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Clinicopathologic and genetic features of primary cutaneous B-cell lymphoma

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CHAPTER 1

General Introduction

Primary cutaneous lymphomas are a group of lymphoproliferative disorders, mostly derived from T-cells or B-cells, which present in the skin with no evidence of extracutaneous disease at time of diagnosis. Whereas most lymphomas arise in lymph nodes, a considerable proportion primarily involves extranodal sites. After the gastrointestinal tract, the skin is the second most common localization of extranodal non-Hodgkin's lymphomas with an estimated annual incidence of 1 per 100 000 individuals.[1] Primary cutaneous lymphomas often have a different clinical behavior and prognosis than nodal lymphomas of the same histological subtype, and require a different type of treatment in most cases. For that reason, recent classification systems for non-Hodgkin's lymphomas, such as the European Organization for Research and Treatment of Cancer (EORTC)-classification and the World Health Organization (WHO)-classification, have included primary cutaneous lymphomas as separate disease entities.[1,2]

Primary cutaneous lymphomas are divided in two main groups: primary cutaneous T-cell lymphomas (CTCL) that account for approximately 75-80% of cases and primary cutaneous B-cell lymphomas (CBCL) accounting for the remaining 20-25%.[1,3] According to the EORTC classification three main types of CBCL can be distinguished: primary cutaneous immunocytoma or marginal zone B-cell lymphoma (PCI/PCMZL), primary cutaneous follicle center cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg). Whereas there is consensus between the EORTC and WHO classification schemes regarding the classification of most types of CTCL, there has been considerable discrepancy on the classification and terminology of CBCL (see Table 1).

This thesis includes a number of clinicopathologic and molecular genetic studies in different groups of CBCL. The aims of these studies were (1) to define more clearly the different types of CBCL, (2) to identify novel diagnostic and prognostic markers and (3) to gain a better understanding of the molecular mechanisms underlying the pathogenesis of these lymphomas. For the understanding of B-cell lymphomas and their classification, knowledge of the genetic, morphological and immunophenotypical changes involved in physiological B-cell differentiation is a prerequisite. For that reason, in this introductory chapter normal B-cell development and its relationship with the pathogenesis of B-cell lymphoma, will be discussed first. Next, the different types of CBCL are presented and current controversies which formed the basis of the studies in this thesis are discussed.

1. Normal B-cell development

B and T lymphocytes are the primary effector cells of the adaptive immune response. Cardinal features of this response type are the specific recognition of a particular antigen, discrimination between self and non-self antigens and the generation of a long-lasting immunologic memory.[4-7]

Table 1. Classification of primary cutaneous B-cell lymphoma according to the Kiel classification, the European Organization for Research and Treatment of Cancer (EORTC) classification and the World Health Organization (WHO) classification.

Kiel classification	EORTC classification	WHO classification
Immunocytoma	Primary cutaneous immunocytoma/ marginal zone B-cell lymphoma	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Centroblastic/centrocytic or centroblastic lymphoma	Primary cutaneous follicle center cell lymphoma	Cutaneous follicle center lymphoma* Diffuse large B-cell lymphoma†
Centroblastic lymphoma Immunoblastic lymphoma	Primary cutaneous large B-cell lymphoma of the leg	Diffuse large B-cell lymphoma
Plasmacytoma	Plasmacytoma	Plasmacytoma
Intravascular large B-cell lymphoma	Intravascular large B-cell lymphoma	Diffuse large B-cell lymphoma (intravascular)

* Cases with a (partly) follicular growth pattern.

† Cases with a diffuse infiltrate of large neoplastic B-cells.

B-cell maturation occurs in steps, first in the bone marrow from hematopoietic precursors to immature B-cells, then in the peripheral lymphoid organs to fully mature B-cells and finally into terminally differentiated plasma cells. At each developmental step, B-cells are instructed by transcription factors on how to further differentiate.[8-10] The sequential developmental stages of B-cells are not only characterized by the particular expression of these transcription factors and other differentiation markers, but also by the specific structure of the antigen receptor on the surface of B-cells, the B-cell receptor (BCR) (see Table 2).[11,12] The BCR is composed of two identical heavy chain and two identical light chain immunoglobulin (Ig) polypeptides that are covalently linked by disulphide bridges. Each Ig chain consist of a variable (V) region, which interact with antigen, and a constant (C) region, which mediate the effector functions of Igs.

Antigen-independent B-cell development.

In the early developmental phase in the bone marrow, B-cells primarily arise from hematopoietic stem cells and differentiate into mature B-cells.[4,11,13] During this phase rearrangement of DNA segments encoding the variable regions (variable (V), diversity (D) and joining (J) regions) of the heavy and light chains takes place to create antigen diversity of the Igs. This molecular process, V(D)J recombination, involves double-stranded DNA-breaks that are initiated by recombinase-activating genes (*RAG1* and *RAG2*) and resolved by the non-homologous end-joining repair apparatus.[12] By V(D)J recombination an immense antibody diversity is generated as each B-cell has its own unique combination of different V-, D- and J-segments of the Ig heavy and light chains.

The first B-cell precursor is the progenitor B-cell or pro-B-cell. In this pro-B-cell stage the rearrangement of the immunoglobulin heavy chain genes is initiated. The next B-cell precursor is termed pre-B-cell. In this cell the recombination of VDJ genes of the heavy chain gene locus has already completed, resulting in cytoplasmic expression of the μ heavy chain. Subsequently, rearrangement of the Ig light chain gene (IgL) locus occurs and leads to the expression of a complete IgM molecule consisting of two μ-chains and two light chains, which is expressed on the cell surface and serves as its receptor for antigen. This third precursor stage is designated as immature B-cell. Immature B-cells give rise to mature naïve B-cells, that as a result of an alternative splicing of Ig heavy chain mRNA, express both IgM and IgD. In contrast to immature B-cells, mature naïve B-cells have the capacity to respond to the binding of a foreign antigen. After negative selection of autoreactivity, the naïve IgM+IgD+ B-cells exit the bone marrow.

The majority of the mature naïve B-cells continues circulating to the peripheral lymphoid tissue, where they, as long-lived IgM+IgD+ follicular B-cells, can form primary B-cell follicles in association with follicular dendritic cells (FDCs). However, a small proportion of the mature naïve B-cells homes to the splenic marginal zone, which is a region at the border of the white pulp, and remains there as naïve non-circulating marginal zone B-cells.

Antigen-dependent B-cell development.

After antigen encounter, naïve follicular B-cells become activated in association with T-cell help and interdigitating cells. They appear to move into the T-cell zones of lymphoid tissues, where they transform into large proliferating blasts (see Figure 1). The daughter cells can either differentiate into plasmablasts and IgM-producing plasma cells outside of the follicle (extrafollicular response), or into B-cells that acquire the capacity to initiate a germinal center reaction. These B-cells move into primary follicles of lymph nodes, where they proliferate and differentiate into centroblasts to form an early germinal center.[4,14] The non-antigen-triggered naïve B-cells in the primary follicle are pushed aside forming the mantle zone of the secondary B-cell follicle.

The ultimate goal of the germinal center reaction is the generation of memory cells and plasma cells that produce Igs with high affinity for the antigen that provoked the immune reaction.[15,16] To achieve this goal, B-cells interact in a dynamic microenvironment with T-cells, FDCs and antigen. Early germinal center B-cells are rapidly proliferating B cell blasts, termed centroblasts, and differentiate further into centrocytes which gather at one end of the follicle. This leads to the formation of two zones within the germinal center: a "dark zone" containing centroblasts and a "light zone" mainly composed of centrocytes. These centroblasts and centrocytes differ from mature B-cells in their immunophenotype and susceptibility to apoptosis: they express CD10 and transcription factor Bcl-6 and down-regulate the expression of surface Ig and of the anti-apoptosis protein Bcl-2.

The rounds of germinal center cell proliferation are accompanied by affinity maturation of immunoglobulins by the processes of somatic hypermutation and antigen selection.[17] In a process of positive selection, germinal center cells introduce single mutations in their genes encoding the variable region of Ig, and those that acquire mutations that maintain or improve the affinity of the Ig for antigen are rescued from the process of apoptosis and can differentiate further. This selection process is made more efficient by the decrease of Ig molecules expressed on the surface of the germinal center cells. The few centrocytes whose mutations have resulted in surface Ig receptors with high affinity for the antigen presented by the FDC will bind to the trapped antigen and receive survival signals from the FDC (positive selection).

Table 2. B-cell development, the expression of differentiation markers and the corresponding lymphomas derived at each stage (adapted from Harris NL, et al[11]).

	B-cells	Immunoglobulin Genes	Somatic Mutations	B-cell Receptor	Markers	Corresponding Lymphoma	
Foreign antigen independent	Stem cell	Germ line	None	None	CD34		**Bone marrow**
↓	Pro B-cell	Germ line	None	None	CD34, TdT, CD19, CD79a, Pax-5, CD10		↓
	Pre B-cell	IgH rearrangement μ-chain	None	Igμ (cytoplasma)	CD34, TdT, CD19, CD79a, Pax-5, CD10, CD45R	**B-LBL/ALL**	
	Immature B-cell	IgL/IgH-rearrangements	None	IgM (membrane)	CD79a, Pax-5, CD10, CD45R, CD19, CD20		↓
Foreign antigen dependent	Mature naïve B-cell	IgH/IgL rearrangements	None	IgM/IgD	CD79a, Pax-5, CD45R, CD19, CD20, CD5	**B-CLL, MCL**	**Peripheral lymphoid tissue**
↓	Germinal center B-cell (CB and CC)	IgH/IgL rearrangements Class switch	Introduction of somatic mutations	Ig minimal or absent	CD79a, Pax-5, CD45R, CD19, CD20, CD10, Bcl-6	**BL, FL, DLBCL, cHL, LPHL**	
	Memory B-cell	IgH/IgL rearrangements	Somatic mutations	IgM	CD79a, Pax-5, CD45R, CD19, CD20	**MZL, B-CLL**	
Terminal Differentiation	Plasma cell	IgH/IgL rearrangements	Somatic mutations	IgG>IgA>IgE	CD38, CD138, Mum1/ IRF4, Blimp-1	**Plasmacytoma, myeloma**	

Abbreviations: CB: centroblasts, CC: centrocytes, Ig: Immunoglobulin, B-LBL: B-cell lymphoblastic lymphoma, B-CLL: chronic lymphocytic leukemia, MCL: mantle cell lymphoma, BL: Burkitt lymphoma, FL: follicular lymphoma, DLBCL: diffuse large B-cell lymphoma, cHL: classic Hodgkin lymphoma, LPHL: lymphocyte-predominant Hodgkin lymphoma, MZL: marginal zone B-cell lymphoma

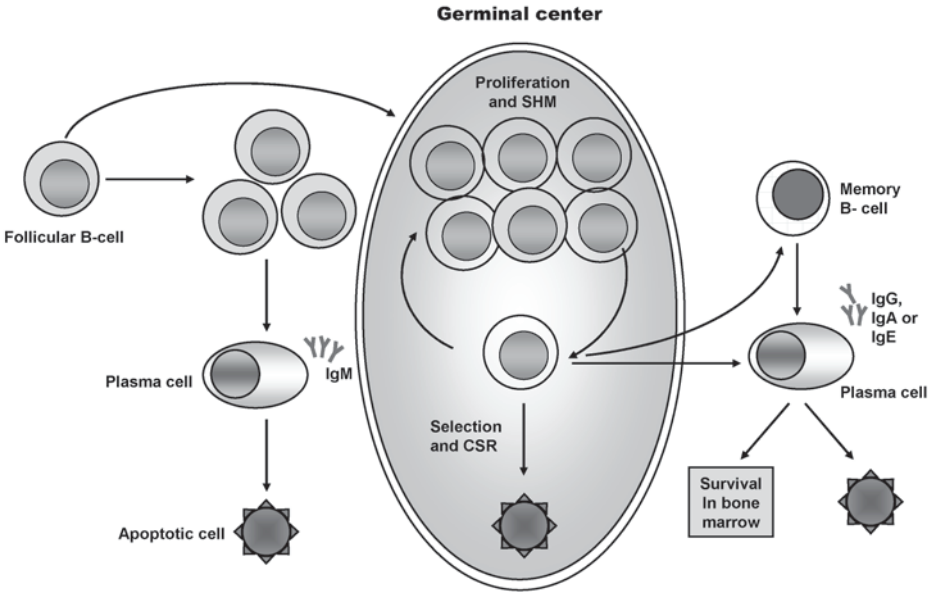


Figure 1. Antigen-dependent development of B-cells.

Recent studies have shown that the somatic hypermutation machinery is not only targeting Ig genes, but also, albeit at a much lower frequency, genes encoding Bcl-6 protein, CD95, and the B-cell receptor accessory proteins CD79a and CD79b.[18-21]

Positively selected B-cells receive growth and differentiation signals that promote class switch recombination and differentiation into memory B-cells or terminally differentiated plasma cells.[11,22] The process of class switch recombination aims to increase the defence efficiency of the secreted antibodies by altering the effector function of the Igs by means of isotype switch: by a DNA rearrangement in which deletion replaces one immunoglobulin heavy chain constant region gene segment (usually μ) with a more downstream located gene segment (γ, ε, or α). The "class-switched" B-cells are those that preferentially mature into plasma cells. During the transitional phase to plasma cells expression of *BCL6* is switched off and expression of plasmacellular transcription factors *MUM1/IRF4* and *BLIMP-1* is switched on.[9,10,12]

Finally, plasma cells and memory B-cells, which mainly have somatically mutated, high affinity BCRs and express switched immunoglobulin isotypes, exit the germinal center.[22] Post-germinal center memory B-cells show little or no expression of IgD and strong expression of IgM, and in case of class switching IgG, IgE or IgA, as well as somatically mutated variable region genes. They retain high-affinity BCR at their cell surface and preferentially home to marginal zones. Upon secondary antigen encounter memory B-cells respond with rapid proliferation and differentiation, generating large numbers of Ig secreting plasma cells. Plasma cells that result from a germinal centre reaction might become long-lived and predominantly survive in the bone marrow and organs directly exposed to foreign antigen, i.e. the lungs and the gastrointestinal tract. They synthesize clonospecific antibodies destinated for secretion, but have down regulated BCR surface expression.

2. Molecular pathogenesis of B-cell lymphoma

In the Western world, about 20 new cases of lymphoma are diagnosed per 100 000 individuals per year.[23] More than 90% of the lymphomas are of B-cell origin, the remaining 10% are T-cell malignancies and a small percentage is derived from NK-cells. It is generally thought that the higher propensity of B-cells for malignant transformation is related to the gene recombination and mutation events that occur during normal B-cell development. In the current WHO classification more than 15 types of B-cell lymphoma are distinguished and most B-cell neoplasms reflect features of the distinct B-cell differentiation stages (Table 2).[2,12,24] The various types are not only characterized by specific histopathological and genetic features, but may also have very different clinical behaviours and therefore require different treatments.

Analogous to other types of cancer, DNA lesions are primarily responsible for the development of B-cell

lymphoma. Alterations in the genome of cancer cells lead to the activation of proto-oncogenes and the inactivation of tumor suppressor genes, which drives the neoplastic process mainly by stimulation of cellular proliferation and by inhibition of apoptosis.[25] Additionally, cancer cells often exhibit genomic instability and capacity to metastasize. The activation of oncogenes can result from chromosomal translocations, amplification or activating mutations. Tumor suppressor genes are mostly inactivated through chromosomal deletions, inactivating mutations or promoter hypermethylation.[26] During evolution of disease, also due to genomic instability, DNA lesions accumulate, cellular heterogeneity develops and more malignant clones emerge.[27]

Historically, the detection of recurrent, non-random chromosomal abnormalities by traditional karyotypic analysis of metaphases has represented the major clue toward the identification and cloning of most genetic alterations of B-cell lymphoma. However, in the past twenty years considerable progress has been made to elucidate the cellular origin of different types of B-cell lymphomas and the identification of key malignant transforming events, in particular the role of chromosomal translocations.[12,28,29]

Chromosomal translocations represent the main mechanism of proto-oncogene activation in B-cell lymphoma. Similar to most types of hematopoietic neoplasms, chromosomal translocations in B-cell lymphoma represent reciprocal and balanced recombination events between two specific chromosomal sites. These translocations are recurrent within a specific clinicopathologic category of B-cell lymphoma and are clonally represented in each tumor case. A hallmark of many types of B-cell lymphoma is a chromosomal translocation involving one of the immunoglobulin loci and a proto-oncogene.[29] As a consequence of such translocations, the oncogene comes under the control of an active immunoglobulin locus, causing deregulated, constitutive expression of the translocated gene. An exception to this model is represented by the t(11;18) of MALT (mucosa associated lymphoid tissue) lymphoma which causes fusion between two genes coding for a chimeric protein (AP12/MLT).[30] The occurrence of chromosomal translocations is associated with genetic remodeling events that take place during normal B-cell development, including V(D)J-recombination, somatic hypermutation and class switch recombination.[12,28] In Table 3 the chromosomal translocations characteristic of the main types of B-cell lymphoma are presented.[2,30-41]

Although chromosomal translocations involving Ig genes are considered as a hallmark of many types of B-cell lymphoma, many other transforming events have also been implicated in the pathogenesis of B-cell lymphomas, such as inactivating mutations of tumor suppressor genes or genomic amplifications. Disruption of tumor suppressor loci in B-cell lymphoma generally leads to biallelic inactivation, most frequently through deletion of one allele and mutation of the other. The tumor suppressor genes most frequently involved in the pathogenesis of B-cell lymphoma are p53, p16 and ATM.[42-45] In addition, specific chromosomal deletions have been reported in B-cell lymphoma, which presumably represent sites of unidentified tumor suppressor genes. These deletions involve most frequently the long arm of chromosomes 6 and 13.[46,47]

Table 3. Chromosomal translocations in the main types of B-cell lymphoma.

Lymphoma	Chromosomal translocation	Involved proto-oncogene	Frequency (%)	References
Mantle cell lymphoma	t(11;14)(q13;q32)	CCND1	>95	2
Follicular lymphoma	t(14;18)(q32;q21)	BCL2	80	2,31
Diffuse large B-cell lymphoma	t(3;14)(q27;q32)	BCL6	25-35	2,32,33
	t(14;18)(q32;q21)	BCL2	20	34
	t(8;14)(q24;q32)	CMYC	10	35
	t(3;14)(p14.1q32)	FOXP1	rare	36
Burkitt's lymphoma	t(8;14)(q24;q32)	CMYC	80	2
MALT lymphoma	t(11;18)(q21;q21)	AP12/MLT	14-42	30,37,38
	t(1;14)(p22;q32)	BCL10	1-2	39
	t(14;18)(q32;q21)	MALT1	15-20	39,40
	t(3;14)(p14.1q32)	FOXP1	10	41

3. Primary cutaneous B-cell lymphoma

The term primary cutaneous B-cell lymphoma (CBCL) refers to a heterogeneous group of non-Hodgkin's B-cell lymphomas primarily presenting in the skin, without evidence of extracutaneous manifestations at time of diagnosis.[1] These CBCL have to be differentiated from systemic B-cell lymphomas involving the skin secondarily. Until recently, the distinction between primary and secondary cutaneous B-cell lymphomas was not made. Malignant lymphomas arising in the skin other than mycosis fungoides, were generally considered as manifestations of systemic disease. However, the introduction of immunohistochemistry in the late seventies was of major importance for the diagnosis and classification of B-cell lymphoproliferative disorders in the skin. The presence of monotypic immunoglobulin light chain expression on frozen or paraffin tissue sections became generally accepted as the golden standard for the diagnosis of a malignant B-cell lymphoma. This did not only facilitate differentiation between malignant B-cell lymphomas and reactive B-cell proliferations (pseudo B-cell lymphomas), but also resulted in the observation that malignant lymphoma can arise in the skin without concurrent systemic disease being detectable. According to the (histologic) criteria of the Kiel classification, most of these CBCL were classified as immunocytoma, centroblastic/centrocytic lymphoma (CB/CC), centroblastic lymphoma (CB) or immunoblastic lymphoma (IB) (see Table 1).[48]

Clinicopathologic studies on well-defined patient groups showed that the different types of CBCL have a distinct clinical presentation and course. Furthermore, it was shown that CBCL often have a completely different clinical behaviour than their morphologically similar systemic counterparts and therefore require a different therapeutic approach. Between 1986 and 1996 three main types of CBCL were defined.

Distinct types of CBCL

CBCL classified as lymphoplasmacytic, lympho-plasmacytoid or polymorphic immunocytoma according to the Kiel classification were collectively called primary cutaneous immunocytoma (PCI). These PCI presented as solitary or multiple (sub)cutaneous tumors preferentially involving the extremities and were characterized by an excellent prognosis.[49]

CBCL classified as CB/CC or CB lymphoma generally presented with tumors on the head or on the trunk, rarely disseminated to extracutaneous sites, were extremely responsive to radiotherapy and appeared to have an excellent prognosis with a 5-year survival of >95%.[50-54] Importantly, no differences in clinical presentation and prognosis were seen between cases classified either as CB/CC or CB lymphoma, neither between cases with a follicular, follicular and diffuse, or diffuse growth pattern. Because the tumor cells of these CB/CC or CB CBCL showed the morphology of cells normally found in the follicle center of lymph nodes, i.e. centrocytes and centroblasts, the term 'primary cutaneous follicle center cell lymphoma' (PCFCCL) was proposed.[50]

Already in the first publication on PCFCCL, it was noticed that patients with tumors on the legs had a more aggressive clinical behaviour. Additional studies confirmed that these tumors particularly develop in elderly females, more often relapse and disseminate to extracutaneous sites, and have an more unfavorable prognosis (5-year survival approximately 50%), as compared to PCFCCL presenting on the head or trunk.[55] Histologically, these lymphomas showed a monotonous population of centroblasts and immunoblasts and in contrast to PCFCCL strongly expressed Bcl-2 protein.[56] For these reasons, these cases were considered as a separate entity and designated 'primary cutaneous large B-cell lymphomas of the leg' (PCLBCL-leg).

Classification of CBCL

Since CBCL often have a different clinical behaviour and prognosis as compared to their nodal counterparts, they should also be treated in a different way. However, existing classification schemes for malignant lymphomas, including the (up-dated) Kiel classification, the Working Formulation and the REAL classification did not recognize these CBCL as distinct entities.[50,57,58] Consequently, patients with indolent types of CBCL were often treated as nodal lymphomas with systemic chemotherapy rather than with radiotherapy. Since the existing classification schemes used for nodal lymphomas were inadequate to categorize CBCL in a clinically meaningful way, the EORTC Cutaneous Lymphoma Study Group proposed a separate classification scheme for primary cutaneous lymphomas. In the EORTC classification three main types of CBCL are distinguished: primary cutaneous immunocytoma or marginal zone B-cell lymphoma (PCI / PCMZL)[49,61-65], primary cutaneous follicle center cell lymphoma (PCFCCL)[51-55,66-74] and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg)[55,56,66-68,70-73]. In addition, plasmacytoma and intravascular large B-cell lymphomas were included as provisional entities (see Tabel 1). In Table 4 the most important clinical and histopathological features of the three main types of CBCL are summarized. The typical clinical manifestations are shown in Figures 2-4.

In 2001 the WHO classification for hematopoietic and lymphoid neoplasms was published as a successor of the REAL classification.[2] This classification scheme adopted the most common types of CTCL, including mycosis fungoides, variants of mycosis fungoides, Sézary syndrome

Table 4. Main clinical and histopathological characteristics of primary cutaneous marginal zone B-cell lymphoma (PCMZL), primary cutaneous follicle center cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg).

	PCMZL	PCFCCL	PCLBCL-leg
Clinical features	- solitary or multiple papules, plaques or nodules preferentially localized on the extremities - sometimes associated with *Borrelia burgdorferi* infection - frequently 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 on the leg(s) - frequently 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	diffuse or (partly) follicular infiltrates composed of centrocytes (small and large cleaved cells) and centroblasts	diffuse infiltrates composed of centroblasts and/or immunoblasts
Immunophenotype	monotypic cIg, CD79a+, CD5-	monotypic sIg or absence of sIg, CD20+, CD79a+, CD5-, Bcl-2-	monotypic sIg or cIg, CD20+, CD79a+, Bcl-2+
Prognosis	5-year survival: >95%	5-year survival: > 95%	5-year survival: 50%
Treatment	radiotherapy	radiotherapy	multiagent chemotherapy



Figure 2. Primary cutaneous marginal zone B-cell lymphoma.

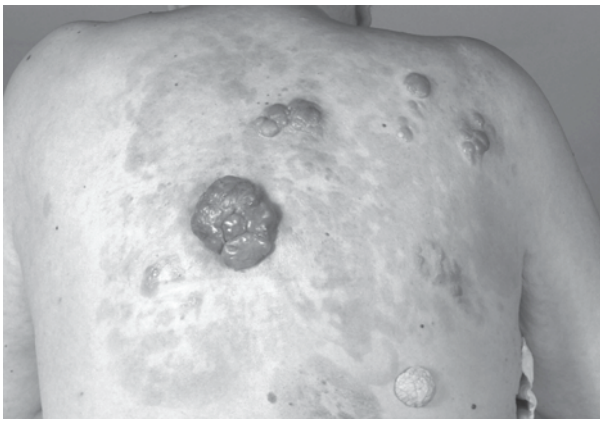


Figure 3. Primary cutaneous follicle center cell lymphoma.



Figure 4. Primary cutaneous large B-cell lymphoma of the leg.

and the group of primary cutaneous CD30 positive lymphoproliferative disorders, together accounting for more than 90% of the CTCL patients. However, the WHO classification did not adopt the different types of CBCL included in the EORTC classification. The PCMZL group of the EORTC classification was included in a broad category of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), although these skin-limited cases may show different clinical behaviour than MALT lymphomas arising at other extranodal sites. The well-defined group of PCFCCL was approached in a rather traditional, i.e. histological way. PCFCCL with a (partially) follicular growth pattern were categorized as cutaneous follicle center lymphoma, a variant of follicular lymphoma (FL), whereas cases with a diffuse infiltrate of large follicle center cells were classified as a diffuse large B-cell lymphoma (DLBCL) (see Table 1).

In recent years the differences in terminology and definition of the three main types of CBCL between the EORTC and WHO classification schemes have resulted in confusion and ongoing debate. These controversies formed the starting point of the studies presented in this thesis and will be discussed in more detail in the next paragraph.

4. Current controversies, aims and outline of the thesis

4.1. The terminology and differential diagnosis of PCFCCL

In retrospect, the most important source of confusion and debate has probably been the introduction of the term 'primary cutaneous follicle center cell lymphoma', which resembles the term follicular lymphoma (FL), but is defined in a different way. The term FL denotes a neoplasm of follicle center B-cells (centrocytes/cleaved follicle center cells and centroblasts/noncleaved follicle center cells), which has at least a partially follicular growth pattern.[2] FL is graded by the proportion of centroblasts. Histological grading of FL is currently assessed by a 3-grade system (grade 1-3), based on the absolute number of centroblasts present in ten neoplastic follicles, expressed per 40x high-power microscopic field. This histological grade correlates with prognosis in FL, with grades 1 and 2 showing an indolent clinical course, and grade 3 being more aggressive and typically treated with multiagent chemotherapy. FL was one of the first lymphomas in which a recurrent chromosomal translocation was identified. In the early eighties the characteristic t(14;18) resulting in overexpression of the anti-apoptotic Bcl-2 protein was recognized and appeared present in 80% of the cases.[2,31]

In contrast, the term PCFCCL was introduced in 1987 as an encompassing term for cutaneous lymphomas composed of cells with the morphology of follicle center cells (centroblasts and centrocytes).[50] In contrast with the follicular growth pattern observed in FL, it was recognized that PCFCCL showed in most cases a diffuse growing infiltrate, whereas a (partially) follicular growth pattern was only observed in a minority of cases.[50-53,69] More important, no difference in clinical behaviour and prognosis was found between cases classified as either CB/CC or CB. Both showed an indolent clinical behaviour and were amenable to local therapy. Since histological grading had no clinical significance, the encompassing term PCFCCL was preferred.

However, because of the rarity of the disease, hematopathologists were in general not familiar with this definition and did not recognize the large cleaved cells in PCFCCL with a diffuse growth pattern as follicle center cells. Moreover, the germinal center cell origin of these lymphomas was questioned when it appeared that most PCFCCL do not express Bcl-2 protein and do not contain the t(14;18), both characteristic of FL.[56,67-69] Some authors even suggested that most, if not all PCFCCL, were derived from marginal zone B-cells.[73]

Recently, new markers allowing differentiation between follicle center cells and marginal zone B-cells have become available. To elucidate the relationship of PCFCCL with both FL and PCMZL, in **chapter 2** well defined groups of PCFCCL, PCMZL, PCLBCL-leg, secondary cutaneous FL and pseudo B-cell lymphomas were investigated for expression of the anti-apoptotic protein Bcl-2 and the germinal center markers Bcl-6 and CD10. The aim of this study was to better define the neoplastic cells of PCFCCL, and to find out whether differences in expression of these markers might be helpful in the differential diagnosis of B-cell lymphoproliferative disorders.

4.2 Clinical and genetic characterization of PCMZL

The results from the study presented in **chapter 2** have led to a better definition of the group of PCMZL. Based on this new definition, clinical and therapeutic characteristics of 50 PCMZL cases included in the registry of the Dutch Cutaneous Lymphoma Working Group were evaluated. The aim of the study was to further characterize the clinical features and outcome and, in particular, to evaluate the currently used therapeutic approach. The results are presented in **chapter 3**.

Recently, the presence of a number of recurring structural and numerical chromosomal aberrations has been reported in MALT lymphomas of different sites of origin, including two cases involving the skin.[37-40] Because the incidence of these genetic alterations is unknown in PCMZL, the presence of chromosomal translocations

BOX 1 An overview of the molecular techniques used in studies included in this thesis.

Array-based comparative genomic hybridization (array-CGH) is a method used to detect copy number aberrations (gains and losses) of specific chromosomal regions on a genome-wide scale. It makes use of microarrays consisting of multiple well-defined genomic clones such as BAC (bacterial artificial chromosomes), PAC, cosmid clones or oligonucleotides. Genomic DNA isolated from tumor tissue and from a reference sample are competitively hybridized to probes on the microarrays. Since these clones or oligonucleotides are complementary to annotated genomic sequences, specific alterations of genes mapped within regions involved in the detected copy number aberrations can be identified.

Fluorescent *in situ* hybridization (FISH) is a technology that utilizes fluorescently labeled DNA probes to detect structural and numerical chromosomal abnormalities. A fluorescently labeled probe is hybridized to denatured sample DNA (metaphase chromosomes or interphase nuclei), upon which the probe signal can be detected by fluorescence microscopy.

Oligonucleotide microarray analysis is a technique to investigate expression levels of thousands of genes simultaneously at the mRNA level. The microarray used in these experiments consists of oligonucleotide (20~80-mer oligos) probes synthesized either *in situ* (on-chip) or by conventional synthesis followed by on-chip immobilization. From isolated sample RNA complementary DNA (cDNA) is synthesized followed by transcription to antisense RNA. Fragmented biotin-labeled antisense RNA is then hybridized to the array and the expression values of the complementary sequences can be determined by specific software programmes quantifying fluorescence signals.

Quantitative real-time polymerase chain reaction (qPCR) is a technique applied for sensitive measurement of mRNA levels of genes of interest. From isolated sample RNA cDNA is prepared, followed by amplification of a specific cDNA in a polymerase chain reaction (PCR) using short oligonucleotide primers. During the multiple cycles of the PCR reaction fluorescence produced by a fluorescent label that is incorporated in the newly formed PCR product is measured simultaneously. Initial cDNA quantity is inferred from the number of PCR cycles required to synthesize a certain measurable amount of the fluorescently labeled PCR product of interest.

t(11;18)(q21;q21), t(1;14)(p22;q32), two different t(14;18)(q32;q21) involving either IGH and MALT1 genes or IGH and BCL2 genes and numerical aberrations of chromosomes 3,7,12 and 18 were investigated in a group of 12 well-defined PCMZL cases by fluorescent *in situ* hybridization (FISH, see Box 1). The results of this study are presented in **chapter 4**.

4.3 Differentiation between PCFCCL with a diffuse growth pattern and PCLBCL-leg

In the EORTC classification PCFCCL, irrespective of growth pattern (follicular, follicular and diffuse, or diffuse) or number of blast cells, is considered as an indolent type of CBCL, which should be treated primarily with radiotherapy. PCLBCL-leg has a more aggressive clinical behaviour and therefore systemic chemotherapy is the treatment of first choice. In the WHO classification both PCFCCL with a diffuse infiltration of large follicle center cells and PCLBCL-leg are classified as a diffuse large B-cell lymphoma, and consequently treated with systemic chemotherapy. In the last decade several studies addressed the question whether these two groups of primary cutaneous large B-cell lymphoma should be considered separately and whether distinction should be made primarily on the basis of site of presentation.[74-77]

In a large multicenter study, the clinical and histological features of 145 cases of primary cutaneous large B-cell lymphoma, including 97 PCFCCL and 48 PCLBCL-leg, were evaluated to identify prognostic factors in this group.[74] Multivariate analysis revealed the round cell morphology and location on the leg as independent adverse prognostic factors. Additionally, the presence of multiple skin lesions at diagnosis was associated with a poor prognosis only in the group of PCLBCL-leg, but not in the group of PCFCCL. In another study, Goodlad *et al* identified location on the leg as the most significant factor in predicting adverse prognosis in 30 patients with primary cutaneous large B-cell lymphoma.[75] Some other studies analyzing prognostic factors in these lymphomas did not demonstrate significant differences in survival, but this is probably due to the small number of cases investigated.[76,77]

An important immunophenotypical difference between PCFCCL and PCLBCL-leg is the strong Bcl-2 expression in the latter, whereas PCFCCL are generally Bcl-2 negative.[56] The Bcl-2 overexpression in PCLBCL-leg is not related to the t(14;18) like in FL and in some DLBCL, but might in some cases result from chromosomal amplification of the BCL2 gene.[67-71] In a study of 80

primary cutaneous large B-cell lymphoma, including both PCFCCL, diffuse large cell, and PCLBCL-leg, Grange *et al* detected Bcl-2 protein expression in 39 out of 80 cases and suggested that Bcl-2 protein expression has prognostic significance in these lymphomas.[78]

Recent studies have started to evaluate the genetic mechanisms involved in the development and progression of primary cutaneous large B-cell lymphoma. Cytogenetic analysis have revealed that the occurrence of chromosomal aberrations is more common in PCLBCL-leg (85% of the cases), than in PCFCCL (30% of the cases).[71] The most frequently detected genetic alterations in PCLBCL-leg were loss of 6q and gain of 18q. Inactivation of P15 and P16 tumor suppressor genes was detected by Child *et al* in 11% and 44% of primary cutaneous large B-cell lymphoma cases.[72] However, the number of studies published to date is limited and specific genetic alterations have not been identified yet. For that reason, we performed microarray analysis to investigate the gene expression profiles of 8 cases of PCFCCL and 13 cases of PCLBCL-leg. Microarray analysis enables to assess the expression values of many genes simultaneously at messenger RNA level (oligonucleotide microarray, see Box 1). The general aim of this study was to find out if these two types of primary cutaneous large B-cell lymphoma have different gene expression profiles, which might provide insight in the molecular pathogenesis of these lymphomas and possibly lead to the identification of novel diagnostic or prognostic markers. The results of the microarray study are presented in **chapter 5**.

As already mentioned, a round cell morphology and the presence of multiple skin lesions at diagnosis were identified as adverse prognostic factors in the group of PCLBCL-leg. Bcl-2 is not a prognostic factor within this group, since both cases with a good prognosis and cases with a poor prognosis strongly express Bcl-2 protein.[56] Chromosomal alterations that determine clinical outcome in this group are still unknown. To further define chromosomal aberrations in PCFCCL and PCLBCL-leg, but particulary to find differences between PCLBCL-leg with a favorable and unfavorable prognosis, a large group of primary cutaneous large B-cell lymphomas including 19 cases of PCFCCL and 12 cases of PCLBCL-leg, was investigated by array-based comparative genomic hybridization (array-based CGH, see Box 1). The PCLBCL-leg cases consisted of 7 cases in which the patients died of lymphoma and 5 cases in which the patients were alive. In **chapter 6** the results of this study are described.

The results of **chapter 2 and chapter 5** revealed the role of B-cell transcription factors in the differentiation between different CBCL subtypes and additionally suggested pathogenetic significance. For this reason, the immunohistochemical expression of an extended panel of B-cell transcription factors, expressed during the whole spectrum of B-cell differentiation, was studied in the three types of CBCL and compared with physiological expression patterns in tonsils and pseudo B-cell lymphomas. The following markers were investigated: Pax-5, PU.1, Oct-2, BOB.1, Bcl-6, Mum1/IRF4, Blimp-1 and FOXP1. The aim of the study was (1) to compare the expression patterns in CBCL with patterns in normal B-cells in order to detect aberrant expression with potential pathogenetic significance and (2) to find out whether expression of some of these B-cell transcription factors is of additional use in the differential diagnosis of these lymphomas. In **chapter 7** the results of this study are presented.

Finally, the results of the studies presented in this thesis are summarized and discussed in **chapter 8**.

References

1. Willemze R, Kerl H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas. A proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer (EORTC). Blood, 1997;90:354-371.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW (Eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon 2001.
3. Fink-Puches R, Zenahlik P, Bäck B, et al. Primary cutaneous lymphomas: applicability of current classification schemes (European Organization for Research and Treatment of Cancer, World Health Organization) based on clinicopathological features observed in a large group of patients. Blood, 2002;99:800-805.
4. Stein H. Lymphocyte differentiation. In Mason DY, Harris NL, eds. Human Lymphoma: Clinical implications of the REAL classification. London: Springer-Verlag, 1999:2.0-2.4.
5. Burrows PD, Cooper MD. B cell development and differentiation. Curr Opin Immunol, 1997;9:239-244.
6. Rudin CM, Thompson CB. B cell development and maturation. Semin Oncol, 1998;25:435-446.
7. Rolink AG, Schaniel C, Andersson J, et al. Selection events operating at various stages in B cell development. Curr Opin Immunol, 2001;13:202-207.
8. Bartoldy B, Matthias P. Transcriptional control of B cell development and function. Gene, 2004;327:1-23.
9. Matthias P, Rolink AG. Transcriptional networks in developing and mature B-cell. Nat Rev Immunol, 2005;5:497-508.
10. Johnson K, Shapiro-Shelef M, Tunyaplin C, et al. Regulatory events in early and late B-cell differentiation. Mol Immunol, 2005;42:749-761.
11. Harris NL, Stein H, Coupland SE, et al. New approaches to lymphoma diagnosis. Hematology, 2001;194-220.
12. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: The dark side of B-cell differentiation. Nat

Rev Immunol, 2002;2:920-932.
13. Ghia P, ten Boekel E, Rolink A, et al. B-cell development: a comparison between mouse and man. Immunol Today, 1998;19:480-485.
14. MacLennan IC. Germinal centers. Ann Rev Immunol, 1994;12:117-139.
15. Guzman-Rojas L, Sims-Mourtada JC, Rangel R, et al. Life and death within germinal centres: a double-edged sword. Immunology, 2002;107:167-175.
16. McHeyzer-Williams LJ, Driver DJ, McHeyzer-Williams MG. Germinal center reaction. Curr Opin Hematol, 2001;8:52-59.
17. MacLennan IC. Somatic mutation. From the dark zone to the light. Curr Biol, 1994;4:70-72.
18. Pasqualucci L, Migliazza A, Fracchiolla N, et al. BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. Proc Natl Acad Sci U S A, 1998;95:11816-11821.
19. Gordon MS, Kanegai CM, Doerr JR, et al. Somatic hypermutation of the B cell receptor genes B29 (Igbeta, CD79b) and mb1 (Igalpha, CD79a). Proc Proc Natl Acad Sci U S A, 2003;100:4126-4131.
20. Shen HM, Peters A, Baron B, et al. Mutation of BCL-6 gene in normal B-cells by the process of somatic hypermutation of Ig genes. Science, 1998;280:1750-1752.
21. Müschen M, Re D, Jungnickel B, et al. Somatic mutation of the CD95 gene in human B-cells as a side effect of the germinal center reaction. J Exp Med, 2000;18:1833-1840.
22. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol, 2005;5:230-242.
23. Fisher SG, Fisher RI. The epidemiology of non-Hodgkin's lymphoma. Oncogene, 2004;23:6524-6534.
24. Küppers R, Klein U, Hansmann ML, et al. Cellular origin of human B-cell lymphomas. N Eng J Med, 1999;341:1520-1529.
25. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. Nat Rev Cancer, 2002;2:331-341.
26. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med, 2004;10:789-799.
27. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell, 2000;100:57-70.
28. Küppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer, 2005;5:251-262.
29. Küppers R, Dalla-Favera R. Mechanisms of chromosomal translocation in B-cell lymphoma. Oncogene, 2001;20:5580-5594.
30. Dierlamm J, Baens M, Wlodarska I, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood, 1999;93:3601-3609.
31. de Jong, D. Molecular pathogenesis of follicular lymphoma: A cross talk of genetic and immunologic factors. J Clin Oncol, 2005;23:6358-6363.
32. Bastard C, Deweindt C, Kerckaert JP, et al. LAZ3 rearrangements in non-Hodgkin's lymphoma: Correlation with histology, immunophenotype, karyotype and clinical outcome in 217 patients. Blood, 1994;83:2423-2427.
33. Lo Coco F, Ye BH, Lista F, et al. Rearrangements of the BCL6 gene in diffuse large cell non-Hodgkin's lymphoma. Blood, 1994;83:1757-1759.
34. Huang JZ, Sanger WG, Greiner TC, et al. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood, 2002;99:2285-2290.
35. Akasaka T, Akasaka H, Ueda C, et al. Molecular and genetic features of non-Burkitt's, diffuse large-cell lymphoma of B-cell type associated with the c-MYC/immunoglobulin heavy chain fusion gene. J Clin Oncol, 2000;18:510-518.
36. Wlodarska I, Veyt E, De Paepe P, et al. FOXP1, a gene highly expressed in a subset of diffuse large B-cell lymphoma is recurrently targeted by genomic aberrations. Leukemia, 2005; 19: 1299-1305.
37. Auer IA, Gascoyne RD, Connors JM, et al. T(11;18) is the most common translocation in MALT lymphomas. An Oncol, 1997;8:979-985.
38. Ye H, Liu H, Attygalle A, et al. Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: Significant association with CagA strains of H pylori in gastric MALT lymphoma. Blood, 2003;102:1012-1018.
39. Farinha P, Gasgcoyne RD. Molecular pathogenesis of Mucosa-Associated Lymphoid Tisuse Lymphoma. J Clin Oncol, 2005;23:6370-6378.
40. Streubel B, Lamprecht A, Dierlamm J, et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood, 2003;101:2335-2339.
41. Streubel B, Vinatzer U, Lamprecht A, et al. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia, 2005;19:652-658.
42. Agarwal ML, Taylor WR, Chernov MV, et al. The p53 network. J Biol Chem, 1998;273:1-4.
43. Liggett WH, Jr, Sidransky D. Role of p16 tumor suppressor gene in cancer. J Clin Oncol, 1998;16:1197-1206.
44. Herman JG, Civin CI, Issa JP, et al. Distinct patterns of inactivation of p15INK4B and p16INK4A characterize the major types of hematological malignancies. Ccer Res, 1997;57:837-841.
45. Westphal CH. Cell-cycle signaling: Atm displays its many talents. Curr Biol, 1997;7:R789-792.
46. Gaidano G, Hauptschein RS, Parsa NZ, et al. Deletions involving two distinct regions of 6q in B-cell non-Hodgkin lymphoma. Blood, 1992;80:1781-1787.
47. Migliazza A, Bosch F, Komatsu H, et al. Nucleotide sequence, transcription map, and mutation analysis of the 13q14 chromosomal region deleted in B-cell chronic lymphocytic leukemia. Blood, 2001;97:2098-2104.
48. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas. Lancet, 1988;1:292-293.
49. Rijlaarsdam JU, van der Putte SCJ, Berti E, et al. Cutaneous immunocytomas: A clinicopathologic study of 26 cases. Histopathology, 1993;23:117-125.
50. Willemze R, Meijer CJLM, Sentis HJ, et al. Primary cutaneous large cell lymphomas of follicular center cell origin. J Am Acad Dermatol, 1987;16:518.
51. Garcia CF, Weiss LM, Warnke RA, et al. Cutaneous follicular lymphoma. Am J Surg Pathol, 1986;10:454-463.
52. Berti E, Alessi E, Caputo R, et al. Reticulohistiocytoma of the dorsum. J Am Acad Dermatol, 1988;19:259-272.

53. Santucci M, Pimpinelli N, Arganini L. Primary cutaneous B-cell lymphoma: A unique type of low-grade lymphoma: Clinicopathologic and immunologic study of 83 cases. Cancer, 1991;67:2311-2326.
54. Rijlaarsdam JU, Toonstra J, Meijer CJLM, et al. Treatment of primary cutaneous lymphomas of follicular center cell origin. A clinical follow-up study of 55 patients treated with radiotherapy or polychemotherapy. J Clin Oncol, 1996;14:549-555.
55. Vermeer MH, Geelen FAMJ, van Haselen CW, et al. Primary cutaneous large B-cell lymphomas of the legs. A distinct type of cutaneous B-cell lymphoma with an intermediate prognosis. Arch Dermatol, 1996;132:1304-1308.
56. Geelen FAMJ, Vermeer MH, Meijer CJLM, et al. Bcl-2 expression in primary cutaneous large B-cell lymphoma is site-related. J Clin Oncol, 1998;16:2080-2085.
57. Harris NL, Jaffe ES, Stein H, et al. A Revised European-American Classification of Lymphoid Neoplasms: A proposal from the International Lymphoma Study Group. Blood, 1994;84:1361-1392.
58. Robb-Smith AH. U.S. National Cancer Institute working formulation of non-Hodgkin's lymphomas for clinical use. Lancet, 1982;2:432-434.
59. Cerroni L, Signoretti S, Höfler G, et al. Primary cutaneous marginal zone B-cell lymphoma: a recently described entity of low-grade malignant cutaneous B-cell lymphoma. Am J Surg Pathol, 1997;21:1307-1315.
60. Bailey EM, Ferry JA, Harris NL, et al. Marginal zone lymphoma (low-grade B-cell lymphoma of mucosa-associated lymphoid tissue) of skin and subcutaneous tissue: a study of 15 patients. Am J Surg Pathol, 1996;20:1011-1023.
61. LeBoit PE, McNutt NS, Reed JA, et al. Primary cutaneous immunocytoma: a B-cell lymphoma that can easily be mistaken for cutaneous lymphoid hyperplasia. Am J Surg Pathol, 1994;18:969-978.
62. Cerroni L, Zöchling N, Pütz B, et al. Infection by Borrelia burgdorferi and cutaneous B-cell lymphoma. J Cutan Pathol, 1997;24:457-461.
63. Goodlad JR, Davidson MM, Hollowood K, et al. Primary cutaneous B-cell lymphoma and Borrelia burgdorferi infection in patients from the Highlands of Scotland. Am J Surg Pathol, 2000;24:1279-1285.
64. Rijlaarsdam JU, Bakels V, van Oostveen JW, et al. Demonstration of clonal immunoglobulin rearrangements in cutaneous B-cell lymphoma and pseudo B-cell lymphomas: Differential diagnostic and pathogenetic aspects. J Invest Dermatol, 1992;99:749-754.
65. Aarts WM, Willemze R, Bende RJ, et al. VH gene analysis of primary cutaneous B-cell lymphoma: Evidence for ongoing somatic hypermutation and isotype switching. Blood, 1998;92:3857-3864.
66. Gellrich S, Rutz S, Golembowski S, et al. Primary cutaneous follicle center cell lymphomas and large B-cell lymphomas of the leg descend from germinal center cells. A single cell polymerase chain reaction analysis. J Invest Dermatol, 2001;117:1512-1520.
67. Child FJ, Russell-Jones R, Woolford AJ, et al. Absence of the t(14;18) translocation in primary cutaneous B-cell lymphoma. Br J Dermatol, 2001;144:735-744.
68. Cerroni L, Volkenandt M, Rieger E, et al. Bcl-2 protein expression and correlation with the interchromosomal (14;18) translocation in cutaneous lymphomas and pseudolymphomas. J Invest Dermatol, 1994;102:231-235.
69. Cerroni L, Arzberger E, Pütz B, et al. Primary cutaneous follicle center cell lymphoma with follicular growth pattern. Blood, 2000;95:3922-3928.
70. Mao X, Lillington D, Child F, et al. Comparative genomic hybridization analysis of primary cutaneous B-cell lymphomas: Identification of common genetic alterations in disease pathogenesis. Genes Chromosomes Cancer, 2002;35:144-155.
71. Hallermann C, Kaune K, Siebert R, et al. Chromosomal aberration patterns differ in subtypes of primary cutaneous B-cell lymphomas. J Invest Dermatol, 2004;122:1495-1502.
72. Child FJ, Scarisbrick JJ, Calonje E, et al. Inactivation of tumor suppressor genes p15ink4b and p16ink4a in primary cutaneous B-cell lymphoma. J Invest Dermatol, 2002;118:941-948.
73. Slater DN. MALT and SALT: the clue to cutaneous B-cell lymphoproliferative disease. Br J Dermatol, 1994;131:557-561.
74. Grange F, Bekkenk MW, Wechsler J, et al. Prognostic factors in primary cutaneous large B-cell lymphomas: a European multicenter study. J Clin Oncol, 2001;19:3602-3610.
75. Goodlad JR, Krajewski AS, Batstone PJ, et al. Primary cutaneous diffuse large B-cell lymphoma. Prognostic significance of clinicopathological subtypes. Am J Surg Pathol, 2003;27:1538-1545.
76. Fernandez-Vasquez A, Rodriguez-Peralto JL, Martinez MA, et al. Primary cutaneous large B-cell lymphoma: the relationship between morphology, clinical presentation, immunohistochemical markers and survival. Am J Surg Pathol, 2001;25:307-315.
77. Hembury TA, Lee B, Gascoyne RD, et al. Primary cutaneous diffuse large B-cell lymphoma: a clinicopathological study of 15 cases. Am J Clin Pathol, 2002;117:574-580.
78. Grange F, Petrella T, Beylot-Barry M, et al. Bcl-2 protein expression is the strongest independent prognostic factor of survival in primary cutaneous large B-cell lymphoma. Blood, 2004;103:3662-3668.