Diagnostic and Prognostic Value of BCL2 Rearrangement in 53 Patients With Follicular Lymphoma Presenting as Primary Skin Lesions

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Key Words: Primary cutaneous follicle center lymphoma; Follicular lymphoma; Translocation t(14;18); BCL2 rearrangement; FISH

ABSTRACT

Objectives: To study the diagnostic value of BCL2 rearrangement in follicle center lymphoma (FCL) presenting as primary skin lesions, evaluate its prevalence and the prognostic value in primary cutaneous FCL (PCFCL), and assess prognostic factors in PCFCL.

Methods: Fifty-three patients with a cutaneous presentation of FCL without a history of nodal lymphoma were selected retrospectively. Clinical and histologic data were collected together with staging and follow-up data. A fluorescence in situ hybridization (FISH) test for BCL2 split probes was performed on skin biopsy specimens.

Results: Initial staging procedures identified 47 PCFCLs and six cases of secondary skin involvement of FCL (SSIFCL). FISH detected seven cases carrying a BCL2 rearrangement: four (8.5%) of 47 PCFCLs and three (50%) of six SSIFCLs. These seven cases coexpressed BCL2 and CD10. In PCFCL, cutaneous relapse rate was 42.6%. A small/medium centrocytic cell population was associated with a higher probability of skin relapse in univariate (P = .008) and multivariate (P = .028) analysis, and BCL2 rearrangement detection was associated with secondary extracutaneous spreading (P = .05).

Conclusions: We observed that BCL2 rearrangement in PCFCL is rare, associated with initial positivity of staging (diagnostic value) or with secondary extracutaneous spreading (prognostic value). In selected cases with BCL2-CD10 coexpression, FISH testing could detect patients with poor outcome and require closer monitoring.

Primary cutaneous lymphomas (PCLs) of B-cell origin account for 25% of all PCLs. Three main types of primary cutaneous B-cell lymphomas are recognized: primary cutaneous follicle center lymphoma (PCFCL), primary cutaneous marginal zone lymphoma (PCMZL), and primary cutaneous diffuse large B-cell lymphoma (PCLBCL), leg type.1,2 PCFCL, representing approximately 55% of all primary cutaneous B-cell lymphomas, is the most common B-cell lymphoma to occur as a primary tumor of skin.3 PCFCL usually involves the head and neck or trunk, with solitary or grouped plaques and tumors, while multifocal skin lesions are possible. Morphologically, PCFCL exhibits a proliferation of neoplastic follicle center cells, usually a mixture of small/medium and large centrocytes (sometimes spindle-shaped type) and a variable proportion of centroblasts. Architectural pattern is variable along a continuum from follicular, nodular, sometimes focally periadnexal to diffuse growth patterns. PCFCL, even with a predominance of large cells (large centrocytes or centroblasts), has an indolent behavior characterized by a 5-year survival rate of 95% as opposed to the 41% rate of PCLBCL, leg type.1,4,5 Multifocal lesions do not have a poorer outcome than solitary lesions.3 PCFCL on the leg displays a poorer prognosis, with 5-year disease-specific survival reported at 41%.4 Skin relapse is observed in 30% to 45% of patients. Recurrence does not affect prognosis and is usually confined to the skin. Secondary extracutaneous dissemination may occur in 5% to 10% of cases.4,5 Furthermore, secondary skin involvement by nodal follicular lymphoma (SSIFCL) may share clinicopathologic similarities with PCFCL, especially if skin lesions are the initial manifestation of a systemic disease. Therefore, a negative staging is required to assert PCFCL.
FISH, fluorescence in situ hybridization; PCFCL, primary cutaneous follicle center lymphoma; PCR, polymerase chain reaction.

Table 1
BCL2 Rearrangement (or t(14;18)) Detection by PCR and/or FISH in PCFCL

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>No. of Patients</th>
<th>t(14;18) Detection by PCR, No./Total No. (%)</th>
<th>t(14;18) Detection by FISH, No./Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geelen et al (1998)</td>
<td>8</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>Child et al (2001)</td>
<td>5</td>
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<tr>
<td>Vergier et al (2004)</td>
<td>30</td>
<td>9/30 (30)</td>
<td>0/17</td>
</tr>
<tr>
<td>Streubel et al (2006)</td>
<td>27</td>
<td>0/27</td>
<td>11/27 (41)</td>
</tr>
</tbody>
</table>

and to rule out SSIFCL since both diseases require a different treatment.5

The hallmark of nodal follicular lymphoma is the t(14;18)(q32;q21) translocation, which consists of a reciprocal translocation between the BCL2 proto-oncogene and the immunoglobulin heavy chain (IgH) gene, leading to the overexpression of BCL2 protein. Therefore, the t(14;18) should be detected either by polymerase chain reaction (PCR) for BCL2-IgH breakpoint amplification or by fluorescence in situ hybridization (FISH) for either BCL2-IgH fusion or BCL2 separation or split. In nodal follicular lymphoma, interphase FISH has detected a higher rate of positive cases for BCL2 rearrangement than the BIOMED-2 PCR protocol.7 While the BCL2 rearrangement is observed in 80% to 90% of cases of follicular lymphoma, this genetic alteration was not detected in several series of PCFCL, including the one from our group using both FISH and PCR with BIOMED-2 techniques.1,7,9 However, Streubel et al10 found a BCL2-IgH fusion by FISH in 41% of PCFCL cases, although they were not able to amplify the BCL2-IgH breakpoint by the BIOMED2 PCR protocol in FISH-positive cases. Alternatively, PCR amplification of the BCL2-IgH breakpoint provided positive results in some series of PCFCL cases, but we clearly demonstrated that molecular detection alone is able to pick up B cells carrying BCL2-JH rearrangement as described in healthy carriers.7-14 Besides the technical aspects, most of the above studies did not provide extensive staging and follow-up data that may account for such discordance in patients with different inclusion criteria.

This prompted us to analyze by FISH the prevalence of the BCL2 rearrangement in follicle center lymphoma (FCL) with skin presentation before staging procedures. The main goal of this study was to estimate the prevalence of BCL2 rearrangement in the largest series of PCFCL cases studied so far and to determine if the detection of a BCL2 rearrangement could represent a biomarker that would help patient management at diagnosis. Thanks to an extended follow-up, we also investigated prognostic factors that would predict relapse or extracutaneous spreading in PCFCL.

Materials and Methods

Patient Selection

Cases were retrieved retrospectively from the Aquitaine database of cutaneous lymphoma and from dermatologic departments of the University Hospital of Bordeaux from 1994 to 2009 (regional referral center for cutaneous lymphomas). The study was performed after patient information and consent, according to the guidelines of the French bioethical law and the Declaration of Helsinki principles.

Patients were consecutively included by the following criteria:

1. Cutaneous lesions of FCL at presentation in patients without a history of B-cell lymphoma. A systematic review of clinical, staging, and histopathologic features was made by an expert panel of dermatologists and pathologists, using the criteria of the World Health Organization classification.

2. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks or frozen material available for molecular studies.

Histologic and Clinical Data Collected

For all cases, H&E-safranin–stained sections and routine immunostaining were reviewed retrospectively. BCL2, BCL6, and CD10 immunostainings were considered positive if 50% or more of tumor cells expressed the protein. Comparison with CD3 staining on consecutive sections allowed us to discard the strong expression of BCL2 by reactive T cells in such PCFCL cases considered BCL2 positive since the staining was moderate and restricted to B cells. In cases predominantly composed of small/medium centrocytic cells, architectural, cytologic, and immunohistochemical features excluded PCMZL. For three cases, small biopsy specimens limited the interpretation of architectural features, but no specific feature of PCMZL was observed (typical colonization of follicular dendritic cell meshwork, immunophenotype, immunoglobulin light chain–restricted plasma cell) on the primary lesion or relapse (n = 2). In cases predominantly composed of a monotonous population of large centrocytes or centroblasts, a “nonactivated B-cell” (germinal center) phenotype permitted us to rule out PCLBCL, leg type.

Staging procedures were performed according to international recommendations,5,7 including detailed history and complete physical examination, routine laboratory tests, computed tomography of the thorax and abdomen, and bone marrow biopsy for most patients (n = 37 [70%], including
all patients with t(14;18)-positive detection). This allowed separating the patients into two groups: PCFCL (n = 47) and SSIFCL with concomitant skin involvement (n = 6). Patients with PCFCL were classified according to the adapted TNM classification. Sex, age at diagnosis, site and size of the skin lesion, initial therapy, cutaneous relapses, secondary extracutaneous spreading, status at last follow-up, and duration of follow-up were recorded. Follow-up was updated in March 2013.

PCR Amplification for B-Cell Clonality Analysis

DNA was extracted from frozen or FFPE skin biopsy specimens (n = 45) and from peripheral blood lymphocytes (n = 44). FR3-JH primers (Ca1/Ca2) were used to amplify the hypervariable third complementarity-determining region (CDR3) of the IgH gene.

FISH Analysis of the BCL2 Locus

The translocation t(14;18)(q32;q21) at both the major breakpoint region or the minor cluster region was detected by interphase FISH with separation probes (BCL2 FISH DNA probe, Split Signal; DAKO, Trappes, France). The 4-µm tissue sections were obtained from FFPE skin tumoral biopsy specimens (n = 50), and FISH analysis was conducted with the histology FISH accessory kit (DAKO). In three cases, frozen skin touch imprints were hybridized as described before. FISH patterns were determined by analyzing 200 nonoverlapped nuclei as reported. Cases with a BCL2 breakpoint were then referred to as t(14;18)-positive FCL, because translocation partners other than IgH are exceedingly rarely affected in FCL.

PCR Amplification of BCL2-JH Rearrangement

The IGH-BCL2 fusion was sought by PCR amplification targeting the JH joining region of the IgH gene and distinct regions of the BCL2 gene. DNA was extracted from peripheral blood lymphocytes and frozen skin biopsy specimens of positive FISH cases. According to the BIOMED-2 protocol, three master mixes were used to identify BCL2 breakpoints MBR (mix A), 3′ MBR (mix B), mcr, and 5′ MCR (mix C).

Response Criteria and Statistical Analysis

Patients with PCFCL were included for statistical analysis of prognosis factors, while patients with a positive extracutaneous staging were excluded. The primary end point was disease-free survival (DFS). In Kaplan-Meier analysis and Cox proportional hazards model of DFS, time was defined as the time elapsed from date of diagnosis to the date of first disease recurrence or death. Time was censored for patients who had not experienced disease recurrence or had not died at the time of last follow-up. Potential prognosis factors examined in our study were patient age (dichotomized in two classes: >60 years vs <60 years) and sex at diagnosis, extent of cutaneous involvement according to the TNM classification (T2/T3 vs T1), tumor size (>5 cm vs <5 cm), type of predominant cell population (large centrocytes and/or centroblasts vs small to medium centrocytes), site of cutaneous lesions, initial therapy, presence of an IgH monoclonal rearrangement, presence of an identical IgH rearrangement in skin and blood, BCL2 expression in immunohistochemistry, BCL2 and CD10 coexpression, and presence of a BCL2 rearrangement. For univariate analysis, survival functions were compared with the nonparametric log-rank test for each prognosis factor. Factors with P values less than .2 in the univariate analysis were entered in a multivariate Cox model to estimate the hazards ratio and sequentially removed from the model if they were not significantly associated with DFS at the .05 level.

To analyze recurrence and estimate survival functions, we secondarily performed univariate and multivariate analysis by using a shared γ-frailty model. In this model, a random effect is added for each subject to adjust to the correlation of recurrence within subjects. In this analysis, time variable ("gap time") was the time since the latest event (diagnosis or recurrence). Time was censored for patients who had no disease recurrence or had not died at the time of last follow-up. Analysis was performed with SAS (version 9.3; SAS Institute, Cary, NC) and R (version 2.12.3; R Project for Statistical Computing, Vienna, Austria) by using the survival and frailty pack packages from the frailty model.

Results

According to inclusion criteria (FCL first presenting in the skin), 60 patients were recorded. Fifty-three gave informative molecular results and constituted the study group, classified as PCFCL (n = 47) or SSIFCL (n = 6) after staging procedures. Their clinical and follow-up data are summarized in Table 2. Nineteen of these 53 patients had been included in a 30-case study from our group.

Clinicopathologic Data at Diagnosis

Image 1 illustrates clinical and histologic features of the PCFCL group. Table 2 summarizes the clinicopathologic data of all patients, including 47 with a final diagnosis of PCFCL and six with SSIFCL. Among the 47 PCFCL cases, there were 20 women and 27 men, with an age range at diagnosis between 27 and 93 years (mean, 57 years; median, 56 years). Skin lesions consisted of cutaneous papules, nodules, tumors, or deeply infiltrated plaques. Most patients (n = 21 [44.7%]) had a solitary lesion and were classified as T1, followed by T2
(multiple skin lesions in a regional skin involvement) \( n = 20 \) (42.5%). Only six patients had cutaneous lesions corresponding to the multifocal or generalized T3 stage (12.8%). The most frequently involved body region was head and neck \( (n = 24 \) [51.1%]), followed by chest or upper back \( (n = 17 \) [36.2%]) and arm(s) \( (n = 2 \) [4.2%]). None of the patients had leg involvement in this study (neither PCFCL nor SSIFCL). In the PCFCL group, radiotherapy was the most common treatment; 17 (36.2%) patients had radiation, and seven (14.9%) patients had surgery followed by radiation. Eight (17%) patients were treated by surgery, eight (17%) received polychemotherapy (with or without rituximab), six (12.8%) had rituximab alone, and one patient received interferon. All patients with SSIFCL had multiple skin lesions with deeply infiltrated plaques or nodules Image 2A. Among them, only one patient had a bone marrow infiltration as the only manifestation of extracutaneous disease. Five of six had node involvement, with bone marrow infiltration in two cases and a negative bone marrow biopsy specimen in three cases.

Among the 47 cases of PCFCL, histopathologic analysis showed that 33 (70.2%) cases had a predominance of small/medium centrocytes (Images 1E, 1G, and 1I) and 14 (29.8%) a predominance of large centrocytes and/or centroblasts, with either a predominance of large centrocytes (including spindle-shaped cells) \( (n = 4 \) or large centrocytes mixed with a variable proportion of centroblasts \( (n = 10 \) (Images 1F, 1H, 1J, and 1K). All cases had follicular (with or without periadnexal distribution) or follicular and/or diffuse growth patterns. Among cases with a predominance of small/medium centrocytes, four cases displayed a very dense follicular growth pattern with ill-defined follicles without periadnexal distribution (type A pattern, as shown in Image 2B), resembling a node in the skin. Other cases were characterized by a less abundant infiltrate with a follicular growth pattern and periadnexal distribution (type B pattern; Image 1E). Among 14 cases with large-cell predominance, four harbored a diffuse growth pattern, and 10 exhibited a nodular to diffuse growth pattern. Expression of BCL2 by more than 50% of neoplastic cells was observed in 25 (53%) cases. Coexpression of BCL2 and CD10 was found in 13 (35.1%) of 37 PCFCL cases. A monoclonal IgH gene rearrangement was detected by PCR on skin biopsy specimens in 30 (67%) of 45 PCFCL cases tested using FR3-JH primers.16 Four (9%) of the 44 PCFCL cases tested had an identical IgH rearrangement in skin and blood. Among the six cases of SSIFCL, one exhibited a large-cell morphology, and five had a predominance of small/medium centrocytes. Architectural patterns were type A in three cases and type B in three cases. All SSIFCLs coexpressed BCL2 and CD10 Image 2C, Image 2D, and Image 2E, and two of six had an identical IgH rearrangement in skin and blood.

**FISH Analysis**

A BCL2 breakpoint was identified in more than 10% of cells (20%-80% of nucleus analyzed) by FISH in seven (13.2%) of the 53 cases. They corresponded to four (8.5%)
Clinical features of patients with a positive FISH test are summarized in Table 3. The presence of a BCL2 breakpoint was significantly associated with extracutaneous involvement at the time of diagnosis (Fisher exact test, \( P = .02 \)) with a specificity at 91% and a negative predictive value at 93%. Interestingly, the single patient diagnosed with SSIFCL on the sole basis of bone marrow infiltration, with otherwise negative staging, displayed a BCL2 rearranged lesion.

PCR Amplification of BCL2-JH Rearrangement

Of the four FISH-positive PCFCL cases, BIOMED-2 PCR amplification was positive in all cases on skin biopsy specimens with mix A (three cases) or mix C (one case) primers, thus showing an MBR-JH breakpoint. We did not detect any corresponding BCL2-JH rearrangement in the blood of these four patients with PCFCL tested by using such a protocol with a detection threshold of 3%. Two of three patients with SSIFCL with FISH positivity had positive PCR amplification with mix A in skin material, and one had a similar band in a concomitant peripheral blood sample.

Relapse Rate and Extracutaneous Spreading in 47 Patients With PCFCL

Among 47 patients with PCFCL, 46 (97.9%) achieved complete remission after initial therapy. Among them, disease relapse occurred in 20 (42.6%) after a median of 35 months (range, 1-170 months). Eight patients experienced two or more cutaneous relapses with a median of four relapses. The time between two events tended to decrease with the number of relapses.

Extracutaneous evolution (nodal involvement) was observed during follow-up in five (10.6%) of 47 patients with PCFCL after a median of 80 months (Table 3; cases 2, 3, 5, 6, and 7). Their initial staging was exhaustive, including a negative bone marrow examination. Among these five patients, three harbored a BCL2-CD10-positive phenotype, and two exhibited a BCL2 rearrangement. Extracutaneous spreading was associated with synchronous cutaneous relapses in these patients.

At the final point, two patients died (one patient, exhibiting a BCL2 rearrangement, died of disease after extracutaneous spreading, and one patient died of another cause). Four were alive with disease, and 43 (91.5%) were alive in complete remission. The median follow-up was 39 months (range, 1-191 months). The 12-, 36-, and 60-months DFS rates were 93%, 66%, and 46%, respectively.

Identification of Factors Predictive of Relapse in 47 Patients With PCFCL

Univariate analysis (using a nonparametric log-rank test, considering the first cutaneous relapse) showed that a predominantly small/medium centrocytic cell population (\( P = .008 \)) was significantly associated with an increased risk of cutaneous relapse Figure 1B. Although not significant, CD10 expression (\( P = .06 \)) and CD10/BCL2 coexpression (\( P = .08 \)) seemed to be potentially related to cutaneous...
Image II (cont) E, G, I. Lymphoid proliferation with nodular or follicular pattern and perianexal distribution (type B pattern); nodules are composed of a mixture of small/medium and a few large follicle center cells (centrocytes and centroblasts) (E, ×10; G, ×100; I, ×400). F, H. Lymphoid proliferation with follicular and diffuse growth pattern, with a predominance of large cells with a mixture of large centroblasts and centrocytes (F, ×20; H, ×100). J. Example of a case harboring follicle center cells with spindle-shaped large centrocytes (×400). K. Example of case exhibiting large centroblast predominance (×400).
relapse. The other factors studied (age older than 60 years, sex, T classification, site involved, first-line therapy, BCL2 expression and rearrangement Figure 1A, presence of a monoclonal IgH rearrangement, and presence of an identical IgH monoclonal rearrangement in skin and blood) did not show any association with the occurrence of cutaneous relapse. In the multivariate analysis, cell morphology (small/medium centrocytes vs large centrocytes and/or centroblasts) was associated with disease recurrence ($P = .028$). Indeed, patients with small/medium centrocytes/cell infiltrate had a higher risk of cutaneous relapse (odds ratio [OR], 8.74; 95% confidence interval [CI], 2.06-37.09) compared with patients with lesions formed by a predominance of large centrocytes and/or centroblasts. When taking into account all cutaneous relapses for each patient (using a shared $\gamma$-frailty model), univariate analysis also showed that a predominance of small to medium centrocytes ($P = .004$) was significantly associated with an increased risk of cutaneous relapse Figure 1D. Although not statistically significant, patients with CD10/BCL2 coexpression showed a higher tendency to have cutaneous relapses ($P = .19$), and BCL2 rearrangement was not associated with cutaneous relapse Figure 1C. In the multivariate analysis using this model, small/medium cell morphology was also associated with cutaneous recurrence ($P = .006$) after adjustment of other criteria. Patients with predominantly small/medium centrocytic cell tumors also had a higher risk of disease recurrence (OR, 7.52; 95% CI, 1.76-32.21) compared with patients with a predominance of large centrocytes and/or centroblast tumors. Secondary extracutaneous spreading was associated with the presence of a $BCL2$ rearrangement (Fisher exact test, $P = .05$) but not with $BCL2$-CD10 coexpression ($P = .13$). Of four patients with PCFCL harboring a $BCL2$ rearrangement, two experienced secondary extracutaneous spreading, and among them, one had transformation of lymphoma and died of disease after 77 months of follow-up. This patient was the only patient with PCFCL in this study who died of lymphoma.
Correlation of Histologic Features With Extracutaneous Disease and BCL2 Rearrangement

To determine if all PCFCL or SSIFCL cases should be tested for BCL2 rearrangement by FISH at diagnosis, we reviewed their histologic features. Among the seven patients with a BCL2 rearrangement, only one harbored large-cell proliferation (predominance of centroblasts). In other cases harboring a predominance of small/medium centrocytes, two types of patterns were observed. Among the seven t(14;18)-positive cutaneous FCLs (four PCFCLs and three SSIFCLs), four (three PCFCLs and one SSIFCL) displayed a type A pattern (Image 2B) and three (one PCFCL and two SSIFCLs) a type B pattern (Image 1E). Only one PCFCL without BCL2 rearrangement also displayed a type A pattern but was associated with BCL2 and CD10 expression. Among the six SSIFCLs, three displayed a type A pattern (with one t(14;18)-positive case), and three showed a type B pattern (with two t(14;18)-positive cases). All SSIFCLs were characterized by BCL2 and CD10 coexpression. Type A pattern was therefore observed more frequently in cases harboring a BCL2 rearrangement (Fisher exact test, \( P = .03; \text{OR}, 17; 95\% \text{ CI}, 1.97-186.45 \)) and in SSIFCL cases with extracutaneous involvement at diagnosis (Fisher exact test, \( P = .02; \text{OR}, 9.91; 95\% \text{ CI}, 1.0042-104.19 \)). The seven cases exhibiting a BCL2 rearrangement expressed BCL2 and CD10. Conversely, nine PCFCL cases that also coexpressed BCL2 and CD10 did not bear a BCL2 rearrangement.

Discussion

In this study, we included 53 patients with FCL presenting in the skin and further evaluated if clinical and morphologic features may be of diagnostic value between PCFCL and SSIFCL in view of the data obtained by further staging procedures, as in the real-life setting. We also evaluated the diagnostic and prognostic value of BCL2 gene rearrangement detected by FISH in seven (13.2\%) of 53 patients,
including four (8.5%) of 47 with PCFCL and three (50%) of six with SSIFCL. Then, the presence of a BCL2 rearrangement was found significantly associated with positivity of initial staging procedures ($P = .02$) but cannot serve as a diagnostic marker between the two entities since it may be observed in some PCFCL cases. The long follow-up period (median, 39 months) also showed that BCL2 rearrangement was associated with a higher risk of secondary extracutaneous spreading in patients with PCFCL ($P = .05$).

Given these results, our study suggests that FISH could be included as an ancillary procedure in FCL presenting in the skin, especially in cases with CD10/BCL2 coexpression. Such coexpression was observed in all SSIFCL cases and in 35% of PCFCL cases, including three of five PCFCL cases with secondary extracutaneous involvement. The single expression of BCL2 observed in more than 50% of PCFCL cases was not found correlated with such clinical evolutive features. In different studies, BCL2 expression in PCFCL has varied considerably, ranging from 0% to 86%.\(^9,15,19,21-23\) Moreover, the presence of T cells strongly immunoreactive for the BCL2 protein requires a careful comparison of BCL2, CD3, and CD20 immunostaining as well as the use of a high cutoff (at 50%) to assess the BCL2 positivity of an FCL case. By comparison with indolent cases, the architectural pattern of the 11 cases with extracutaneous involvement was not found specific with either a follicular growth pattern with dense infiltration of the dermis (type A pattern) or a focal periadnexal infiltrate (type B pattern), even if the type A pattern was observed more frequently in SSIFCL. Five of these 11 patients, however, were harboring a BCL2 rearrangement. Therefore, only combined BCL2 and CD10 expression could be relevant to select patients for FISH testing among any other histologic criteria. Analyzing a surgical biopsy specimen including the reticular dermis and fat rather than a punch biopsy specimen, as recommended by others,\(^3\) permitted us to increase the reliability of histopathologic analysis of the above criteria and FISH testing on a representative tumor sample.

Moreover, bone marrow biopsy has been considered an “optional” staging procedure in PCFCL according to the International Society for Cutaneous Lymphoma/European Organization for Research and Treatment of Cancer recommendations.\(^6\) Indeed, in patients with PCFCL and otherwise negative staging, bone marrow biopsy is often not performed in our national practice.\(^24\) Only one of our patients (of 43 patients with bone marrow examination) displayed a bone marrow infiltration as the only evidence of extracutaneous disease. Interestingly, this case displayed a BCL2/CD10 phenotype associated with BCL2 rearrangement. In the largest series reported so far by Senff et al,\(^25\) 22 (11%) of 193 patients with FCL first presenting in the skin showed a bone marrow infiltration. However, it was the only evidence of extracutaneous disease in nine of these 22 patients (nine of 193 [4.7%]), in accordance with the rate (2.3%) observed here. Moreover, the 5-year overall and disease-specific survival of these nine patients was 44% and 63%, respectively, compared with 84% and 95%, respectively, in 157 patients without extracutaneous disease. These data may support that bone marrow examination should be considered a systematic staging procedure in patients with FCL presenting in the skin. Nevertheless, isolated bone marrow involvement would not directly change the therapeutic management, since hematologists recommend a “watch-and-wait strategy” for FCL without clinical symptoms or cytopenias.\(^26\) Our data therefore suggest evaluating the presence of BCL2/CD10 coexpression and BCL2 rearrangement to determine if these features are recurrently observed by other groups in FCL cases with extracutaneous spreading, primarily or secondarily. The small subset of PCFCL cases exhibiting a BCL2 rearrangement seems to harbor a disease closer to nodal FCL, since
Table 3  Clinicopathologic Features of Patients With BCL2 Rearrangement and/or Secondary Extracutaneous Spreading

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y/Sex</th>
<th>TNM</th>
<th>Bone Marrow</th>
<th>Diagnosis</th>
<th>Site</th>
<th>Therapy</th>
<th>CR</th>
<th>Skin Relapse</th>
<th>Secondary Extracutaneous Spreading (Time to Onset, mo)</th>
<th>Follow-up, mo</th>
<th>Status at Last Follow-up (mo)</th>
<th>Cell Morphology</th>
<th>Phenotype BCL2/CD10 ( % Nucleus )</th>
<th>FISH BCL2 (% Nucleus)</th>
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<td>HN</td>
<td>RT</td>
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<td>No</td>
<td>No</td>
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<td>150</td>
<td>CR (150)</td>
<td>Small/medium</td>
<td>+/-</td>
<td>Positive (45)</td>
</tr>
<tr>
<td>7</td>
<td>69/F</td>
<td>T3bN0M0</td>
<td>Negative</td>
<td>PCFCL</td>
<td>Multiple</td>
<td>PC</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (134)</td>
<td>147</td>
<td>CR (147)</td>
<td>Small/medium</td>
<td>+/-</td>
<td>Positive (50)</td>
</tr>
<tr>
<td>8</td>
<td>56/M</td>
<td>T2aN0M1</td>
<td>Positive</td>
<td>SSIFCL</td>
<td>UB</td>
<td>R-PC</td>
<td>No</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>AWG (11)</td>
<td>Small/medium</td>
<td>+/-</td>
<td>Positive (20)</td>
</tr>
<tr>
<td>9</td>
<td>49/M</td>
<td>T2bN3M1</td>
<td>Positive</td>
<td>SSIFCL</td>
<td>UB</td>
<td>R-PC</td>
<td>No</td>
<td>—</td>
<td>—</td>
<td>26</td>
<td>CR (26)</td>
<td>Small/medium</td>
<td>+/-</td>
<td>Positive (50)</td>
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<tr>
<td>10</td>
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<td>T3bN3M1</td>
<td>Positive</td>
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<td>PC</td>
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<td>NA</td>
<td>NA</td>
<td>103</td>
<td>CR (103)</td>
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<td>+/-</td>
<td>Positive (50)</td>
</tr>
</tbody>
</table>

AWD, alive with disease; CR, complete response; DA, died of another cause; DD, died of disease; FISH, fluorescence in situ hybridization; HN, head and neck; NA, not available; PC, polychemotherapy; PCFCL, primary cutaneous follicle center lymphoma; R-PC, rituximab + polychemotherapy; RT, radiotherapy; RTX, rituximab; SSIFCL, secondary skin involvement of follicle center lymphoma; UB, upper back; +, positive; –, negative; —, not applicable.

Figure 1  Survival function in patients with primary cutaneous follicle center lymphoma (n = 47). A, Kaplan-Meier survival function (log-rank test) considering the first cutaneous relapse, according to the presence of BCL2 rearrangement. B, Kaplan-Meier survival function (log-rank test) considering the first cutaneous relapse, according to the size of cells. C, Survival function (shared γ-frailty model), considering all cutaneous relapses, according to the presence of BCL2 rearrangement. D, Survival function (shared γ-frailty model), considering all cutaneous relapses, according to the size of cells.
they seem to be at risk for developing secondary extracutaneous spreading and should require adequate clinical monitoring.

FISH-positive cases have been further analyzed by PCR amplification of BCL2-JH rearrangement using the BIOMED-2 protocol. Here, molecular detection provided concordant data on skin specimens with FISH in all cases but one. Moreover, the absence of BCL2 rearrangement in peripheral blood samples of patients with PCFCL permitted excluding skin contamination with BCL2-rearranged circulating B cells, as described in healthy individuals but also in patients with FISH-negative PCFCL. For such reason, we would recommend performing FISH on skin sections rather than PCR to assess the presence of BCL2 rearrangement within a significant proportion of tumoral cells to avoid false positivity due to bystander cells, as previously reported.

Besides BCL2 rearrangement detection, we also investigated other potential prognostic factors for cutaneous relapse and extracutaneous spreading. In this study, 20 (42.6%) of 47 patients had cutaneous relapse, a rate observed in other studies. As reported by others, clinical features such as age or sex did not influence the DFS in PCFCL. We observed that predominant small/medium centrocytic cell morphology was statistically associated with a higher probability of cutaneous relapse(s) in the univariate and multivariate analysis. We assessed this prognosis factor using both the usual nonparametric log-rank test that considers only the first cutaneous relapse for each patient and the shared γ-frailty model that considers all cutaneous relapses for each patient. We also believe that such a model could be particularly relevant to the study of disease progression in different types of cutaneous lymphomas in which the number of relapses and their time interval may represent an indicator of the disease aggressiveness, not otherwise modeled so far in this field.

Finally, our study identified that BCL2 rearrangement is a rare event in PCFCL (8.5%) but may identify patients at risk of extracutaneous involvement together with other associated features, such as type A architectural pattern and BCL2-CD10 coexpression. Alternatively, we suggest searching a BCL2 rearrangement by FISH using separation probes in cases harboring the above features. Moreover, small/medium centrocyte morphology, and potentially BCL2/CD10 coexpression, seems to be predictive for a higher risk of cutaneous relapse.

References


