INVESTIGATIVE REPORT

Diagnosis of Primary Cutaneous B-cell Lymphoma by Immunohistochemical and *in situ* Hybridization Methods

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A diagnosis of primary cutaneous B-cell lymphoma is mainly supported by identification of a monoclonal B-cell population in the skin. This identification is made either by immunohistochemistry techniques using monoclonal antibodies towards the lambda or kappa chains, or by PCR for the heavy chain of immunoglobulins. Immunohistochemistry has a low sensitivity in detecting monoclonality, whereas the PCR technique is sensitive, but does not permit the localization of tumour cells in the skin. The aim of this study was to determine whether a monoclonal population in the skin could be detected by *in situ* hybridization. Thirty-eight skin biopsies from patients with primary cutaneous B-cell lymphoma were studied. Monoclonality was observed in 24/38 (63%) biopsies using immunohistochemical methods and 32/38 (84%) when combining this with *in situ* hybridization. It is concluded that kappa or lambda chain *in situ* hybridization is an interesting complementary technique to detect monoclonal B cells, if the immunohistochemical technique is negative. *Key words: cutaneous lymphoma; immunohistochemistry; hybridization.*

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Primary cutaneous B-cell lymphoma (PCBCL) is defined as a B-cell lymphoma proliferation developing only in the skin at diagnosis and for the subsequent 6 months. The overall annual incidence of cutaneous lymphomas is estimated to be 0.5 to 1 per 100,000 (1) and of these 20 to 25% originate from B cells (2, 3). Most of the cutaneous B-cell malignancies belong to the group known as follicle centre cell lymphoma (FCCL) or marginal zone lymphoma (MZL) (4).

The diagnosis of PCBCL is often difficult and has to be supported by clinical, histological, immunohistology and genotyping analyses, demonstrating a monoclonal B-cell population. Monoclonal expression of kappa (κ) or lambda (λ) light chains is considered to reflect a malignant process in contrast to the polyclonal expression in reactive hyperplasia (5). However, it has now been shown that clones can be identified in cutaneous benign lesions, so clonality is not diagnostic for malignancy.

In PCBCL, clonal rearrangement of the immunoglobulin (Ig) chain can be demonstrated either by using immunohistochemical (IHC) analysis with monoclonal antibodies or by immunogenotyping using Southern blot or PCR (6). The IHC technique has the advantage of localizing the monoclonal cells in the skin, but lacks sensitivity and is often associated with background staining, which can limit its sensitivity. The Southern blot technique allows for detection of clonal cells representing more than 5% in the lesion, whereas PCR is much more sensitive with a level of detection as low as 0.1% of clonal cells (7, 8). However, these techniques do not permit localization of the tumour cells in the infiltrate.

Our purpose was to test the sensitivity of an *in situ* hybridization (ISH) technique with kappa and lambda probes in the diagnosis of B-cell monoclonality in PCBCL by comparing the results with the IHC analysis for detecting a monotypic Ig light chain expression.

MATERIAL AND METHODS

Thirty-eight patients with primary cutaneous B-cell lymphoma confirmed by histological examination were selected from the Department of Dermatology and 4 B-cell pseudolymphomas were used as controls. The staging procedure included a thoraco-abdominal scan, an abdominal ultrasound and a bone marrow biopsy. None of the patients had extra cutaneous spreading in the 6 months following the initial investigations. The study group included 23 males and 15 females with a median age at diagnosis of 63.7 years, range 21–95 years.

At diagnosis the tumour was localized to the trunk (18 cases, 47.3%), lower extremity (13 cases, 34.2%) associated with upper extremity lesions in one case, upper extremity (6 cases, 15.8%) associated with trunk localizations in one case, face (2 cases, 5.3%) and scalp (2 cases, 5.3%). Lesions were initially multiple in 60.5% of patients (23/38). The 4 B-cell pseudolymphomas were isolated nodular lesions located on the arm (2 patients) and the trunk (2 patients). The median follow-up time was 7.8 years. Five patients developed extracutaneous lesions (4 in the lymph nodes and one in the mediastinum) and all of them had a fatal course. The PCBCL was classified according to the EORTC criteria.

Two skin biopsies were obtained from each patient: one was frozen for IHC analysis and the other was paraffin embedded for ISH.

IHC analysis was performed using a streptavidine/biotin

immunoperoxidase system (DAKO, Copenhagen) using primary and secondary antibodies against B-cell antigens (CD19, CD20, CD21, CD22). AEC substrate solution (3-amino, 9-ethylcarbazole and hydrogen peroxide) and a counter-colorization with haemalun (Mayer, Bordeaux) was used for cell visualization. A negative control was prepared by using a skin biopsy of the PCBCL of one of the patients without adding antibodies.

ISH was done on 5 μm sections of biopsies. Pretreatment was done with xylene, alcohol diluted at 100%, 70%, 50%, and finally a buffer solution (Tris buffered saline (TBS) with Tween 20; SIGMA, St Louis, USA).150 μl K proteinase was added to the slides, which were then rinsed in TBS and alcohol 95%. ISH was done using the DAKO kit, with FITC probes (kappa or lambda) incubated for 2.5 h at 55°C. The slides were then immersed in a stringent solution (Dako kit) diluted 1/60 in distilled water for 25 min at 55°C and 10 s in TBS.

Detection was obtained by adding an anti-FITC antibody on each slide for 30 min. The substrate used for visualization was DAKO fast red for 30 min in the dark, followed by a counter-colorization with haematoxylin. Negative and positive probes are included in the DAKO kit as controls.

RESULTS

According to histology of the lymphomas 23 specimens (60.5%) were classified as FCCLs, 12 specimens (31.6%) as large B-cell lymphomas of the leg (LBCLLs), and 3 specimens (7.9%) as MZLs.

The IHC analysis showed that 63.1% (24/38) of patients expressed a monotypic immunoglobulin, where a kappa light chain was present in 29.2% (7/24) of cases and a lambda chain in 70.8% (17/24) (Table I and Fig. 1). The histological classification of tumours with clonal cells is listed in Table I: 13 FCCL, 8 LBCLL and 3 MZL.

Among the 24 patients expressing a monotypic immunoglobulin on immunochemistry, ISH with kappa and lambda chains confirmed the same monotypic immunoglobulin in 45.8% (11/24) patients, but was negative in 54.2% (13/24) patients. Fig. 2 represents a positive κ monoclonality in B cells, where the IHC technique was negative.

Using the IHC technique, we found that 14 specimens yielded no expression of monotypic immunoglobulin. These cases were classified as non-secreting B lymphomas. The histological examination showed that 10 were FCCLs and 4 were LBCLLs. ISH of these
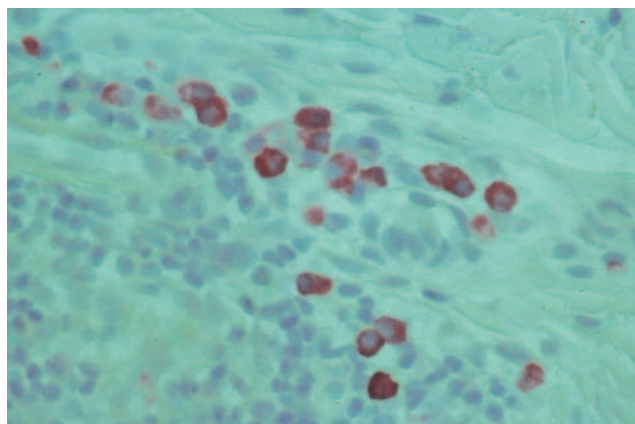

Fig. 1. Monotypic immunoglobulin revealed by the immunohistochemical method in a biopsy of primary cutaneous B-cell lymphoma. Expression of kappa light chain.
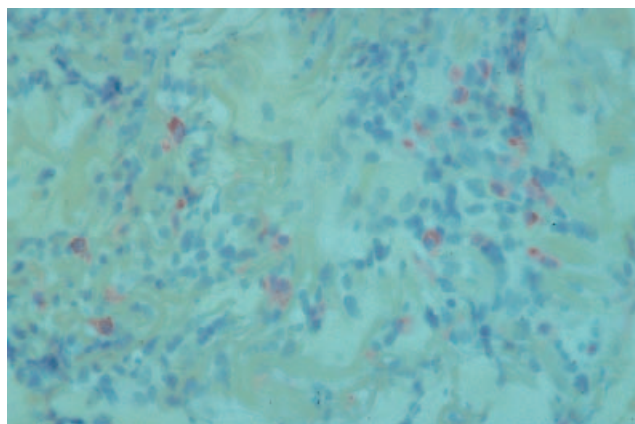

Fig. 2. Positive monoclonality into B cells of primary cutaneous B-cell lymphoma by the *in situ* hybridization method. Expression of kappa light chain.

specimens showed a monoclonality (κ/λ) among 8/14 (57.2).

Thus, with the two techniques, monoclonality was demonstrated in 82.6% of FCCL (19/23) and 83.3% (10/12) of LBCLL cases. For MZL, all of them presented a positive light chain restriction using the IHC method, but none with ISH.

The 4 B-cell pseudolymphoma biopsies had both

Table I. *Summary of the results of immunochemistry and in situ hybridization*

Diagnostic	Immunohistochemistry			In situ hybridization		
	Negative	Kappa +	Lambda +	Negative	Kappa +	Lambda +
FCCL (n=23)	10	4	9	8	6	9
LBCLL (n=12)	4	3	5	8	0	4
MZL (n=3)	0	0	3	3	0	0
BCPL (n=4)	0	4	4	0	4	4

FCCL: Follicular centre cutaneous lymphoma; LBCLL: large B-cell lymphoma of leg; MZL: marginal zone lymphoma; BCPL: B-cell pseudolymphoma.

kappa and lambda light chains detected by IHC and ISH, and thus no clonality.

DISCUSSION

Our 38 patients had a median age of 63.7 years comparable to what has been reported in the literature (9). PCBCL is reported to be slightly more common in females, with a ratio of 2:1 (3), but this was not the case in our study (M:F ratio: 1.7). Extracutaneous spreading of the disease has been reported to occur in 3 to 18% of cases with visceral or lymph node involvement (6). We observed 15% with extra-cutaneous spreading (5/38) and all had a fatal course. These patients all had LBCLLs, which is in agreement with other observations (6, 10, 11).

The demonstration of a monoclonal B-cell population supports the diagnosis of lymphoma (12–17), which is performed by using IHC staining with kappa and lambda monoclonal antibodies. This technique is easy to carry out, but is often associated with a high background, which makes the diagnosis of monoclonality difficult. The advantage of the technique is its ability to locate the tumour cells in the tissue. Quantitation is difficult, however. A clonal rearrangement of the JH gene by PCR can detect a low percentage of monoclonal cells (11), but it does not give the localization of tumour cells in the skin. It is also expensive and is not yet a routine technique in all departments of pathology. In this context, ISH, which is easy to use, may be of benefit when the IHC technique is negative or uncertain.

In the literature the detection of clones in PCBCL varies from 8% to 75% (18–20) with a predominant monoclonal expression of the lambda light chain (70.8%). In our study the IHC method itself had a detection range of 63.1%. The most common subtype of PCBCL is FCCL lymphoma, which is found in 40% of PCBCL (9, 21). In this subtype, the tumour cells express monotypic surface immunoglobulin and a clonal rearrangement of JH genes can be demonstrated in the majority of cases (11). In our study, FCCL represented the predominant histological subtype (60.5%) and a monoclonality by IHC was noted in 56.5% of biopsies. Addition of ISH permitted detection of clonality in 82.6% of the specimens.

Concerning the large B-cell lymphoma, the literature shows a percentage of detection of kappa or lambda chains at the surface of cells (11) by IHC varying from 5% to 10% (3, 9, 17). In our study, the immunochemistry technique permitted the diagnosis of monoclonality in 66.6% of cases increasing this figure to 83.3% with the combined techniques. Expression of immunoglobulin light chain in the MZL subtype of PCBCL is detected in 50% to 75% of biopsies according the different studies (11, 17, 22, 23). In our study, the detection of light chain was positive for all patients using the combined technique, but there were only 3 specimens.

There are several cases of B-cell lymphoproliferative disorders, in which the cells do not express immunoglobulins, and the frequency of immunoglobulin-negative B-cell proliferations appears to be higher in the skin than in lymph nodes (24). Therefore, ISH can be helpful in differentiating a reactive polyclonal infiltrate of B cells and a neoplastic process.

In conclusion, when a monotypic immunoglobulin light chain expression is seen by the IHC technique, ISH is not necessary. In contrast, the technique is indicated when the IHC technique fails to establish the diagnosis of PCBCL, because half of those patients do have clonal cells, as seen by the ISH technique. Thus, ISH appears to be a complementary method to IHC in the genotypic detection of a clonal rearrangement in PCBCL.

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