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Damage of Collagen and Elastic Fibres by Borrelia Burgdorferi – Known and New Clinical and Histopathological Aspects

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Abstract: Lyme Borreliosis, or Lyme's disease, manifests itself in numerous skin conditions. Therapeutic intervention should be initiated as soon as a clinical diagnosis of erythema migrans is made. The histopathology of some of the skin conditions associated with Lyme Borreliosis is characterised by structural changes to collagen, and sometimes also elastic fibres. These conditions include morphea, lichen sclerosus et atrophicus and acrodermatitis chronica atrophicans. More recently, further skin conditions have been identified by the new microscopic investigation technique of focus floating microscopy: granuloma annulare, necrobiosis lipoidica, necrobiotic xanthogranuloma, erythema annulare centrifugum, interstitial granulomatous dermatitis, cutaneous sarcoidosis and lymphocytic infiltration; these conditions also sometimes cause changes in the connective tissue. In the case of ligaments and tendons, collagen and elastic fibres predominate structurally. They are also the structures that are targeted by Borrelia. The resultant functional disorders have previously only rarely been associated with Borreliosis in clinical practice. Ligamentopathies and tendinopathies, spontaneous ruptures of tendons after slight strain, dislocation of vertebrae and an accumulation of prolapsed intervertebral discs as well as ossification of tendon insertions can be viewed in this light.

Keywords: Lyme Borreliosis, collagen fibres, elastic fibres, skin, connective tissue, tendons, ligaments, diverticulum.

INTRODUCTION

Lyme Borreliosis causes skin symptoms at all stages of the disease [1-3]. The most common skin conditions associated with Lyme Borreliosis are morphea [1, 4-10], lichen sclerosus et atrophicus [LSA; 1, 4-6, 10-12], and acrodermatitis chronica atrophicans [ACA; 1, 6-8, 13], which possess an acute inflammatory stage followed by a chronic atrophic stage (Table **1-3**). These skin conditions associated with Lyme Borreliosis are characterised by pronounced histopathological changes of the collagen fibres, but also occasionally the elastic fibres in their connective tissue structures [6,1,9,12,13]. The following also display similar disorders of their connective tissue: granuloma annulare, necrobiosis lipoidica and necrobiotic xanthogranuloma [1]. With the aid of a new histopathological technique, these skin conditions have also been found to contain Borrelia [13-17]. In some cases, LB was also detected in the connective tissue of patients suffering from pseudopelade of Brocq [18], Sudeck's dystrophy [19], hemifacial atrophy [20] and scleroedema of Buschke [21].

There are also other skin conditions that do not display any particular connective tissue changes and optionally may occur as reactive skin conditions in Lyme Borreliosis. These include urticaria [22], erythema multiforme [23], erythema annulare centrifugum [13], microbial eczema [Müller: unpublished], erythema nodosum [3; Müller unpublished], pityriasis lichenoides chronica [24], acrodermatitis papulosa [Gianotti-Crosti syndrome], pityriasis rosea [21] perifolliculitis [24], panniculitis [25,26], lymphocytoma [synonym: lymphadenosis cutis benigna [Bäfverstedt's syndrome; 27-29], benign lymphocytic infiltration [Jessner-Kanof syndrome; [30,31], sarcoidosis [32], B-cell lymphoma [33], pseudolymphoma [Müller: unpublished], Raynaud's syndrome [34] and vasculitis racemosa [Müller; unpublished]. This paper will deal with skin conditions that cause histopathological changes to the collagen and elastic fibres.

Focus Floating Microscopy (FFM)

After the introduction of FFM, a number of dermatological conditions could be attributed to Lyme Borreliosis. FFM is a modified immunohistochemical investigation technique in which several strategies are combined in order to be able to identify microorganisms in tissue sections. In FFM, tissue sections are investigated in two planes: horizontally in a serpentine-like pattern, as is usual in cytology, and vertically, focussing through the entire thickness of the section (normally 3-4 µm). The tissue section is stained bright red with aminoethylcarbazol (AEC) and a light source is used that shines through the specimen approximately 10 times more strongly than usual. Thus a good contrast can be established between the microorganisms and the bright yellow collagen, optimising identification of the pathogen [14].

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Table 1. Pathohistology of Morphea

Early Phase	Sclerotic Stage
Oedematous swelling of collagen fibres	Homogenised collagen fibre bundle
Cell infiltration of lymphocytes, eosinophils and plasma cells	Decrease in strength of fibrils
	Reduction in cell infiltrates
	Slit-like narrowing of vessels
	Loss of accessory structures of the skin
	Hyaline sclerotic transformation of corium

Table 2. Pathohistology of Acrodermatitis Chronica Atrophicans

Inflammatory Stage	Atrophic Stage
Oedematous swelling of hyalinised collagen fibres	Atrophy with disappearance of elastic fibres
Undulating or ribbon-like perivascular lymphocytic infiltrates	Nests of Borrelia can be found in the collagen fibre bundles
Subepidermal haemorrhagic blisters due to infiltration of blood	
Deposit of IgM, IgG, IgA, complement and fibrin	

Table 3. Pathohistology of Lichen Sclerosus et Atrophicus

Initial Phase	Late Phase
Oedematous swelling of hyalinised collagen fibres	Atrophy with disappearance of elastic fibres
Undulating or ribbon-like perivascular lymphocytic infiltrates	Foci of Bb can be found in the collagen fibre bundles
Subepidermal haemorrhagic blisters due to infiltration of blood	
Deposit of IgM, IgG, IgA, complement and fibrin	

Cutaneous Manifestations of Lyme Borreliosis with Involvement of the Connective Tissue

Morphea

Clinical finding: At the beginning a spot-like focus can be seen with slight inflammatory erythema radiating outwards on all sides. It can occur in isolation or in groups. The erythema gradually completely disappears in the centre into a disc-like, ivory-coloured hardened area, that is surrounded by a bluish-violet, lilac-coloured ring [1,7]. Atrophy develops in the late phase, which is a hyper-pigmented dirty greyish-brown colour at the edges but usually without pigment in the centre. During this phase there may be a loss of hair and sebaceous glands. Morphea tends to occur on the trunk, less commonly on the extremities. The foci may develop individually and be disseminated but also merge into patches or become generalised and are then difficult to differentiate from scleroderma [1, 4-6, 10, 35, 36]. Atrophoderma of Pierini and Pasini [37, 38] is now considered to be an abortive form of morphea [39, 40].

Histopathology (Table **1**): Two phases can be distinguished histologically. The early phase is associated with an oedematous swelling of the collagen fibres and cell infiltration of lymphocytes, eosinophilic granulocytes and plasma cells. This is followed by the second, sclerotic stage with homogenised collagen fibre bundles, a reduction in fibril strength, decrease in cell infiltration, slit-like vessels and loss of accessory structures of the skin in a hyaline sclerotic corium, in which only the arrector pili muscles are maintained [1, 6, 35]. Borrelia were detected in foci of morphea using FFM [9].

Acrodermatitis Chronica Atrophicans (ACA; Herxheimer's Disease)

Clinical finding: ACA tends to occur over the large joints and distal parts of the extremities. It can also be seen on the face and trunk. It initially causes an oedematous skin swelling with slight erythema. Thread-like erythema may also develop on the extremities, characterised as ulnar striations on the forearms and tibial striations on the legs. As there are usually no subjective symptoms during this phase, early ACA of stage 1 Borreliosis usually goes unnoticed. In stage 2 there is pronounced atrophy of the skin, which becomes thin, flaccid and creases like cigarette paper. The following symptoms then develop: hair loss, telangiectasias, varicose veins and altered pigmentation [1, 3, 8, 35].

Histopathology (Table **2**): ACA starts with an inflammatory oedematous stage of the corium associated with perivascular lymphohistiocytic cell infiltration and plasma cells. This turns into an atrophic stage, where swelling and ho-

mogenisation of the fibres and disappearance of collagen and elastic fibres is observed. This stage enables a differential diagnosis to systemic scleroderma to be made, where the elastic fibres are not affected. There is also narrowing of the corium with infiltration of lymphocytes, histiocytes and a large number of plasma cells [1,3]. Borrelia were detected in 50 out of 51 patients with ACA [15]. FFM was more sensitive than Polymerase Chain Reaction (PCR; 96.0% versus 45.2%) and almost as specific (99.4% versus 100%) in fresh bites, EM, lymphocytoma and ACA.

Lichen Sclerosus et Atrophicus (LSA)

Clinical finding: Initially one can detect small porcelain-coloured to bluish-white round to oval atrophic foci, which later merge into irregularly-configured larger areas. Older foci develop fine parchment-like folds on the surface with follicular hyperkeratosis and cone-shaped papules. Preferred sites for LSA in men and women are the side of the neck, shoulders, the flexor side of the forearms, submammary region, and anal and perianal area as well as the genitals. A short frenulum is often the commonest sign of LSA in men. Shrinking of the foreskin can lead to secondary phimosis in men, the worst variant of which is balanitis xerotica obliterans. The equivalent advanced form of genital signs of LSA in women is kraurosis vulvae, which are lesions with tendency to malignancy [1, 3, 4, 6, 10, 35].

Histopathology (Table **3**): Initially the hyalinised collagen fibres and cell undulating and ribbon-like infiltrates become impregnated with oedema. Blood infiltration leads to subepidermal haemorrhagic blisters. Deposits of immunoglobulins IgA, IgG and IgM and complement and fibrin are found. The lymphatic vessels are dilated. During the late phase, disappearance of elastic fibres leads to atrophy [1, 3, 6, 35]. B. burgdorferi sensu lato was detected in 63% of patients with LSA [12]. Detection was significantly higher in the inflammatory initial phase of LSA (80%) than in the atrophic late phase (33.3%).

Necrobiosis Lipoidica (NL)

Clinical finding: Irregularly-configured, sharply delineated, disc-like, atrophic plaques criss-crossed with teleangiectasias with a yellow to brownish-reddish-yellow sclerotic hard centre. Several foci may merge. In 30% of cases ulcers may develop which are difficult to heal; these have a greasy yellow necrotic background and indurated edges. As these heal the skin becomes atrophied and the integumentary appendage is lost [1, 35].

Histopathology: The epidermis shows no specific changes. In the middle and lower dermis, and sometimes also the subcutaneous adipose tissue, nodular or striated granulomatous inflammation can be found consisting of histiocytes, epitheloid cells and polynuclear giant cells as well as focal lymphocyte and plasma cell nests. Individual lymph follicles may also form. In addition to these inflammatory components, extensive changes may occur to the collagen, which are described as collagen necrobiosis. The walls of the vessels may swell and the vessels close [1]. Borrelia were detected with FFM in 42 (75%) of the 56 patients with NL investigated [15]. Where there was a stronger inflammatory reaction, Borrelia were detected significantly more frequently - in 92.7% of cases (38/41) - than in the atrophic late stage where there was less inflammation – 26.7% of cases (4/15).

Granuloma Annulare (GA)

Clinical finding: One can see small, sharply-delineated, slightly reddened papules that are slightly shiny. As these grow they become bow or ring-shaped and the middle sinks level with the skin. Secondary changes such as erosions and ulcers develop. Preferred sites are the backs of the hands and feet, but also joints of the hands, elbows, joints of the feet and buttocks. A distinction is made between the erythematous, subcutaneous, disseminated, perforating and plaque forms [1, 3, 35]. The presence of antibodies against Bb in cases of GA had been published in 1987 [41].

Histopathology: Histologically one can determine two distinct variants: foci of necrobiotic degenerated collagen, sometimes surrounded by histiocytes in palisade-like formation. In the more discrete (mild) course the histiocytes are embedded in the collagen. Histiocytic pseudorosettes were described in GA associated with a Borrelia infection [42, 43]. These are free-floating bundles of collagen that are surrounded by histiocytes. In 86.66% of cases that were investigated histologically (13/15), histiocytic pseudorosettes were detected with degeneration of the collagen. The fragmented collagen fibres are surrounded by CD68-expressing granulomatous cells [42]. Borrelia were detected in 80.9% of biopsies (127/157) of GA investigated [17]. In 85.2% of the cases investigated, the pathogen was detected in the localised form, i.e. much more commonly than in the diffuse form.

Necrobiotic Xanthogranuloma (NXG)

Clinical finding: One can find reddish-yellow to brown papules, annular plaques, scars and teleangiectasias that tend to predominate in the periorbital region, but are also found on the trunk and proximal extremities [44, 45]. NXG is frequently associated with paraproteinaemia, other neoplasms and lymphoproliferations [46, 47]. It has also been described in connection with scleroderma [48].

Histopathology: The biopsies show granuloma without a palisade-like formation with numerous polynuclear giant cells and extensive necrobiotic areas over the entire dermis. In the central atrophic region of annular plaques there may be complete loss of elastic fibres [1]. In 6 out of 7 cases of NXG, Borrelia were found on their own, in pairs and in clusters. In 4 out of 6 positive cases, PCR was positive. In 2 cases it was normal [16].

Pseudopelade of Brocq

Clinical finding: Irregularly-configured, merging foci which remain sharply delineated compared with the unaffected skin. The affected skin usually glistens whitish-yellow and is atrophically thinned like cigarette paper and without follicular openings. Many hairs are lost, some tufts may remain [1]. A link with Borreliosis has been reported [18].

Histopathology: There is sclerosis with destruction of the elastic fibres and follicular openings. The former follicle areas are characterised by collagen fibres that run perpen-

dicular to the surface of the skin and arrector pili muscles that were maintained. The perifollicular and perivascular lymphocytic inflammations are only moderately pronounced [1, 35].

Structure and Function of Tendons and Ligaments

Tendons are support tissues which anchor the skeletal muscle in the periosteum. They are formed predominantly from collagen, to a lesser extent elastic fibres as well as intercellular substance. In short tendons the bundles of fibres run parallel with one another. In long tendons they wind around one another in steep spirals. Bundles of tendon fibres increase cross-cohesion and strength against shearing. The formation of fine waves enables the tendon to lengthen by approximately 4% under stress. This causes a certain loss of transferred force [49]. However, it enables a more gentle exertion of the muscles and reduces the risk of injury. The tendon develops gradually from the muscle and attaches with tendon fibres (Sharpey´s fibres) to the periosteum or perichondrium. In addition to collagen fibres, tendons that radiate into soft tissue like those of the mimetic muscles, the skin or the tongue muscles have numerous elastic fibres that extend like bristles into the soft tissue [49]; (Table **4**).

Ligaments are high-tensile connective tissue ribbon-like structures that sensibly limit the range of movement. Their collagen fibres are not branched. They are not able to distend, they increase tensile strength and are flexible. A healthy ligament only tears at a load of between 6 and 10 kg/mm². The elastic fibres of ligaments have varying thicknesses, have sharp contours and branch unlike collagen fibres; they also form networks. They can resist acids and alkaline chemicals. They can only be made visible on fixed specimens by staining with orcein and resorcin-fuchsin. Elastic fibres have low resistance to distension. Once the extension force finishes they return to their original state. Distension of up to 2.5 times the original state is reversible [49,50].

The combination of elastic and collagen fibres has a functional significance. Collagen fibres, when arranged in parallel, become gathered. If the fibre bundle is stretched longitudinally the elastic fibres involved become taut. As soon as the stretching force diminishes, the taut elastic fibres pull the collagen fibres back to their original state. They are therefore critical to returning the ligament to the midpoint and prevent the collagen fibres from becoming entangled [49].

Reticular fibres are a third type of fibre that can be differentiated histologically from collagen fibres by their ability to become stained with silver. That is why they are also described as argyrophilic fibres. They are usually very delicate in structure, never occur in great quantities or thick bundles and are optically anisotropic. They have a thin skin and their task is to encase. They can be found at the edges bordering the connective tissue and adjacent non-connective tissue. The general consensus is that reticular cells produce collagen fibres [49].

Interaction of Borrelia Burgdorferi with Structures in Tendons and Ligaments

Borrelia are capable of breaking down soluble and insoluble ground substance within the extracellular matrix [51]. They activate metalloproteases, cause collagen to dissolve and can colonise as microcolonies in collagen fibres [52]. They inhibit the regeneration of collagen promoted by fibronectin, and hence delay the healing process or prevent it completely [53]. Binding to adhesins such as glucosamine glycan-binding protein [54], fibronectin-binding protein [55] and the proteoglycan decorin [11, 56, 57] are described. The last is probably responsible for the destruction of collagen, since direct binding to collagen I and II has not been detected. Mice with decorin deficiency were resistant to Borrelia [58]. Binding of the surface protein BmpA, BmpB, BmpC and BmpD to laminin inhibits cell-cell contact [59]. The same was established for the surface protein ErpX [60]. The involvement of gene conversion has also been established [61], as has the link to autoimmune disorders [62,63]. The persistence of Bb in human ligaments was described as early as 1993 [64]. In primates, Borrelia have been detected in the connective tissue of the aorta, the atria and the ventricles of the heart [65]. In endomysium biopsy Bb was detected in dilative cardiomyopathy in humans too [66]. Using FFM, Bb was also determined in the intestinal wall of an own patient who had to undergo surgery for acute diverticulitis. Serological tests revealed no antibodies [AB] in this case, even the immunoblot test was normal. This is not surprising since antibodies may not develop [11]. Activation of the conventional and alternative pathways of complement activation and the lysis of Bb triggered by this is absent in those cases [11]. The lymphocyte transformation test [LTT] was used

Table 4. Structure and Task of Tendons and Ligaments

	Tendon	Ligament
Structure	Tendon cells	Unbranched collagen fibres which give tensile strength in the longitudinal direction
	Intercellular substance	Good flexibility
	Tendon fibres made from collagen	Slight distensibility
		In addition net-like structured branched elastic fibres with good distensibility
		Stable with acids and alkalis
Task	Support tissue to anchor the skeletal muscle with the periosteum	High-tensile connective tissue ribbon-like structure for sensible functional restriction of range of movement

diagnostically [2, 67, 68]. The lympho-proliferative immune response in EM, ACA, lymphocytoma and morphea was established as early as 1995 [9]. In agreement with the FFM result, LTT was positive to Borrelia antigens. There is a clinical link between Borreliosis and colon elongatum [Müller: unpublished cases].

Clinically, the interaction of Borrelia with the glucosamine glycan hyaluronic acid is very significant. This is an acid, high-viscous mucopolysaccharide that binds strongly to water and occurs in ground substance as the free form, the ester or bound to protein. It is used as a lubricant, to regulate water-binding and control permeability and diffusion. 1 g hyaluronic acid can bind with up to 6 l water. This glucosamine glycan is also able to prevent the penetration of infectious microorganisms. The reproduction of Borrelia might also be promoted by hyaluronidase. Binding of surface proteins to hyaluronic acid causes a reduction in pH, inactivation of proteoglycans, a decrease in cross-linking and a reduction in viscosity. Later on there may be displacement of gap junctions whose task it is to regulate the transport of organic and inorganic ions in tissues that are poorly supplied with blood [69]. This relates to tendons and ligaments in particular. If this occurs, the initial oedematous swelling of the tendons will disappear, and finally the ligament fibres will dry out. The risk of tendon rupture increases in association with damage to the collagen fibres. The extent to which elastic fibres are also damaged was previously unknown. The function of porins is also altered by Bb [70].

Clinical Symptoms of Lyme Borreliosis in Tendons and Ligaments

While it is generally recognised that arthritis is a possible condition associated with Lyme Borreliosis and osteoarthritis is frequently the first sign in a joint, the involvement of tendons and ligaments was previously not given much consideration although the high affinity of Borrelia to collagen and elastic fibres was already recognised in the histopathological changes of skin conditions associated with Lyme Borreliosis. It is notable clinically that the symptoms in tendons and ligaments frequently develop independently of special strain or trauma to the affected tendons and/or ligaments and that there is an apparent contradication between the symptoms experienced and that triggering event [71].

Insertional tendinopathies are often observed clinically. The most common locations are the epicondylitis humeri [tennis elbow], the meeting point of the tendons of the sartorius, gracilis and semitendinosus muscles in the medial region of the knee joint [pes anserinus superficialis]. The symptoms may radiate from there a hands-width cranially to the upper leg. The Achilles [or calcaneal] tendon or plantar fascia ligament may also be affected. Ruptures of the extensor tendons of the fingers, the quadriceps tendon, of cruciate ligaments and Achilles tendon and spontaneous vertebral dislocations have been seen clinically at low levels of strain in patients who were suffering from Lyme Borreliosis. In addition, carpal tunnel syndrome [CTS] and calcifications of the tendon insertions [periarthropathia homeroscapularis, calcifications of the patellar ligament, achillodynia and Haglund's heel] have also been observed in this connection. Eosinophilic fasciitis [Shulman's syndrome, Shulman's fasciitis] is described in connection with LB [21].

Dysfunction of the Atlaido-occipital Joint

The atlaido-occiopital joint has been reported to be another common and clinically significant site for symptoms [71]. The structural change of the ligaments which fix the atlas to the base of the skull [alar ligaments] or stabilise the dens axis [transverse atlantis liagments], have special significance owing to their complex effects on craniomandibular function, vegetative control through sympathetic and parasympathetic nerves, the function of various centres of the brain stem, the induction of increased nitrogen oxide and peroxynitrite formation [72, 73], increased production of pro-inflammatory cytokines and cyclooxygenases [74], and finally also due to dislocation of vertebrae and intervertebral discs and accompanying myopathies [72].

Dysfunction of the atlaido-occipital joint without traumatic damage but correlated to chronic Lb has been described inducing a variety of symptoms [71]. 12 [18,8%] of the 64 patients reported had positive IgG antibodies in ELISA and 17 [26,6%] in immunoblot. IgM antibodies had not been found in any of the cases. In 57 (89,1%) patients the stimulation index (SI) in lymphocyte transformation test (LTT) was pathologically increased (SI > 3,0) in one of the four antigens investigated (B. burgdorferi, B. afzelii, B. garinii, Ospc). A functional magnetic resonance imagin (MRI) scan was carried out on 28 (49,1 %) of the 57 patients with pathological SI in LTT. Structural damage has been described to the alar ligament, the transverse axis ligament included involvement of the spinal cord medulla with mechanically triggered myelopathy as it has been reported in cases following traumatic damage [75]. As published before [76], the number of CD57+ natural killer cells (NK-cells) was determined. 36 (78,3 %) of the examined 46 patients showed significant reduction of this cell type.

CONCLUSIONS

Morphological changes of the skin are histologically characterised by very similar pathological changes to the collagen and also elastic fibres in a whole range of skin conditions that occur with Lyme Borreliosis. Using FFM Bb was detected in the tissue within a clear time interval relative to disease transmission. Using this technique, it was possible to confirm skin conditions already known to be associated with Borreliosis and recognise new ones such as necrobiosis lipoidica or necrobiotic xanthogranuloma. The histologically-detected persistence of the pathogen does not correlate reliably with PCR or serological parameters.

Collagen and elastic fibres are essential structural elements of tendons and ligaments. Few publications to date have dealt with the possibility of their being affected in the context of Lyme Borreliosis, even though the first indications of this were published over almost two decades ago [64]. There is evidence that the presence of decorin in tendons and ligaments plays a critical role in infestation. Decorin-deficient mice were resistant to Bb. The extent to which the genetic expression of decorin plays a role has not been investigated to date. From own results on two brothers who contracted Lyme Borreliosis one after the other with pronounced symptoms in the tendons and ligaments, elements of individual susceptibility in addition to the special characteristics of the pathogen can be anticipated to play a

role in the symptoms in individual cases. Spontaneous ruptures of tendons and ligaments that are triggered by no or only slight trauma are clinically suspicious of Lyme Borreliosis. It would be good if, when treating tendons and ligament damage surgically, the possibility of Lyme Borreliosis would be considered more often and tissue obtained for histopathological examination using FFM.

Various factors are now known that imply that an isolated case of LB will not be overcome either by the individual power of the immune system or by the treatment implemented, but will take a chronic course. Animal studies have shown that even with adequate formation of antibodies only those cell populations that were carriers of surface markers were eliminated. Elimination of the entire population was not achieved [57]. Moreover, only a proportion of patients develop sufficient antibodies. In cases such as this even the complement activation that leads to lysis of the pathogen does not occur [11]. In neuron and astrocyte cultures as well as in the brains of three patients, pleomorphic and cystic forms of Borrelia that indicate resistance to pathogens were detected extra and intracellularly using dark field microscopy, histochemistry and immunohistochemistry. The findings were similar to those obtained with Treponema pallidum [77].

Tendons and ligaments are a structurally and topographically ideal retreat for Borrelia. Their involvement is a further factor to indicate a chronic course of the disease. Serological antibody diagnostic techniques proved to be inadequate in cases such as this, possibly because the formation of antibodies is inhibited by the pathogen [11]. Immunological investigation on immune cells has widened the diagnostic spectrum. The sensitivity of LTT was superior to serological investigation of antibodies in the ELISA or immunoblot tests and correlated well with clinical symptoms. FFM was able to detect the pathogen directly in numerous skin conditions associated with LB, regardless of the results of serology or of PCR. LTT is a useful addition to the haematological diagnostic spectrum, especially for the diagnostic of LB of tendons and ligaments, as histopathology in those cases rarely can be done.

ABBREVIATIONS

AB	=	antibodies
ACA	=	acrodermatitis chronic atrophicans
AEC	=	aminoethylcarbazol
Bb	=	borrelia burgdorferi
Bmp	=	borrelia membrane protein
CD	=	cluster of differentiation
CTS	=	carpal tunnel syndrome
EM	=	erythema migrans
Erp	=	OspE/F related protein
FFM	=	focus floating microscopy
GA	=	granuloma annulare
IFN	=	interferon
LSA	=	lichen sclerosus et atrophicus
LB	=	Lyme Borreliosis
LTT	=	lymphocyte transformation test
NK	=	natural killer celles
NL	=	necrobiosis lipoidica
NXG	=	necrobiotic xanthogranuloma
Osp	=	outer surface protein
PCR	=	polymerase chain reaction
TNF	=	tumor necrosis factor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Braun-Falco O, Plewig G, Wolff HH: Dermatologie und Venerologie. Berlin: Springer Verlag 1995.
[2] Hopf-Seidel P. Krank nach Zeckenstich. München: Knaur Taschenbuchverlag 2008.
[3] Satz N. Klinik der Lyme Borreliosis. Bern: Verlag Hans Huber 2002.
[4] Aberer E, Stanek G: Histological evidence for spirochetal origin of morphea and lichen sclerosus et atrophicans. Am J Dermatopathol 1987; 9: 374-9.
[5] Aberer E, Stanek G: Evidence of spirochetal origin of circumscript scleroderma (Morphea). Acta Dermatol Verol 1987; 67 (3): 225-31.
[6] Altmeyer P: Zur Klassifikation der zirkumskripten Sklerodermie (CS): Multizentrische Untersuchung an 286 Patienten. Hautarzt 1990; 41: 16-21.
[7] Büchner SA: Morphea – eine zeckenübertragene Borreliosis der Haut? Ein Beitrag zur zirkumskripten Sklerodermie. H + G Zeitsch Hautkr 1989; 64: 661-9.
[8] Büchner SA, Lautenschlager S, Itin P, Bircher A, Erb P: Lymphoproliferative Responses to Borrelia burgdorferi in Patients with Erythema Migrans, Acrodermatitis Chronica Atrophicans, Lymphadenosis Benigna Cutis and Morphea. Arch Dermatol 1995; 131(6): 673-7.
[9] Einsendle K, Grabner T, Zelger B. Morphea – a manifestation of infection with borrelia species? Br J Dermatol 2007; 157:1189-98.
[10] Neubert U, Aberer U, Rufli T. Localized Scleroderma and Lichen Sclerosus et Atrophicus: Manifestation of Borrelia burgdorferi Infection? In Aspects of Lyme Borreliosis. Edited by Weber K, Burgdorfer W. Berlin: Springer Verlag 1992; pp. 240-7.
[11] Aberer E. Lyme Borreliosis - Update. JDDG 2007; 5: 406-13.
[12] Einsendle K, Grabner T, Kutzner H, Zelger B. Possible role of Borrelia burgdorferi sensu lato infection in lichen sclerosus. Arch dermatol 2008; 144(5): 591-8.
[13] Einsendle K, Zelger B. The expanding spectrum of cutanous borreliosis. G Ital Venereol 2009; 144(2):157-71.
[14] Einsendle K, Grabner T, Zelger B. Focus floating microscopy "gold standard" for cutanous borreliosis? Am J Clin Pathol 2007; 127: 213-22.
[15] Einsendle K, Baltaci M, Kutzner H, Zelger B. Detection of spirochetal microorganisms by focus floating microscopy in necrobiosis lipoidica patients from central Europe. Histopathol 2008; 52(7): 877-84.
[16] Zelger B, Einsendle K, Mensing C, Zelger B: Detection of spirochetal microorganisms by focus floating microscopy in necrobiotic xanthogranuloma. J Am Acad Dermatol 2007, 57:1026-30.

[17] Zhang JR, Norris SJ. Variations of the Borrelia burgdorferi Gene vIsE Involves Cassette-specific, Segmental Gene Conversion. Infect Immun 1998; 66(8): 3698-704.
[18] Schwarzenbach R, Djawari DJ. Pseudopelade Brocq – mögliche Folge einer Borreliosis III. Hautarzt 1998; 49: 835-7.
[19] Neumann RA, Aberer E, Stanek K. Evidence for spirochetal origin of Sudeck´s atrophy (algodystrophy, reflex sympathetic dystrophy). Arch Orthop Trauma Surg 1989; 108(5): 314-6.
[20] Ventura F, Gomes J, Vilarinho C, *et al*. Parry-Romberg syndrome: A possible association with Lyme borreliosis. J Infect Dis Immun 2011; 3(3): 36-9.
[21] Trevisan G. Atypical dermatological manifestations of Lyme borreliosis. Acta Dermatoven Alpina, Pannonica et Adriatica 2001;10(4):1581-2979.
[22] Steere AC, Bartenhagen NC, Craft JE. The early clinical manifestation of Lyme Disease. Ann Intern Med 1983; 99: 76-82.
[23] Schuttelaar ML, Laeijendecker R, Heinhuis RJ, Van Joost T. Erythema multiforme and persistent erythema as early cutanous manifestations of Lyme disease. J Acad Dermatol 1997; 37: 873-5.
[24] Menni S, Pistritto G, Gelmetti C, Stanta G, Travisan G. Pityriasis lichenoides-like lesions with perifolliculitis in Lyme Borreliosis. Europ J Pediat Dermatol 1994, 4:77-80.
[25] Hassler D, Zorn J, Zöllner L. Noduläre Pannikulitis. Eine Verlaufsform der Lyme Borreliosis? Hautarzt 1992; 43:134-8.
[26] Kramer N, Rickert RR, Brodkin RH, Rosenstein ED. Septical panniculitis as a manifestation of Lyme disease. Am J Med 1986; 81: 149-52.
[27] Picken RN, Strle F, Ruzic-Sabiljic E, *et al*. Molecular subtyping of Borrelia burgdorferi sensu lato isolates from five patients with solitary lymphocytoma. J Invest Dermatol 1997;108(1): 92-7.
[28] Strle F, Pleterski-Rigler D, Stanek G, Pejovnik-Pustinek A, Ruzie E. Cimperman J: Solitary borrelial lymphocytoma: report of 36 cases. Infection 1992; 20: 201-6.
[29] Weber K, Schierz G, Wilske B, Prac-Mursic V: Das Lymphozytom – eine Borreliosis? Zentralbl Hautkr 1985; 60: 1585-98.
[30] Abele DC, Anders KH, Chandler FW: Benign lymphocytic infiltration (Jessner-Kanof: Another manifestation of Borreliosisis. J Am Acad Dermatol 1989; 21: 795-7.
[31] Ziemer M, Grabner T, Einsendle K, Baltaci M, Zelger B: Granuloma annulare - a manifestation of infection with borrelia species? J Cutan Pathol 2008, 35(11): 1050-7.
[32] Derler AM, Einsendle 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 2009; 36(12):1262-8.
[33] Goodlad JR, Davidson MM, Hollowood K, *et al*. Primary cutanous B-cell lymphoma and Borrelia burgdorferi infection in patients from the highlands of Scotland. Am J Surg Pathol 200; 24: 1279-85.
[34] Kristof V, Bozsik BP, Szirtes M, Simonyi J. Lyme borreliosis and Raynaud´s syndrome. Lancet 1990; I: 975-6.
[35] Altmeyer P, Bacharach-Buhles M. Springer Enzyklopädie Dermatologie, Allergologie, Umweltmedizin. Berlin: Springer Verlag 2002.
[36] Ross SA, Sánchez JL, Taboas JO: Spirochetal forms in dermal lesions of morphea and lichen sclerosus et atrophicus. Am J Dermatopathol 1990; 12: 597-602.
[37] Pasini A: Atrofodermia idiopatica progressiva. G Ital Dermatol 1923; 58: 785.
[38] Pierini L, Vivoli D. Atrofodermia progressiva (Pasini). G Ital Dermatol 1936; 77: 403-9.
[39] Büchner SA, Rufli T. Atrophoderma of Pasini and Pierini. Clinical and histological findings and antibodies to Borrelia burgdorferi in thirty-four patients. J Am Acad Dermatol 1994; 30(3): 441-6.
[40] Kohler J, Wokalek H, Thoden U. Atrophodermia Pasini-Pierini mit lokalem Schmerzsyndrom – eine Borreliainfektion? Der Schmerz 1988; 2(1): 44-5.
[41] Kuske B, Schmidly J, Hurzika T, Cueni M, Rufli T. Antibodies against Borrelia burgdorferi in patients with granuloma annulare. Congress in Lyme Borreliosis Update: Baden-Vienna 1987.
[42] Fernandez-Flores A, Ruzic-Sabljic E. Granuloma annulare displaying pseudorosettes in Borrelia infection. Acta Dermatoven 2008;17(4):171-6.
[43] Moreno C, Kutzner H, Palmedo G, Gorttler E, Carrasco I. Interstitial granulomatous dermatitis with histiocytic pseudorosettes: a new histiopathologic pattern in cutanous borreliosis. Detection of Borrelia burgdorferi DNA sequences by a highly sensitive PCR-ELISA. J Acad Dermatol 2003; 48: 376-84.
[44] Kossard S, Winkelmann RK. Necrobiotic xanthogranuloma. Austral J Dermatol 1980; 21: 85-8.
[45] Mehregan DA, Winkelmann RK. Necrobiotic xanthogranuloma. Arch Dermatol 1992; 128: 94-100.
[46] Ito Y, Nashimura K, Yamanaka K, *et al*. Necrobiotic xanthogranulomaa with paraproteinemia; an atypical Case. J Dtsch Dermatol Ges 2008; 6(1): 40-3.
[47] Kossard S. Winkelmann: Necrobiotic xanthogranuloma with paraproteinemia. J Am Acad Dermatol 1980; 40: 257-70.
[48] Russo GG: Necrobiotic xanthgranuloma with scleroderma. Cutis 2002; 70: 311-16.
[49] Benninghoff A, Goerttler K: Lehrbuch der Anatomie. Bd.1: AllgemeineAnatomie und Bewegungsapparat. München: Urban & Schwarzenberg 1964.
[50] Schmidt W: Bewegungssystem – Grundlagen. In: Bob A, Bob K, Eds. Anatomie – Duale Reihe. Stuttgart: Thieme Verlag 2006; 197-224.
[51] Coleman JL, Roemer EJ, Benach JL. Plasmin-coated Borrelia burgdorferi degrades soluble and insoluble components of the mammalian extracellular matrix. Infect Immun 1999; 67(8): 3929-36.
[52] Zambrano MC, Beklemisheva AA, Bryksin AV, Newman SA, Cabello FC. Borrelia burgdorferi binds to, invades, and colonizes native Type I collagen Lattices. Infect Immun 2004, 72(6): 3138-46.
[53] Grab DJ, Kennedy R, Philipp MT. Borrelia burgdorferi posses a collagenolytic activity. FEMS Microbiol Lett 1996;144(1): 39-45.
[54] Parveen N, Leong M. Identification of a candidate glycosaminglycan-binding adhesin of Lyme disease spirochete Borrelia burgdorferi. Mol Microbiol 2000; 35(5): 1220-34.
[55] Brisette CA, Bykowski T, Cooley AE, Bowman A, Stevenson B. Borrelia burgdorferi RevA Antigen Binds Host Fibronectin. Infect Immunol 2009; 77(7): 2802-12.
[56] Guo BP, Norris SJ, Rosenberg LC, Höök M. Adherence of Borrelia burgdorferi tot he Proteoglycan Decorin. Infect Immun 1995; 63(9): 3467-72.
[57] Liang FT, Jacobs MB, Boers LC, Philipp MT. An immune evasion mechanism for spirochetal persitence in Lyme borreliosis. J Exp Med 2002; 195(4): 415-22.
[58] Brown EL, Wooten RM, Johnson BJB, *et al*. Resistance to Lyme disease in decorin-deficient mice. J Clin Invest 2001; 107(7): 845-52.
[59] Verma A, Bristte CA, Bowman A, Stevenson B. Borrelia burgdorferi BmpA is Laminin-Binding Protein. Infect Immun 2009; 77(11): 2802-12.
[60] Brisette CA, Verma A, Bowman A, Cooley A, Stevenson B. The Borrelia burgdorferi outer-surface protein ErpX binds mammalian laminin. Microbiology 2009; 155: 863-72.
[61] Ziemer M, Einsendle 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 2009, 161(3):583-90.
[62] Sarafova S. Borrerlia burgdorferi – Evasion of the Immun System. Available at: http://www.bio.davidson.edu/people/sosarafova/Assets/Bio307/meprasse/page06.html
[63] Singh SK, Girschick HJ: Lyme borreliosis: from infection to autoimmunity. Clin Microbiol Infect 2004; 10(7): 598-614.
[64] Häupl T, Hahn G, Rittig M, *et al*. Persistence of borrelia burgdorferi in ligamentous tissue from a patient with lyme borreliosis. Arthitis Rheum 1993; 36(11): 1621-6.
[65] Cadavid D, Bai Y,Hodzic E, Narayan K, Barthold SW, Pachner AR. Cardiac involvement in non-human primates infected with the Lyme diease spirochete Borrelia burgdorferi. Lab Invest 2004; 84(11):1439-50.
[66] Palecek T, Kuchynka P, Hulinska D, *et al*. Presence of Borrelia burgdorferi in endomyocard biopsies in patients with new-onset unexplained dilated cardiomyopathy. Med Microbiol Immunol 2010; 199(2): 139-43.
[67] Valentine-Thon E, Ilsemann K, Sandkamp MA. A novel lymphocyte transformation test (LTT-MELISA®) for Lyme borreliosis. Diagn Microbiol Infect Disease 2006; 57: 27-34.
[68] Von Baehr, V. Die Labordiagnostik der Borreliainfektion.Umweltmedizin-gesellschaft 2009; 22(2):119-24.

[69] Breves S. Borreliose. Colmberg: Medea-Verlag 2007.
[70] Thein M, Bonde M, Bunikis I, *et al*. DipA, a pore-forming protein in outer membrane of lyme disease Borrelia exhibits specificity for permeation of dicarboxylates. 8th Anual Meeting of Deutsche Borreliosis-Gesellschaft. Wuppertal Germany, 2011. Submitted for publication.
[71] Müller KE. Erkrankungen der elastischen und kollagenen Fasern von Haut, Sehnen und Bänder bei Lyme-Borreliose. umwelt-medizin-gesellschaft 2009; 22(2):112-8.
[72] Kuklinski B. Das HWS-Trauma. Zwickau: Aurum-Verlag 2006.
[73] Pall ML. Explaining Unexplained Illnesses. Binghampton: Harrington Press 2007.
[74] Müller KE. Störung der immunoneurogenen Kommunikation. OM – Zs Orthomol Med 2008; 3: 9-13.
[75] Volle E. Diagnostische Methoden bei Verletzung am kraniozervikalen Übergang. In: Moskopp D, Wassmann, Eds. Neurochirurgie. Stuttgart: Schattauer 2007; pp. 555-60.
[76] Stricker RB: Decreased CD57+ lymphocyte subset in patients with chronic Lyme disease. Immunol Lett 2001; 76: 43-8.
[77] Miklossy J, Kasa S, Zurn AD, McCall S, Yu S, McGeer PL. Persisting atypical and casting forms of Borrelia burgdorferi and local inflammation in Lyme neuroborreliosis. J Neuroinflamm 2008; 40(5):1742-2094.

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