G ITAL DERMATOL VENEREOL 2009;144:157-71

The expanding spectrum of cutaneous borreliosis

K. EISENDLE, B. ZELGER

Department of Dermatology and Venerology
Innsbruck Medical University, Innsbruck, Austria

The known spectrum of skin manifestations in cutaneous Lyme disease is continuously expanding and can not be regarded as completed. Besides the classical manifestations of cutaneous borreliosis like erythema (chronicum) migrans, borrelial lymphocytoma and acrodermatitis chronica atrophicans evidence is growing that at least in part also other skin manifestations, especially morphea, lichen sclerosus and cases of cutaneous B-cell lymphoma are causally related to infections with *Borrelia*. Also granuloma annulare and interstitial granulomatous dermatitis might be partly caused by *Borrelia burgdorferi* or similar strains. There are also single reports of other skin manifestations to be associated with borrelial infections like cutaneous sarcoidosis, necrobiosis lipoidica and necrobiotic xanthogranuloma. In addition, as the modern chameleon of dermatology, cutaneous borreliosis, especially borrelial lymphocytoma, mimics other skin conditions, as has been shown for erythema anulare centrifugum or lymphocytic infiltration (Jessner Kanof) of the skin.

KEY WORDS: Borrelia infections - Lymphoma, B-cell - Granuloma annulare - Immunohistochemistry - Lyme disease - Necrobiosis lipoidica.

The germ *Borrelia (B.) burgdorferi* is a slowly growing microaerophil gram negative spirochete. The generation time is about 7-12 hours.[1, 2] At the time 13 different species are included in the *B. burgdorferi sensu latu* (s.l.) complex. They show different geographic distributions and are associated with different vectors and hosts. For example *B. garinii* and *B. afzellii* are frequently found in Europe but not in the United States. The different species of *Borrelia* also show different patterns of pathogenicity.[3] Only *B. burgdorferi sensu strictu*, *B. garinii*, *B. afzelii* and *B. spielmanii* are clearly known to cause disease in the human host. Different *Borrelia* species also show a different tissue preference in humans (tissue tropism). While *B. burgdorferi sensu strictu* mainly affects the joints, *B. garinii* shows a preference for the nervous system, while *B. afzelii* most frequently affects the skin.[4] The pathogenicity for other species like *B. valaisiana, B. lusitaniae* und *B. lonestari* is not definitely clear. All the other species, like *B. bisetii, B. japonica* or *B. californiensis* seem not to cause disease in the human host. In addition there are a few atypical and not yet classified strains of *B. burgdorferi* s.l. in Europe and in the USA.[5, 6]

Abbreviation list.—ACA: acrodermatitis chronica atrophicans; BL: borrelial lymphocytoma; EM: erythema migrans; FFM: focus floating microscopy; LS: lichen sclerosus

Fundings.—None.
Conflict of interests.—None.

Corresponding author: K. Eisendle, Department of Dermatology and Venerology, Innsbruck Medical University, Anichstr 35, 6020 Innsbruck, Austria. E-mail: Klaus.eisendle@uki.at

Overview about the diseases caused by B. burgdorferi

Lyme disease is the most frequent tick born disease in the northern hemisphere.[7] *Borrelia* are mainly transmitted in Europe by *Ixodes (I.) ricinus*, in Asia by *I. persulcatus* and in the United States by *I. scapularis*,

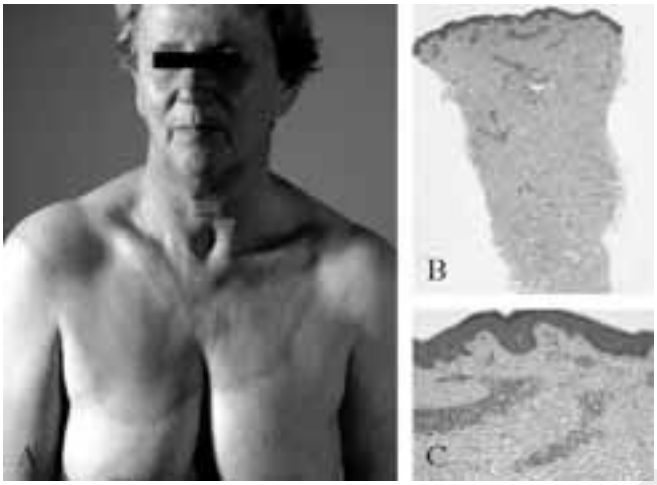

Figure 1.—A) Characteristic clinical manifestation of erythema migrans; B) histological examination (H&E, x20) reveals superficial and middermal dense perivascular infiltrate of (C) lymphocytes, some plasma cells, and an increase of fibroblasts and mucin between collagen bundles (H&E, x200).

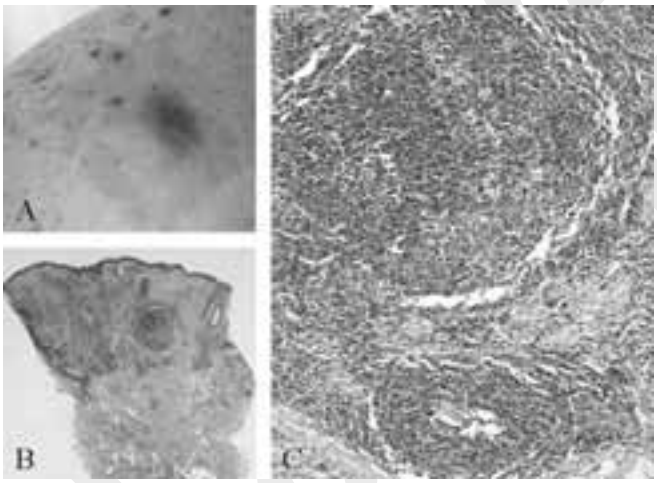

Figure 2.—A) Borrelial lymphocytoma with concomitating erythema migrans with characteristic histological features (B) of dense infiltrates of lymphocytes (H&E, x10) with (C) follicle formation (H&E, x200).

I. pacificus or *Amblyomma americanum,* also named "lone star tick" for the prominent white dot on the back of the adult female.[6, 8] *Borrelia* cause characteristic diseases and, similar to syphilis, borreliosis has been separated into three stages. Stage I (stage of first manifestation) comprises the *erythema migrans* (EM, Figure 1A) and the early borrelial lymphocytoma (B, Figure 2A), which develop weeks to months after the tick bite and are accompanied by mild influenza like symptoms. The second stage (stage of dissemination) includes the involvement of the musculoskeletal system (acute Lyme arthritis with additional painful muscles, tendons, bursae and bones), the nervous system (meningitis, *lymphocytic meningoradiculoneuritis*, also called Bannwarth syndrome, mild encephalitis and myelitis), the heart (atrioventricular block, myopericarditis, pancarditis), the skin with late borrelial lymphocytoma, acute inflammatory *acrodermatitis chronica atrophicans* (ACA Figure 3A) and multiple *erythemata migrantia*, as well as the involvement of all other organs (lymphadenopathy, splenomegaly, hepatitis, mild haematuria, conjunctivitis, iritis and ophthalmitis). The third stage ("stage of chronicity") is limited to organ diseases with irreversible organic or functional damage in joints, nervous system or in the skin. This are in the case of skin diseases chronic-atrophic stages of ACA and in part morphea (Figure 4A) and lichen sclerosus. These chronic changes develop after months to years.[1, 9] Single stages might overlap or be completely missing.[10, 11]

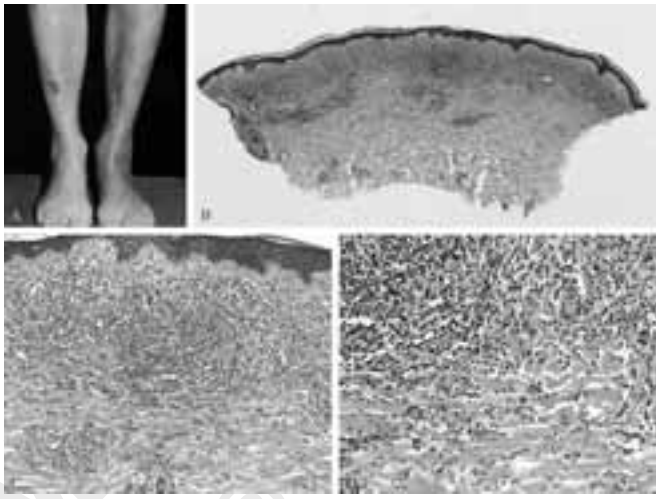

Figure 3.—A) Acrodermatitis chronica atrophicans of the left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins!); B) histology (H&E) reveals a dense lichenoid and middermal perivascular infiltrate with (C) hints of follicle formation composed of (D) lymphocytes, some plasma cells, and an increase of firbroblasts between fibrosclerotic collagen bundles (H&E, x200).

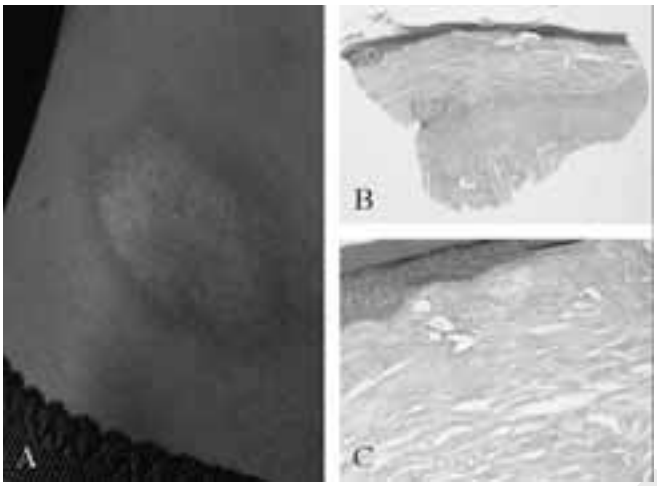

Figure 4.—A) Late inflammatory poor "burned out" morphea; B, C) histopathology showing nearly absence of inflammatory infiltrates, atrophy of epidermis and marked sclerosis (H&E, b x10, c x100).

History of cutaneous borreliosis

In the newer literature infections with *B. burgdorferi sensu latu* are named after the city Lyme in Connecticut although first descriptions have occurred long before in Europe.[12] Already Afzelius wrote in 1909 in the *Archiv für Dermatologie und Syphilis* about an EM caused by the tick *Ixodes reduvius* (*Ixodes scapularis* in the new taxonomy) and recognized already the tick as vector for this disease.[13] The first description and nomination of ACA occurred even earlier in the year 1902 by Herxheimer and Hartmann.[14] The BL was first described as *lymphadenosis cutis benigna* by Brävferstedt in 1943, Brävferstedt also suspected insect bites, mainly tick bites, as triggers of the disease.[15] Finally, Willy Burdorfer discovered the spirochetal etiology of borreliosis (Lyme disease) in 1982 and the pathogenic agent was named *B. burgdorferi* in his honor.[12, 16]

Histopathologic patterns in classical cutaneous borreliosis

The dermatohistopathological changes in cutaneous borreliosis are a consequence of the continuous antigenic stimulus by persisting *Borrelia* in the tissue. The consequence are histopathologic similarities in between the different cutaneous manifestations, like structural changes in the collagen texture and the frequent presence of B lymphocytes, especially plasma cells. B lymphocytes can be easily shown by staining with anti-CD20 antibodies. The different clinical and histological manifestations can be explained by the location and duration of the infection and by the different known borrelial strains, on the other hand also by the dominating immune response involving B and T cells, as well as the number of inoculated bacteria and the genetic predisposition of the infected host (HLA type, disposition to autoimmune reactions). The damage in the connective tissue collagen is on one hand the result of the inflammatory infiltrate ("cytokine storm", collateral damage), on the other hand *Borrelia* show collagenotropism and might directly influence and damage the collagen structure.[7]

Histopathology of classical cutaneous borrelioses is characteristic, but unfortunately non specific, meaning that the diagnosis is first of all a clinical one. In the case of EM histopathology shows perivascular lymphocytic infiltrations not always containing plasma cells in all the dermal layers, sometimes with admixture of eosinophilic granulocytes and macrophages, as well as slight changes in the structure of the collagen texture in the connective tissue (Figure 1 B,C).[17] In the case of ACA (Figure 3 B,C), depending on the duration of the disease, the changes include more or less pronounced atrophy of the epidermis, dermis and subcutaneous tissue with ectatic capillaries in the upper corium. The inflammatory infiltrate is accentuated perivascular or even bandlike and contains plasma cells. In addition there is a loss of elastic fibers with the development of fibrosis or incipient sclerosis of the papillary and reticular dermis.[18] Some authors consider BL as special form of an EM. Histopathology shows an unremarkable epidermis with sharp defined partially confluent lymphocytic infiltrates (Figure 2 B,C). There are two histopathologic types of BL with (follicular type) or without (diffuse/nodular type) follicular structures, resembling the germinal centers of lymph nodes. Combinations between the two types can be observed. Plasma cells and sometimes eosinophils and multinucleated giant cells can additionally be found at the border of the infiltrate. An unaltered grenz zone between the epidermis and the lymphocytic infiltrate can regularly be observed.[19, 20] Table I shows the expression of leukocyte differentiation antigens based on a score of 0-3+ in lesional skin

TABLE I.—*Expression of leukocyte differentiation antigens based on a score of 0-3+ in lesional skin of patients with various manifestations of dermatoborrelioses (EM erythema migrans, BL borrelial lymphocytoma, ACA acrodermatitis chronica atrophicans).*[19]

Score: 0-3+ macrophages	CD68+ T cells	CD3+ T cells	CD4+ T cells	CD8+ B cells	CD20+
EM (N=12)	++	++	+/-	+++	+
BL (N=5)	+++	++	+/-	++	+++
ACA (N=10)	++	++	+/-	++	++

of patients with classical manifestations of dermatoborrelioses.[19]

Diagnostic aids for cutaneous borreliosis

After initial enthusiasm,[21] the detection of microorganisms has turned out to be difficult, frequently unreliable, and almost always extremely time-consuming by different procedures, including histochemical stains (Gram, Wright, Wright-Giemsa, and polychromes), fluorochromes (thioflavine-T, acridine orange, and rhodamine), silver impregnation techniques (Warthin-Starry, modified Dieterle, modified microwave-Dieterle, and Bosma-Steiner) in the 1980s,[22-26] and immunohistochemical analysis in the 1990s.[22, 23, 27-29] Serologic techniques (immunofluorescence, enzyme linked immuno sorbent assay [ELISA], and immunoblot) are similarly unsatisfying, with false-negative (20-80%) and false positive results occasionally due to cross-reactions with *Treponema pallidum* or, more commonly, to a positive endemic background of 20% to 30% in many parts of Europe.[23, 30, 31] Cultures with specified media such as modified Pettenkofer-Kelly or Barbour-Stoenner-Kelly can detect *Borrelia* in all clinical forms, but these techniques are limited to special laboratories and are unreliable, with less than 50% sensitivity.[32] Moreover the time delay to get a positive culture can be up to four weeks.[4, 33] Molecular techniques initially seemed to solve the riddle,[34-36] but in due course, it became clear that sensitivity varies (30-90%) according to the *Borrelial* strains, the material (fresh frozen tissue or paraffin material), and the applied primers. There is further a risk of contamination leading to false positive results.[4, 37-40] So, cutaneous borreliosis remains a diagnosis based on circumstantial evidence combining clinicopathologic and laboratory information and clinical response to therapy.

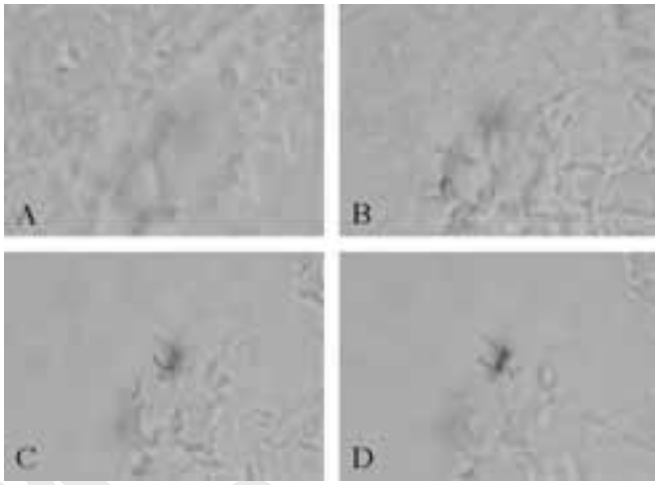

Figure 5.—Immunhistochemistry for Borrelia. Four photomicrographs of the same area focusing through the thickness of this section (focus floating microscopy). Note the (A) absence to varying appearance (B-D) of a cluster of Borrelia (Acris BP 1002, no counterstain, x1000).

Immunhistochemistry and focus floating microscopy

The histopathologic diagnosis was recently made easier by the direct detection of the pathogen by immunhistochemistry and focus floating microscopy (FFM).[41] The method is an advancement to older immunhistochemistry techniques employing a polyclonal anti-borrelial antibody, which recognizes all different borrelial strains. FFM combines several strategies to detect minuscule organisms in tissue sections. The key point to this technique is an almost holoscopic approach to the slide by tuning the focus of the microscope through the thickness of the slide (3-4 μm). So with FFM the section is scanned through in two planes: horizontally in serpentines as in routine cytology, and, simultaneously, vertically at a magnification of 200 to 400 times.[42] This approach allows detection of *B. burgdorferi* (diameter 0.2 μm compared to 2 μm of collagen bundles) which pass through the section at various angles and accordingly may appear as undulated, comma-like to dot forms (Figure 5). In addition omission of counter stain as well as bright illumination of the scanning field proves to be helpful as the bright red color of the 3-amino-9-ethylcarbazole-stained microorganisms best contrasts with the faint yellow color of unstained collagen bundles as well as other tissue structures. The technique can be applied successfully on fresh material, nitrogen-frozen material and paraf-

TABLE II.—*Detection of Borrelia in classical cutaneous Lyme disease by focus floating microscopy (FFM) only and by direct comparison of FFM with PCR.*

Diagnosis	Borrelia positive cases [%]		
	FFM only	FFM	PCR
Ticks	47/71 [66.2]	NA	NA
Tick bites	34/66 [51.5]	NA	NA
Erythema chronicum migrans	15/17 [88.2]	15/15 [100]	7/15 [46.7]
Borrelial lymphocytoma	22/24 [91.7]	19/19 [100]	4/19 [21.1]
Acrodermatitis chronica atrophicans	22/23 [95.7]	28/28 [100]	17/28 [60.7]
Controls*	0/109 [0]	1/60 [1.7]**	0/66 [0]+
Total number of cases	310	122	128

NA (not available). *including atopic and stasis dermatitis, prurigo, insect bite, scabies, (false positive) secondary syphilis**, drug eruption, psoriasis, pityriasis rubra pilaris, lichen planus, erythema multiforme, urticaria, polymorphous light eruption, lupus erythematodes, systemic scleroderma, pemphigus seborrhoicus, Wells syndrome, rheumatoid nodule, ganglion, foreign body reaction due to ruptured infundibular cyst, folliculitis, acne, rosacea, lichen nitidus, (hypertrophic) scar, keloid, dermatofibroma, fibroma molle, connective tissue naevus, melanocytic naevi, lentigo senilis, seborrheic keratoses, actinic keratoses, squamous and basal cell carcinomas, mycosis fungoides. +in six cases no material was left on paraffin blocks for FFM.

fin-embedded material and it is an easy, quick and inexpensive method to reliably detect *Borrelia* in cutaneous tissue sections.

FFM proved to be more sensitive than PCR- and ELISA-PCR in cases of classical borreliosis. Cases of prominent *Borrelia* detection by FFM are usually positive by PCR, which becomes negative in later stages as the number of microorganisms drop. So, it was possible to detect *Borrelia* by FFM in 47 of 71 ticks, in 34 of 66 tick bites, in 30 of 32 cases of EM, in 41 of 43 cases of BL (Figure 5) and in 50 of 51 cases of ACA. With a sensitivity of over 90% FFM was more sensitive than PCR with a sensitivity of 45% (P<0.001) and nearly equally specific (99% *versus* 100%). All control cases, except one case of false-positive secondary syphilis, were negative with FFM (Table II).[42]

Detection of B. burgdorferi in morphea

Morphea is an inflammatory connective tissue disorder of unknown etiology. The involvement of *B. burgdorferi* as a causative agent was first proposed by Aberer *et al.* in 1985.[43] Since then conflicting results have been obtained by different studies using serological, immunohistochemical, culture and PCR approaches.[44-58] *Borrelia* has been frequently detected in Europe and Asia patients, but not in cases from the United States or Scotland.[59-77] Studies reporting a positive association between *B. burgdorferi* infection and morphea found evidence of the organism in 26-100% of cases (Table III); on the other hand there are at least 10 reports where no positive cases could be

TABLE III.—*Literature with direct detection of Borrelia in morphea.*

Author	Country	Year	Method	Results pos./n
Aberer *et al.*[44]	Austria	1987	Immunoperoxidase	7/21
Aberer *et al.*[45]	Austria	1987	Culture	1/4
Weber *et al.*[57]	Germany	1988	Culture	1/1
Aberer *et al.*[46]	Austria	1988	Immunoperoxidase	3/9
Ross *et al.*[48]	Puerto Rico	1990	Silver stain	10/25
Aberer *et al.*[47]	Austria	1991	Culture	1/11
Schempp *et al.*[49]	Germany	1993	PCR	9/9
Schempp *et al.*[58]	Germany	1993	PCR/immunohistochemistry	1/1
Weidenthaler *et al.*[74]	Germany	1994	PCR	1/1
Granter *et al.*[66]	USA	1994	PCR	1/1
Trevisan *et al.*[51]	Italy	1996	PCR	6/10
Fujiwara *et al.*[61]	Japan	1997	PCR	2/5
	Germany	1997	PCR	3/4
Breier *et al.*[75]	Austria	1999	Culture	1/1
Özkan *et al.*[54]	Turkey	2000	PCR	3/10
Hercogova [69]	Czech Republic	2002	PCR	1/1
Eisendle *et al.*[77]	Germany/Austria	2007	PCR	1/29
			FFM	84/122

Pos. positive; N number

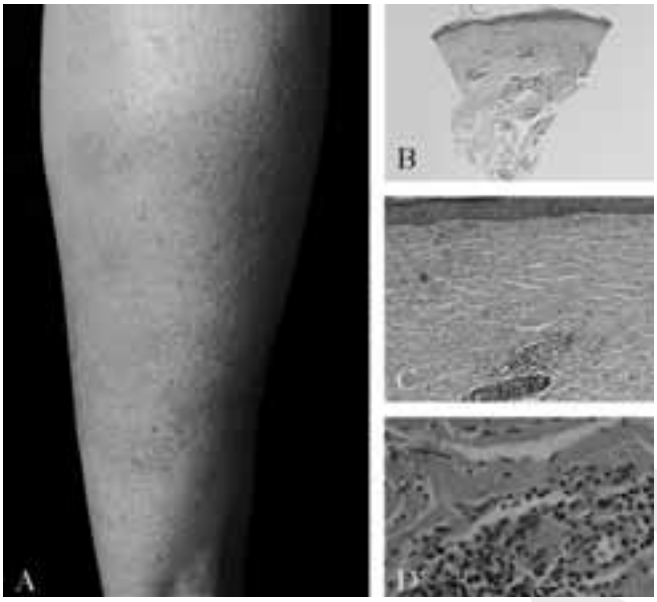

Figure 6.—Morphea (A) Clinical picture of early plaque morphea; B,C) histopathology of morphea at different magnifications showing dense infiltrates of lymphocytes and plasma cells and fibrosclerosis (H&E, b x4, c x100, d x200).

identified. The different studies concerning the detection of *Borrelia* in morphea have been reviewed by Weide *et al.* 2000 [63] and Goodland *et al.* 2002.[55] One major difficulty in assessing the association between morphea and borreliosis is the challenge of reliably detecting *Borrelia* in tissue specimens. These conflicting results at least in part reflect the difficulties of the various techniques used to prove/document the participation of *Borrelia* in the disease process.

Some patients with morphea show clinical similarities to classical borreliosis (Figure 6A). Early stages of morphea with a prominent lilac ring are not always easy to differentiate from EM with its active border. In late stages red-purple-brown macules of burned out morphea may mimic ACA. Remarkably, these clinical entities can occur at the same time in the same patient, and co-existent morphea has been described in patients with EM, BL, ACA and Lyme arthritis.[64-69] Moreover, there are descriptions of new patterns in borreliosis such as interstitial granulomatous dermatitis, which clinically most closely resembles morphea.[70] There are also similarities in the histopathological findings (variable infiltrates of lymphocytes, macrophages and plasma cells; slight vacuolar degeneration of the basal layer in late stages; an increase of fibrocytes/fibroblasts with variable fibrosis to sclerosis) of EM and ACA on the one hand and morphea on the other.[71] A bacterial etiology is further suggested because some cases of morphea respond well to antibiotic therapy,[72, 73] such as penicillin and ceftriaxone.[45, 74, 75] D-penicillamine, discussed in older text books as inhibiting cross connections between collagen fibers, was likely to have been effective because of its metal-chelating activity which deprived the bacteria of essential trace elements such as manganese, zinc and magnesium.[76]

In a recent large study of 122 morphea cases *Borrelia* could be detected in more than 68% of all cases with a significantly higher percentage (P=0.018) in active (75.0%) than inactive morphea (52.9%). This might reflect intentional or coincidental antibiotic exposure in longer existing cases and/or the natural course of disease with repression of the microorganisms by the immune system. The presence of B lymphocytes as determined by positive staining with CD20 proved to be a good diagnostic predictor for the presence of *Borrelia*, with a positive correlation of 0.85 (P<0.001). This is not surprising as the presence of B lymphocytes reflects an immune response against bacterial infections like borreliosis. The low percentage of detection of borrelial DNA with PCR in this study with one positive case in 30 (3.3%) indicates the problematic role of this technique to reliably detect *Borrelia* in tissue specimens.[77] The reason for the inconsistent results in PCR studies (positive, Table III, negative see [50, 52, 53, 55, 57, 78]) could be the low number of microorganisms found in the tissue with the detection threshold being beyond for this technique. Other explanations include previous antibiotic treatment, old stage of disease, wrong biopsy site (*e.g.* from negative sclerotic area), or wrong fixation of tissue specimens leading to DNA cross-linking e.g. with inadequately buffered formalin. Further — except for the studies done by Ranki 1994,[50] Dillon 1995 [59] and Wienecke 1995 [52] — most other negative PCR studies are lacking appropriate positive controls in terms of detection of borrelial DNA in tissue specimens from classical borreliosis such as EM, BL and ACA.[53, 78, 79] Thus, the reliability of the DNA extraction method for small DNA amounts or the PCR technique used in these studies remains somewhat debatable.

Another explanation for negative PCR results is that *B. burgdorferi* sensu latu includes *B. burgdorferi* sensu strictu, *B. garinii* and *B. afzelii* VS461, but also

newer *Borrelia* species have been identified. The pathogenic significance of these species, such as *B. valaisiana*, *B. hermsii*, *B. turicatae*, *B. parkeri* and most recently *B. spielmanii* is not yet fully answered. While *B. burgdorferi* sensu strictu is the only well-established cause of Lyme disease in the United States, *B. afzelii*, *B. garninii* and probably *B. valaisiana* additionally cause "Lyme disease" in Europe and Asia. Relapsing fever borreliosis by *B. hermsii*, *B. turicatae* and *duttonii* and EM by *B. spielmanii* have been described.[80, 81] The study by van Dam suggests that different *B. burgdorferi* genotypes have different pathogenic potentials.[82] This is well documented for the classical borrelial manifestations, so ACA rarely occurs in the United States but is commonly seen in Europe where *B. afzelii* and *B. garinii* are more prevalent.[83] Maybe, subspecies variations dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of morphea.[55, 61] Thus, another explanation for the moderate results by PCR might be that these techniques use primers highly specific for known human pathogenetic strains, while FFM uses immunhistochemistry with a less specific polyclonal antibody that probably detects most different borrelial species.

So, borreliosis is a vector transmitted disease whose causative agents, *B. burgdorferi* and variants, share collagenotropism, whereas other spirochaetes or spiral-shaped bacteria are epithelio- & endotheliotropic (*e.g. T. pallidum*) or mucotropic (*e.g. H. pylori*). Fibronectin binding proteins of *B. burgdorferi* promote bacterial attachment to glycosaminoglycans which are most prominent in connective tissue between collagen bundles.[84, 85] While the immune system can cope with the organisms in EM and BL comparatively quickly, in ACA and morphea the situation seems more complicated. The low level of microorganisms probably indicates that the disease is not only due to the effect of the infectious agent, but also reflects the challenge for the immune system due to the location of the microorganisms and or even a compromised immune reaction in morphea patients themselves, where *Borrelia* might trigger a subsequent autoimmune reaction.[86] This could also explain why not all patients benefit from antibiotic therapy. Indeed, a recent study published in 2009 by Prinz JC found evidence for the induction of autoimmunity by *Borrelia* infection. The authors examined the relationship between *Borrelia* exposure, serologic autoimmune phenomena and age at disease onset in morphea patients. In 90 morphea patients the presence of *Borrelia*-specific serum antibodies was correlated to the age at disease onset and the presence and titers of antinuclear antibodies. A statistically highly significant association between morphea, serologic evidence of *Borrelia* infection, and high-titer antinuclear antibodies was observed, when disease onset was in childhood or adolescence. The conclusion was that *B. burgdorferi* infection may be relevant for the induction of a distinct autoimmune type of scleroderma, which the authors suggested to be called "*Borrelia*-associated early onset morphea". This condition is characterized by the combination of disease onset at younger age, infection with *B. burgdorferi*, and evident autoimmune phenomena as reflected by high-titer antinuclear antibodies.[87]

Detection of *Borrelia* in patients with *lichen sclerosus*

Lichen sclerosus (LS), frequently reported in the dermatologic literature as *lichen sclerosus et atrophicus*, is a chronic inflammatory skin disease of unknown etiology leading to substantial discomfort and morbidity. It commonly affects adult woman in the genito-anal region (Figure 7A) but also occurs elsewhere (Figure 7B).[88-91] LS has clinical and histological similarities with morphea and some investigators consider this entity a superficial variant of morphea, an opinion supported by its frequent coincidence with morphea.[92, 93] LS further shares similarities and common features with ACA, a chronic form of borreliosis, in particular including histological findings such as an infiltrate of lymphocytes admixed with some plasma cells, an increase of fibrocytes and fibroblasts and a diffuse dermal fibrosis to sclerosis (Figures 3B,C, 7C,D).[94] These observations have led several investigators to consider the possibility of *B. burgdorferi* as a common etiologic factor for both diseases. The involvement of *B. burgdorferi* as a causative agent for LS was first proposed by Aberer *et al.* in 1987 [44] and subsequently further supported at least in part by several other studies (Table IV) [46-50, 54, 59, 61, 62, 78, 79, 95-99] A bacterial etiology is further suggested because several cases of LS respond well to antibiotic therapy, such as dirithromycin, penicillin and ceftriaxone.[96-98] *Borrelia* have frequently been detected in Europe, but not

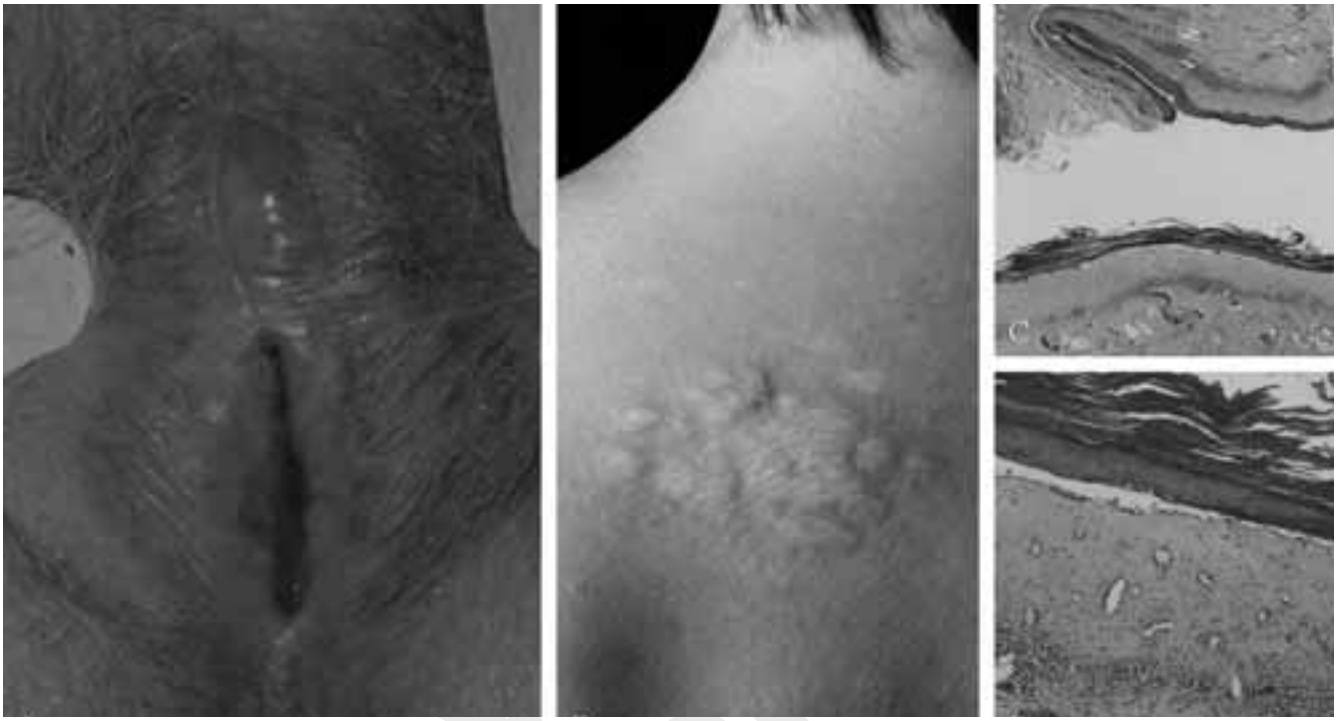

Figure 7.—Clinical photograph of lichen sclerosus in the female genital area (A) and on the upper back of a young female (B). Histopathology at different magnifications (C,D) shows infiltrates of lymphocytes admixed with some plasma cells, an increase of fibrocytes and a diffuse dermal sclerosis (H&E, x100 b, x200 c)

TABLE IV.—*Results of studies investigating B. burgdorferi in lichen sclerosus patients. For the PCR involving studies the amplified gene is shown in parenthesis.*

Author	Country	Year	Method	Results pos./N
Aberer *et al.*[44]	Austria	1987	Immunoperoxidase	6/13
Aberer *et al.*[46]	Austria	1988	Immunoperoxidase	1/2
Ross *et al.*[48]	Puerto Rico	1990	silver stain	10/21
Schempp *et al.*[49]	Germany	1993	PCR (Flagellin)	6/6
Ranki *et al.*.[50]	Finland	1994	PCR (OspA)	0/1
Dillon *et al.*[59]	USA	1995	PCR (Flagellin)	0/10
De Vito *et al.*.[79]	USA	1996	PCR (Clone 2H1)	0/7
Fujiwara *et al.*[61]	Japan	1997	PCR (16s-RNA)	2/3
	Germany		PCR (16s-RNA)	1/10
	USA		PCR (16s-RNA)	0/21
Colomé-Grimmer *et al.* [62]	USA	1997	PCR (Flagellin)	0/10
Alonso-Llamazares *et al.*[63]	Spain	1997	PCR (OspA)	0/8
	Culture	0/1		
Aberer *et al.*[95]	Austria	1999	PCR (Flagellin)	13/1
Özkan *et al.*[95]	Turkey	2000	PCR (Flagellin)	6/12
Breier *et al.*[96]	Austria	2001	Culture	1/1
Eisendle *et al.*[99]	Austria	2006	PCR (23s-RNA)	0/11
			FFM	38/60

Pos. positive, N number

in cases from the United States. Studies reporting a positive association between *B. burgdorferi* infection and LS found evidence of the organism in 10-68% of cases; on the other hand there are reports where no positive cases could be identified (Table IV).

In a recent large study on 61 cases of LS *Borrelia* could be detected in more than 60% of all LS cases, with a significantly higher percentage (P=0.001) in early (79.5%) than late LS (33.3%), while it made no difference if LS was associated with morphea or not (Table V). This might reflect intentional or coincidental antibiotic exposure in longer existing cases or the natural course of disease with repression of the microorganisms by the immune system. The negative detection of borrelial DNA with PCR in this study again indicates the problematic role of PCR to reliably detect *Borrelia* in tissue specimens. The low number of microorganisms beyond the detection threshold [100] could be one explanation for the inconsistent results in PCR studies. Other explanations again include old stage of disease, wrong biopsy site (*e.g.* from negative fibrosclerotic parts), or wrong fixation of tissue specimens leading to DNA cross linking e.g. with inadequately buffered formalin. Further as explained above - negative PCR studies are frequently lacking appropriate positive controls in terms of detection of borrelial DNA in tissue specimens from classical borreliosis.

As in the case for morphea there is another explanation for negative PCR results as beside *B. burgdorferi* sensu latu newer *Borrelia* species have been identified, as mentioned above with unclear pathogenic significance. So, subspecies variations might dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of LS. Thus, like in the case for morphea, an other explanation for the moderate results by PCR is the specificity of the primers used working only for known human pathogenetic strains, while immunhistochemistry with a less specific polyclonal antibody detects most different borrelial species.

In any case, detection of spirochetes in pure LS and LS associated with morphea seems to be a common denominator which indicates the nosologic relationship of these skin disorders.[44, 61] Moreover, the infectious hypothesis with spirochetes helps to explain the most common stereotypical presentation of LS, namely in the genitoanal area. Subclinical dissemination with spread of *Borrelia* to kidneys and urine occurs in early borrelial infection.[101, 102] Favored by the moist and frequently traumatized conditions of genitalia, this might allow a superficial *Borrelia* infection in the perigenital region, *i.e.* LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where *Borrelia* antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry.[86] So, thyroid autoantibodies have been described in 36% of LS patients.[103] *B. burgdorferi* has been proposed as environmental trigger of autoimmune thyroiditis through amino acid sequence homologies between proteins of *B. burgdorferi* and all thyroid autoantigens, like human thyrotropin receptor, human thyroglobulin, human thyroperoxidase and human sodium iodide symporter, or segments thereof.[104, 105] As in the case of morphea the induction of autoimmunity also might explain why not all patients benefit from antibiotic therapy and make an early antibiotic treatment reasonable.

In summary, there are histopathologic similarities between classical borrelioses, morphea and LS. All this diseases show fibrosclerosis of the connective tissue and plasma cells in early inflammatory rich stages (Figures 1-3,6,7). Also clinically it is not always easy to distinguish cases of EM or ACA from early morphea (Figure 6A). Further there are clinical reports of improvement after antibiotic therapy for morphea and

TABLE V.—*Detection of Borrelia by FFM and PCR in LS and controls. Data are given as number positive/total tested (percentage).*

Classification of LS	FFM	PCR
LS ("pure")	33/52 (63.5)	0/5 (0.0)
LS with morphea	5/8 (62.5)	0/6 (0.0)
Stage (activity) of LS		
Inflammatory-rich ("early")	31/39 (79.5)	0/7 (0.0)
Inflammatory-poor ("late")	7/21 (33.3)§	0/4 (0.0)
Total	38/60 (63.3)	0/11 (0.0)
Controls		
Negative*	0/60 (0.0)	0/15 (0.0)
Positive**	61/68 (89.7)§§	25/68 (36.8)§§§

FFM, focus-floating microscopy; PCR, polymerase chain reaction, LS lichen sclerosus

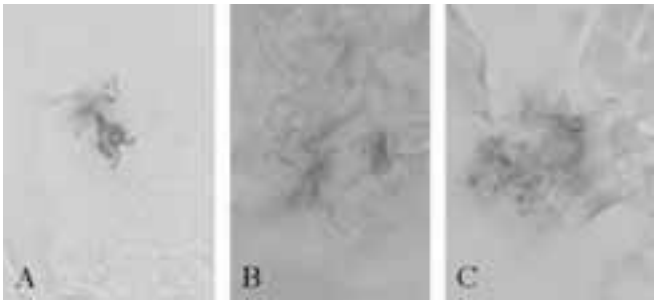

Figure 8.—A) Focus-floating microscopy and immunohistochemical staining (Acris BP 1002, no counterstain, x1000) for various borrelial colony forms. Medusa-like cluster of 'planktonic microorganisms' in a case of acrodermatitis chronica atrophicans. B) Colony of degenerating fragmented/small granular "dying" spirochetes in a case of morphea. C) Putative biofilm formation of a borrelial colony with a mixture of medusa-like and granular spirochetal aggregations with cystic rounded forms, tubular elements or swollen granules covered by a reddish veil in a case of lichen sclerosus.

LS. For these reasons and the reliable detection of spirochetal microorganisms in morphea and LS, one must conclude that at least some cases of morphea and LS should be integrated in the spectrum of cutaneous borrelioses.

Biofilms of *B. burgdorferi sensu latu* in chronic or recurrent cutaneous borreliosis?

The hypothesis that *B. burgdorferi* might form biofilm structures in BL and ACA was recently proposed based on the finding of large colonies of *Borrelia* in classical cutaneous borrelioses shown by immunohistochemicistry and FFM.[106, 107] So, *Borrelia* can grow in a "medusa colony" or in a "granular colony with a reddish veil" [42] (Figure 8 A-C). These forms of borrelial growth were first described in vitro by Aberer and Duray [22] and such colonies reveal striking similarities to previously published biofilm pictures.[108] It is a fascinating hypothesis to compare large borrelial aggregations in the tissues with biofilms and speculate that such biofilms of *B. burgdorferi* might be responsible for a partial resistance to antibiotic therapy in some patients with Lyme disease. Subsequently, the potential that *Borrelia* may shed from these biofilms, might thus provide a possible explanation for chronic relapsing courses of some borrelial infections. Of note, biofilm formation in the human host has already been described for other spirochetes like *Treponema denticula*,[109] and biofilm formation has been associated with antibiotic resistance in *Helicobacter pylori* infections.[110] Bacterial biofilms are responsible for several chronic diseases (*e.g.* periodontitis and chronic lung infection in cystic fibrosis patients) that are very difficult to treat because they show much greater resistance to antibiotics than their free-living counterparts.[111] The biofilm resistance is very unique in a sense that it requires multiple mechanisms such as incomplete penetration of the antibiotics into the matrix, inactivation of antibiotics by altered chemical microenvironment within the biofilm and an altered, protected phenotypic "spore like" state of the resistant bacteria population.[112] If *B. burgdorferi* is indeed capable forming biofilms, it will change the way, how we think about Lyme disease especially in patients,

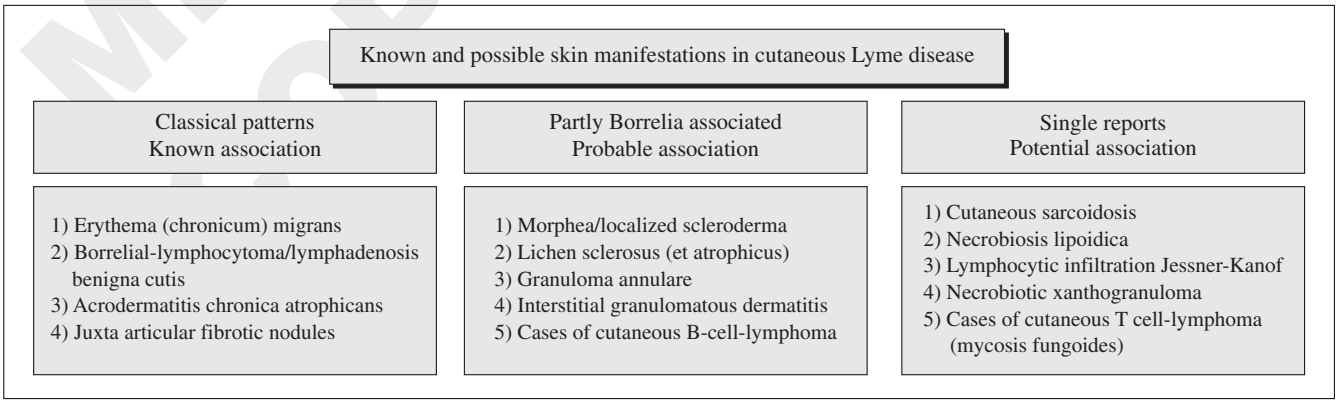

Figure 9.—Summary of the various known and possible skin manifestations in cutaneous borreliosis as described in the literature. The skin diseases are subdivided for known, probable and possible borrelial etiology.
173 voci biblio in editing da Catia.

where it seems to be persistent despite antibiotics treatment.[113] It would be interesting to see if patients with big borrelial colonies that might resemble biofilms are the ones that are more resistant to antibiotic treatment. More research on this topic will answer these questions.

Conclusions

The classical manifestations of cutaneous borreliosis are EM, BL — a cutaneous B-cell-pseudolymphoma (or *lymphadenosis cutis benigna*) — ACA and iuxtaarticular fibrinoid nodules which are related to ACA.[114, 115] At least in part also other skin manifestations – as described above — especially morphea,[43-49, 77, 87, 116, 117] *lichen sclerosus* [44, 49, 58, 75, 96, 99, 118] and cases of cutaneous B-cell lymphoma [119-126] are causally related to infections with *Borrelia*. In the case of cutaneous B-cell lymphoma the pathogenic mechanism seems to be analogous to the one discussed for MALT-lymphomas which are induced by chronic *Helicobacter pylori* infection.[127-138] The evidence is also growing that granuloma annulare [139-147] and interstitial granulomatous dermatitis [148-150] might be partly caused by *B. burgdorferi* or similar strains. There are single reports which connect other skin diseases to *Borrelia*, for example cutaneous sarcoidosis, especially in the Chinese literature,[151-164] then necrobiosis lipoidica,[165] necrobiotic xanthogranuloma [166] and cases of mycosis fungoides,[167] but the evidence for the latter skin diseases is not unambiguous. In addition, as the modern chameleon of dermatology, cutaneous borrelioses, especially BL, mimic other skin conditions, as has been shown for erythema anulare centrifugum or lymphocytic infiltration of the skin (Jessner-Kanof).[168-173] In summary, it can be concluded that the known spectrum of skin manifestations in cutaneous borreliosis is continuously expanding and can not be regarded as completed (Figure 7).

Riassunto

Lo spettro in espansione della borreliosi cutanea

Lo spettro conosciuto delle manifestazioni cutanee della malattia di Lyme è in continua espansione e non può essere considerato come completato. Accanto alle classiche manifestazioni della borreliosi cutanea, quali l'eritema (cronico) migrante, il linfocitoma borrelioso e l'acrodermatite cronica atrofizzante, sta crescendo l'evidenza che almeno in parte anche altre manifestazioni cutane siano correlate all'infezione da *Borrelia*, specialmente la morfea, il lichen sclerosus ed alcuni casi di linfoma cutaneo a cellule B. Anche il granuloma annulare e la dermatite granulomatosa interstiziale potrebbero in parte essere provocati da *Borrelia burgdorferi* o da ceppi simili. Vi sono anche segnalazioni isolate di altre manifestazioni cutanee associate alle infezioni da *Borrelia* quali la sarcoidosi cutanea, la necrobiosi lipidica e lo xantogranuloma necrobiotico. Inoltre, come un camaleonte dermatologico, la borreliosi cutanea, specialmente nel caso del linfocitoma borrelioso, mima altre patologie cutanee, come è stato dimostrato per l'eritema annulare centrifugo o l'infiltrazione linfocitica (Jessner Kanof) della cute.

Parole chiave: Infezioni da Borrellia - Linfoma a cellule B - Granuloma annulare - Immunoistochimica - Malattia di Lyme - Necrobiosi lipoidica.

References

1. Southwick F. Infectious diseases: a clinical short course. NewYork: McGraw Hill; 2007.
2. Strle F. Principles of the diagnosis and antibiotic treatment of Lyme borreliosis. Wien Klin Wochenschr 1999;111:911-5.
3. Gern L. *Borrelia burgdorferi sensu lato*, the agent of lyme borreliosis: life in the wilds. Parasite 2008;15:244-7.
4. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 2007;49:13-21.
5. Postic D, Garnier M, Baranton G. Multilocus sequence analysis of atypical *Borrelia burgdorferi sensu lato* isolates: description of *Borrelia californiensis* sp. nov., and genomospecies 1 and 2. Int J Med Microbiol 2007;297:263-71.
6. James AM, Liveris D, Wormser GP, Schwartz I, Montecalvo MA, Johnson BJ. *Borrelia lonestari* infection after a bite by an *Amblyomma americanum* tick. J Infect Dis 2001;183:1810-4.
7. Aberer E. Lyme borreliosis: an update. J Dtsch Dermatol Ges 2007;5:406-14.
8. Steere AC. Lyme disease. N Engl J Med 2001;345:115-25.
9. Duray PH. Clinical pathologic correlations of Lyme disease. Rev Infect Dis 1989;11 Suppl 6:S1487-S1493.
10. Wormser GP. Clinical practice: early Lyme disease. N Engl J Med 2006;354:2794-801.
11. Stanek G, Strle F. Lyme borreliosis. Lancet 2003;362:1639-47.
12. Norbert S. Klinik der Lyme-Borreliose. Bern: Hans Huber; 2002.
13. Afzelius A. Verhandlungen der dermatologischen Gesellschaft zu Stockholm. Arch Derm Syph 1910;101:404.
14. Herxheimer K, Hartmann D. Über Acrodermatitis chronica atrophicans. Arch Dermatol Syph 1902;61:57-76.
15. Bävferstedt B. Über Lymphadenosis benigna cutis. Eine klinische und pathologisch-anatomische Studie. Acta Derm Venerol (Stockh) 1943;24 Suppl 11:1-102.
16. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease: a tick-borne spirochetosis? Science 1982;216:1317-9.
17. Elder D, Elenitsas R, Jaworsky C, Johnson B. Lever's histopathology of the skin. Philadelphia: Lippcott-Raven; 1997.
18. Kempf W, Hantschke M, Kutzner H, Burgdorf W. Dermatopathologie. Darmstadt: Steinkopff; 2007.
19. Weger W, Müllegger RR. Histopathology and immunohistochemistry of dermatoborrelioses. Acta Dermatoven APA 2001;10: 135-42.

20. Grant-Kels J. Color atlas of dermatopathology. New York: Informa Healthcare; 2007.
21. de Koning J, Hoogkamp-Korstanje JA. Diagnosis of Lyme disease by demonstration of spirochetes in tissue biopsies. Zentralbl Bakteriol Mikrobiol Hyg [A] 1986;263:179-88.
22. Aberer E, Duray PH. Morphology of *Borrelia burgdorferi*: structural patterns of cultured *Borreliae* in relation to staining methods. J Clin Microbiol 1991;29:764-72.
23. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin Microbiol Rev 2005;18:484-509.
24. Albrecht S, Hofstadter S, Artsob H, Chaban O, From L. Lymphadenosis benigna cutis resulting from *Borrelia* infection (*Borrelia* lymphocytoma). J Am Acad Dermatol 1991;24:621-5.
25. Frithz A, Lagerholm B. Acrodermatitis chronica atrophicans, erythema chronicum migrans and lymphadenosis benigna cutis - spirochetal diseases? Acta Derm Venereol 1983;63:432-6.
26. de Koning J, Bosma RB, Hoogkamp-Korstanje JA. Demonstration of spirochetes in patients with Lyme disease with a modified silver stain. J Med Microbiol 1987;23:261-7.
27. Aberer E, Kersten A, Klade H, Poitschek C, Jurecka W. Heterogeneity of *Borrelia burgdorferi* in the skin. Am J Dermatopathol 1996;18:571-9.
28. Park HK, Jones BE, Barbour AG. Erythema chronicum migrans of Lyme disease: diagnosis by monoclonal antibodies. J Am Acad Dermatol 1986;15:406-10.
29. Granter SR, Barnhill RL, Hewins ME, Duray PH. Identification of *Borrelia burgdorferi* in diffuse fasciitis with peripheral eosinophilia: borrelial fasciitis. JAMA 1994;272:1283-5.
30. Plörer A, Sepp N, Schmutzhard E, Krabichler S, Trobos S, Schauer G *et al*. Effects of adequate *versus* inadequate treatment of cutaneous manifestations of Lyme borreliosis on the incidence of late complications and late serologic status. J Invest Dermatol 1993;100:103-9.
31. Schmutzhard E, Stanek G, Pletschette M, Hirschl AM, Pallua A, Schmitzberger R *et al*. Infections following tickbites. Tick-borne encephalitis and Lyme borreliosis: a prospective epidemiological study from Tyrol. Infection 1988;16:269-72.
32. Picken MM, Picken RN, Han D, Cheng Y, Ruzic-Sabljic E, Cimperman J *et al*. A two year prospective study to compare culture and polymerase chain reaction amplification for the detection and diagnosis of Lyme borreliosis. Mol Pathol 1997;50:186-93.
33. Berger BW, Kaplan MH, Rothenberg IR, Barbour AG. Isolation and characterization of the Lyme disease spirochete from the skin of patients with erythema chronicum migrans. J Am Acad Dermatol 1985;13:444-9.
34. Melchers W, Meis J, Rosa P, Claas E, Nohlmans L, Koopman R *et al*. Amplification of *Borrelia burgdorferi* DNA in skin biopsies from patients with Lyme disease. J Clin Microbiol 1991;29:2401-6.
35. Guy EC, Stanek G. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. J Clin Pathol 1991;44:610-1.
36. Moter SE, Hofmann H, Wallich R, Simon MM, Kramer MD. Detection of *Borrelia burgdorferi sensu lato* in lesional skin of patients with erythema chronicum migrans and acrodermatitis chronica atrophicans by ospA-specific PCR. J Clin Microbiol 1994;32:2980-8.
37. Pachner AR, Ricalton N, Delaney E. Comparison of polymerase chain reaction with culture and serology for diagnosis of murine experimental Lyme borreliosis. J Clin Microbiol 1993;31:208-14.
38. von Stedingk LV, Olsson I, Hanson HS, Asbrink E, Hovmark A. Polymerase chain reaction for detection of *Borrelia burgdorferi* DNA in skin lesions of early and late Lyme borreliosis. Eur J Clin Microbiol Infect Dis 1995;14:1-5.
39. Brettschneider S, Bruckbauer H, Klugbauer N, Hofmann H. Diagnostic value of PCR for detection of *Borrelia burgdorferi* in skin biopsy and urine samples from patients with skin borreliosis. J Clin Microbiol 1998;36:2658-65.
40. Wienecke R, Neubert U, Volkenandt M. Molecular detection of *Borrelia burgdorferi* in formalin-fixed paraffin-embedded lesions of Lyme disease. J Cutan Pathol 1993;20:385-8.
41. White KP, Barry CI, Patterson JW. Focus-floating microscopy for detecting *borrelia* species in tissue sections: back to basics. Arch Dermatol 2008;144:662-3.
42. Eisendle K, Grabner T, Zelger B. Focus floating microscopy: "gold standard" for cutaneous borreliosis? Am J Clin Pathol 2007;127:213-22.
43. Aberer E, Neumann R, Stanek G. Is localised scleroderma a *Borrelia* infection? Lancet 1985;2:278.
44. Aberer E, Stanek G. Histological evidence for spirochetal origin of morphea and lichen sclerosus et atrophicans. Am J Dermatopathol 1987;9:374-9.
45. Aberer E, Stanek G, Ertl M, Neumann R. Evidence for spirochetal origin of circumscribed scleroderma (morphea). Acta Derm Venereol 1987;67:225-31.
46. Aberer E, Kollegger H, Kristoferitsch W, Stanek G. Neuroborreliosis in morphea and lichen sclerosus et atrophicans. J Am Acad Dermatol 1988;19:820-5.
47. Aberer E, Klade H, Stanek G, Gebhart W. *Borrelia burgdorferi* and different types of morphea. Dermatologica 1991;182:145-54.
48. Ross SA, Sanchez JL, Taboas JO. Spirochetal forms in the dermal lesions of morphea and lichen sclerosus et atrophicans. Am J Dermatopathol 1990;12:357-62.
49. Schempp C, Bocklage H, Lange R, Kölmel HW, Orfanos CE, Gollnick H. Further evidence for *Borrelia burgdorferi* infection in morphea and lichen sclerosus et atrophicus confirmed by DNA amplification. J Invest Dermatol 1993;100:717-20.
50. Ranki A, Aavik E, Peterson P, Schauman K, Nurmilaakso P. Successful amplification of DNA specific for Finnish *Borrelia burgdorferi* isolates in erythema chronicum migrans but not in circumscribed scleroderma lesions. J Invest Dermatol 1994;102:339-45.
51. Trevisan G, Stinco G, Nobile C. Detection of *Borrelia burgdorferi* in skin biopsies from patients with morphea by polymerase chain reaction. J Eur Acad Dermatol Venereol 1996;6:15-9.
52. Wienecke R, Schlupen EM, Zochling N, Neubert U, Meurer M, Volkenandt M. No evidence for *Borrelia burgdorferi*-specific DNA in lesions of localized scleroderma. J Invest Dermatol 1995;104:23-6.
53. Weide B, Schittek B, Klyscz T, Schuz K, Stark M, Rassner G *et al*. Morphoea is neither associated with features of *Borrelia burgdorferi* infection, nor is this agent detectable in lesional skin by polymerase chain reaction. Br J Dermatol 2000;143:780-5.
54. Özkan S, Atabey N, Fetil E, Erkizan V, Günes AT. Evidence for *Borrelia burgdorferi* in morphea and lichen sclerosus. Int J Dermatol 2000;39:278-83.
55. Goodlad JR, Davidson MM, Gordon P, Billington R, Ho-Yen DO. Morphoea and *Borrelia burgdorferi*: results from the Scottish Highlands in the context of the world literature. Mol Pathol 2002;55:374-8.
56. Palacios R, Torres A, Trujillo R. IgG antibody reactivity to *Borrelia burgdorferi sensu stricto* antigens in patients with morphea in Colombia. Int J Dermatol 2003;42:882-6.
57. Weber K, Preac-Mursic V, Reimers CD. Spirochetes isolated from two patients with morphea. Infection 1988;16:25-6.
58. Schempp C, Bocklage H, Owsianowski M, Lange R, Orfanos CE, Gollnick H. [*In vivo* and *in vitro* detection of *borrelia* infection in morphea-like skin changes with negative *Borrelia* serology]. Hautarzt 1993;44:14-8.
59. Dillon WI, Saed GM, Fivenson DP. *Borrelia burgdorferi* DNA is undetectable by polymerase chain reaction in skin lesions of morphea, scleroderma, or lichen sclerosus et atrophicus of patients from North America. J Am Acad Dermatol 1995;33:617-20.
60. Fan W, Leonardi CL, Penneys NS. Absence of *Borrelia burgdorferi* in patients with localized scleroderma (morphea). J Am Acad Dermatol 1995;33:682-4.
61. Fujiwara H, Fujiwara K, Hashimoto K. Detection of *Borrelia*

burgdorferi DNA (*B. garinii* or *B. afzelii*) in morphea and lichen sclerosus et atrophicans tissue of German and Japanese but not of US patients. Arch Dermatol 1997;133:41-4.
62. Colome-Grimmer MI, Payne DA, Tyring SK, Sanchez RL. *Borrelia burgdorferi* DNA and *Borrelia hermsii* DNA are not associated with morphea or lichen sclerosus et atrophicus in the Southwestern United States. Arch Dermatol 1997;133:1174.
63. Weide B, Walz T, Garbe C. Is morphoea caused by *Borrelia burgdorferi*? A review. Br J Dermatol 2000;142:636-44.
64. Asbrink E, Hovmark A, Olsson I. Clinical manifestations of acrodermatitis chronica atrophicans in 50 Swedish patients. Zentralbl Bakteriol Mikrobiol Hyg [A] 1986;263:253-61.
65. Buchner SA. [Morphea--a tick transmitted borreliosis of the skin? A contribution to the pathogenesis of circumscript scleroderma]. Z Hautkr 1989;64:661-4, 667-9.
66. Granter SR, Barnhill RL, Hewins ME, Duray PH. Identification of *Borrelia burgdorferi* in diffuse fasciitis with peripheral eosinophilia: *borrelial fasciitis*. JAMA 1994;272:1283-5.
67. Duray PH, Asbrink E, Weber K. The cutaneous manifestations of human Lyme disease: a widening spectrum. Adv Dermatol 1989;4:255-75.
68. Asbrink E, Hovmark A, Olsson I. Lymphadenosis benigna cutis solitaria- *Borrelia* lymphozytoma in Sweden. Zentralbl Bakeriol Mikrobiol Hyg [A] 1989; 263 Suppl 18:159-63.
69. Hercogova J. *Borrelia burgdorferi*: a protagonist in Lyme disease, a bystander in morphoea? J Eur Acad Dermatol Venereol 2002;16:98-9.
70. Moreno C, Kutzner H, Palmedo G, Goerttler E, Carrasco L, Requena L. Interstitial granulomatous dermatitis with histiocytic pseudorosettes: a new histopathologic pattern in cutaneous borreliosis. Detection of *Borrelia burgdorferi* DNA sequences by a highly sensitive PCR-ELISA. J Am Acad Dermatol 2003;48:376-84.
71. Aberer E, Klade H, Hobisch G. A clinical, histological, and immunohistochemical comparison of acrodermatitis chronica atrophicans and morphea. Am J Dermatopathol 1991;13:334-41.
72. Buchner SA, Rufli T. [Morphaea profunda]. Hautarzt 1990;41:155-7.
73. Abele DC, Bedingfield RB, Chandler FW, Given KS. Progressive facial hemiatrophy (Parry-Romberg syndrome) and borreliosis. J Am Acad Dermatol 1990;22:531-3.
74. Weidenthaler B, Roux M, Moter SE, Schulze HJ, Kramer MD. Sclerodermiform skin changes in *Borrelia burgdorferi* infection. Diagnostic use of polymerase chain reaction. Hautarzt 1994;45:171-5.
75. Breier FH, Aberer E, Stanek G, Khanakaha G, Schlick A, Tappeiner G. Isolation of *Borrelia afzelii* from circumscribed scleroderma. Br J Dermatol 1999;140:925-30.
76. Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. Science 2000;288:1651-3.
77. Eisendle K, Grabner T, Zelger B. Morphoea: a manifestation of infection with *Borrelia* species? Br J Dermatol 2007;157:1189-98.
78. Alonso-Llamazares J, Persing DH, Anda P, Gibson LE, Rutledge BJ, Iglesias L. No evidence for *Borrelia burgdorferi* infection in lesions of morphea and lichen sclerosus et atrophicus in Spain. A prospective study and literature review. Acta Derm Venereol 1997;77:299-304.
79. De Vito JR, Merogi AJ, Vo T, Boh EE, Fung HK, Freeman SM *et al*. Role of *Borrelia burgdorferi* in the pathogenesis of morphea/scleroderma and lichen sclerosus et atrophicus: a PCR study of thirty-five cases. J Cutan Pathol 1996;23:350-8.
80. Foldvari G, Farkas R, Lakos A. *Borrelia spielmanii* erythema migrans, Hungary. Emerg Infect Dis 2005;11:1794-5.
81. Rebaudet S, Parola P. Epidemiology of relapsing fever borreliosis in Europe. FEMS Immunol Med Microbiol 2006;48:11-5.
82. van Dam AP, Kuiper H, Vos K, Widjojokusumo A, de Jongh BM, Spanjaard L *et al*. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin Infect Dis 1993;17:708-17.
83. Nadelman RB, Wormser GP. Lyme borreliosis. Lancet 1998;352:557-65.
84. Parveen N, Caimano M, Radolf JD, Leong JM. Adaptation of the Lyme disease spirochete to the mammalian host environment results in enhanced glycosaminoglycan and host cell binding. Mol Microbiol 2003;47:1433-44.
85. Fischer JR, LeBlanc KT, Leong JM. Fibronectin binding protein BBK32 of the Lyme disease spirochete promotes bacterial attachment to glycosaminoglycans. Infect Immun 2006;74:435-41.
86. Behar SM, Porcelli SA. Mechanisms of autoimmune disease induction. The role of the immune response to microbial pathogens. Arthritis Rheum 1995;38:458-76.
87. Prinz JC, Kutasi Z, Weisenseel P, Pótó L, Battyáni Z, Ruzicka T. "*Borrelia*-associated early-onset morphea": a particular type of scleroderma in childhood and adolescence with high titer antinuclear antibodies? Results of a cohort analysis and presentation of three cases. J Am Acad Dermatol 2009;60:248-55.
88. Meffert JJ, Davis BM, Grimwood RE. Lichen sclerosus. J Am Acad Dermatol 1995;32:393-416.
89. Powell JJ, Wojnarowska F. Lichen sclerosus. Lancet 1999;353:1777-83.
90. Marini A, Blecken S, Ruzicka T, Hengge UR. Lichen sclerosus. [New aspects of pathogenesis and treatment]. Hautarzt 2005;56:550-5.
91. Val I, Almeida G. An overview of lichen sclerosus. Clin Obstet Gynecol 2005;48:808-17.
92. Shono S, Imura M, Ota M, Osaku A, Shinomiya S, Toda K. Lichen sclerosus et atrophicus, morphea, and coexistence of both diseases. Histological studies using lectins. Arch Dermatol 1991;127:1352-6.
93. Peterson LS, Nelson AM, Su WP. Classification of morphea (localized scleroderma). Mayo Clin Proc 1995;70:1068-76.
94. Asbrink E, Brehmer-Andersson E, Hovmark A. Acrodermatitis chronica atrophicans: a spirochetosis. Clinical and histopathological picture based on 32 patients; course and relationship to erythema chronicum migrans Afzelius. Am J Dermatopathol 1986;8:209-19.
95. Aberer E, Schmidt BL, Breier F, Kinaciyan T, Luger A. Amplification of DNA of *Borrelia burgdorferi* in urine samples of patients with granuloma annulare and lichen sclerosus et atrophicus. Arch Dermatol 1999;135:210-2.
96. Breier F, Khanakah G, Stanek G, Kunz G, Aberer E, Schmidt B *et al*. Isolation and polymerase chain reaction typing of *Borrelia afzelii* from a skin lesion in a seronegative patient with generalized ulcerating bullous lichen sclerosus et atrophicus. Br J Dermatol 2001;144:387-92.
97. Shelley WB, Shelley ED, Grunenwald MA, Anders TJ, Ramnath A. Long-term antibiotic therapy for balanitis xerotica obliterans. J Am Acad Dermatol 1999;40:69-72.
98. Shelley WB, Shelley ED, Amurao CV. Treatment of lichen sclerosus with antibiotics. Int J Dermatol 2006;45:1104-6.
99. Eisendle K, Grabner T, Kutzner H, Zelger B. Lichen sclerosus et atrophicus: a manifestation of infection with *Borrelia* species? Arch Dermatol. 2008 May;144(5):591-8.
100. Schewe C, Rizzello M, Dietel M, Hauptmann S. [PCR based diagnosis in pathology]. Pathologe 2000;21:218-28.
101. Brettschneider S, Bruckbauer H, Klugbauer N, Hofmann H. Diagnostic value of PCR for detection of *Borrelia burgdorferi* in skin biopsy and urine samples from patients with skin borreliosis. J Clin Microbiol 1998;36:2658-65.
102. Bergmann AR, Schmidt B, Derler AM, Aberer E. Importance of sample preparation for molecular diagnosis of Lyme Borreliosis from urine. J Clin Microbiol 2002;40:4581-4.
103. Dickie RJ, Horne C, Sutherland H, Bewsher PD, Stankler L. Direct evidence of localised immunological damage in vulvar lichen sclerosus et atrophicus. J Clin Pathol 1982;35:1395-7.
104. Benvenga S, Santarpia L, Trimarchi F, Guarneri F. Human thyroid autoantigens and proteins of *Yersinia* and *Borrelia* share amino acid sequence homology that includes binding motifs to HLA-DR molecules and T-cell receptor. Thyroid 2006;16:225-36.

105. Vaccaro M, Guarneri F, Borgia F, Cannavo SP, Benvenga S. Association of lichen sclerosus and autoimmune thyroiditis: possible role of *Borrelia burgdorferi*? Thyroid 2002;12:1147-8.
106. Sapi E, MacDonald A. Biofilms of *Borrelia burgdorferi* in chronic cutaneous borreliosis. Am J Clin Pathol 2008;129:988-9.
107. Eisendle K, Mueller H, Zelger B. Biofilms of *Borrelia burgdorferi* in chronic cutaneous borreliosis. Am J Clin Pathol 2008;129:989-90.
108. Kjelleberg S. Giskov M. The biofilm mode of life: mechanisms and adaptations. Norwick, UK: Horizon Scientific Press; 2007.
109. Yamada M, Ikegami A, Kuramitsu HK. Synergistic biofilm formation by *Treponema denticola* and *Porphyromonas gingivalis*. FEMS Microbiol Lett 2005;250:271-7.
110. Graham DY. Antibotic resistance in *Helicobacter pylori*: implications for therapy. Gastroenterology 1998;115:1272-7.
111. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms, a common cause of persistent infections Science 1999;284,1318-22.
112. Stewart P, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-8.
113. Liegner KB, Shapiro JR, Ramsay D, Halperin AJ, Hogrefe W, Kong L. Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting *Borrelia burgdorferi* infection. J Am Acad Dermatol 1993;28(2 Pt 2):312-4.
114. Kluge K, Krahl D, Kramer K, Yaguboglu R. [Juxta-articular fibroid nodules and acrodermatitis chronica atrophicans in late stage Lyme borreliosis]. Hautarzt 2000;51:345-8.
115. Kerl H, Garbe C, Cerroni L, Wolff H. Histopathologie der Haut. Berlin: Springer; 2003.
116. Aberer E, Ertl M, Neumann R, Stanek G. Morphea another manifestation of Lyme disease? Zentralbl Bakteriol Mikrobiol Hyg [A] 1986;263:266-7.
117. Stanek G, Konrad K, Jung M, Ehringer H. Shulman syndrome, a scleroderma subtype caused by *Borrelia burgdorferi*? Lancet 1987;1:1490.
118. Breier P, Klade H, Stanek G, Poitschek C, Kirnbauer R, Dorda W *et al*. Lymphoproliferative responses to *Borrelia burgdorferi* in circumscribed scleroderma. Br J Dermatol 1996;134:285-91.
119. Monari P, Farisoglio C, Calzavara Pinton PG. *Borrelia burgdorferi*-associated primary cutaneous marginal-zone B-cell lymphoma: a case report. Dermatology 2007;215:229-32.
120. Schöllkopf C, Melbye M, Munksgaard L, Smedby KE, Rostgaard K, Glimelius B *et al*. *Borrelia* infection and risk of non-Hodgkin lymphoma. Blood 2008;111:5524-9.
121. Ziemer M, Bauer HI, Fluhr JW, Kaatz M, Elsner P. Primary cutaneous follicle center lymphoma –'crosti lymphoma': what can we learn? Am J Clin Dermatol 2008;9:133-6.
122. Portlock CS, Hamlin P, Noy A, Chey W, Gaydos CA, Palomba L *et al*. Infectious disease associations in advanced stage, indolent lymphoma (follicular and nonfollicular): developing a lymphoma prevention strategy. Ann Oncol 2008;19:254-8.
123. Monari P, Farisoglio C, Calzavara Pinton PG. *Borrelia burgdorferi*-associated primary cutaneous marginal-zone B-cell lymphoma: a case report. Dermatology 2007;215:229-32.
124. Ferreri AJ, Zucca E. Marginal-zone lymphoma. Crit Rev Oncol Hematol 2007;63:245-56.
125. Batinac T, Petranovic D, Zamolo G, Petranovic D, Ruzic A. Lyme borreliosis and multiple sclerosis are associated with primary effusion lymphoma. Med Hypotheses 2007;69:117-9.
126. Cho-Vega JH, Vega F, Rassidakis G, Medeiros LJ. Primary cutaneous marginal zone B-cell lymphoma. Am J Clin Pathol 2006;125 Suppl:S38-49.
127. Guidoboni M, Ferreri AJ, Ponzoni M, Doglioni C, Dolcetti R. Infectious agents in mucosa-associated lymphoid tissue-type lymphomas: pathogenic role and therapeutic perspectives. Clin Lymphoma Myeloma 2006;6:289-300.
128. Munksgaard L, Obitz ER, Goodlad JR, Davidson MM, Ho-Yen DO, Hamilton-Dutoit S *et al*. Demonstration of *B. burgdorferi*-DNA in two cases of nodal lymphoma. Leuk Lymphoma 2004;45:1721-3.
129. Colli C, Leinweber B, Müllegger R, Chott A, Kerl H, Cerroni L. *Borrelia burgdorferi*-associated lymphocytoma cutis: clinicopathologic, immunophenotypic, and molecular study of 106 cases. J Cutan Pathol 2004;31:232-40.
130. de la Fouchardiere A, Vandenesch F, Berger F. *Borrelia*-associated primary cutaneous MALT lymphoma in a nonendemic region. Am J Surg Pathol 2003;27:702-3.
131. Hofbauer GF, Kessler B, Kempf W, Nestle FO, Burg G, Dummer R. Multilesional primary cutaneous diffuse large B-cell lymphoma responsive to antibiotic treatment. Dermatology 2001;203:168-70.
132. Slater DN. *Borrelia burgdorferi*-associated primary cutaneous B-cell lymphoma. Histopathology 2001;38:73-7.
133. Goodlad JR, Davidson MM, Hollowood K, Batstone P, Ho-Yen DO. *Borrelia burgdorferi*-associated cutaneous marginal zone lymphoma: a clinicopathological study of two cases illustrating the temporal progression of *B. burgdorferi*-associated B-cell proliferation in the skin. Histopathology 2000;37:501-8.
134. Goodlad JR, Davidson MM, Hollowood K, Ling C, MacKenzie C, Christie I *et al*. Primary cutaneous B-cell lymphoma and *Borrelia burgdorferi* infection in patients from the Highlands of Scotland. Am J Surg Pathol 2000;24:1279-85.
135. Jeliç S, Filipoviç-Ljeskoviç I. Positive serology for Lyme disease borrelias in primary cutaneous B-cell lymphoma: a study in 22 patients; is it a fortuitous finding? Hematol Oncol 1999;17:107-16.
136. Cerroni L, Zöchling N, Pütz B, Kerl H. Infection by *Borrelia burgdorferi* and cutaneous B-cell lymphoma. J Cutan Pathol 1997;24:457-61.
137. Garbe C, Stein H, Dienemann D, Orfanos CE. *Borrelia burgdorferi*-associated cutaneous B cell lymphoma: clinical and immunohistologic characterization of four cases. J Am Acad Dermatol 1991;24:584-90.
138. Garbe C, Stein H, Gollnick H, Taud W, Orfanos CE. [Cutaneous B cell lymphoma in chronic *Borrelia burgdorferi* infection. Report of 2 cases and a review of the literature]. Hautarzt 1988;39:717-26.
139. Strle F, Preac-Mursic V, Ruzic E, Wilske B, Cimperman J. Isolation of *Borrelia burgdorferi* from a skin lesion in a patient with granuloma annulare. Infection 1991;19:351-2.
140. Ziemer M, Grabner T, Eisendle K, Baltaci M, Zelger B. Granuloma annulare: a manifestation of infection with *Borrelia*? J Cutan Pathol 2008;35:1050-7.
141. Smoller BR, Madhusudhan KT, Scott MA, Horn TD. Granuloma annulare: another manifestation of *Bartonella* infection? Am J Dermatopathol 2001;23:510-3.
142. Svecova D, Buchvald J. [*Borrelia burgdorferi* antibodies in scleroderma circumscripta, lichen sclerosus et atrophicus, erythema nodosum, granuloma annulare, erythema annulare and chronic urticaria]. Bratisl Lek Listy 2000;101:194-9.
143. Aberer E, Schmidt BL, Breier F, Kinaciyan T, Luger A. Amplification of DNA of *Borrelia burgdorferi* in urine samples of patients with granuloma annulare and lichen sclerosus et atrophicus. Arch Dermatol 1999;135:210-2.
144. Strle F, Preac-Mursic V, Ruzic E, Wilske B, Cimperman J. Isolation of *Borrelia burgdorferi* from a skin lesion in a patient with granuloma annulare. Infection 1991;19:351-2.
145. Duray PH. Clinical pathologic correlations of Lyme disease. Rev Infect Dis 1989;11 Suppl 6:S1487-93.
146. Halkier-Sørensen L, Kragballe K, Hansen K. Antibodies to the *Borrelia burgdorferi* flagellum in patients with scleroderma, granuloma annulare and porphyria cutanea tarda. Acta Derm Venereol 1989;69:116-9.
147. Berger BW. Dermatologic manifestations of Lyme disease. Rev Infect Dis 1989;11 Suppl 6:S1475-81.
148. Moreno C, Kutzner H, Palmedo G, Goerttler E, Carrasco L, Requena L. Interstitial granulomatous dermatitis with histiocytic pseudorosettes: a new histopathologic pattern in cutaneous borreliosis. Detection of *Borrelia burgdorferi* DNA sequences by a highly sensitive PCR-ELISA. J Am Acad Dermatol 2003;48:376-84.

149. Fernandez-Flores A, Ruzic-Sabljic E. Granuloma annulare displaying pseudorosettes in Borelia infection. Acta Dermatovenerol Alp Panonica Adriat 2008;17:171-6.
150. Gualco F, Zaccaria E, Drago F, Rebora A. Interstitial granuloma annulare and borreliosis: a new case. J Eur Acad Dermatol Venereol 2007;21:1117-8.
151. Ishihara M, Ohno S, Ono H, Isogai E, Kimura K, Isogai H *et al*. Seroprevalence of anti-*Borrelia* antibodies among patients with confirmed sarcoidosis in a region of Japan where Lyme borreliosis is endemic. Graefes Arch Clin Exp Ophthalmol 1998;236:280-4.
152. Derler AM, Eisendle K, Baltaci M, Obermoser G, Zelger B. High prevalence of *Borrelia* like organisms in skin biopsies from sarcoidosis patients from Western Austria. J Cutan Pathol. In press 2009.
153. Derler AM, Eisendle K, Baltaci M, Obermoser G, Zelger B. High prevalence of "*Borrelia*-like" organisms in skin biopsies of sarcoidosis patients from Western Austria. J Cutan Pathol. In press 2009.
154. Spiegel IB, White SD, Foley JE, Drazenovich NL, Ihrke PJ, Affolter VK. A retrospective study of cutaneous equine sarcoidosis and its potential infectious aetiological agents. Vet Dermatol 2006;17:51-62.
155. Klint H, Siboni AH. Borreliosis associated with Lofgren's syndrome. Ugeskr Laeger 2000;162:4154-5.
156. Xu Z, Ma D, Luo W, Zhu Y. Detection of *Borrelia burgdorferi* DNA in granulomatous tissues from patients with sarcoidosis using polymerase chain reaction in situ technique. Chin Med Sci J 1996;11:220-3.
157. Ishihara M, Ishida T, Isogai E, Kimura K, Oritsu M, Matsui Y *et al*. Detection of antibodies to *Borrelia* species among patients with confirmed sarcoidosis in a region where Lyme disease is nonendemic. Graefes Arch Clin Exp Ophthalmol 1996;234:770-3.
158. Lian W, Luo W. *Borrelia burgdorferi* DNA in biological samples from patients with sarcoidosis using the polymerase chain reaction technique. Chin Med Sci J 1995;10:93-5.
159. Morris JT, Longfield RN. Sarcoidosis and ELISA for *Borrelia burgdorferi*. South Med J 1994;87:590-1.
160. Arcangeli G, Calabro S, Cisno F, Zambotto FM, Drigo R, Ferraresso A. Determination of antibodies to *Borrelia burgdorferi* in sarcoidosis. Sarcoidosis 1994;11:32-3.
161. Chen YQ, Miao JZ, Zhang XZ. Determination of antibody of *Borrelia burgdorferi* in the serum of patients with sarcoidosis and its significance. Zhonghua Nei Ke Za Zhi 1994;33:15-7.
162. Liu HG. Spirochetes in the cheilitis granulomatosa and sarcoidosis. Zhonghua Yi Xue Za Zhi 1993;73:142-4, 189-90.
163. Hua B, Li QD, Wang FM, Ai CX, Luo WC. *Borrelia burgdorferi* infection may bet he cause of sarcoidosis. Chin Med J (Engl) 1992;105:560-3.
164. Montemurro L, Rizzato G. Is sarcoidosis a borreliosis? Sarcoidosis 1991;8:134-5.
165. Eisendle K, Baltaci M, Kutzner H, Zelger B. Detection of spirochaetal microorganisms by focus floating microscopy in necrobiosis lipoidica in patients from central Europe. Histopathology 2008;52:877-84.
166. Zelger B, Eisendle K, Mensing C, Zelger B. Detection of spirochetal micro-organisms by focus-floating microscopy in necrobiotic xanthogranuloma. J Am Acad Dermatol 2007;57:1026-30.
167. Tothova SM, Bonin S, Trevisan G, Stanta G. Mycosis fungoides: is it a *Borrelia burgdorferi*-associated disease? Br J Cancer 2006;94:879-83.
168. Kaatz M, Zelger B, Norgauer J, Ziemer M. Lymphocytic infiltration (Jessner-Kanof): lupus erythematosus tumidus or a manifestation of borreliosis? Br J Dermatol 2007;157:403-5.
169. Bagot M, Revuz J. Jessner-Kanof lesion and *Borrelia* infection. J Am Acad Dermatol 1990;23(4 Pt 1):772-3.
170. Abele DC, Anders KH. The many faces and phases of borreliosis II. J Am Acad Dermatol 1990;23(3 Pt 1):401-10.
171. Abele DC, Anders KH, Chandler FW. Benign lymphocytic infiltration (Jessner-Kanof): another manifestation of borreliosis? J Am Acad Dermatol 1989;21(4 Pt 1):795-7.
172. Ziemer M, Eisendle K, Zelger B. New concepts on erythema annulare centrifugum: a clinical reaction pattern that does not represent a specific clinicopathological entity. Br J Dermatol 2009;160:119-26.
173. Ziemer M, Eisendle K, Müller H, Zelger B. Lymphocytic infiltration of the skin (Jessner-Kanof) but not reticular erythematous mucinosis occasionally represent clinical manifestations of *Borrelia*-associated pseudolymphoma. Br J Dermatol in press.