and small cohort studies. Despite these limitations, there was consensus among the members of the multidisciplinary expert panel that these recommendations reflect the state of the art management as currently practised in major cutaneous lymphoma centres. They may therefore contribute to uniform staging and treatment and form the basis for future clinical trials in patients with a CBCL.

Introduction

In the last two decades it has become clear that some subtypes of B-cell non-Hodgkin lymphoma can exclusively present in the skin. These primary cutaneous B-cell lymphomas (CBCL) are much less common than primary cutaneous T-cell lymphomas (CTCL) and represent approximately 20-25% of all primary cutaneous lymphomas.[1] For many years there has been confusion regarding the terminology and classification of CBCL (see Table 1), which may have come to an end with the publication of the new WHO-EORTC classification for cutaneous lymphomas.[1] This classification distinguishes three main types of CBCL: primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle centre lymphoma (PCFCL) and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT). The characteristic clinical, histological and immunophenotypical features of these three subgroups are presented in Table 1 and characteristic clinical presentations in Figures 1-3. In the forthcoming update of the WHO classification (to be published in the WHO Blue Book Series in June 2008), PCFCL and PCLBCL, LT will be included as separate entities, while PCMZL will be included in the broader category of extranodal marginal zone B-cell lymphomas.

It is generally recognized that PCMZL and PCFCL are indolent types of CBCL, which should not be treated primarily with systemic chemotherapy. Nonetheless, many patients with a PCFCL, - in particular patients with a diffuse infiltration of large cleaved cells that were previously classified as diffuse large B-cell lymphoma (DLBCL) in the WHO 2001 classification[2] -, are often still treated unnecessarily with systemic chemotherapy. Moreover, staging procedures and treatment strategies vary between different cutaneous lymphoma centres, which may be due to the fact that, in contrast to CTCL[3;4], consensus guidelines for the management of CBCL have never been published.

Based on an extensive literature search and discussions at meetings of the EORTC Cutaneous Lymphoma Group (EORTC-CLG) and the International Society for Cutaneous Lymphoma (ISCL), the present report aims to provide uniform recommendations for the staging and treatment of the three main types of CBCL. Rare types of CBCL that occasionally present in the skin without detectable extracutaneous disease (e.g. intravascular large B-cell lymphoma and plasmablastic or lymphoblastic lymphoma) will not be discussed in this review.

Table 1. Overview of previously and currently used classification systems for cutaneous lymphomas and clinicopathologic features of the different CBCL entities.

Previous and current classifications			
EORTC 1997	PCI/ PCMZL	PCFCCL	PCLBCL of the leg
WHO 2001	EMZL	cFCL DLBCL	DLBCL
WHO-EORTC 2005	**PCMZL**	**PCFCL**	**PCLBCL, LT**
WHO 2008	**EMZL**	**PCFCL**	**PCLBCL, LT**
Clinicopathologic features			
Clinical features	• solitary or multiple papules, plaques or nodules preferentially localized on the extremities • sometimes associated with Borrelia burgdorferi infection • frequent cutaneous relapses • rarely extracutaneous dissemination	• solitary or grouped tumors presenting on the head or on the trunk • cutaneous relapses in 20% • extracutaneous dissemination in 5-10%	• solitary or multiple tumors presenting mainly on the leg(s) and rarely at other sites • frequent relapses and extracutaneous dissemination
Histopathology	patchy or diffuse infiltrates composed of small B-cells, including marginal zone (centrocyte-like) cells, lymphoplasmacytoid cells and plasma cells	follicular, follicular and diffuse or diffuse infiltrates composed of neoplastic follicle center cells , usually a mixture of centrocytes and variable numbers of centroblasts	diffuse infiltrates with a predominance or confluent sheets of of centroblasts and immunoblasts
Immunophenotype	monotypic cIg, CD79a+, Bcl-2+, CD5-, cyclin D1-, Bcl-6-, CD10-, MUM-1+ (on plasma cells)	monotypic sIg or absence of sIg, CD20+, CD79a+, Bcl-6+, Bcl-2-, MUM-1-, CD10+/-, FOXP1- (+/-)	monotypic sIg and/or cIg, CD20+, CD79a+, Bcl-6 +/(-), CD10-, Bcl-2+, MUM-1+, FOXP1+
Prognosis	5-year survival: > 95%	5-year survival: 95%	5-year survival: 50%

Development process of recommendations

At a meeting of the ISCL Group in March 2006 representatives of the Dutch Cutaneous Lymphoma Group were asked to formulate a proposal for recommendations for the management of CBCL. Based on the results of an extensive literature search, concept recommendations were made, which were discussed and agreed upon with minor modifications at an ISCL meeting in Buenos Aires in September 2007. The final text was agreed upon by an expert group of dermatologists, hematologists/oncologists and radiation oncologists, selected from the membership of the EORTC-CLG and ISCL group for their expertise in research and clinical practice of CBCL.

Review of the literature

A search of PubMed, EMBASE and Web of Science was conducted to identify relevant studies (final update 7 December 2007). Relevant articles were identified by combining terms and phrases related to cutaneous or skin, B-cell lymphoma(s) and treatment or therapy. Relevant articles were selected and reviewed by two reviewers (NJS; RW). Articles were selected for inclusion if they described treatment results in patients with CBCL or if they contained a table with clinical and follow-up data of CBCL patients, containing at least information on initial treatment and treatment results. Because of the low incidence of CBCL and the fact that (randomised) controlled trials and systematic reviews were not available, case reports and small retrospective cohort studies were also included. Papers describing other entities besides the three main groups of primary cutaneous B-cell lymphoma (PCMZL, PCFCL and PCLBCL, LT), papers without relevance to treatment, reviews merely summarizing existing literature, meeting abstracts and most non-English literature were excluded. Many other papers were of limited value because the type of CBCL was not specified or treatment results were not reported separately for different diagnostic subgroups. If the same cohort was described in several subsequent papers, only the last publication was used in our analysis. From the original 318 retrieved references, 92 were selected for further analysis. These included 32 case reports (35%) and 60 case series or retrospective cohort studies including 2-5 patients (20 papers; 22%), 6-10 patients (9 papers; 10%), 11-50 patients (23 papers; 25%) or more than 50 patients (8 studies; 9%). Papers were scored according to the levels of evidence as described by the Oxford Centre for Evidence-Based Medicine (available from: http://www.cebm.net/levels_of_evidence.asp. Accessed 3 December 2007). However, since only case reports, case series and retrospective cohorts were available and as a result all evidence was scored as level 4 or 5 and grades of recommendation were mostly of the D category, levels of evidence are not further discussed.

Diagnosis and staging of cutaneous B-cell lymphomas

Diagnosis

When a cutaneous B-cell lymphoma is clinically suspected, adequate histological and immunohistochemical studies are required to confirm or discard the diagnosis. A definite diagnosis can only be made on a representative biopsy of adequate length and diameter (preferably an excisional biopsy, but in case of punch biopsies the diameter should be at least 4 mm whenever possible). Besides considering the morphology of the neoplastic B-cell population and the growth pattern of the malignant infiltrate, immunohistochemical studies are required to make a definite diagnosis. Since a detailed description of markers used in the differential diagnosis of cutaneous B-cell infiltrates is beyond the scope of this review, only a short-list of relevant immunostainings is provided:

- CD3, CD20 and/or CD79a for confirmation of B-cell lineage and assessment of the amount of admixed reactive T-cells.
- Surface and cytoplasmic immunoglobulins (sIg; cIg) on frozen and paraffin sections, respectively, to confirm B-cell lineage and to detect monotypic Ig expression.
- CD35 or CD21 to visualize reactive follicles or (remnants of) dendritic networks.
- Ki-67 to determine the proliferative fraction and to aid in the differentiation between neoplastic and reactive follicles.[5]
- Bcl-2, Bcl-6, CD10, MUM-1 and FOXP1 may aid in the distinction between different types of CBCL, pseudo B-cell lymphoma and secondary cutaneous B-cell lymphoma (see Table 1).[6-9] Strong expression of Bcl-2, Bcl-6 and CD10 in follicular structures should always raise suspicion of a systemic follicular lymphoma with secondary skin involvement and the presence of the interchromosomal translocation t(14;18) should be examined.
- In selected cases use of CD5 and cyclin D1 is useful to differentiate PCMZL (CD5-, cyclin D1-) from mantle cell lymphomas (CD5+, cyclin D1+) and skin localizations of B-cell lymphocytic leukaemia (CD5+, cyclin D1-).

Although successful application of flow cytometry in the diagnosis of CBCL has been reported[10], it is not widely used and can not be considered as a substitute for immunohistochemistry. Disadvantages of this approach are the difficulties to obtain sufficient viable single cell suspensions, due to the vulnerability of the cutaneous B-cells, the lack of architectural information and the need of additional fresh tissue material. Demonstration of clonal IgH gene rearrangements, using the standard BIOMED-2 primers and protocol[11-14], may be a useful aid in the diagnosis of CBCL, but the results should always be considered in conjunction with clinical, histological and immunohistochemical data.

Staging procedures

ISCL/EORTC recommendations for staging in cutaneous lymphomas other than mycosis fungoides/Sézary syndrome have recently been published.[15] Proper clinical staging evaluation should begin with a complete history and review of systems (e.g. B-symptoms, organ-specific signs) and a thorough physical examination. Laboratory studies include a complete blood cell count with differential and a comprehensive blood chemistry measurement including lactate dehydrogenase (LDH), and in selected cases, serum electrophoresis to exclude a monoclonal gammopathy and/or flow cytometry on peripheral blood. Since an association between Borrelia burgdorferi infection has been reported in a significant minority of European cases of PCMZL, but not in Asian cases or cases from the United States[16-19], in European areas with endemic *B. burgdorferi* infection, the presence of *B. burgdorferi* should be investigated by serology and PCR techniques on skin biopsy specimens. Adequate imaging studies (contrast enhanced CT-scan with or without PET, or whole-body integrated PET/CT[15]) should be performed of at least chest, abdomen and pelvis, and in cases of skin lesions in the head and neck area, of the neck as well. In the recently published ISCL/EORTC guidelines, bone marrow biopsy and aspirate are required in PCLBCL, LT, but considered optional in indolent CBCL (PCMZL and PCFCL).[15] However, a recent study demonstrated bone marrow involvement in 22 of 193 (11%) patients with a follicle centre lymphoma (FCL) first presenting in the skin, as compared to two of 82 patients with a marginal zone lymphoma (MZL)[20] In nine of these 22 FCL patients this was the only evidence of extracutaneous disease. The 5-year overall and disease-specific survivals of these nine patients were 44% and 63%, respectively, compared to 84% and 95% in 157 patients without extracutaneous disease. These results indicate that bone marrow examination should be considered as an essential part of staging procedures in patients with a FCL first presenting in the skin and that, from a clinical point of view, bone marrow examination appears to have limited value in patients with a MZL presenting in the skin. Since currently there is no uniform consensus whether bone marrow biopsy is required in follicle center lymphomas presenting in the skin, the clinician is advised to follow the standard of care of his or her regional practice.

Recommendations for the treatment of primary cutaneous B-cell lymphomas

The different treatment options emerging from our literature search are summarized in Table 2. Therapy options most often reported included radiotherapy, systemic chemotherapy, rituximab and excision. For the other treatment modalities relative few studies were available for review. Table 2 also includes therapies which are still under investigation as well as some therapies, for which only anecdotal reports are available. Most of these will not further be discussed. Cumulative complete remission (CR) and relapse rates for the different treatment modalities used in patients with PCMZL, PCFCL and PCLBCL, LT were synthesized from the reviewed literature and are provided in Table 3. Data on relapse free survival or progression free survival are hardly available and will

Figure 1. Primary cutaneous marginal zone lymphoma

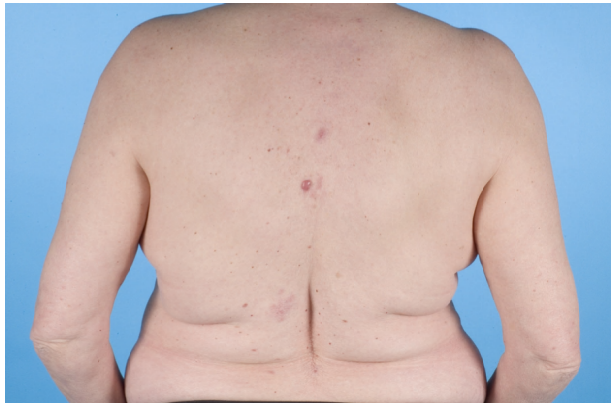


Figure 2. Primary cutaneous follicle centre lymphoma

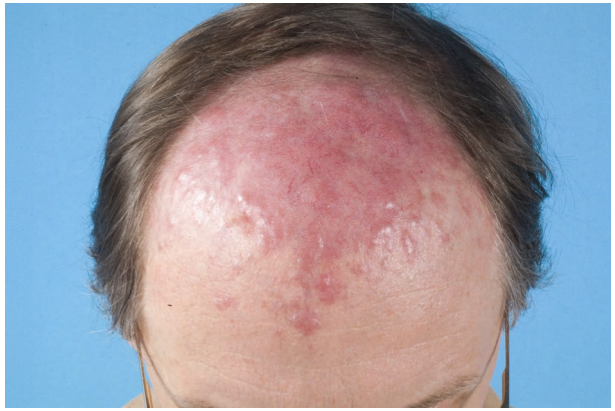
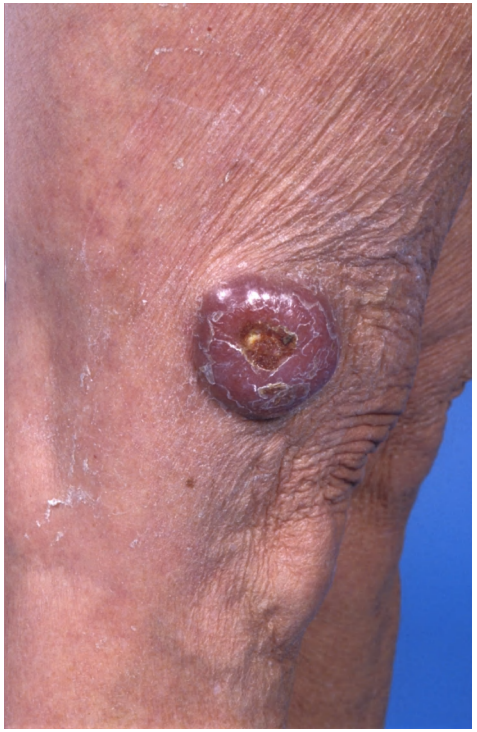


Figure 3. Primary cutaneous diffuse large B-cell lymphoma, leg type



therefore not be discussed. In the following paragraphs the results of these different treatment options are described in more detail and recommendations for the management of these CBCL are formulated.

Table 2. Overview of reported therapies for primary cutaneous B-cell lymphomas.

Therapy	References
First and second-line therapies	
Local radiotherapy	5;21-31;40;45;50;52-61;80;81;87;102-108
Systemic multiagent chemotherapy	21;25;28-30;40;45;52;53;56;78-81;84;85;93;104;105;109
Systemic rituximab (Mabthera®)	34-36;38;68-77;88-92
Chorambucil (Leukeran®)	23;25;31;39;60;110
Excision	5;21;25;28-32;53-55;60;81;104;111
Antibiotics	29;31;45-48;112
Radiochemotherapy	28;45;52;54-56;94;95;113
Investigational therapies	
Intralesional IFNα	33;46;62-64;114-116
Intralesional Rituximab	34;36;37;65-67
Adenovirus-mediated IFNγ gene transfer	99-101
Anecdotal therapies	
Photodynamic therapy	117;118
Intralesional Cisplatin	119
Mechlorethamine/ clobetasol	120
Topical imiquimod	121;122
Topical hexadecylphosphocholine	123

Table 3. Therapy results.

Cumulative studies	Patients, N	CR, no. (%)	Relapse, no. (%)
PCMZL			
Radiotherapy	132	130/132 (99%)	60/130 (46%)
Excision	75	74/75 (99%)	32/74 (43%)
Interferon alpha	8	8/8 (100%)	2/8 (25%)
Rituximab i.l.	9	8/9 (89%)	5/8 (62%)
Rituximab i.v.	3	2/3 (67%)	1/2 (50%)
Chlorambucil	14	9/14 (64%)	3/9 (33%)
Antibiotics	14	6/14 (43%)	1/5 (20%)*
Multi-agent chemotherapy	33	28/33 (85%)	16/28 (57%)
PCFCL			
Radiotherapy	460	457/460 (99%)	216/457 (47%)
Multi-agent chemotherapy	104	88/104 (85%)	42/83 (51%)*
R-CHOP	2	1/2 (50%)	0/1 (0%)
Interferon alpha	7	7/7 (100%)	2/7 (29%)
Rituximab i.l.	12	10/12 (83%)	4/10 (40%)
Rituximab i.v.	28	21/28 (75%)	4/19 (21%)*
Excision	93	91/93 (98%)	36/91 (40%)
Chemoradiotherapy	7	7/7 (100%)	1/7 (14%)
PCLBCL, LT			
Radiotherapy	101	89/101 (88%)	52/89 (58%)
Multi-agent chemotherapy	32	26/32 (81%)	14/24 (58%)*
R-CHOP	12	11/12 (92%)	1/11 (9%)#
Chemoradiotherapy	6	4/6 (67%)	1/2 (50%)*
Rituximab i.v.	13	5/13 (39%)	0/4 (0%)*

* Data on relapse rate were not available in all patients.
# Short follow-up period.

Radiotherapy

In the literature a total of 132 PCMZL patients are described who were treated with radiotherapy.[21-30] In nearly all cases (130/132; 99%) a CR after initial therapy was reached. Sixty patients (46%) showed one or more relapses, which were mostly confined to the skin. Extracutaneous progression was reported in only three of 132 patients, one of whom died of lymphoma.[26] Reported cumulative doses per irradiation field were mostly between 30 and 45 Gy. However, doses as low as 10 Gy and as high as 50 Gy have been reported.[22;29] Most studies included a margin of clinically normal skin in the radiation field, ranging from 1 to more than 5 cm, partly depending on the affected body site.

Excision

In daily practise, surgical excision is a first choice of treatment in patients presenting with one or few small skin lesions. In the literature 75 patients treated with surgical excision have been reported.[21;25;29-32] A CR was reached in all but one[30] cases. However, 32 patients (43%) developed new skin lesions. Information whether these represent local recurrences or true relapses (new skin lesions at other skin sites) is not provided. In addition, no information was given regarding the margins of resection or whether dissemination to extracutaneous sites had occured.

Interferon alpha (IFNα) intralesionally (i.l.)

Only one paper describes the use of IFNα in PCMZL.[33] In contrast to its systemic use in CTCL, in this study eight patients received intralesional injections of 3 million IU, 3 times per week. All patients reached a CR after a median of 8 weeks (range 3-20 weeks). Two patients experienced a local recurrence, which went again in CR after treatment with IFNα. No extracutaneous relapses were reported and side effects were generally mild.

Rituximab i.l. or intravenously (i.v.)

Five reports describe the use of rituximab in PCMZL.[34-38] In three patients it was administered systemically (375 mg/m^2 i.v. once weekly, for 4 or 8 weeks; two out of these three patients had multifocal disease)[35;36;38], and in nine patients intralesionally (5-30 mg once or 3x/week;8/9 patients had ≤ 4 skin lesions).[34;36;37] Two out of three (67%) systemically treated patients and eight out of nine (89%) intralesionally treated patients reached a CR, with the remaining patients reaching a partial remission (total response rates 100%). Relapse rates were 50% (1/2) and 62% (5/8), respectively, and no extracutaneous relapses were reported.

Single agent and combination chemotherapy

With respect to single agent chemotherapy, four reports describe the results of chlorambucil, a nitrogen mustard derivative, which has been used for many years in the treatment of low-grade systemic non-Hodgkin's lymphomas and leukemias. There was a total number of 14 patients with a PCMZL in these studies, all with multifocal skin lesions.[23;25;31;39] The response rate was 100% with a CR in nine patients (64%) and a partial remission in the other five patients. Of the nine patients with a CR, three patients showed relapses (33%), one of whom also with extracutaneous localizations. Reports on other agents used in indolent B-cell NHL, such as fludarabine and 2-chlorodeoxyadenosine (2-CDA) are lacking.

Multiagent chemotherapy (mostly CHOP) was administered to 33 PCMZL patients reported in the literature, again mostly patients with multifocal skin lesions.[21;25;30;40] Complete remission rate was 85% and a relapse rate of 57% was found.

Antibiotics

Analogous to the treatment of gastric MALT lymphomas with antibiotic therapy to eradicate *Helicobacter pylori*[41-43], recent reviews suggest that PCMZL associated with *B. burgdorferi* infection should first be treated with antibiotics before more aggressive therapies are employed.[44] However, the efficacy of antibiotic treatment in *B. burgdorferi*-associated PCMZL is poorly documented.[29;31;45-48] Six of 14 (43%) reported patients achieved CR after various antibiotic regimens. Data on eight patients for whom antibiotic treatment was specified, suggest that systemic treatment with cephalosporins is superior to oral treatment with high dose tetracyclins.

Recommendations (see Table 4.)

Patients presenting with solitary or few scattered skin lesions can best be treated with local radiotherapy (20-36 Gy) or excision. For patients presenting with disseminated skin lesions several management strategies can be considered. A strategy not supported by data from literature, but generally practised by the expert panel members as the management of choice, is a wait and see strategy, similar to what is often used for indolent non-cutaneous B-cell lymphomas and leukemias. This strategy implies that patients are carefully followed and only symptomatic lesions are treated ("treat as needed" concept). Individual, symptomatic skin lesions can be treated with surgery, topical or intralesional steroids or low dose radiotherapy. Intralesional administration of IFNα or rituximab may be interesting alternatives, but further studies are required.

In patients with very extensive skin lesions, chlorambucil is still often used in European centres, - in particular in older patients and for a limited period of time (maximal 3 months) -, but it is uncommonly used in the United States. Other single agent or combination chemotherapy regimens appropriate for systemic low-grade B-cell lymphomas may be

considered, but published results are not available. Alternatively, systemic rituximab can be considered. Multi-agent chemotherapy is rarely indicated in this type of CBCL.
Cutaneous relapses do not signify a worse prognosis and can be treated in the same way as the initial skin lesions.
Theoretically, it is attractive to suggest that PCMZL associated with a *B. burgdorferi* infection should first be treated with antibiotics before other therapies are employed. However, although some CR have been reported, in particular in patients treated with systemic cephalosporins, additional studies are required to establish which patients may benefit from antibiotic treatment and to assess which type, dose and duration of antibiotic treatment should be recommended.

Primary cutaneous follicle centre lymphoma

Radiotherapy
Already in 1951 Crosti described 7 cases of "reticulohistiocytoma of the dorsum" that were markedly sensitive to radiation therapy.[49] This entity was later reclassified as PCFCL.[50;51] Most recent reports on treatment of PCFCL describe local radiotherapy as the first choice of treatment, since it is highly effective and it has few side effects. In the literature we found a total number of 460 PCFCL patients initially treated with RT.[5;22;23;26;27;29;30;45;50;52-61] These were mostly patients with solitary or localized skin lesions, but some reports also include patients with multifocal skin disease, necessitating the use of multiple radiation fields.[22;26;27;58] In one study specifically addressing such patients, it was found that radiotherapy of all visible skin lesions was equally effective as treatment with multi-agent chemotherapy.[40] A CR was reached in nearly all cases, irrespective of the extent of the disease (457/460; 99%). Three major studies on RT in PCFCL (combined including 147 patients; median follow-up 52, 62 and 41 months, respectively) report a relapse rate of approximately 30%[22;26;27], which contrasts with the 76% in an Italian study (104 patients; median follow-up, 62 months).[58] This latter group also reported in-field and marginal recurrences which were not seen in the other studies. These differences might be due to the variance in techniques used and, most important, the margins of healthy looking skin included in the radiation field (ranging from 0,5 to more than 5 cm.). The cumulative dose per irradiation field was generally more than 30 Gy (range, 20-54 Gy).

Excision
A total of 93 patients was found in the literature that were initially treated with complete surgical excision.[5;28-30;53-55;60] Most patients reached CR (91/93; 98%) and less than half of them (36/91; 40%) developed a skin relapse. No details are provided concerning excision margins or specific sites of local recurrence or relapse at distant skin sites.

Interferon alpha (IFNα) i.l.

Intralesional treatment of PCFCL patients with IFNα has been reported in only seven cases, with dosages varying from 1 million IU, 3x per week to 6 million IU, 3x per week for larger lesions.[46;62-64] All seven patients reached a CR. Two patients experiencing a clinical relapse were again successfully treated with a second course of IFNα.[62] However, since the follow-up periods were rather short, no firm conclusions regarding relapse rate or long-term efficacy can be made.

Rituximab i.l. + i.v

Both intralesional and systemic rituximab have been used in PCFCL. Ten of 12 patients treated intralesionally reached CR and the other two patients had a partial remission.[34;36;65-67] Dosages and treatment regimens varied between 10 and 30 mg per lesion per application for two or three times a week. Duration of treatment was more variable, ranging from one or two injections in total, to treatment for up to six months. Four of 10 patients with CR developed new skin lesions; two in the originally treated site and two at distant skin sites. No extracutaneous relapses were seen.

The 28 patients systemically treated with rituximab, all received doses of 375 mg i.v./m^2 body surface area once weekly.[34-36;68-77] Duration of treatment was mostly four weeks, but ranged from one to eight weeks. Twenty-one patients reached CR, six patients showed partial remission and one patient[76] had progressive disease, but went into complete remission following eight CHOP courses. In four of the 21 patients with CR, relapses were reported and these were all confined to the skin.

Multi-agent chemotherapy

Relatively few data exist in the literature concerning treatment of PCFCL patients with multi-agent chemotherapy, whilst one would expect that many of these patients, -in particular those with a diffuse growth pattern, classified as DLBCL according to the WHO classification -, were treated in this way. Moreover, two of the largest studies could not be used for analysis since they describe mixed groups of patients based on older classification schemes.[78;79] Cumulative data from eight papers on 104 patients show a CR rate of 85% (88/104 patients) and a relapse rate of 48% (42/88 patients).[28;30;40;52;53;56;80;81] These were mostly patients with disseminated cutaneous lesions or heavy tumour burden. Most patients had been treated with CHOP or CHOP-like courses, and only five patients with COP, which however was reported to be less effective.[78;80]

In DLBCL presenting with localised disease (Ann Arbor stage I-E) a combination of chemotherapy (3-4 cycles of CHOP) and involved field radiotherapy (IFRT) has been suggested to be superior to one of either modalities alone.[79] Reports on this combined treatment in patients with PCFCL are few.[28;52;54-56] Cumulative data on seven patients show a CR in all patients, with a reported relapse in one of them.

While a combination of rituximab and CHOP (R-CHOP) has become standard treatment for DLBCL for more than five years[82;83] and many cases of PCFCL would have been classified as DLBCL using the WHO 2001 classification, only two patients with PCFCL who were treated with R-CHOP have been published thus far. One patient went into CR and had no relapse in the following 12 months.[84] The second patient had only a PR following six cycles of R-CHOP. Further progression of the skin lesions was noted 1.5 years later, and single agent treatment with rituximab resulted in a CR, which was ongoing for over 19 months.[70] A third patient, treated with a combination of cyclophosphamide and rituximab, showed a reduction of less than 30% of skin lesions.[85]

Recommendations (see Table 4)

In patients presenting with solitary or localized skin lesions, RT with a dose of at least 30 Gy and a margin of clinically uninvolved skin of at least 1-1,5 cm, is the preferred mode of treatment. Solitary lesions that are small and well-demarcated can be treated with surgical excision.

In patients with an indolent PCFCL presenting with few scattered lesions both radiotherapy of all visible skin lesions as well as a wait and see policy with treatment of only symptomatic skin lesions, similar as recommended for PCMZL, can be considered for initial management. In patients with very extensive skin lesions systemic rituximab is the first choice of treatment. Combination chemotherapy (R-COP; R-CHOP) should be considered only in exceptional cases, such as patients with progressive disease not responding to rituximab or patients developing extracutaneous disease.

Relapses occur in approximately 30% of patients, are mostly confined to the skin and do not signify a worse prognosis. For the treatment of cutaneous relapses a similar approach as for the initial skin lesions is recommended. Also in these cases multi-agent chemotherapy is rarely indicated.

Finally, recent studies showed that PCFCL presenting on the leg(s) have a considerably worse prognosis, comparable to that of PCLBCL, LT.[9;86] Although reports are few, it seems safe to propose that such patients should receive the same treatment as recommended for PCLBCL, LT (see below).

Primary cutaneous diffuse large B-cell lymphoma, leg type

Radiotherapy

Radiotherapy is less effective in PCLBCL, LT than in the indolent CBCL. The CR rate in 101 reported cases was 88% (89/101 patients) and the relapse rate was 58% (52/89 patients), while also in-field and marginal recurrences have been reported.[22;23;26;27;29;30;56;58;87] Extracutaneous progression was reported in approximately 30% of patients.

Rituximab i.v.

Treatment with rituximab i.v. as single agent therapy (375 mg i.v./m^2 body surface area once weekly for four to eight weeks) is reported in 13 patients, in seven of them as second or third line treatment.[35;76;77;88-92] A CR was obtained in only five of 13 patients, including three of six patients, who received rituximab as initial treatment. None of the four patients with CR of whom follow-up was reported had a relapse, but the duration of follow-up was short (median seven months).

Multi-agent chemotherapy

Recent reviews suggest that PCLBCL, LT should be treated as systemic DLBCL with multi-agent chemotherapy with or without rituximab.[1;44] However, only 32 patients receiving CHOP(-like) (n = 29) or COP (n = 3) have been reported.[29;30;40;45;56;80;81;93] Collectively, 26 of 32 patients (81%) reached a CR and 14 of 26 CR patients (54%) had a relapse. A combination of CHOP and IFRT was used in a further six patients and resulted in a CR in four of them.[28;45;94;95]

One recent study describes 12 patients treated with various combinations of anthracycline-containg chemotherapies and rituximab. All but one patient reached a complete remission (92%) and only one patient relapsed (9%). The authors suggest that patients with PCLBCL, LT treated with various combinations of age-adapted, anthracycline-containing chemotherapies and rituximab, have a better outcome than patients receiving other treatments, however, follow-up of the group treated with chemotherapy and rituximab was insufficient (mean follow-up period less than two years) to determine a statistically significant difference.[96] In addition, another report described a CR in one of two patients treated with cyclophosphamide and rituximab.[85]

Recommendations (see Table 4.)

Since PCLBCL, LT patients have morphological, phenotypical and molecular genetic features as well as a clinical behaviour similar to that of systemic DLBCL, they should be treated consistently. Nowadays, R-CHOP with or without IFRT is considered as the first line of treatment in these lymphomas, but the efficacy of this approach in patients with PCLBCL, LT is still poorly documented. In case the condition of the patient does not allow such an aggressive treatment, local radiotherapy of all visible skin lesions or perhaps rituximab as single agent therapy can be considered. Whether radiotherapy should be considered as first choice of treatment in patients presenting with a small solitary tumour is a matter of debate. In order to gain more knowledge on the most favourable treatment for this rare patient group with a poor prognosis, it is important to treat patients in the setting of controlled clinical trials.

As there are insufficient studies on relapsed PCLBCL, LT it is recommended that these patients be treated as relapsed DLBCL, using local protocols.

Table 4. Recommendations for initial management of the 3 main types of CBCL.

Disease type and extent	First line therapy	Alternative therapies
PCMZL		
Solitary/ localized	• Local radiotherapy • Excision • Antibiotics[1]	• IFN alpha i.l. • Rituximab i.l. • Intralesional steroids
Multifocal	• Wait-and-see • Local radiotherapy • Chlorambucil[2] • Rituximab i.v • Antibiotics [1]	• IFN alpha i.l. • Rituximab i.l. • Topical or intralesional steroids
PCFCL		
Solitary/ localized	• Local radiotherapy • Excision	• IFN alpha i.l. • Rituximab i.l.
Multifocal	• Wait-and-see • Local radiotherapy • Rituximab i.v.	• R-CVP/CHOP [3]
PCLBCL, LT		
Solitary/ localized	• R-CHOP +/- IFRT	• Local radiotherapy • Rituximab i.v.
Multifocal	• R-CHOP	• Rituximab i.v.

[1] in case of evidence for *B. burgdorferi* infection.
[2] or other single or combination regimens appropriate for low-grade B-cell lymphomas.
[3] in exceptional cases or for patients developing extracutaneous disease.

Conclusion

Based on an extensive review of available literature and discussions within the EORTC and ISCL groups, the present report provides consensus recommendations for the management of CBCL. It should be emphasized that, since systematic reviews and (randomized) controlled trials were not available, the treatment recommendations are mainly based on retrospective studies and small cohort series. In addition, in most of these studies information on relapse-free survival or progression-free survival was not included and in many studies follow-up was too short to draw conclusions on long-term efficacy. Despite these limitations, there was consensus among the members of the multidisciplinary expert panel that these recommendations reflect the state of the art treatment of CBCL, as currently practised in major cutaneous lymphoma centres. These recommendations may therefore contribute to uniform staging and treatment and may prevent in particular overtreatment of subgroups of CBCL patients. These recommendations may also guide the design of future clinical trials in CBCL, which are highly necessary and, in view of the rarity of these conditions, should be multicenter studies, preferably within the frame work of the EORTC Cutaneous Lymphoma Group and/or ISCL. Trials most often suggested by the expert panel members were, rituximab against current best practice (wait and see strategy with radiotherapy when needed) in both PCMZL and PCFCL, and radiotherapy versus R-CHOP + IFRT, in patients with PCLBCL, LT presenting with a solitary lesion. In addition, comparison between systemic and intralesional treatment with rituximab in PCFCL could be of interest, since also in patients treated intralesionally a complete disappearance of B-cells in the peripheral blood has been noted, indicating a systemic effect.[34;65] In selected cases intralesional rituximab might prove to be an equally effective, but much cheaper alternative for systemic rituximab. Cutaneous lymphoma groups with experience in treating *B. burgdorferi*-associated PCMZL with (systemic) antibiotics are encouraged to publish their treatment results, either positive or negative, to find out if further studies are required. Controlled multicenter studies are also required to assess the efficacy of new therapies, such as yttrium-90 ibritumomab tiuxetan (Zevalin®) or 131I-tositumomab (Bexxar®) radioimmunotherapy[97] and pegylated liposomal doxorubicin[98] (plus rituximab) in PCLBCL, LT and gene therapy with adenovirus-mediated transfer of IFN-gamma in PCMZL and PCMZL.[99-101] Today, only the last mentioned approach is investigated in a prospective trial. Finally, patients with a CBCL can best be managed in centres where a close collaboration between dermatologist, haematologist/oncologist and radiation oncologist exists. This is probably the most important recommendation and the best guarantee for optimal management.

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Chapter 8.

Summary and Discussion

In the last decade there has been an ongoing debate whether the EORTC or the WHO classification should be used for classification of primary cutaneous B-cell lymphomas (CBCL). The discrepant points of view were not only a matter of academic dispute, but also had major therapeutic consequences. With the publication of the WHO-EORTC classification in 2005, this controversy may have come to an end. However, a number of outstanding issues remained, that needed to be addressed.

The main issues concerned (1) the clinical usefulness of the WHO-EORTC classification in daily practice, (2) the validity of prognostic parameters reported in previous studies and identified in patient groups classified according to previously used classification schemes and (3) optimal management for the different types of CBCL as defined in the WHO-EORTC classification. In this concluding chapter, these three issues will be discussed based on the data described in this thesis and data from the literature.

The WHO-EORTC classification for CBCL in daily practice

In **Chapter 2**, the clinical significance of the WHO-EORTC classification was assessed on 300 CBCL cases, present in the database of the Dutch Cutaneous Lymphoma Group (DCLG), and compared to the previously used EORTC and WHO classification schemes. Furthermore, we aimed to more clearly define which cases should be assigned to the group of primary cutaneous diffuse large B-cell lymphoma, other (PCLBCL, other).

Using the criteria of the WHO- EORTC classification, primary cutaneous marginal zone B-cell lymphoma (PCMZL) and primary cutaneous follicle centre lymphoma (PCFCL) showed a 5-year disease-specific survival (DSS) of 98% and 95%, respectively, whereas primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT) had a 5-year DSS of only 50%. These results are in agreement with the results of recent studies and confirm that the new WHO-EORTC classification adequately distinguishes between CBCL with an indolent and CBCL with a more aggressive clinical behaviour.[1-3] Most patients with PCMZL presented with multifocal skin lesions mainly on the trunk and extremities. Although the frequency of skin relapses was high and extracutaneous dissemination was observed in 9% of patients, only one patient died due to lymphoma. In the group of PCFCL, most patients characteristically presented with skin lesions on the head or trunk and only a small minority (6%) presented with lesions on the leg(s). Expression of the so called, activated B-cell (ABC) markers, Bcl-2, MUM-1 and FOXP1, was found in only 11%, 10% and 4% of cases respectively. Extracutaneous progression was seen in 11% of patients, while ultimately 5% of patients died due to their lymphoma. Patients with PCLBCL, LT most often presented with lesions on the leg(s) and only 12% of patients had skin lesions restricted to another skin site. The neoplastic B-cells strongly expressed Bcl-2, MUM-1 and FOXP1 in the majority of cases (90%, 90% and 81% respectively), both in patients with lesions on the legs and elsewhere. This group showed a high rate of extracutaneous progression (47%) and 45% of patients died of lymphoma.

In contrast to the EORTC classification, in which differentiation between primary cutaneous follicle centre cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg) was based on site (non-leg or leg), in the WHO-EORTC differentiation between PCFCL and PCLBCL,LT is based primarily on cell morphology, i.e. cleaved cells (centrocytes) versus noncleaved or round cells (centroblasts and immunoblasts). Differentiation on the basis of morphology is known to be associated with a considerable inter-observer variation.[4;5] While cases with a clear (partly) follicular growth pattern will be easily classified as PCFCL, in cases with a diffuse growth pattern this classification might be more difficult. In such difficult cases, the presence of a considerable proportion of admixed T cells, the presence of a stromal reaction as well as demonstration of (remnants of) follicular dendritic cell networks by staining with appropriate antibodies (CD35 or CD21) may serve as useful additional criteria suggesting a diagnosis of PCFCL.[6] Moreover, since it was found that most PCLBCL, LT cases expressed Bcl-2, MUM-1 and FOXP1, this phenotypic profile might also be a useful adjunct, supporting a diagnosis of PCLBCL, LT.[1;2;6] However, since Bcl-2, MUM-1, and to a lesser extent FOXP1 are also expressed by a small minority of PCFCL and a small minority of PCLBCL, LT do not express these markers, they cannot be used as a golden standard to differentiate between both conditions.

Comparing the three classification schemes demonstrated no important differences in the classification of PCMZL and PCFCL with a follicular or follicular and diffuse growth pattern. However, 65% of cases classified as diffuse large B-cell lymphoma (DLBCL) using the WHO classification, were reclassified as PCFCL using the WHO-EORTC scheme, which implies that these patients can be managed by local therapies instead of more aggressive systemic therapies. This illustrates that the WHO-EORTC classification is a major step forward as compared to the WHO classification and that it contributes to a more appropriate treatment in patients with CBCL. The reclassification of 10% of cases formerly classified by the EORTC classification, concerned seven PCFCCL patients with a predominant round-cell morphology, that were now classified as PCLBCL, LT and nine PCLBCL-leg patients with a cleaved-cell morphology that were now included as PCFCL. For the seven PCFCCL patients with round-cell morphology this reclassification proved clinically relevant since they showed the same intermediate prognosis as other cases with the same morphology presenting on the leg. However, the reclassification of the nine cleaved-cell cases presenting on the leg as PCFCL proved less fortunate, since they showed a significantly worse prognosis as compared to PCFCL cases presenting with skin lesions on other body regions.

Recent studies used the term PCLBCL, other in different ways. Some authors assigned all cases with a predominance of large round cells without Bcl-2 expression to the group of PCLBCL, other[2;7], while we and others classified such cases as PCLBCL, LT, irrespective of the location of skin lesions and Bcl-2 expression.[3;6] In our study, comparison of Bcl-2–positive and Bcl-2–negative PCLBCL, LT showed no significant difference in 5-

year DSS or 5-year overall survival (OS), which is consistent with the results of Kodama et al.[2] Similarly in both studies, no significant differences in 5-year OS and DSS were found between PCLBCL, LT with or without expression of MUM-1 or FOXP1. These data indicate that distinction between patients with or without expression of Bcl-2, MUM-1, or FOXP1 is not useful, and that categorization of Bcl-2–negative patients as PCLBCL, other, as suggested previously[2;7], is not justified. This term should be reserved for rare morphological variants of diffuse CBCL, that do not fit the criteria of either PCFCL or PCLBCL, LT.

Prognostic factors for the newly defined CBCL groups

Prognostic factors for CBCL described in previous studies, are currently not useful anymore or need to be confirmed, since they were identified on patient groups defined with older classification systems or by studying mixed diagnostic populations. Prognostic factors were studied in three chapters of this thesis.

Clinical, histological and immunophenotypical markers: a multivariate analysis

In **Chapter 2** we aimed to define prognostic parameters within the redefined categories PCFCL and PCLBCL, LT. We analyzed various clinical, histological and immunophenotypical markers using univariate and multivariate analyses.

Both in the total group of PCFCL and in the group of PCFCL with a diffuse infiltrate of large cleaved cells, patients presenting with skin lesions on the leg(s), had a significantly worse prognosis than PCFCL presenting at other sites. This finding is consistent with other recent studies and implies that within this entity further distinction should be made based on the site of presentation.[2;4;7] However, since these results are based on a relatively small number of PCFCL patients with lesions on the leg, this finding needs further validation.

Expression of Bcl-2, MUM-1 and FOXP1 was not associated with prognosis in PCFCL cases with a diffuse growth pattern. However, in the total group of PCFCL, both weak and strong expression of FOXP1 was associated with an inferior prognosis as compared to patients without expression of FOXP1. While in CBCL this marker has been reported to be associated with a round cell morphology, i.e. a diagnosis of PCLBCL, LT, prognostic significance within one of the CBCL entities has not been described so far. In systemic DLBCL, it is a matter of debate whether FOXP1 is associated with a bad prognosis or not.[8-10] These contradictory results might be explained by recently reported findings, describing that the JC12 monoclonal antibody, used to determine FOXP1 protein expression, does not distinguish between the full-length FOXP1 protein and smaller isoforms, whilst it appears that these smaller FOXP1 isoforms, rather than the full-length protein, have a potentially oncogenic role in B-cell non-Hodgkin lymphomas.[11] The exact role and prognostic significance of this marker in PCFCL needs further clarification. Other

parameters, such as age, extent of skin lesions or growth pattern did not have independent prognostic significance.

In the PCLBCL, LT group localization of skin lesions or presence or absence of Bcl-2, MUM-1 or FOXP1 was not associated with prognosis. While in univariate analysis of OS both age and extent of skin lesions were associated with a poor prognosis, in multivariate analysis no independent prognostic markers could be identified.

The TNM classification system for primary cutaneous lymphomas other than mycosis fungoides (MF) and Sézary syndrome (SS)

In 2007 a new clinical staging system was proposed for primary cutaneous lymphoma other than MF and SS.[12] This TNM based system was meant to document extent of disease in a consistent matter, thereby facilitating comparison between different research centres and study populations. At the time of publication it was unknown if the system had prognostic significance in CBCL. In **Chapter 4** we applied this system to the 300 CBCL that had been reclassified according to the WHO-EORTC classification, described in **Chapter 2**. The results of **Chapter 4** show that the TNM system can easily be applied to the three main groups of CBCL. With regard to prognostic significance, it was found that increasing T-score was associated with decreased survival in the group of PCLBCL, LT. Although the association was not statistically significant, this is in accordance with two other recent reports on PCLBCL, LT. These studies report a better prognosis for PCLBCL, LT patients presenting with a solitary tumour as compared to patients presenting with multiple tumours on one or both leg(s).[3;4] These results are also in line with the findings described in **Chapter 2**. Here we found extent of skin lesions to be associated with an unfavourable prognosis only in univariate analysis. For both indolent groups of CBCL, PCMZL and PCFCL, the TNM classification did not provide prognostic significance.

In addition, we compared the proposed TNM classification system with the scoring of the DCLG, which has been in use for many years and was used to report disease extent in the study described in **Chapter 2**. There was a discrepancy in the classification of 20 cases, all indolent types of CBCL. This concerned cases with multiple skin lesions at two contiguous body sites. As there is no anatomical or biological relation between such separate lesions, in the DCLG system such lesions are classified as multifocal or generalized disease. However, as the T3 category in the TNM system is defined as involvement of two non-contiguous body regions or three or more body regions, such cases are classified as T2, which denotes regional skin involvement. In both indolent types of lymphoma, classification as T2 is of minor clinical importance, since they can be treated with non-aggressive therapies, irrespective of disease extent. However, in other, more aggressive cutaneous lymphomas this discrepant classification of multiple distant skin lesions restricted to two adjacent skin sites as T2 might have important therapeutic consequences. Since in our study the staging of PCLBCL, LT did not show any discrepancies between both systems, the applicability of the definitions for T2 and T3

should be evaluated in other cohorts of (more aggressive) cutaneous lymphomas, in order to avoid undesirable therapeutic consequences.

Inactivation of CDKN2a as a prognostic marker in PCLBCL, LT

A recent study suggested inactivation of CDKN2A as a prognostic marker in the PCLBCL, LT group.[13] Since this was only investigated in a small number of cases, we sought to confirm these findings on a larger patient group. For that purpose, we needed a technique that could be applied on DNA derived from formalin-fixed paraffin-embedded (FFPE) material, since this is more readily available. We tested a recently described technique, called Multiplex Ligation-dependent Probe Amplification (MLPA), that was reported to be less sensitive to DNA degradation and shows reliable results when applied to DNA derived from FFPE material.[14-17] Indeed, we found that comparison between fresh-frozen and FFPE material obtained from the same tumour in two patients, showed identical results. In an EORTC Cutaneous Lymphoma Group (CLG) multicentre study, described in **Chapter 3**, we analyzed tumour DNA of 64 PCLBCL, LT patients, which is the largest group described so far. We were able to confirm inactivation of CDKN2A in a large part (75%) of PCLBCL, LT patients, which was correlated with reduced survival (5-year DSS for patients with versus without inactivation of CDKN2A: 43% versus 70%, respectively). However, our results were not mutually exclusive, in the sense that the study group contained patients with inactivation of CDKN2A that had a good prognosis, but more importantly, it contained five patients (8% of the total study group) that did not show aberrations in CDKN2A but nonetheless died of their lymphoma. The latter patients run the risk of being undertreated when management would be solely based on CDKN2A status. So, while inactivation of this gene is associated with a worse prognosis, caution is warranted before these results are incorporated into clinical decision making. Regardless of these results, MLPA has proven to be a valuable technique providing new possibilities for molecular studies on larger patient groups of rare diseases, using the candidate gene approach.

In summary, the result of Chapters 2, 3 and 4 have not provided an independent prognostic marker for PCLBCL, LT which is useful at the time of diagnosis. It seems worthwhile to further investigate other possible prognostic markers for this rare group, by exploring newer genetic and epigenetic parameters. For instance, new promising research in the field of cancer prognostication is formed by studying microRNAs. It has been suggested that microRNA expression can distinguish between the germinal centre B-cell (GCB)-like and the activated B-cell (ABC)-like subtypes of DLBCL.[18] More recently it was shown that elevated levels of tumour-associated microRNAs can be detected in the serum of patients with DLBCL and that specific microRNAs may have prognostic relevance.[19] Since microRNAs proof to be relatively resistant to RNase degradation and can be successfully isolated from FFPE tissues[18], they might form an ideal target for studies in CBCL samples.

Analysis of microRNAs in patient serum may provide new, non-invasive possibilities for finding relevant prognostic factors.

Management and treatment of patients with a (primary) cutaneous B-cell lymphoma.
Staging

A diagnosis of primary cutaneous lymphoma can only be made after adequate staging investigations have been conducted. However, whether or not bone marrow biopsies should be performed routinely in patients with skin lesions that show histological features consistent with an indolent lymphoma has recently been debated.[12] Since there are no studies that have addressed this question for this particular group of lymphomas, in the study described in **Chapter 5** we evaluated the results of bone marrow examinations in a large group of patients who presented with skin tumours, histologically suggestive of an indolent B-cell lymphoma, either marginal zone lymphoma (MZL) or follicle centre lymphoma (FCL).

In the total group of 275 patients, 24 patients (9%) showed a positive bone marrow histology, while in 10 patients (4%; one MZL and nine FCL) this was the only evidence of extracutaneous disease. If bone marrow examination had not been performed, these 10 patients would have been wrongfully classified as CBCL. For patients with a histology of MZL the clinical consequences of this "misdiagnosis" are expected to be limited, since treatment of MZL with skin lesions and isolated bone marrow involvement will not be different from that of MZL presenting with only skin involvement (i.e. PCMZL). However, the nine patients with the histologic features of a FCL and isolated bone marrow involvement had a significantly worse prognosis as compared to genuine PCFCL (5-year DSS 63% versus 95% respectively), which may have therapeutic consequences. In summary, the results of the study showed that bone marrow investigation is an essential component of the staging procedure in patients with an FCL first presenting in the skin and that bone marrow examination appears to have limited value in patients with MZL presenting in the skin.

These conclusions are in line with other staging recommendations such as the most recent National Comprehensive Cancer Network (NCCN) clinical practice guidelines (version 2.2008). In these guidelines, bone marrow investigation is considered essential in indolent follicular lymphomas (FL), while in extranodal MZL (including PCMZL) it is only considered useful in selected cases. Interestingly, in previous versions of the NCCN guidelines (versions 2.2006, 1.2007 and 3.2007), also bone marrow examination in indolent FL was only considered useful in selected cases. In line with these previous NCCN guidelines, but not substantiated by any published data, some cutaneous lymphoma centres in the United States still argue that bone marrow examination in FCL patients should be considered optional and should only be performed in selected cases, e.g. patients with other positive staging assessments. Since survival of these patients is significantly different and

they may thus require a different management strategy, this outstanding question needs further clarification.

Treatment

In **Chapters 6 and 7** we evaluated the results of radiotherapy in the newly defined groups of CBCL and subsequently aimed to provide consensus recommendations for the management of these diseases.

Local radiotherapy (RT) is a well known and effective treatment modality in the field of CBCL. It is widely given to patients with indolent CBCL with curative intent. Since it was expected that a number of patients would be assigned to a different prognostic category, using the criteria of the new WHO-EORTC classification, we sought to evaluate the results of this treatment in the newly defined categories. The results, described in **Chapter 6**, show that the large majority of tumours is very sensitive to RT. All but two of 153 patients (99%) responded to initial RT with a complete remission (CR). The two patients that did not reach CR were both PCLBCL, LT patients. Relapse rates for PCMZL, PCFCL and PCLBCL, LT were 60%, 29% and 64% and the 5-year DSS was 95%, 97% and 59% respectively. PCFCL patients who presented with skin lesions on the leg(s) had a higher relapse rate (63%) and a much lower 5-year DSS (44%) as compared to PCFCL patients presenting with skin lesions at other sites (relapse rate 25% and 5-year DSS 99%).

In summary, the results of this large retrospective study indicate that RT is a safe and effective treatment for patients with PCMZL and PCFCL with solitary or localized skin lesions. Patients with multifocal skin disease showed a tendency towards higher relapse rate (PCMZL and PCFCL) and extracutaneous dissemination (PCFCL), suggesting that other treatment modalities might be considered in such patients. Moreover, for PCFCL patients presenting with skin lesions on the leg and PCLBCL, LT patients, RT should not be the first choice of treatment.

For the paper described in **Chapter 7,** we integrated the results of **Chapters 4, 5 and 6** and performed an extensive literature study. Together with the results of discussions among a multidisciplinary group of dermatologists, haematologists/oncologists and radiation oncologists , selected from the International Society of Cutaneous Lymphoma (ISCL) and the EORTC Cutaneous Lymphoma Group (EORTC-CLG), we were able to formulate consensus recommendations for the management of the three main types of CBCL, which are summarized in Table 4 of **Chapter 7**. Major limitations in reviewing the literature on the treatment of CBCL were that (1) there was a complete lack of systematic reviews and large (randomized) controlled trials, (2) information on relapse-free survival or progression –free survival was often not included and (3) in many studies follow-up was too short to draw conclusions on long-term efficacy. Moreover, most of the reported studies so far have been based on formerly used classification schemes or heterogeneous study groups of different types of CBCL. Despite these limitations, there was consensus among

the members of the multidisciplinary expert panel that these recommendations reflect the state of the art management as currently practised in major cutaneous lymphoma centres.

In brief, the main conclusions from **Chapter 7** are, that for both types of indolent CBCL with solitary or localized disease, non-aggressive therapies such as excision or local RT can be applied. In PCMZL patients with multifocal or generalized skin lesions, a wait-and-see policy seems justified. In contrast, patients with PCLBCL, LT follow a more aggressive clinical course and should be treated accordingly.

Regarding PCFCL patients with generalized skin lesions, optimal treatment remains to be determined. Currently employed management strategies vary from a wait-and-see policy to RT of all visible skin lesions, while multi-agent chemotherapy is only considered in exceptional cases. Apart from RT, favourable results have recently been described for both intralesional and intravenous administration of rituximab in the treatment of CBCL.[20-25] Rituximab is a chimeric (human-mouse) monoclonal antibody directed against the CD20 molecule present on the surface of all mature B-cells. Binding of rituximab to the B-cells expressing CD20, results in the elimination of all B-cells in the body. Systemic use of this agent has improved outcome rates in nodal FL and DLBCL significantly. [26-30] It seems interesting to explore the long-term efficacy of this agent in generalized PCFCL. Moreover, comparison between systemic and intralesional treatment deserves further investigation, since also in patients treated intralesionally a complete disappearance of B-cells in the peripheral blood has been noted, indicating a systemic effect.[20;31] Thus, intralesional rituximab might prove to be an equally effective, but much cheaper alternative for systemic rituximab in PCFCL patients with extensive skin lesions.

Besides its use in generalized PCFCL, the addition of rituximab to the standard chemotherapy regiments in PCLBCL, LT warrant future controlled trials in order to confirm recent promising findings.[3;32] Controlled multicentre studies are also required to assess the efficacy of several other new therapies, such as intralesional interferon alpha for indolent CBCL[33-36], yttrium-90 ibritumomab tiuxetan (Zevalin®) or 131I-tositumomab (Bexxar®) radioimmunotherapy[37], pegylated liposomal doxorubicin[38](plus rituximab) and gene therapy with adenovirus-mediated transfer of IFN-gamma.[39-41]

Concluding remarks and future perspectives

In summary, the WHO-EORTC proved clinically very relevant and delineates distinct disease entities with different prognoses. It is therefore fortunate that both PCFCL and PCLBCL, LT will be incorporated as new distinct disease entities into the updated WHO classification for tumours of hematopoietic and lymphoid tissues, which will be published in 2008. PCMZL is incorporated into the broad category of extranodal marginal zone lymphomas, which is unfortunate in view of the major pathogenetic differences that exist in terms of translocations and antigenic stimuli involved in the development of marginal zone lymphomas at different sites.[42-44] When PCMZL is no longer distinguished from other

extranodal marginal zone lymphomas this may hamper elucidation of the pathogenesis of genuine PCMZL.

The recognition of PCFCL and PCLBCL, LT as distinct disease entities in the WHO classification 2008 will definitely aid in the wider recognition of CBCL by hematopathologists and hematologists/oncologists worldwide. This will hopefully result in more uniform diagnosis and more appropriate management, leading to more homogeneous patient groups available for further studies.

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Nederlandse samenvatting

Maligne lymfomen zijn kwaadaardige woekeringen van verschillende typen witte bloedcellen. Deze aandoeningen ontstaan meestal in lymfklieren, maar in ongeveer 40% van de patiënten presenteert het lymfoom zich in een zogenaamd extranodaal orgaan, zoals bijvoorbeeld de huid. De term primair cutaan lymfoom wordt gereserveerd voor lymfomen die zich primair in de huid manifesteren en waarbij op moment van diagnose geen lokalisaties buiten de huid (extracutaan) worden gevonden. Ongeveer 20-25% van alle primair cutane lymfomen bestaan uit proliferaties van maligne B-cellen, ook wel B-lymfocyten genaamd. In de rest van de patiënten betreft dit vooral woekeringen van maligne T-lymfocyten. De studies beschreven in dit proefschrift hebben allen betrekking op cutane B-cel lymfomen.

In de sinds 2005 gebruikte WHO-EORTC classificatie voor primair cutane lymfomen worden 3 hoofdgroepen van primair cutane B-cel lymfomen (CBCL) onderscheiden: het primair cutaan marginale zone lymfoom (PCMZL), het primair cutaan follikel centrum lymfoom (PCFCL) en het primair cutaan difuus grootcellig B-cel lymfoom , been type (PCLBCL, LT).
Het PCMZL wordt klinisch gekenmerkt door rode tot livide papels, plaques en nodi welke met name op de romp en de extremiteiten voorkomen. De prognose van deze lymfomen is gunstig met een 5-jaarsoverleving van bijna 100%. Histopathologisch bestaat deze entiteit uit een proliferatie van kleine B-lymfocyten , waaronder de marginale-zone B-cellen, lymfoplasmacytoïde cellen en plasmacellen.
Het PCFCL wordt klinisch gekenmerkt door solitaire of gegroepeerde plaques en tumoren, voornamelijk gelokaliseerd op het hoofd en de romp. Ook de prognose van deze groep is gunstig met een 5-jaarsoverleving van rond de 95%. Histopathologisch kan dit lymfoom een folliculaire, folliculair en diffuse of diffuse groeiwijze hebben en het infiltraat bestaat uit grote B-lymfocyten, welke een “gekliefd” aspect hebben (centrocyten en bijgemengde centroblasten).
Het PCLBCL, LT tot slot, wordt gekenmerkt door nodi en tumoren welke zich vrijwel altijd op het been bevinden. In uitzonderlijke gevallen komen deze tumoren voor op andere delen van de huid. Histopathologisch wordt hierbij een diffuus infiltraat gezien bestaande uit grote, “ronde” B-lymfocyten, namelijk centroblasten en immunoblasten.

Tot voor kort bestond veel discussie over de classificatie van primair cutane lymfomen. Verschillende classificatiesystemen werden naast elkaar gebruikt, zoals de EORTC classificatie voor primair cutane lymfomen uit 1997 en de WHO classificatie voor hematologische maligniteiten uit 2001. Met name de classificatie van primair cutane grootcellige B-cel lymfomen (PCFCL en PCLBCL, LT) was reden tot veel ophef. De discrepantie in het classificeren van deze lymfomen had belangrijke klinische en therapeutische consequenties. Met de publicatie van de WHO-EORTC classificatie voor

cutane lymfomen in 2005 werd een consensus bereikt tussen aanhangers van de verschillende classificatiesystemen.
Er bleven echter enkele vragen onbeantwoord en deze hebben wij in dit proefschrift getracht te behandelen. Globaal kunnen drie aspecten onderscheiden worden: 1) Wat is de klinische toepasbaarheid van de nieuwe WHO-EORTC classificatie?, 2) Hoe valide zijn de prognostische parameters welke beschreven zijn in eerdere studies en welke geïdentificeerd werden in patiëntengroepen die gebaseerd waren op eerder gebruikte classificatiesystemen? en 3) Wat is het optimale management van de verschillende soorten CBCL zoals gedefinieerd in de WHO-EORTC classificatie?

In hoofdstuk 2 werden de klinische en histopathologische karakteristieken van 300 CBCL, welke opgenomen waren in de database van de Nederlandse Werkgroep Cutane Lymfomen en die geclassificeerd waren volgens de EORTC classificatie, gereviseerd en geherclassificeerd volgens zowel de WHO 2001 als de WHO-EORTC classificatie. Daarnaast werd met behulp van multivariabele analyse gezocht naar onafhankelijke prognostische factoren binnen de groepen primair cutane grootcellige B-cel lymfomen. Uit deze studie bleek dat de WHO-EORTC classificatie klinisch goed toepasbaar is en dat het relevante klinische en histopathologische parameters beschrijft voor de verschillende subgroepen. De nieuwe classificatie maakt een zinvol onderscheid in patiëntengroepen met een indolent en meer agressief klinisch beloop. Vergelijking met de eerder gebruikte classificaties toonde aan dat de consensus classificatie bijdraagt tot een adequatere indeling en dientengevolge meer uniforme behandeling van deze patiënten. Als belangrijkste prognostische factor binnen de groep van PCFCL werd gevonden dat tumoren gelokaliseerd op het been, in tegenstelling tot de tumoren elders op het lichaam, geassocieerd zijn met een significant slechtere prognose.
Binnen de groep van PCLBCL, LT werden geen klinische of histopathologische parameters gevonden die onafhankelijk geassocieerd waren met prognose. Echter, in hoofdstuk 3 werd getracht eerdere veelbelovende resultaten te bevestigen, die beschreven dat verlies van een deel van chromosoom 9 geassocieerd is met een slechtere prognose in deze groep patiënten. In een Europese multi-centre studie werd met behulp van een nieuwe techniek, DNA van 64 PCLBCL, LT patiënten geanalyseerd. Hierbij werd gevonden dat er inderdaad bij een groot deel van deze patiënten verlies is van een stukje van de korte arm van chromosoom 9. Dit stukje bevat het CDKN2A gen, dat codeert voor 2 eiwitten die dienen als tumor-suppressor gen, namelijk p16 en p14/ARF. Patiënten met verlies van dit stukje DNA hadden een slechtere 5-jaarsoverleving dan patiënten zonder afwijkingen in dit gebied, namelijk 38% ten opzichte van 69% respectievelijk.
In hoofdstuk 4 werd gekeken naar de klinische toepasbaarheid en eventuele prognostische waarde van een nieuw beschreven classificatie systeem voor het rapporteren van ziekte-uitgebreidheid in cutane lymfomen. Dit systeem werd gebaseerd op het Tumor, Node, Metastasis (TNM-) systeem, zoals dat ook gebruikt wordt bij verschillende solide tumoren

en melanomen. Wij pasten dit systeem toe op het geherclassificeerde cohort uit hoofdstuk 2 en vonden dat het voorgestelde TNM-systeem goed toepasbaar is op de drie groepen CBCL en dat het de clinicus een handig instrument geeft voor het uniform registreren van uitgebreidheid van ziekte. Voor PCMZL en PCFCL heeft de T-score geen prognostische waarde. Voor patiënten met PCLBCL, LT is een oplopende T-score geassocieerd met een slechtere prognose.

In het laatste deel van het proefschrift werd gekeken naar twee aspecten van het management van verschillende groepen CBCL te weten, stagering en behandeling.
Met betrekking tot stagering werd in hoofdstuk 6 nagegaan of het zinvol is bij patiënten met in het huidbiopt de histologie van een indolent cutaan B-cel lymfoom (dus marginale zone lymfoom (MZL) of follikel centrum lymfoom (FCL)), in alle gevallen beenmergonderzoek te verrichten. Hiertoe werden de resultaten van beenmergonderzoek van 275 patiënten met in het huidbiopt kenmerken van een indolent B-cel lymfoom retrospectief geanalyseerd. Deze resultaten werden gecorreleerd met uitkomsten van overige stagringsonderzoeken (laboratorium- en beeldvormend onderzoek) en overlevingsdata. In de groep van 82 MZL hadden twee patiënten een positief beenmergbiopt bij diagnose, waarbij bij één patiënt dit de enige aanwijzing was voor extracutane ziekte. Deze patiënt had net als de patiënten zonder aanwijzingen voor extracutane ziekte een 5-jaarsoverleving van 100%. Echter, in de groep van 193 FCL patiënten, hadden 22 patiënten een positief beenmerg en bij negen van hen was dit de enige extracutane lokalisatie. Deze negen patiënten hadden echter wel een significant slechtere 5-jaarsoverleving in vergelijking met de patiënten zonder extracutane ziekte (respectievelijk 63% versus 95%). Uit deze resultaten werd geconcludeerd dat beenmergonderzoek een essentieel onderdeel is van het stageringsonderzoek bij patiënten met een FCL in de huid en dat voor patiënten met een MZL in de huid, een beenmergbiopt alleen overwogen zou hoeven te worden wanneer andere onderzoeken aanwijzingen geven voor aanwezigheid van extracutane ziekte.

Tot slot werd onderzoek gedaan naar de optimale behandeling van de drie CBCL entiteiten. Aangezien radiotherapie (RT) al sinds de eerste beschrijvingen van lymfomen in de huid, gebruikt wordt voor de behandeling van deze ziekte, werden in het onderzoek beschreven in hoofdstuk 6, de resultaten van dit type behandeling geanalyseerd op de nieuw gedefinieerde entiteiten. Uit dit retrospectieve onderzoek bleek dat alle drie de subgroepen een goede initiële reactie vertonen op bestraling, met een complete remissie in bijna 100% van de gevallen. Echter, een aanzienlijk deel van de mensen met een PCLBCL, LT vertoont extracutane progressie na RT behandeling en uiteindelijk overlijdt 41% aan de gevolgen van het lymfoom. In de groep van PCFCL moet een onderscheid gemaakt worden tussen patiënten met huidafwijkingen op het been en elders op het lichaam. Patiënten met afwijkingen op het been hebben namelijk een significant hoger recidiefpercentage, vaker extracutane disseminatie en een veel lagere 5-jaars ziekte-specifieke overleving.

Concluderend kan gezegd worden dat radiotherapie een veilige en effectieve behandeling is bij PCMZL en PCFCL, maar dat het geen eerste keus behandeling is voor mensen met PCLBCL, LT en mensen met PCFCL gelokaliseerd op het been.
In hoofdstuk 7 werden de resultaten van de studies beschreven in hoofdstukken 4, 5 en 6 geïntegreerd en aangevuld met de resultaten van een uitgebreide literatuur studie en discussies binnen een multidisciplinair panel van experts op het gebied van cutane lymfomen. Op deze manier was het mogelijk consensus richtlijnen op te stellen voor de behandeling van de drie CBCL entiteiten. Deze richtlijnen staan beschreven in Tabel 4 van hoofdstuk 7.

Samenvattend tonen de studies beschreven in dit proefschrift dat de WHO-EORTC classificatie een vooruitgang heeft betekend in de classificatie van patiënten met een CBCL en dat het bijdraagt tot adequate en meer uniforme behandeling van deze zeldzame groep patiënten. De definities van PCFCL en PCLBCL, LT zijn dan ook ongewijzigd overgenomen in de recent gepubliceerde nieuwe versie van de WHO classificatie voor hematologische tumoren. Het PCMZL wordt helaas onderdeel van de bredere groep van extranodale marginale zone lymfomen en wordt niet langer als aparte entiteit erkend.

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Submitted

Curriculum Vitae

Nancy Senff werd geboren op 5 oktober 1977 te Arnhem. Zij doorliep haar middelbare school aan het Stedelijk Gymnasium te Arnhem. Na het behalen van het eindexamen in 1996, begon zij in datzelfde jaar aan de studie geneeskunde aan de Universiteit Maastricht. Tijdens haar studie was zij gedurende twee jaar werkzaam als student-assistent bij de vakgroep Algemene Heelkunde op een onderzoek naar de progressie van atherosclerotisch vaatlijden in relatie tot de aanwezigheid van chlamydia pneumoniae in atherosclerotische plaques, onder leiding van Prof. Dr. P.J.E.H.M. Kitslaar. In 2001 werd het doctoraalexamen behaald. Aan het einde van haar coschappen volgde zij gedurende zes weken een keuzecoschap Dermatologie in het Sint Lucas Andreas ziekenhuis te Amsterdam. De coschappen werden afgesloten met een wetenschapsstage van 12 weken in het Perinatal Research Centre van de Universiteit van Alberta in Edmonton, Canada. Onder leiding van Prof. Dr. B.F. Mitchell participeerde zij daar in onderzoek naar de regulering van baarmoeder contractiliteit tijdens de zwangerschap.
Bij terugkomst in Nederland werd in januari 2004 het artsexamen behaald. Zij was in dat jaar gedurende 6 maanden werkzaam als ANIOS (Arts Niet In Opleiding tot Specialist) op de afdeling Interne Geneeskunde van het Kennemer Gasthuis te Haarlem onder leiding van Prof. Dr. R.W. ten Kate. In oktober 2004 werd zij aangesteld als AIOSKO (Arts In Opleiding tot Specialist en Klinisch Onderzoeker) op de afdeling Dermatologie van het Leids Universitair Medisch Centrum en werd onder leiding van Prof Dr. R. Willemze aangevangen met het promotieonderzoek zoals beschreven in dit proefschrift. Op 1 mei 2007 is zij gestart met de opleiding tot dermatoloog.

Nawoord

De studies beschreven in dit proefschrift zouden nooit tot stand zijn gekomen zonder de hulp van en de prettige samenwerking met velen.

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