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CLINICAL REPORT

# Primary Cutaneous B-cell Lymphomas of the Lower Limbs: A Study of Integrin Expression in 11 Cases

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**Primary cutaneous B-cell lymphoma is a rare disease. Among the cutaneous B lymphomas, B-cell lymphomas of the lower limbs appear as a special subgroup with a prognosis that is possibly worse than that of primary cutaneous B-cell lymphomas located on the trunk, arms or head, with more frequent relapses. In addition, some recent studies indicate that the level of expression of integrins on tumour cells could be related to the clinical course of the disease. This study reports on 14 cases of primary cutaneous B-cell lymphomas of the lower limbs and their clinical course. A study of integrin expression by tumour cells was performed in 11 of these cases. With a mean follow-up of 31 months, the study confirmed the worse prognosis of lymphomas with a predominance of centroblasts and immunoblasts (3 deaths) compared with lymphomas with a predominance of centrocytes, as well as their higher rate of recurrence (7/11). A correlation was confirmed between the course of the disease and the level of expression of lymphocyte function-associated antigen-1, intercellular adhesion molecule-1 and very late antigen-4 by tumour cells. *Key words: B-cell lymphoma; skin; limbs; integrins.***

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Primary cutaneous B-cell lymphomas (CBCL) are infrequent cutaneous diseases (1, 2). Some recent series (3–5) consider large cell lymphomas of lower limbs as a special subgroup of primary CBCL more likely to develop in elderly subjects, mainly women. Their prognosis is considered as intermediary in the classification of the European Organization for Research and Treatment of Cancer (6). However, this designation is still contested (7). Recent studies have suggested that the expression of adhesion molecules could have a prognostic value for the clinical course of CBCL in any location (8–10). This study reports on 14 cases of primary CBCL of the lower limbs and their clinical course. In addition, a study of integrin expression by tumour cells was performed for 11 of these cases.

## PATIENTS AND METHODS

The 14 patients studied had purplish cutaneous nodules located only on the lower limbs. The diagnosis of primary CBCL of the lower limbs was based on clinical, histological and immunophenotypic criteria. Assessment of initial disease extension, including a medullary biopsy, thoracoabdominal computed tomographic (CT) scan and abdominopelvic ultrasonography, was strictly negative for all patients.

Skin biopsies were performed in all patients, one fragment being kept in formaldehyde for histology and another in liquid nitrogen for immunohistochemistry and studies of adhesion molecules. The immunophenotype was determined using a large panel of anti-B-cell monoclonal antibodies (CD20-22, Dako, Copenhagen, Denmark; CD19-21, Beckton-Dickinson, Franklin Lakes, USA); anti-T (CD1, Immunotech, Marseilles, France; CD2-3-4-5-8, Dako) and activation markers (CD30 and Ki 67, Dako). Screening for a kappa–lambda monotype was performed using 2 methods: immunohistochemistry with anti-kappa–lambda monoclonal antibodies (Dako) on frozen sections, and *in situ* hybridization with PNA probes (Dako) on paraffin sections to detect kappa or lambda mRNA. The screening techniques for adhesion molecules and Bcl-2 oncogene were performed on frozen sections for 11 patients using anti-intercellular adhesion molecule-1 (ICAM-1; CD54), anti-lymphocyte function-associated antigen-1 (LFA-1; CD11a), anti-$\beta_1$-integrin (CD29), anti-very late antigen-3 (VLA-3; CD49c) and anti-VLA-4 (CD49d) monoclonal antibodies from Immunotech and anti-Bcl2 from Dako. For each monoclonal antibody, the percentage of positive cells in the infiltrate and the intensity of labelling (+ weak, + + moderate, + + + strong) was determined in 3 fields ($\times 40$).

## RESULTS

Nine men and 5 women were studied (median age 71 years, range 37–95), whose clinical and follow-up data are summarized in Table I. The skin lesions were single nodules or tumours in 5 cases, multiple nodules or tumours in 5 cases, and plaques in 4 cases. They appeared on the lower legs in 12 cases and on the thighs in 2 cases. Initial involvement in all patients concerned only a single limb. Histological studies showed in 8 cases a predominance of large round cells with typical centroblasts and immunoblasts and in 6 cases a predominance of large centrocytes in addition to centroblasts and rare immunoblasts. The tumour cells expressed CD19 (3/14), CD20 (14/14) and CD22 (13/14) B antigens, but not CD21. Screening for a kappa–lambda monotype by the immunoperoxidase method showed a kappa monotype in 4 cases, lambda in 6 cases and a non-secreting lymphoma in 3 cases. *In situ* hybridization detected mRNA kappa in one case and lambda in another among the 3 non-secreting lymphomas.

The tumour cells expressed Bcl-2 (11/11), ICAM-1 (10/11, with only 10% of positive cells in 2 cases), $\beta_1$-integrin (10/11), VLA-3 (1/11), VLA-4 (8/11) and LFA-1 (6/11). Among the 5 cases with LFA-1-negative tumour growth, 4 had a bad course (2 deaths and 2 recurrences which did not respond to treatment) (Table II). Moreover, there was no expression of ICAM-1 in 1 of these 4 cases and only 10% of tumour cells in 2 other cases; VLA-4 was not expressed in 3 cases. However, no relation was found between VLA-4, $\beta_1$-integrin or VLA-3 expression and the disease course of these patients.

Table I. *Clinical characteristics and follow-up data of 14 patients with primary cutaneous B-cell lymphomas of the lower limbs*

| Patient | Age (years) | Gender | Presenting symptoms | Immunophenotype | Initial therapy | Site of relapse | Therapy |
|---|---|---|---|---|---|---|---|
| 1 | 82 | M | 1995: multiple nodules on a lower limb | CD20-22$^+$, 19-21$^-$, kappa$^+$ | ERT | 2 lower limbs + pharynx | ERT – IFN – VP16 |
| 2 | 74 | M | 1997: single nodule on a leg 4 cm | CD20-22$^+$, 19-21$^-$, lambda$^+$ | ERT | 0 | 0 |
| 3 | 72 | M | 1997: single plaque on a thigh 3 × 4 cm | CD20-22$^+$, 19-21$^-$, lambda$^+$ | Spontaneous remission | 0 | 0 |
| 4 | 47 | M | 1996: single nodule on a leg 2.5 cm | CD19-20-22$^+$, 21$^-$, lambda$^+$ | ERT | 0 | 0 |
| 5 | 50 | M | 1996: single nodule on a leg 2 cm | CD20-22$^+$, 19-21$^-$, kappa$^+$ | ERT | 1997: plaque on a leg | ERT |
| 6 | 73 | F | 1998: papules and nodules on a leg | CD20-22$^+$, 19-21$^-$, lambda$^+$ | Spontaneous remission | 0 | 0 |
| 7 | 71 | F | 1993: nodule+ plaque on a leg | CD19-20-22$^+$, 21$^-$, kappa$^+$ | ERT | 1996: nodules on a leg | ERT – IFN – COP – VP16 |
| 8 | 83 | M | 1996: single nodule on a leg | CD20-22$^+$, 19-21$^-$, non-secreting | ERT | 1998: 2 plaques on thigh+ leg | ERT |
| 9 | 81 | M | 1998: circumferential plaque on a leg | CD20-22$^+$, 19-21$^-$, non-secreting | ERT | 1999: nodules on a leg | ERT |
| 10 | 37 | M | 1998: single plaque on a thigh | CD19-20-22$^+$, 21$^-$, lambda$^+$ | IFN | 0 | 0 |
| 11 | 80 | F | 1998: single nodule on a leg 5 cm | CD20-22$^+$, 19-21$^-$, non-secreting | IFN+ ERT | 1998: multiple nodules on a leg | ERT – VP16 |
| 12 | 78 | F | 1998: single nodule on a leg 4 cm | CD19-20-22$^+$, 21$^-$, kappa$^+$ | ERT | 0 | 0 |
| 13 | 77 | M | 1999: multiple nodules on a leg | CD20-22$^+$, 19-21$^-$, lambda$^+$ | ERT | 0 | 0 |
| 14 | 95 | F | 1999: multiple nodules on a lower limb | CD20-22$^+$, 19-21$^-$, lambda$^+$ | ERT | Nodules on lower limb | ERT |

ERT: external radiotherapy; IFN: interferon-α; VP16: vepeside; COP: cyclophosphamide, oncovin, prednisone.

Initial treatment consisted of external radiotherapy (ERT) with a dose of 20 Gy in 10 cases, interferon-α (IFN) alone ($3 \times 10^6$IU × 3/week) in 1 case, and ERT associated with IFN in 1 case. Initial complete remission was observed in 13/14 patients (10 ERT, 1 ERT+ IFN, 2 spontaneous remissions). Seven patients (5 lymphomas with a predominance of centroblasts and immunoblasts, and 2 lymphomas with a predominance of large centrocytes in addition to centroblasts) experienced a relapse (6 ERT, 1 ERT+ IFN). Three patients died of their lymphoma (all histologically confirmed lymphomas with a predominance of centroblasts and immunoblasts). Mean follow-up was 31 months (range 9 – 78 months).

## DISCUSSION

The main clinical studies (3 – 5) of primary CBCL of the lower limbs conducted up to now suggest that these lymphomas have to be distinguished from other primary CBCL since they occur at a more advanced age and show high rates of

Table II. *Expression of oncogene Bcl-2 and adhesion molecules*[a] *by tumour cells and the outcome in 11 patients*

| Patient | Bcl-2 | ICAM-1 | LFA-1 | $\beta_1$-Integrin | VLA-3 | VLA-4 | Current status |
|---|---|---|---|---|---|---|---|
| 1 | + + | + (10%) | – | – | – | – | Dead |
| 2 | + | + | + | + | – | + + | CR |
| 5 | + + | – | – | + | – | + | CR |
| 6 | + | + + | + + | + + | – | + | CR |
| 8 | + | + + | + + | + + | – | + | CR |
| 9 | + | + (10%) | – | + | – | – | Dead |
| 10 | + | + | + | + + | – | + + | CR |
| 11 | + + | + + | – | + + | + + | + + | Relapse |
| 12 | + | + + | + | + + | + + | + + | CR |
| 13 | + | + + | + + | + + | + + | + + | CR |
| 14 | – | – | – | + + | + + | – | Relapse |

[a]Intercellular adhesion molecule-1 (ICAM-1; CD54), lymphocyte function-associated antigen-1 (LFA-1; CD11a), $\beta_1$-integrin (CD29), very late antigen-3 (VLA-3; CD49c) and VLA-4 (CD49d).
CR: complete remission.

recurrence and mortality. This study of 14 primary CBCL of the lower limbs gives similar results with a median age at the time of diagnosis. However, a male predominance (9/14, 64%) was noted, in contrast to a female predominance in the previous studies (10/14, 71% and 13/22, 59%).

The survival rate was 78% (3 patients died after a mean follow-up of 31 months), which is similar to that reported in previous studies (77% after 2 years, and 58% after 5 years) (3–5).

Histologically, while in the 2 previous studies all primary CBCL of the lower limbs were diffuse large cell lymphomas, in the present study 2 different cell types were noted: 8 cases of lymphomas with a predominance of centroblasts and immunoblast cells and 6 cases of lymphomas with large centrocyte and centroblast cells. The centroblast and immunoblast subtype appears to be related to a worse prognosis. Thus, this study indicates that the less favourable prognosis for primary CBCL of the lower limbs is related more to the histological type than to the site of lesions. Concerning treatment, it was found that ERT with or without IFN-$\alpha$ induces a rapid response with few side-effects in most cases and thus appears to be a less aggressive treatment than the polychemotherapy used in previous studies (4–6).

In addition, this study shows that kappa and lambda *in situ* hybridization may help in the diagnosis of monotype, notably in non-secreting lymphoma at the protein level. Indeed, 1 kappa and 1 lambda monotype were identified at the mRNA level among 3 non-secreting lymphomas.

Recent studies (11–13) based on large series of non-cutaneous diffuse large cell lymphomas suggest that prognosis is worse for those expressing the Bcl-2 oncogene. Geelen et al. (8) showed that primary CBCL of the lower limbs were Bcl-2 positive (9/9) while primary CBCL of the head and trunk were all Bcl-2 negative (14/14). The present results are similar and suggest differences in lymphogenesis that tend to make these 2 types of CBCL distinct entities.

Other studies have shown a relation between abnormal expression of adhesion molecules and the clinical course of follicular B-cell lymphoma (14–17). Beljaards et al. (10) demonstrated that primary CBCL of the lower limbs and secondary CBCL expressed ICAM-1 and LFA-1 less often than did primary CBCL of the head and trunk. Moreover, dissemination in extracutaneous sites and deaths were only observed in patients whose neoplastic cells were negative for ICAM-1 and LFA-1. These results suggest that the expression of adhesion molecules by tumour B-cells could be an important prognostic factor. This study confirms the results of Beljaards et al. (10), since 2 deaths and 2 progressions occurred among the 5 patients with primary CBCL of the lower limbs whose tumour cells did not express LFA-1, whereas 6 other patients with lymphomas expressing LFA-1 are still in complete remission. The low expression of ICAM-1 and VLA-4 in this study also appears to be related to progressive disease. These results need to be confirmed in a larger series, particularly to determine whether the aberrant expression of adhesion molecules by tumour B-cells is an independent prognostic factor.

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