Prevalence of *Borrelia burgdorferi* Infection in a Series of 98 Primary Cutaneous Lymphomas

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ABSTRACT

Borrelia burgdorferi has been variably associated with different forms of primary cutaneous lymphoma. Differences in prevalence rates among reported studies could be a result of geographic variability or heterogeneity in the molecular approaches that have been employed. In the present study, we investigated the prevalence of *Borrelia burgdorferi sensu lato* DNA in diagnostic tissue samples from fresh cutaneous biopsies of 98 primary cutaneous lymphomas and 19 normal skin controls. Three different polymerase chain reaction (PCR) protocols targeting the *hbb*, *flagellin*, and *Osp-A* genes were used. Direct sequencing of both sense and antisense strands of purified PCR products confirmed the specificity of the amplified fragments. Sequence specificity was assessed

using the Basic Local Alignment Search Tool, and MultAlin software was used to investigate the heterogeneity of target gene sequences across the different samples.

Borrelia DNA was not detected in 19 controls, 23 cases of follicular lymphoma, 31 cases of extranodal marginal zone lymphoma, or 30 cases of mycosis fungoides. A single case of 14 diffuse large B-cell lymphoma cases was positive for *B. burgdorferi*.

This study does not support a pathogenic role of *B. burgdorferi* in primary cutaneous B- and T-cell lymphomas from areas nonendemic for this microorganism and the consequent rationale for the adoption of antibiotic therapy in these patients. *The Oncologist* 2011;16:1582–1588

INTRODUCTION

Borrelia burgdorferi, the causative agent of Lyme disease, infects its human targets when transmitted by the bite of *Ixodes* ticks,

which are found mostly in certain endemic areas of North America and Central Europe. Different *Borrelia* strains have been variably associated with primary cutaneous lymphomas [1]. This

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association has been suggested by the correlation occurring between acrodermatitis chronica atrophicans, a cutaneous manifestation of Lyme disease, and cutaneous B-cell lymphomas, and confirmed by serologic tests and molecular studies; these results are almost exclusively reported under the form of case studies or small retrospective series of primary and secondary cutaneous B-cell lymphomas [2–7]. *B. burgdorferi* DNA has been detected in 10%–42% of patients with cutaneous mucosa-associated lymphoma tissue (MALT) lymphomas, in 15%–26% of cases of cutaneous follicular and diffuse large B-cell lymphomas, and in 18% of mycosis fungoides diagnosed in some European countries [2, 3, 7, 8]. At variance with these figures, other American [9], European [10], and Asian [11] studies did not show these observations. Lyme disease can be successfully treated with *Borrelia* eradication with specific antibiotics, which is particularly evident for Borreliac lymphocytoma, a cutaneous manifestation of Lyme disease that is usually responsive to antibiotics [12]. These observations suggested that *Borrelia* infection should be investigated and, if present, eradicated in patients with cutaneous lymphomas. Nevertheless, only a few cases of cutaneous MALT lymphoma responding to antibiotics have been reported [1–4, 6, 7, 13–15]. Taken together, this incomplete evidence presently impairs our ability to show a definitive, significant association between *B. burgdorferi* and cutaneous lymphoma as well as the related rationale for the adoption of antibiotic therapy as an antilymphoma strategy. On these grounds, to overcome the limitations of small series and to perform a more comprehensive analysis with a molecular viewpoint, we extensively characterized a series of 98 consecutive cases of cutaneous B- and T-cell lymphoma of four different histotypes using three different *Borrelia*-specific PCR protocols.

PATIENTS AND METHODS

Case Selection

Ninety-eight consecutive patients with a histopathological diagnosis of cutaneous lymphoma of the B-cell or T-cell immunophenotype were included in the study. All cases were selected and independently reviewed by one expert dermatopathologist and at least two hematopathologists; cases with discordant diagnoses were discussed in a consensus meeting. Cases were classified as follicular lymphoma, marginal zone B-cell lymphoma, diffuse large B-cell lymphoma, and mycosis fungoides according to the European Organization for Research and Treatment of Cancer–World Health Organization classification [16]. The clinicopathological characteristics of the patients, who were not treated with antibiotics, are summarized in Table 1. DNA collected from fresh-frozen skin biopsies was available for all cases analyzed. Histologically normal cutaneous specimens obtained during breast surgery from 19 consecutive surgical specimens were included as negative controls.

PCR Amplification

DNA from fresh-frozen skin biopsies was analyzed using three different PCR protocols. The first PCR protocol allowed the amplification of a 152-bp fragment of the *hbb* gene sequence for the detection of *B. burgdorferi sensu lato*,

Table 1. Clinical, histopathologic, and therapeutic features of the present series of 98 patients with cutaneous lymphomas

Characteristic	n
Median age (range), yrs	53 (29–94)
Sex	
Male	68
Female	30
Histotype	
Diffuse large B-cell lymphoma	14
Follicular B-cell lymphoma	23
Marginal zone B-cell lymphoma	31
Mycosis fungoides	30
Stage ^a	
IA	24
IB	14
IIA	6
IIB	19
IIIA	3
IIIB	1
Therapy	
Radiotherapy	39
Interferon	7
Oral methotrexate	6
Oral cyclophosphamide	11
Chlorambucil	2
Retinoids	10
Oral steroids	3
Local steroids	20
Oral psoralen + UVA	8
Cyclophosphamide, doxorubicin, vincristine, and prednisone	6
Allogeneic stem cell transplant	5

^aInternational Society for Cutaneous Lymphomas–European Organization for Research and Treatment of Cancer staging system [24].

according to a previously published protocol [17]. The upstream forward primer displayed a sequence corresponding to a conserved region from nucleotide –16 to +19 (5'-GTAAGGAAATTAGTTTATGTCTTTT-3') of the gene. The reverse primer showed a reverse sequence complementary to the *hbb* gene, spanning nucleotides 113–136 (5'-TAAGCTCTTCAAAAAAGCATCTA-3'). PCR was performed in a 50-μL final mixture containing 500 ng DNA template, 5 μL 10X Fast Start Buffer containing 2.5 mM MgCl₂, 0.2 mM deoxyribonucleotide triphosphates (dNTPs), and 2.0 U of Fast Start Taq, and 10 pmol of each primer (all from Roche Diagnostics, Basel, Switzerland). The amplification program was carried out with a “hot-start” procedure using the following conditions: a first denaturation/activation step at 95°C for

Table 2. Case reports of cutaneous lymphomas treated with antibiotics

Case	Histology	Age	Country	Bb Serology	Culture	PCR DNA	Antibiotic	Objective response
Garbe et al. [4]	B-NHL, small–medium size, cleaved nuclei, low grade	72	Germany	Pos	ND	ND	Penicillin i.m.	NR
Garbe et al. [4]	Centroblastic–centrocytic	67	Germany	Pos	ND	ND	Penicillin i.m.	NR
Garbe et al. [4]	B-NHL, small size, cleaved nuclei	63	Germany	Pos	ND	ND	Penicillin i.m.	NR
Garbe et al. [4]	B-NHL, medium size, cleaved nuclei	82	Germany	Pos	ND	ND	Penicillin i.m. Tetracycline orally	NR
Kütting et al. [6]	MZL	28	Austria	Lightly pos	Pos	Pos	Ceftriaxone i.v. Doxycycline orally Cefotaxime i.v.	CR
Kütting et al. [6]	FCL	38	Austria	Pos	Pos	Neg	Ceftriaxone i.v. Doxycycline orally Cefotaxime i.v.	NR
Roggero et al. [15]	MZL	70	Switzerland	Pos	ND	Pos (<i>hbb</i>)	Ceftriaxone i.v.	PR
Grange et al. [14]	FCL	26	France	Highly pos	ND	Pos (<i>flagellin</i>)	Amoxicillin orally	CR
Grange et al. [14]	DLBCL	67	Austria	Highly pos	ND	Pos (<i>flagellin</i>)	Ceftriaxone i.v.	CR
de la Fouchardiere et al. [7]	MZL	NA	France	NA	NA	Pos	Yes	CR
Aberer et al. [23]	MZL	88	Austria	Pos	ND	Pos (<i>Osp-A</i>)	Ceftriaxone i.v.	CR

Abbreviations: CR, complete response; DLBCL, diffuse large B-cell lymphoma; FCL, follicular lymphoma; MZL, marginal zone lymphoma; NA, not available; ND, not done; NHL, non-Hodgkin's lymphoma; NR, no response.

specific DNA sequences of *B. burgdorferi* in one lymphoma case (diffuse large B-cell lymphoma of the leg type) (Fig. 1), whereas all the other lymphoma categories and controls were negative (Fig. 2). PCR targeting of the *Osp-A* gene was negative in all cases assessed and in controls. The prevalence of *B. burgdorferi* DNA among diffuse large B-cell lymphomas was not statistically different from the prevalence in controls (7% versus 0%; $p = .66$).

DISCUSSION

We herein report the largest available study aimed to evaluate the prevalence of *Borrelia* infection in patients with primary cutaneous lymphomas. According to our figures, the prevalence of *Borrelia* infection is extremely low within the most common forms of cutaneous lymphoma diagnosed in patients resident in northern Italy. The choice of three distinctive PCR protocols represented the most practical, best presently available comprehensive approach used to minimize the risk for false-negative results. In fact, in this study we employed robust, broadly used procedures for the detection of DNA from *B. burgdorferi sensu stricto*, the main causative agent of

Lyme disease in North America, and also DNA from *B. azfeli* and *B. garinii*, two strains prevalent in European countries [19]. The present data point toward a lack of association between cutaneous lymphoma and these infectious agents and, most importantly, preclude the use of antibiotic therapy in these malignancies. Accordingly, treatment against *Borrelia* infection should not be adopted outside areas endemic for this microorganism.

The main limitations of the present study are its retrospective nature, the lack of analysis extended to possible additional multifocal simultaneous lesions, and the unavailability of serological data in investigated patients. The presentation and behavior of cutaneous lymphomas are variable and sometimes represented by a history of simultaneous multifocal lesions that could be present both at diagnosis and at relapse. Importantly, all samples investigated herein were collected at diagnosis, whereby the DNA analyzed was from a single biopsy, regardless of the possible simultaneous presence of additional lesions; this potential bias, however, could be attenuated if we speculate that, once it is hypothesized that a local *Borrelia* infection could play a role in sustaining the growth of the lym-

Table 3. Molecular studies focused on the putative association between *Borrelia* infection and cutaneous lymphomas

Study	n	Geographic area	PCR target gene	Lymphoma subtypes	PCR positive	Sequence specificity
Takino et al. [20]	60	Taiwan-Japan (n = 25) Germany (n = 23) U.S. (n = 12)	<i>hbb</i>	MZL (n = 60)	0 (0%)	ND
Goteri et al. [10]	73	Central Italy	<i>Bb</i> -specific gene [21]	FCL (n = 33) DLBCL (n = 24) MZL (n = 16)	0 (0%) 0 (0%) 0 (0%)	ND
Li et al. [11]	24	Taiwan	<i>hbb</i>	MZL (n = 24)	0 (0%)	ND
de la Fouchardiere et al. [7]	16	France (Rhodes Alps)	<i>flagellin</i>	MZL (n = 16)	3 (19%)	ND
Wood et al. [9]	38	U.S.	<i>Bb</i> -specific gene [21] 224 bp of <i>Ly-1</i> [22]	FCL (n = 7) DLBCL (n = 14) MZL (n = 7)	0 (0%) 0 (0%) 0 (0%)	ND
Goodlad et al. [2]	20	Scottish Highlands	<i>flagellin</i>	FCL (n = 5) DLBCL (n = 3) MZL (n = 12)	1 1 5 (42%)	ND
Cerroni et al. [3]	50	Austria	<i>Bb</i> -specific gene [21]	FCL (n = 20) DLBCL (n = 6) MZL (n = 20) Lp (n = 4)	3 (15%) 1 2 (10%) 3	ND
Aberer et al. [23]	1	Austria	<i>Osp-A</i>	MZL	1	Yes
Present series	98	Northern Italy	<i>Osp-A</i> <i>hbb</i> <i>flagellin</i>	FCL (n = 23) DLBCL (n = 14) MZL (n = 31) MF (n = 30)	0 (0%) 1 (7%) 0 (0%) 0 (0%)	Yes

Abbreviations: ATB, antibiotic therapy; DLBCL, diffuse large B-cell lymphoma; FCL, follicular lymphoma; Lp, lymphoplasmacytoid lymphoma; MF, mycosis fungoides; MZL, marginal zone lymphoma; ND, not done; PCR, polymerase chain reaction.

phoma, we should expect the DNA from this bacterium to be detectable in any involved tissue. Unfortunately, serological data from the studied patients were not available and it is, therefore, not possible, once again theoretically, to exclude the possibility that a proportion of our lymphoma patients could actually have been infected by *Borrelia* despite the fact that this microorganism could not be detected in the lymphomatous biopsy at a molecular level; however, it is important to underline that, at least in the last 20 years, a good concordance rate between serology and PCR aimed to diagnose *Borrelia* infection does exist (Table 2). On the other hand, the above-mentioned limitations as well as patients' and disease characteristics present in our study are mostly overlapping with those described in previously reported studies. On these grounds, the possibility that *Borrelia* infection is associated with the most common forms of cutaneous lymphomas appears unlikely.

Previously reported studies assessing the possible association between cutaneous lymphoma and infection by *Borrelia* strains at the molecular level are summarized in Table 3. All

these studies but one investigated a single target gene using PCR and were invariably performed on formalin-fixed, paraffin-embedded specimens. Studies performed in Japan, Taiwan, the U.S., Germany, and central Italy [10] excluded a *Borrelia*-lymphoma association, whereas two studies, mostly performed in areas endemic for some *Borrelia* genospecies and/or Lyme disease such as Austria and the Scottish Highlands, showed a prevalence of this infection in cutaneous lymphomas in the range of 18%–35% [2, 3]. The highest frequency was found in the MALT histotype (10%–42%), followed by follicular lymphoma (15%–20%) and diffuse large B-cell lymphoma (17%–33%). Moreover, *Borrelia* DNA was detected in 19% of 16 cases of primary cutaneous MALT lymphomas diagnosed in a nonendemic region of France [7]. These studies exhibited two major limitations: PCR amplicons were not sequenced to confirm *Borrelia* identity and the adopted PCR protocols were unable to discriminate among involved genospecies.

At variance with the literature, our study includes the largest series of cutaneous lymphoma ever tested so far for the

presence of *B. burgdorferi sensu lato* DNA, in a comprehensive series analyzing the most frequent histologies. To further reduce methodological pitfalls, we exclusively used DNA extracted from fresh-frozen samples of diagnostic cutaneous biopsies and, for the first time, three different PCR protocols able to distinguish the major *Borrelia* genospecies. As a result of this rigorous approach, only one lymphoma contained *B. burgdorferi* DNA, thus suggesting that this association is markedly less frequent than hypothesized.

Another crucial and practical topic involves the importance of antibiotic therapy in lymphomas of the skin. Available data on treatment in our series clearly show that an antibiotic approach was not among our therapeutic choices (Table 1), but regression of cutaneous lymphoma after antibiotic therapy in a few anecdotal case reports represents a tempting argument, which corroborates a role for *Borrelia* in sustaining tumor growth (Table 2). Available evidence supporting this principle actually relies on only 11 cases: these patients were treated with a variable combination of antibiotics and often followed up for only a short period, with the intrinsic difficulty of drawing any conclusion about treatment efficacy. In fact, only half of the reported cases showed a tumor response; most importantly, because these cases were not reported within a clinical trial, it is likely that unsuccessfully treated cases were not reported, leading to activity overestimation. Along this line, antibiotic treatment should be indicated only in areas endemic for *Borrelia* infection, using regimens containing i.v. ceftriaxone or oral amoxicillin (Table 2).

CONCLUSION

The prevalence of *Borrelia* infection in tumor tissue is extremely rare in patients with cutaneous lymphoma diagnosed

in areas nonendemic for this microorganism and/or Lyme disease. In these areas, *Borrelia* probably has no pathogenetic role in cutaneous lymphomas; accordingly, the search for it is expensive and has no clinical relevance, rendering antibiotic therapy useless. Conversely, in areas endemic for *Borrelia* infection, the use of antibiotics like ceftriaxone would constitute a rational choice to induce objective clinical responses in patients with these malignancies.

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Maurilio Ponzoni and Andrés J. M. Ferreri contributed equally to this work. Emilio Berti and Riccardo Dolcetti share senior authorship.

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