Primary cutaneous large B-cell lymphomas.

Clinicopathologic features, classification, and prognostic factors in a large series of patients.

Short title: Primary cutaneous large B-cell lymphomas

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Abstract

In the new World Health Organization/European Organization for Research and Treatment of Cancer (WHO/EORTC) classification of cutaneous lymphomas, large B-cell lymphomas (LBCLs) are divided into 3 groups: LBCL, leg-type (LBCLLT); follicle center lymphoma, diffuse type (FCLDT); LBCL, others (LBCLO). We studied a large number of primary cutaneous LBCLs in order to test the validity of the classification, and to identify prognostic factors for these patients. Ninety-three cases of primary cutaneous LBCL were analyzed for clinicopathologic features, expression of several markers including Bcl-2, Bcl-6, MUM-1, and FOX-P1, in-situ-hybridization for Epstein-Barr virus, and molecular analyses of IgH gene rearrangement and of Borrelia burgdorferi and Human herpesvirus 8 DNA. Patients were classified into the following categories: FCLDT: 44 cases; LBCLLT: 40 cases. LBCLO: 9 cases. Statistical analyses showed that the groups of LBCLLT and FCLDT were clearly distinct in terms of clinicopathologic features and survival. The group of LBCLO had features in-between those of LBCLLT and FCLDT. Our study shows that accurate morphologic and phenotypic analyses allow to stratify most patients into the prognostically different categories of LBCLLT and FCLDT. The definition of a third category of LBCLO requires further studies to clarify whether these cases show indeed distinct clinicopathologic features.
Introduction

In the new World Health Organization/European Organization for Research and Treatment of Cancer (WHO/EORTC) classification of cutaneous lymphomas, large B-cell lymphomas (LBCL) are divided into 3 groups: large B-cell lymphoma, leg-type (LBCLLT) is defined as a cutaneous B-cell lymphomas with predominance of large round cells (centroblasts, immunoblasts) that are positive for Bcl-2. Cases of LBCL with a predominant cleaved cell morphology (large centrocytes) are included within the follicle center lymphomas, diffuse type (FCLDT). These cases would be included in the categories of diffuse LBCL in the WHO classification of tumors of hematopoietic and lymphoid tissues, and of cutaneous follicle center cell lymphomas in the first EORTC classification of primary cutaneous lymphomas, respectively. Rare other cases not fitting within these 2 categories are included in a group of large B-cell lymphoma, others (LBCLO). Although the new WHO/EORTC classification is the result of a consensus among representatives of the WHO and EORTC groups, at present there are no clinicopathologic data supporting the inclusions of primary cutaneous LBCLs into the 3 aforementioned groups. In addition, prognostic factors for these patients are largely unknown.

Recently, we could confirm the clinical value of the classification of cutaneous lymphomas proposed in 1997 by the EORTC. In the present study we analyzed a large group of patients with primary cutaneous LBCL, classifying patients according to the new WHO/EORTC classification, and evaluating the prognostic value of several factors including classification, age of patients, anatomical site of onset, number of lesions at presentation, cell morphology, and expression of Bcl-2, MUM-1 and FOX-P1 proteins.

Patients and methods

Biopsy specimens from 93 cases of primary cutaneous LBCL were retrieved from the database files of the Division of Dermatopathology, Department of Dermatology, Medical University of Graz. The cases had been registered between 1960 and 2004. Primary cutaneous LBCL was defined as large cells constituting >80% of the infiltrate and absence of extracutaneous dissemination after staging investigations. Staging procedures were performed with standard methods available at time of first diagnosis including, when applicable, physical examination, blood cell count, chest radiograph, computed tomographic (CT) scan, abdominal...
ultrasound sonography, sonography of superficial lymph nodes, and bone marrow biopsy. Unfortunately, we do not have exact data on therapy for all patients. First-line treatment strategies, however, were similar, with radiotherapy generally preferred to chemotherapy, with the exception of cases presenting with disseminated skin involvement.

The histopathological features were reviewed by at least three independent investigators (KK, CM and LC). We only included cases characterized by diffuse infiltrates of large cells with morphologic features of large centrocytes, centroblasts and/or immunoblasts. Cases with a follicular growth pattern were excluded, as well as cases with presence of remnants of lymphoid follicles as detected by immunohistologic staining for follicular dendritic cells (CD21). Cases arising in immunosuppressed individuals were excluded as well.

All cases were classified according to the new WHO/EORTC classification of cutaneous lymphomas. FCLDT was defined as a tumor with predominance of large cleaved cells, irrespective of Bcl-2 expression. LBCLLT was defined as a tumor with predominance of large round cells and positivity for Bcl-2. LBCLO was defined as a tumor with predominance of large round cells and absence of Bcl-2 expression.

**Histology & Immunohistochemistry**

Biopsy specimens were fixed in 10% buffered formalin and subsequently embedded in paraffin. Sections were stained with hematoxylin-and-eosin and Giemsa stains for routine histopathologic evaluation. Detailed immunophenotypic analysis was performed on routinely-fixed, paraffin-embedded tissue sections according to a standard immunoperoxidase technique, using a broad panel of monoclonal antibodies including CD20, CD21, CD30, CD138, Bcl-2, Bcl-6, MIB-1, MUM-1, and ALK-1 (all from Dakopatts, Glostrup, Denmark), CD3 and CD10 (Novocastra, Newcastle upon Tyne, UK). The FOX-P1 antibody was kindly provided by Dr. Alison Banham, Nuffield Department of Clinical and Laboratory Sciences, Oxford, United Kingdom. Second and third antibodies were obtained from Dakopatts. Heat-induced antigen retrieval with microwave was used for all of the antibodies.

Staining for CD10, Bcl-2 and Bcl-6 were scored as positive when the majority of the neoplastic cells expressed the protein. Similarly to a previous study, staining for FOX-P1 was scored into 3 groups as follows: positive (nearly all tumor cells showed strong, uniform expression of the protein), +/- (weak expression in a variable proportion of tumor cells), and
negative (only occasional positive cells were observed). The staining for MUM-1 was 
evaluated in a manner similar to that of FOX-P1.

**Molecular biology**

Polymerase chain reaction (PCR) analyses of DNA of *Borrelia burgdorferi* and human herpes 
 virus-8 (HHV-8), and of immunoglobulin heavy chain (IgH) gene were performed as 
described previously.6-8

**Oligonucleotide primers for Borrelia burgdorferi PCR:** Oligonucleotide primers for PCR 
amplification reactions were designed by Wienecke et al.9 on the basis of the *Borrelia 
burgdorferi*-specific gene described by Rosa et al.10 The outer primer pair (Bb1 and Bb2) is 
flanking a 171-base pair (bp) fragment (nucleotide position/np 143 - np313), while the inner 
primer pair (Bb3 and Bb4) spans a 92-bp amplification product (np160 - np251). For internal 
hybridization control, oligo Bb-hyb, annealing between nucleotides 182 and 217, was used.

**PCR amplification:** For analysis of *Borrelia burgdorferi* DNA we used a nested PCR 
technique.7 The two-step nested PCR procedure substantially increases both sensitivity and 
specificity of the assay. An aliquot of the first PCR product, produced by the outer primer pair, 
is amplified by an internal set of primers annealing to *Borrelia burgdorferi* specific sequences. 
In this way only *Borrelia burgdorferi*-specific PCR products obtained in the first assay are 
further amplified, considerably increasing the specificity of the technique. Briefly, 3 µl of each 
DNA sample served as template for PCR. The reaction cocktail (25 µl) contained 10mM 
TrisHCl pH 8,3, 50mM KCl, 1,5mM MgCl2, 200 µM each of deoxyribonucleotide 
triphosphate (dATP, dCTP, dGTP, dTTP), 1 unit of Taq Gold DNA Polymerase (Perkin Elmer 
Corporation) and 0,2µM each of the outer primers Bb1 and Bb2. After initial denaturation at 
95°C for 12 min, samples were subjected to 40 cycles of PCR at 95°C for 1 min, 50°C for 30 
seconds and 72°C for 30 seconds. Subsequently, 2 µl of external PCR product were submitted 
to nested PCR for 40 cycles using inner primers Bb3 and Bb4 under the same temperature 
profile as above. Inner PCR products were analyzed by an ethidium bromide stained 2, 5% 
agarose gel and visualized by UV light.

For detection of HHV-8-DNA sequences, a nested PCR technique was used as follows: in the 
first step, a 233bp product was amplified using the KS330_{233} primers.11 In the second step an 
additional internal 160bp fragment was amplified using primers NS1 and NS2.12 The β-actin
gene was successfully amplified in all cases. Specificity of PCR products was confirmed by P32 Southern blot hybridization using a 25 base-pair internal oligonucleotide probe as described previously.11

The IgH gene was analyzed using a semi-nested polymerase chain reaction (PCR) technique as described by Wan et al.13 with minor modifications.7 For the first PCR one µl of template DNA was used in a 50 µl PCR reaction containing deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, dTTP) (200 µM each), primers Fr3A and LJH (0,1 µM each), MgCl2 (1,0 mM), KCl (50 mM), and TrisHCl pH 8.3 (10 mM). After initial denaturation at 94°C for 5 min, thirty cycles of the reaction were carried out (denaturation: 94°C, 60 seconds; annealing: 57°C, 60 seconds; extension: 72°C, 60 seconds) followed by a 7 min final extension step at 72°C. A second PCR was performed using 1 µl from the first PCR as template in 25 µl of the same buffer except that primer VLJH was used instead of LJH. Twenty-five cycles were carried out using the same temperature profile as in the first PCR.

For visualization a 10 µl aliquot of the PCR products was applied to a commercially available gel (GeneGel Excel 12.5cm/24 slots; Amersham Biosciences, Little Chalfont, UK) and run for 1 hour at 15°C. The running conditions were as follows: voltage 600V, current 25mA, power 15W. Finally, the gels were stained using a DNA silver staining kit (Amersham Biosciences). The beta-actin gene was analyzed as amplification control in all cases.

**In-situ-hybridization for Epstein-Barr virus (EBV)**

In-situ-hybridization for EBV was performed according to standard procedures using the EBER-1 in-situ hybridization kit by Kreatech Diagnostic (Amsterdam, The Netherlands).

**Statistical analyses**

Statistical testing with \( \chi^2 \) analysis was used to examine relationships between variables. The paired t-test was used to analyze age differences between the groups. Survival time was defined as the time from first diagnosis until death or last follow-up. For evaluation of disease-specific survival death unrelated to lymphoma was not considered an event and was censored. Survival curves were estimated by the Kaplan-Meier method, using log rank to analyze the differences between groups.
Results

Ninety-three patients were included in the study (M: F = 42:51; median age: 71; range: 33-98). Cases were classified into the following groups:

*Follicle center lymphoma, diffuse type (FCLDT):* 44 cases (M:F = 25:19; median age: 66.5; range: 33-89). Five cases were located solely on the leg, 15 on the back, 9 on the head-neck, 6 on the trunk, one on the upper extremities, one on the buttock, and 7 at multiple sites (head-neck, upper extremities and trunk: 4; leg and upper extremities: 1; leg, head-neck, upper extremities and trunk: 1; leg and buttocks: 1) (Figure 1). A single lesion was observed in 12 patients, whereas 32 had multiple lesions.

*Large B-cell lymphoma, leg-type (LBCLLT):* 40 cases (M:F = 15:25; median age: 79; range: 46-98). Thirty-two cases were located on the leg, 2 on the back, one on the head-neck, 3 on
the trunk, and 2 at multiple sites (trunk and upper extremities: 1, leg, trunk and upper extremities: 1) (Figure 2). Twenty patients presented with a single lesion, the other 20 had multiple lesions.

Large B-cell lymphoma, other (LBCLO): 9 cases (M:F = 2:7; median age: 70; range: 58-86). Five cases were located only on the leg, one on the head-neck, one on the trunk, one on the upper extremities, and one at multiple sites (leg and head-neck). Lesions were solitary in 7 patients and multiple in 2 of them.

All cases were positive for CD20 and negative for CD3. All tested cases were negative for ALK-1 and CD138 (LBCLLT 0/29 and 0/33; FCLDT 0/15 and 0/17; LBCLO 0/5 and 0/7, respectively). CD30 was focally positive only in two cases of LBCLLT (LBCLLT 2/33; FCLDT 0/33; LBCLO 0/7). Proliferation rate measured by MIB-1 antibody was high in the majority of cases irrespective of classification (LBCLLT: mean 71.3%, median 70%, range 5-90%; FCLDT: mean 55.3%, median 60%, range 5-90%; LBCLO: mean 62.7%, median 80%,
range 5-90%). Expression of CD10, Bcl-2, Bcl-6, MUM-1 and FOX-P1 in the different groups is summarized in Table 1 (Figures 3-5).

Figure 3

Figure 4
Figure 5
PCR analysis of IgH gene rearrangement performed in 33 cases revealed a monoclonal band in 23 cases (FCLDT: 9; LBCLLT: 14), and a polyclonal smear in 10 cases (FCLDT: 7; LBCLLT: 3). In-situ hybridization for EBV and PCR analysis of *Borrelia burgdorferi* and HHV-8 DNA showed only one case of LBCLLT positive for HHV-8 (EBV: LBCLLT 0/36; FCLDT 0/19; LBCLLO 0/7; *Borrelia burgdorferi*: LBCLLT 0/33, FCLDT 0/19, LBCLLO 0/4; HHV-8: LBCLLT 1/33; FCLDT 0/18; LBCLLO 0/4).

**Follow-up**

Follow-up data were available for 75 cases (FCLDT: n=37, median follow-up time: 47 months; LBCLLT: n=31, median follow-up time: 16 months; LBCLLO: n=7, median follow-up time: 20 months). Fifteen patients died of disease after a period variable between 6 and 93 months (median: 12 months) (FCLDT: n=4, 8-15 months, median: 12,5 months; LBCLLT: n=10, 6-93 months, median: 12,5 months; LBCLLO: n=1, 36 months). Five died of unrelated causes after 35-165 months (median: 48 months) (FCLDT: n=4, 47-165 months, median: 83 months; LBCLLT: n=1, 35 months). Thirty-four are alive with disease after 1-241 months (median: 21,5 months) (FCLDT: n=15, 2-241 months, median: 63 months; LBCLLT: n=14, 2-59 months, median: 15,5 months; LBCLLO: n=5, 1-39 months, median: 20 months). Twenty-one are alive and well after 3-166 months (median: 37 months) (FCLDT: n=14, 2-166 months, median: 48,5 months; LBCLLT: n=6, 10-73 months, median: 31 months; LBCLLO: n=1, 17 months).

The summary of prognostic features related to several parameters is shown in Tables 1 and 2.

**Statistical analyses**

Statistical analyses are summarized in Tables 1 and 2. The gender distribution between LBCLLT and FCLDT did not reach statistical significance ($\chi^2$ 3,135, p = 0,0766). The age distribution was significantly different among these two groups (t value 5,510, p < 0,0001), as well as the location on the leg or at other anatomical sites ($\chi^2$ 40,050, p < 0,0001). Expression of MUM-1 and FOX-P1 were clearly different among the two groups ($\chi^2$ 36,259 and 33,907, respectively; p < 0,0001). Expression of Bcl-6 was of borderline significance ($\chi^2$ 3,926; p = 0,0476), and of CD10 was not significantly different among the two groups ($\chi^2$ 2,310, p = 0,1286). Bcl-2 expression was highly different between the two groups ($\chi^2$ 51,9, p = 0,0001), due also to the definition of LBCLLT as a Bcl-2+ tumor.

Comparison of the LBCLLO group with the other two revealed differences between LBCLLO
and LBCLLT concerning the age (t value: 2.622, p = 0.0305), and no statistically significant
differences concerning gender distribution, anatomical site, FOX-P1, MUM-1, Bcl-6 and
CD10 expression. Although gender distribution, Bcl-6 and CD10 expression were not
statistically different between the groups of LBCLO and FCLDT, the groups differed
concerning age (t value 2.808, p = 0.0229), anatomical site (χ² 9.532, p = 0.002), FOX-P1
expression (χ² 6.470, p = 0.011), and MUM-1 expression (χ² 15.320, p < 0.0001).

Discussion

In this study we clearly demonstrated that the WHO/EORTC classification is applicable to the
great majority of patients with cutaneous LBCL. The difference in survival between the
groups of LBCLLT and FCLDT is stastically significant (p = 0.02), confirming the clinical
value of the stratification of patients with primary cutaneous LBCL into these two groups.
Gender distribution between the two groups was dissimilar (although did not reach
significance); age distribution, anatomical site, and FOX-P1, MUM-1 and Bcl-6 protein
expression were significantly different, supporting the concept that the two groups represent
indeed different entities. In terms of clinicopathologic features, phenotype and prognosis it
seems that LBCLO represents an intermediate group between cases of LBCLLT and those of
FCLDT (with more similarities to the first than to the second), and at present it is unclear
whether LBCLO represents truly a distinct group of cutaneous LBCLs, or if it should be
considered as either a phenotypic (Bcl−2−) variant of the LBCLLT, or as a morphologic (round
cell) variant of the FCLDT. In fact, it seems likely that the group of LBCLO consists of
phenotypic and/or morphologic variations of both LBCLLT and FCLDT.

Although the vast majority (90.3%) of the cases could be classified as either LBCLLT or
FCLDT, it should be mentioned that a few of the patients show overlapping clinicopathologic
features. In fact, three patients with FCLDT had lesions located solely on the leg and that were
Bcl-2+/MUM-1− (2 cases) or Bcl-2−/MUM-1+ (1 case), thus showing similarities to LBCLLT.
On the other hand, three patients with LBCLLT had lesions located at sites other than the legs
and that were MUM-1−, thus showing similarities to FCLDT. The existence of such
overlapping features underlines the difficulty of classifying accurately some of the cases.

In the past, prognosis of cutaneous LBCLs has been linked to anatomical site (location on the
leg vs. non-leg), age of onset, and number of lesions at presentation in one large study,14 and
to expression of Bcl-2 in another study. In both studies cases with cleaved and round cell morphology were lumped together. On the other hand, location on the leg and Bcl-2 expression had no prognostic value in another study that lumped together different groups of cutaneous LBCL. In our study the number of lesions had no prognostic value, neither overall, nor in the specific diagnostic groups. Analysis of all patients with primary cutaneous LBCL showed that prognosis was strongly linked to age of onset, anatomical site, cell morphology, Bcl-2 expression, MUM-1 expression, and FOX-P1 expression. Older age had a prognostic implication when all LBCLs were analyzed together, consistent with previous results by Grange et al. and Goodlad et al. When age was analyzed separately for the groups of LBCLLT and FCLDT, however, it had no statistically significant prognostic value for the LBCLLTs, although it retained a significant value for FCLDTs. This discrepancy is most likely related to the different median age of the two groups, as 82.5% of LBCLLT patients was ≥71 at first diagnosis.

Lesions of LBCLLT were indeed located solely on the legs in the great majority of cases (80%), thus justifying the term large B-cell lymphoma of the leg proposed by Vermeer et al in 1996 and adopted in the EORTC classification of cutaneous lymphomas in 1997, and of LBCLLT introduced in the new WHO/EORTC classification. The term LBCLLT is better than the old denomination of "large B-cell lymphoma of the leg", as it reflects more accurately the predominant but not exclusive anatomic location of these tumors. However, tumors confined to the legs only were found also in 55.6% and 11.4% of LBCLOs and FCLDTs, respectively, confirming that the anatomical site alone is not sufficient for classification of the cases. In addition, in 5 further patients lesions were located both on the legs and at other anatomical sites (FCLDT: 6.8%; LBCLLT: 2.5%; LBCLO: 11.1%). Interestingly, location on the legs only had a strong prognostic value when all LBCLs were considered together, and had statistical significance for the group of FCLDT as well, suggesting that for cases of FCLDT located on the legs the anatomical site may be more important then morphologic-phenotypic classification when assessing prognosis.

Similarly to the study by Grange et al., Bcl-2 expression had a bad prognostic implication when cases were not stratified into precise diagnostic categories. However, it did not retain a statistically significant value when the analysis was performed separately for the two groups with cleaved (FCLDT) and round cell morphology (including LBCLLTs and LBCLOs). Thus, it does not seem that Bcl-2 expression has any prognostic value in cases that are appropriately
classified. Interestingly, although in a previous report Bcl-2 expression was strictly related to the anatomical site (cases on the legs were always positive, whereas those on the head-neck and/or trunk always negative), we did not find such a strict association, as 19% of cases located only on the legs were Bcl-2−, and 28.9% of those located only on the head-neck or trunk were Bcl-2+. Thus, it does not seem that expression of this protein is strictly related to any anatomical site.

MUM-1/IRF4 is a transcription factor that is considered to play a crucial role in lymphoid differentiation and development. In normal lymphoid tissue, it is mainly expressed by plasma cells and a subset of germinal center B lymphocytes. MUM-1 expression was found in all cases of cutaneous LBCL of the leg classified according to the first EORTC classification in one study, and in the new WHO/EORTC classification of cutaneous lymphomas it is suggested that MUM-1 expression is found constantly in LBCLLT and is absent in FCLDT. In our study, specificity and sensitivity of MUM-1 expression for diagnosis of LBCLLT were 85.7% and 75.9%, respectively. Strong MUM-1 positivity was found in 7% of cases of FCLDT, showing that expression of this marker is not constantly absent in this diagnostic group. In addition, 7 cases of LBCLLT (24.1%) showed a lower degree of positivity for MUM-1, similar to that found in 13 cases of FCLDT (30.2%). Thus, although strong positivity for MUM-1 is mostly consistent with a diagnosis of LBCLLT or LBCLO, a weaker expression can be observed in all diagnostic groups. Strong MUM-1 expression was linked to a worse prognosis when all cases of LBCL were analyzed together, most likely due to the predominant expression of this marker in cases with round cell morphology. Interestingly, a worse prognosis (though not statistically significant) was found also for MUM-1+ cases of FCLDT. In FCLDT, strong MUM-1 expression, like location on the leg, may point at a more aggressive behaviour of the disease. The prognostic implications of these two parameters in patients with FCLDT underline once again the overlapping features of some cases of cutaneous LBCL.

FOX-P1 (forkhead box protein P1) is a member of the FOX-P subfamily of transcription factors, and it has been demonstrated predominantly in the non-germinal center type of nodal diffuse LBCL. Previous studied in nodal lymphomas showed that FOX-P1 is expressed predominantly in cases with non-germinal center phenotype. Although expression of FOX-P1 had no prognostic value in one study, two other reports found an association with a worse outcome. We found a strong FOX-P1 expression in 22 of our cases (FCLDT: 10%; LBCLLT: 72.4%; LBCLO: 50%). When all primary cutaneous LBCLs were analyzed together,
strong expression of this protein was clearly linked to a worse prognosis. However, when data were analyzed separately for each diagnostic group expression of the protein failed to reach a statistically significant prognostic value. Thus, it seems likely that in cutaneous LBCLs the prognostic value of FOX-P1 is mainly linked to the predominant expression of this protein in cases with round cell morphology.

Bcl-6 and CD10 are markers of germinal center B-cells,24,25 and have been used for characterization of LBCLs of germinal center origin in the appropriate context.26-29 We could demonstrate Bcl-6 positivity in the vast majority of cases, irrespective of anatomical site, cell morphology, and classification (FCLDT: 93.2%; LBCLLT: 75%; LBCLO: 100%). By contrast, CD10 was expressed only in a minority of cases, and slightly more frequently in FCLDTs (FCLDT: 17.9%; LBCLLT: 6.1%; LBCLO: 14.3%). A similar phenotype was observed in a recent study by Hoefnagel et al.30 Thirteen of our cases were negative for both markers (FCLDT: 6.8%; LBCLLT: 25%). Interestingly, 2 out of 3 cases of FCLDT that were CD10−/Bcl-6− were positive for MUM-1, suggesting an activated rather than germinal center B-cell phenotype, and confirming again that a few cases of FCLDT reveal overlapping phenotypic features with LBCLLT. A recent study on 14 patients with primary cutaneous LBCL suggested a favourable prognostic significance of bcl-6 expression.31 In our study expression of Bcl-6 had no impact on prognosis overall and in the two groups of LBCLLT and FCLDT (data not shown), and the Bcl-6/CD10 double negative phenotype had no prognostic implications as well, but especially for cases of FCLDT this may be due also to the small number of such cases.

The etiology of cutaneous LBCLs is unknown. We analyzed our cases for presence of infectious agents including HHV-8, EBV and Borrelia burgdorferi, but could find only one case of LBCLLT positive for HHV-8. HHV-8 is well known as the causative agent of Kaposi’s sarcoma and it is detected in 95-100% of cases in this condition.32 Recently, HHV-8 has also been identified in cases of primary effusion lymphoma, which is a subtype of diffuse LBCL.33,34 Borrelia burgdorferi was demonstrated in 15-20% of cases of primary cutaneous B-cell lymphoma in three independent studies in the past,6,35,36 and EBV is a virus with a well known association with some human lymphomas. However, our results suggest that these 3 microorganisms are not implicated in the pathogenesis of primary cutaneous LBCLs, irrespective of classification.

At extracutaneous sites, some authors showed the presence of a subgroup of diffuse LBCL
positive for ALK-1 and negative for CD30. In our study we could find positivity for CD30 in 2/72 cases only (both of them were cases of LBCLLT showing focal positivity), and negativity for ALK-1 in all tested cases (49/49). Thus, these two antibodies have no relevance in diagnosis and classification of primary cutaneous LBCLs.

Recently, microarray studies suggested that nodal diffuse large B-cell lymphomas can be subclassified according to the molecular signature of neoplastic cells. Studies of cases of cutaneous B-cell lymphoma showed that subdivision of primary cutaneous LBCLs into the groups of FCLDT and LBCLLT was supported by the molecular profile of neoplastic cells. Our findings clearly show that a clinically relevant classification of primary cutaneous LBCLs into two main groups (FCLDT, LBCLLT) can be achieved in most cases with morphologic and phenotypic analyses. Although prognosis is mainly related to specific classification, cases of FCLDT arising on the legs and/or showing an activated B-cell phenotype (strong MUM-1 expression and/or double negativity for CD10 and Bcl-6) should be approached carefully, as they may have a worse prognosis than other cases of this group. The definition of a third category of cutaneous LBCL characterized by predominance of round cells and absence of Bcl-2 expression, currently included into the group of LBCLO in the WHO/EORTC classification, requires further studies to clarify whether these cases show indeed peculiar clinicopathologic features or, more likely, represent only a morphologic and/or phenotypic variant of FCLDTs and LBCLLTs.

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<td><strong>Site [leg/total ( %)]</strong></td>
<td>32/40 (80%)</td>
<td>5/9 (55,6%)</td>
<td>5/44 (11,4%)</td>
</tr>
<tr>
<td>Disease-specific survival according to anatomical site (leg vs. non-leg)</td>
<td>p = 0,6</td>
<td>(*)</td>
<td>p = 0,02</td>
</tr>
<tr>
<td><strong>Bcl-2 [+/total (%)]</strong></td>
<td>40/40 (100%)</td>
<td>0/9 (0%)</td>
<td>10/44 (22,7%)</td>
</tr>
<tr>
<td>Disease-specific survival according to Bcl-2 expression</td>
<td>-</td>
<td>-</td>
<td>p = 1</td>
</tr>
<tr>
<td><strong>FOX-P1 [+/total (%)]</strong></td>
<td>23/29 (72,4%)</td>
<td>3/6 (50%)</td>
<td>4/40 (10%)</td>
</tr>
<tr>
<td>Disease-specific survival according to FOX-P1 expression</td>
<td>p = 0,5</td>
<td>(*)</td>
<td>p = 0,6</td>
</tr>
<tr>
<td><strong>MUM-1 [+/total (%)]</strong></td>
<td>22/29 (75,9%)</td>
<td>4/6 (66,7%)</td>
<td>3/43 (7%)</td>
</tr>
<tr>
<td>Disease-specific survival according to MUM-1 expression</td>
<td>p = 0,9</td>
<td>(*)</td>
<td>p = 0,07</td>
</tr>
</tbody>
</table>
Bcl-6 [+]/total (%) | 30/40 (75%) | 7/7 (100%) | 41/44 (93.2%)
---|---|---|---
CD10 [+]/total (%) | 2/33 (6.1%) | 1/7 (14.3%) | 7/39 (17.9%)
---|---|---|---
Bcl-6−/CD10− phenotype [−]/total (%) | 10/40 (25%) | 0/7 (0%) | 3/44 (6.8%)
---|---|---|---
Disease-specific survival according to CD10/Bcl-6 expression | p = 0.5 | (*) | p = 0.2
---|---|---|---

(#) LBCLLT vs. FCLDT: p = 0.02; LBCLLT vs. LBCLO: p = 0.6; FCLDT vs. LBCLO: p = 0.7

($) LBCLLT vs. FCLDT: p = 0.01; LBCLLT vs. LBCLO: p = 0.5; FCLDT vs. LBCLO: p = 0.7

(*) size of the group too small for statistical analysis
Table 2. Summary of prognostic features in cutaneous large B-cell lymphomas.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5-year disease-specific survival</th>
<th>10-year disease-specific survival</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round cells predominate (LBCLLT+LBCLO)</td>
<td>58.2%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Cleaved cells predominate (FCLDT)</td>
<td>86.7%</td>
<td>86.7%</td>
<td>0.02</td>
</tr>
<tr>
<td>Age &gt; 70</td>
<td>61.4%</td>
<td>46.0%</td>
<td></td>
</tr>
<tr>
<td>Age &lt;= 70</td>
<td>88.7%</td>
<td>88.7%</td>
<td>0.007</td>
</tr>
<tr>
<td>Site: leg</td>
<td>52.6%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Site: non-leg or multiple</td>
<td>84.9%</td>
<td>84.9%</td>
<td>0.001</td>
</tr>
<tr>
<td># of lesions = 1</td>
<td>72.3%</td>
<td>72.3%</td>
<td></td>
</tr>
<tr>
<td># of lesions ≥ 2</td>
<td>75.8%</td>
<td>68.2%</td>
<td>1</td>
</tr>
<tr>
<td>Bcl-2⁺</td>
<td>66.6%</td>
<td>44.4%</td>
<td></td>
</tr>
<tr>
<td>Bcl-2⁻</td>
<td>84.6%</td>
<td>84.6%</td>
<td>0.05</td>
</tr>
<tr>
<td>FOX-P1⁺</td>
<td>57.9%</td>
<td>43.4%</td>
<td></td>
</tr>
<tr>
<td>FOX-P1⁻</td>
<td>85.9%</td>
<td>85.9%</td>
<td>0.03</td>
</tr>
<tr>
<td>MUM-1⁺</td>
<td>52.4%</td>
<td>52.4%</td>
<td></td>
</tr>
<tr>
<td>MUM-1⁻</td>
<td>84.8%</td>
<td>77.8%</td>
<td>0.05</td>
</tr>
<tr>
<td>CD10 and/or Bcl-6 pos.</td>
<td>77.9%</td>
<td>70.8%</td>
<td></td>
</tr>
<tr>
<td>CD10 and Bcl-6 both neg.</td>
<td>57.8%</td>
<td>57.8%</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1.** Various clinical features of FCLDT: *a*) multiple red-brown plaques and large tumors on the lower back and buttock; *b*) two distinct reddish tumors on the upper arm; *c*) confluent erythematous plaques and flat tumors involving large part of the trunk ("Crosti's lymphoma"); *d*) confluent reddish tumors on the lower leg.

**Figure 2.** Different morphologic expressions of LBCLLT: *a*) small solitary tumor on the lower leg; *b*) confluent large tumors on knie; *c*) distinct large tumors on the lower leg on the background of chronic venous insufficiency; *d*) large tumor involving almost the entire frontal aspect of the lower leg; *e*) large ulcerated lesion involving almost the entire lower leg; *f*) erythematous tumor and small plaque on the chest.

**Figure 3.** Histopathologic features and phenotypic variations of FCLDT: *a*) large cleaved cells predominate; *b*) variable, weak expression of FOX-P1; *c*) strong, uniform expression of FOX-P1; *d*) negativity for Bcl-2; please note internal positive controls; *e*) strong positivity for Bcl-2; *f*) positive staining for Bcl-6; *g*) staining for MUM-1 showing only scattered positive cells; *h*) strong positivity for MUM-1 in the great majority of neoplastic cells; *i*) expression of CD10 by the large lymphocytes.

**Figure 4.** Histopathologic features and phenotypic variations of LBCLLT: *a*) large round cells predominate; *b*) strong positivity for Bcl-2; *c*) positive staining for Bcl-6; *d*) strong, uniform expression of FOX-P1; *e*) strong, uniform positivity for MUM-1; *f*) staining for MUM-1 showing only a weak expression of the protein.

**Figure 5.** Histopathologic features and phenotypic variations of LBCLO: *a*) large round cells predominate; *b*) only a proportion of the large cells are positive for MUM-1; *c*) variable expression of Bcl-6 in the majority of neoplastic cells; *d*) negative staining for Bcl-2; please note positive internal controls (small lymphocytes);
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