REVIEW

Tired of Lyme borreliosis

Lyme borreliosis in the Netherlands

J. Coumou[1,2], T. van der Poll[1,3], P. Speelman[3], J.W.R. Hovius[1,3*]

[1]Center for Experimental and Molecular Medicine (CEMM), Academic Medical Center (AMC), University of Amsterdam (UvA), the Netherlands, [2]Public Health Service of Amsterdam (GGD), the Netherlands, [3]Department of Internal Medicine/Infectious Diseases/Tropical Medicine/AIDS, Academic Medical Center (AMC), University of Amsterdam (UvA), the Netherlands, *corresponding author: lyme@amc.uva.nl

ABSTRACT

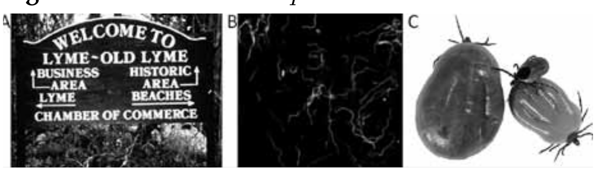
Lyme borreliosis has become the most common vector-borne illness in North Eastern USA and Europe. It is a zoonotic disease, with well-defined symptoms, caused by *B. burgdorferi* sensu lato, and transmitted by ticks. Lyme borreliosis is endemic in the Netherlands with a yearly incidence of approximately 133 cases/100,000 inhabitants. Similar to another spirochetal disease, syphilis, it can be divided into three stages; early, early disseminated and late disseminated manifestations of disease, of which the specific clinical presentations will be discussed in detail. The diagnosis of Lyme borreliosis is based on a history of potential exposure to ticks and the risk of infection with *B. burgdorferi* s.l., development of specific symptoms, exclusion of other causes, and when appropriate, combined with serological and/or other diagnostic tests. The specific indications for, but also the limitations of, serology and other diagnostic tests, including the polymerase chain reaction (PCR), are detailed in this review. Lyme borreliosis is treated with antibiotics, which are usually highly effective. Recent literature discussing the indications for antibiotic treatment, the dosage, duration and type of antibiotic, as well as indications to withhold antibiotic treatment, are reviewed. This review presents the most recent, and when available Dutch, evidence-based information on the ecology, pathogenesis, clinical presentation, diagnosis, treatment and prevention of Lyme borreliosis, argues against the many misconceptions that surround the disease, and provides a framework for the Dutch physician confronted with a patient with putative Lyme borreliosis.

Keywords: Lyme borreliosis, *B. burgdorferi,* clinical signs, diagnostics, treatment

INTRODUCTION

Lyme disease, or Lyme borreliosis, has become the most common tick-borne disease in North Eastern USA and Europe.[1] The disease is named after the town Old Lyme, Connecticut, USA (*figure 1A*), where the link between a tick-borne disease and a group of children suspected of juvenile arthritis was noted in the mid-1970s.[2] Seven years later, the causative agent was discovered by Burgdorfer.[3] In Europe, syndromes, reported as early as 1883, among which Bannwarth syndrome (painful radiculitis, cranial neuritis and lymphocytic meningitis), can retrospectively be designated as manifestations of Lyme borreliosis.[4,5] Lyme borreliosis is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) group *(figure 1B)*.[3] In the USA, *Borrelia burgdorferi* sensu stricto, from here on referred to as *B. burgdorferi*, is the sole causative agent, whereas in Europe *Borrelia garinii* and *Borrelia afzelii* are the predominant causative agents and to a lesser extent *B. burgdorferi* and more recently also *Borrelia bavariensis* and *Borrelia spielmanii*.[6,7] More *Borrelia* species, for example *Borrelia valaisiana* and *Borrelia lusitaniae,* have been identified in Europe; however, for most of these species the pathogenicity to humans is not as clear.[8] In 2009,

Figure 1. *Introduction to Lyme borreliosis*



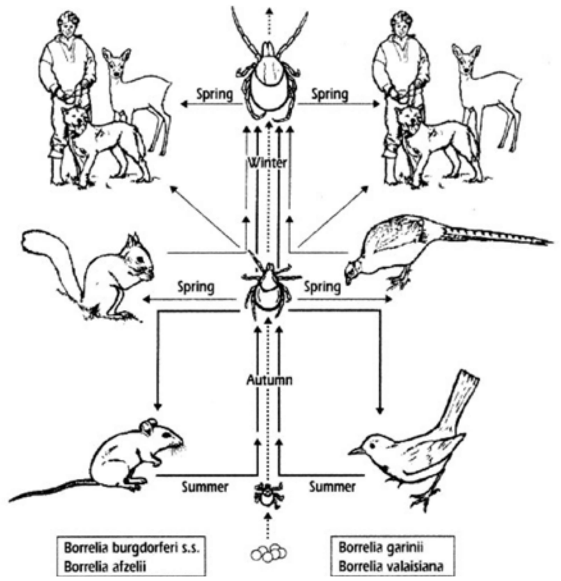
A. The little town, Old Lyme, Connecticut, USA. *B. Fluorescence* microscope image of the spirochete B. burgdorferi. C. Flat, partially fed and fully engorged *Ixodes ricinus* ticks.

Dutch general practitioners (GPs) were estimated to have diagnosed early Lyme borreliosis 22,000 times, corresponding with approximately 133 new cases of erythema migrans (see below) per 100,000 inhabitants per year. This number increased from 6500 in 1994, 13,000 in 2001 to 17,000 in 2005.[9,10]

ECOLOGY

In the USA *B. burgdorferi* is transmitted by the deer tick, *Ixodes scapularis*, whereas the European *Borrelia* species are transmitted by the sheep tick, *Ixodes ricinus (figure 1C)*. In general, uninfected tick larvae acquire the bacterium by feeding on infected animals. Ticks remain infected during their consecutive moulting periods, enabling both nymphal and adult ticks to transmit spirochetes to other (larger) animals, including humans. After their final blood meal adult female ticks, which have already mated, usually lay uninfected eggs *(figure 2)*.[11] The number of visits to Dutch GPs for tick bites rose from 371 per 100,000 in 2001, to 446 and 564 in 2005 and 2009, respectively.[9] In the Netherlands roughly 20% of adult ticks are infected, compared with 10% of nymphs, as shown by a European meta-analysis in 2005.[12] In 2007, 38% of all tick bites in the Netherlands happened in forests, 36% in gardens and 10% in dunes.[13]

Figure 2. *Simplified diagram of the transmission cycle of B. burgdorferi sensu lato species in the Netherlands*



Borrelia **transmission is tightly interwoven with the tick reproductive cycle, which is estimated to take two years in the temperate climate zone. Uninfected eggs (vertical transmission, represented by the thin dashed line, seldom occurs) hatch in summer and autumn and larvae feed in autumn before, or in spring, after winter diapause. In early spring the largest cohort of nymphal ticks emerges, which may yield adult ticks in summer and autumn. In spring young hosts are infected by the emerged nymphs creating the possibility for larvae to acquire an infected blood meal which in its turn augments the abundance of infected nymphs (thick continuous lines 'spring' and 'summer'). On the right side of the diagram the cycle depicts the flow of** ***Borrelia*** **though avian populations (mainly** ***B. garinii*** **and** ***B. valaisiana*****), while on the left side the flow of** ***B. burgdorferi*** **sensu stricto and** ***B. afzelii*****, both with preference for mammals, is shown. However, all four species may be transferred by nymphal and adult ticks (thin continuous lines) to large hosts such as deer, dogs and humans. We thank Dr KE Hovius for providing the figure.**

PATHOGENESIS

Borrelia encounters different environments during its enzootic life cycle,[14] for which differential expression of outer surface proteins (Osp's) is crucial. In unfed ticks, spirochetes express OspA, which binds to the tick receptor of OspA (TROSPA), ensuring attachment of the spirochete to the tick gut.[15] In feeding ticks, approximately 24 to 48 hours after attachment, *Borrelia* down-regulates OspA, expresses OspC and migrates to the salivary glands.[16,17] Here, OspC binds a tick salivary gland protein of 15 kDa (Salp15), shielding the spirochete from complement-dependent (antibody-mediated) killing when transmitted to the host.[18-20] Furthermore, we have previously shown that Salp15 exerts immunosuppressive activity;[21] inhibiting murine T-cell activation and suppressing human dendritic cell (DC) function,[22] which could facilitate both tick feeding as well as *Borrelia* transmission. Numerous other tick proteins, which interact with other host defence mechanisms, facilitate tick feeding and/or enhance the transmission of *Borrelia* or other tick-borne pathogens from the tick to the host, have been identified, as we have previously reviewed.[23] Furthermore, a number of adhesins, proteins on the outer membrane of *B. burgdorferi* s.l. that are involved in the anchoring and interaction with host cells, have been identified, and are important for the establishment and dissemination of infection.[24]

A striking feature of *Borrelia* is its ability to evade host immune response. One mechanism to evade host immune responses is the recombinant gene expression of the variable major protein-like sequence *(vls)* locus.[25] This results in altered antigenicity of the lipoprotein VlsE and thus protection against anti-VlsE antibodies.[26] Also, *Borrelia* can express complement regulator-acquiring surface proteins (CRASPs), preventing complement-mediated killing.[27,28] Recently, another protein, Lmp1, was suggested to be important for evasion of the host adaptive immune responses. Yang *et al.* showed that the N-terminal region of the protein increased pathogen survival.[29] Importantly, *B. afzelii* is associated with skin manifestations, *B. garinii* with neurological involvement and *B. burgdorferi* with infection of the large joints; however, there is a fair amount of overlap between the tropisms of the different genospecies.[30]

CLINICAL MANIFESTATIONS

Tick bite

In a Dutch study with 167 tick bite cases in a GP population, only one case (0.7%) developed Lyme borreliosis upon follow-up serology. Notably, this tick was attached longer than 24 hours.[31] In general, ticks attached shorter than 24 hours do not transmit *Borrelia*.[32,33] Erythematous skin lesions smaller than 5 cm starting within two days after detachment of the tick are most likely a tick bite hypersensitivity reaction. Tick bite hypersensitivity should disappear within one to two days. Diagnostic tests or treatment after a possible tick bite, without symptoms of early Lyme borreliosis (see below), are not recommended in the Netherlands.[34] A recent meta-analysis suggested that, in highly endemic areas in the United States, one case of Lyme borreliosis is prevented for every approximately 50 individuals who are prophylactically treated with antibiotics.[35] Importantly, asymptomatic infection is thought to be much more frequent in Europe than in the USA,[36] arguing against the standard use of prophylactic antibiotics after a tick bite in Europe. However, in individual cases, when the tick was acquired in a highly endemic area of the Netherlands (see www.rivm.nl/cib/infectieziekten-A-Z/infectieziekten/Lyme-borreliose), was attached for a longer period of time, i.e. more than 24 to 48 hours, and the patient presents within three days after the tick bite, prophylactic doxycycline (200 mg once) can be considered. Patient instructions to be alert for typical symptoms of (early) Lyme borreliosis (see below) might be an equally effective alternative.

Early Lyme borreliosis (days to weeks)

Typical erythema migrans is an expanding erythematous skin lesion with central clearing located at the site of tick bite starting after three to 30 days, typically after seven to 14 days, which can vary from 5 to 75 cm (median 15 cm) (*figure 3A*).[37] Both systemic symptoms, such as fever, myalgias and arthralgias, and local symptoms among which itching, burning, and mild pain can accompany EM. A borrelial lymphocytoma is seldom diagnosed, and only in Europe, and described as a bluish red tumour-like skin infiltrate, often located at the earlobe (*figure 3B*) or nipple. It is more common in children and can spontaneously resolve.[6,38] Early Lyme borreliosis symptoms respond well to antibiotic therapy.[39] In Europe, 77 to 89% of all Lyme manifestations are erythema migrans and 2 to 3% borrelial lymphocytoma.[40,41]

Early disseminated Lyme borreliosis (weeks to months)

When the infection is untreated, the spirochete can disseminate and cause early neuroborreliosis (3-16% of Lyme manifestations), Lyme arthritis (5-7%), and seldom a (myo)carditits with (partial) atrioventricular block (<1%).[40,41] Notably, since the late 1980s, increasing awareness for EM and better effective antibiotic regimes have probably made these clinical manifestations become even less common.[42] The European Union Concerted Action on Lyme borreliosis (EUCALB, www.eucalb.com) has established criteria for these manifestations for clinical purposes, which are used in the Dutch guideline developed by the Dutch Institute for Healthcare Improvement (CBO) in 2004,[34,43] which is currently being updated. Early neuroborreliosis can present with lymphocytic meningitis, which is more common in the United States, cranial nerve paresis, usually the facial nerve (*figure 3C*), painful radiculitis, which is more common in Europe, or all of the above, which is equivalent to the Bannwarth syndrome.[44,45] In most patients, acute neurological symptoms improve or resolve in several weeks to months, even without antibiotic treatment.[42] In the early disseminated phase of Lyme borreliosis, infection of the joints is oligoarticular and 50% occur in the knee (*figure 3D*). This manifestation is mostly observed in the United States, where 60% of untreated patients developed arthritis.[42] Cardiac involvement in early Lyme borreliosis in adults is rare and symptoms are usually related to atrioventricular conduction abnormalities.[36]

Figure 3. *Clinical symptoms of Lyme borreliosis*

A. Erythema migrans B. Borrelial lymphocytoma C. Facialis paresis D. Lyme arthritis. E. Acrodermatitis Chronica Atrophicans late (atrophic) stage. We are grateful to Prof. Dr. A.C. Steere and Dr. D.J. Tazelaar for the pictures.

Late Lyme borreliosis (months to years)

One could divide late Lyme manifestations into two groups, manifestations in which persistent *Borrelia* infection is causative for the ongoing symptoms, e.g. acrodermatitis chronica atrophicans (ACA), persistent (untreated) Lyme arthritis and neuroborreliosis, and manifestations in which other mechanisms, e.g. autoimmune phenomena or irreversible tissue damage, might play a role, such as antibiotic-refractory Lyme arthritis, encephalopathy (a subgroup of late neuroborreliosis) and dilated cardiomyopathy.[36] Importantly, over half of the patients with late manifestations of Lyme borreliosis do not remember an EM.[44,46]

ACA can develop up to ten years after infection and is described as a bluish-red atrophic skin lesion, initially combined with oedema, in later stages with atrophy (*figure 3E*) and is predominantly located on the plantar sites of hands and feet or distal parts of the legs. Periarticular nodules, sclerotic lesions and sensory polyneuropathy can be observed.[47] ACA can be confused with vascular conditions, such as venous insufficiency, particularly when the legs are affected.[48] It generally occurs in women older than 40 years,[49] but has been described incidentally in children.[50]

Late neuroborreliosis is rare and includes encephalomyelitis, encephalopathy and axonal polyneuropathy, for a period of at least six months.[42] Encephalomyelitis can present as a slowly progressive myelopathy beginning with an ataxic gait, a gradually worsening spastic paraparesis or tetraparesis or with hearing loss and is accompanied by relatively severe cerebrospinal fluid (CSF) pleocytosis or evident intrathecal anti-*Borrelia* antibody production.[44,51] In contrast, symptoms of encephalopathy are mainly cognitive, in combination with aspecific symptoms, such as fatigue, malaise and myalgia. In most of these patients, there is no evidence of inflammation due to *Borrelia* in the central nervous system (CNS), and therefore, an encephalopathy might actually be an indirect effect of systemic (non-CNS) infection accompanying typical clinical findings of disseminated Lyme borreliosis.[52] Finally, an European study showed that isolated chronic polyneuropathy, without the presence of other late Lyme borreliosis manifestations, such as ACA, is rarely caused by *B. burgdorferi* s.l. infection.[53]

Joint manifestations can occur months to years after exposure, with intermittent recurrent attacks that persist for days, weeks, or months and are typically asymmetrical and pauciarticular in nature and involve one or two larger joints and almost invariably the knee.[54] Most Lyme arthritis patients respond well to conventional antibiotic treatment strategies, such as doxycycline, but a small percentage will continue to have chronic joint inflammation, not due to persistence of the spirochete. This is called antibiotic-refractory Lyme arthritis and occurs more often in the United States than in Europe[42] and has recently been associated with polymorphisms in toll-like receptor (TLR)-1[55] and autoantibodies[56] (and personal communication Prof. Dr. A.C. Steere, Harvard Medical School, Boston, Massachutes, USA), genetic predisposition, i.e. the presence of certain HLA-DR alleles,[57] specific *B. burgdorferi* genotypes[58] and T cell responses, i.e. Th17 responses[59] and low number of regulatory T cells.[60]

In dilated cardiomyopathy, a very rare manifestation of late Lyme borreliosis, spirochetes have rarely been isolated by culture. This might indicate that symptoms could be due to past infection and myocardial scarring rather than ongoing inflammation due to the presence of the spirochete.[61]

A large proportion of treatment-naive individuals present with serological evidence of exposure to *B. burgdorferi* s.l. and symptoms that are aspecific and highly prevalent in the normal population, such as fatigue, myalgia, headache and joint pain. These are not considered specific symptoms or signs of late Lyme borreliosis. However, a selection of these individuals might have an increased risk of Lyme borreliosis, for example a history of previous EM or abundant tick infestations, or live in a highly endemic region of the Netherlands. Although these aspecific symptoms are highly prevalent in the normal population, as are antibodies against *Borrelia* (see below), when other causes have been thoroughly excluded the diagnosis Lyme borreliosis could be considered in a minority of these individuals.

Persistent aspecific symptoms after treatment

A minority of patients, approximately 10 to 20%,[62] experience aspecific symptoms after adequate treatment with antibiotics. This complex of aspecific symptoms might best be referred to as post-Lyme disease syndrome (PLDS).[63] PLDS, for which a definition has been postulated,[37] has been linked to a broad array of symptoms that are highly prevalent in the normal population,[64] similar to those described in the last paragraph of the previous section. This, in combination with the fact that specific antibodies against *Borrelia* occur in approximately 4 to 8% of the normal Dutch population,[65] and in even up to 20% in highly endemic areas in other European countries,[66] and the fact that in this group additional antibiotics after previous adequate treatment have no substantial beneficial effects compared to placebo,[67-70] strongly suggests that persistent *Borrelia* infection is not the cause of the symptoms. Indeed, in animal models (mouse and dog), *B. burgdorferi*-infected animals readily become culture negative upon antibiotic treatment.[71,72] In these studies, which all have major pharmacodynamic concerns, persistence of *B. burgdorferi* DNA has been reported, but has not been associated with disease.[36,73] In humans, mostly in studies of questionable quality and in studies that did not use recommended courses of antibiotic treatment, treatment failure – importantly often associated with persistence or development of specific symptoms – has been described.[74-77] In contrast, in well-designed studies using recommended therapies treatment failure is only seldom reported.[39,78,79] Therefore, the term 'chronic Lyme disease' for persistent aspecific symptoms after adequate treatment for Lyme borreliosis seems to be a misnomer and should be avoided.[63]

DIAGNOSTICS

Considerations before diagnostic tests are performed

The diagnosis of Lyme borreliosis is predominantly based on clinical symptoms and serological tests. The diagnosis can be readily considered in case of symptoms which have been associated with *Borrelia* infection and serological evidence for *Borrelia* infection *(table 1)*. However, in the

Table 1. *Overview of recommended diagnostics and treatment for the different manifestations of Lyme borreliosi*

Clinical manifestation	Serum antibodies sensitivity[I]	Other helpful diagnostics	Sensitivity other diagnostics[VI]	Treatment CBO (2004) and IDSA (2006) first choice	Treatment alternatives
Erythema migrans	38-88% > 6 weeks: 100%[137]	*In case of atypical EM*[IV] PCR Culture	 60-80%[90-92] 40-88%[93,94]	Doxycycline bid 100 mg for 10-14 days	Amoxicillin tid 500 mg for 14 days[VII] or azithromycin qd 500 mg 5 days
Borrelial lymphocytoma	70%	Histopathology PCR Culture	 ND 24%[95]	Doxycycline bid 100 mg for 10-14 days	Amoxicillin tid 500 mg for 14 days[VII] or azithromycin qd 500 mg 5 days
Early neuroborreliosis	80% > 6 weeks: 100%	Intrathecal antibodies (AI) LP cell count (pleocytosis) LP culture LP PCR	55-80%[98,99] 95-100%[44,138] 13%[110] 10-50%[102-104]	Ceftriaxone qd 2 gram[IV] for 14 days	Penicillin-G 2-3 ME 6 times a day, for 14 days or Doxycycline bid 200 mg for 14 days[97]
Late neuroborreliosis	100%	Intrathecal antibodies (AI) LP cell count (pleocytosis)	100%[44] 100%[34,44,139]	Ceftriaxone qd 2 gram[IV] for 30 days	Doxycycline bid 100 mg for 30 days[VIII]
Lyme arthritis	100%[II]	Synovial fluid PCR	46-88%[89,107]	Doxycycline bid 100 mg for 30 days	Ceftriaxone qd 2 gram iv for 14 days
Lyme carditis	80-100%[III]	ECG[V]	ND	Doxycycline b.i.d 100 mg for 21 days	Ceftriaxone q.d 2 gram[IV] for 14 days
Acrodermatitis chronica atrophicans (ACA)	100%	Histopathology PCR Culture	 68-92%[91,92] 22-60%[88]	Doxycycline b.i.d 100 mg for 21-30 days	Ceftriaxone q.d 2 gram[IV] for 14 days[127]

I: Based on the CBO guideline 2004, IDSA guideline 2006. Since 4-8% of the normal population has antibodies against Borrelia, the theoretical specificity of serology is limited to 92-96%. II: Borrelia serology only recommended if the knee is involved. III: Estimated to become 100% during the course of the disease based on other disseminated manifestations. IV: Serology is recommended at least 6-8 weeks after onset. V: Only perform in case of proven early disseminated Lyme borreliosis. VI: In theory, specificity of culture is 100%, of PCR (when appropriate measures are undertaken) 93-100%, and AI 63-97%. VII: In case of pregnancy, photosensibility or allergy for doxycycline. VIII: In case of absence of pleiocytosis. ND: no data. This table does not include treatment recommendations for children younger than 9 years. EM = erythema migrans; PCR = polymerase chain reaction; ND = no data; AI = antibody index; LP = lumbar puncture; qd = once daily; bid = twice daily, tid = three times a day; iv = intravenous.

absence of specific clinical symptoms, the presence of anti-*Borrelia* antibodies does not necessarily indicate the presence of an active *Borrelia* infection, since 4 to 20% of the normal Western European population have detectable antibodies,[65,66,80] most likely due to an (asymptomatic) *Borrelia* infection in the past. When antibodies against *Borrelia* are detected in individuals without specific clinical symptoms, these could be considered 'false positive', since they are not predictive of disease. Therefore, international guidelines, including the CBO 2004 guideline,[34,37] recommend not to test for antibodies against *Borrelia* when there is only a small suspicion on Lyme borreliosis. This has been recently reviewed by others[81] and is enumerated in *table 2*. Despite these recommendations, we have recently shown that of all serological tests (n=312) requested by Dutch physicians in the Amsterdam area, 72% are from individuals with aspecific symptoms. Not surprisingly, in 6% of these sera we demonstrated antibodies against *B. burgdorferi* s.l. (unpublished data), which equals the seroprevalence of the Dutch population.

Table 2. *Testing for antibodies against B. burgdorferi in the normal population*

Test	Lyme disease	No Lyme disease	Total
Positive	40	497	537
Negative	10	9453	9463
Total	50	9950	10,000

False positives = (497/537) *100 = **92.5%**
False negatives = (10/9463) *100 = **0.1%**

Sensitivity: 80%; specificity 95%, pre-test probability: 0.5%. Sensitivity and specificity are based on published literature (see text). The pre-test probability is an estimation based on recent RIVM data and the percentual distribution of symptoms of Lyme borreliosis.

General considerations on diagnostic tests

Detection of antibodies in serum directed against *B. burgdorferi* s.l. is the most common diagnostic approach. Several guidelines recommend that (at least second generation) *B. burgdorferi* s.l. Enzyme-linked Immuno Sorbent Assays (ELISAs or EIAs) should be used as a screening test and, when reactive, should be confirmed by an immunoblot or Western blot (two-tier testing).[34,82] The spectrum of *Borrelia* proteins recognised on Western blot expands with the duration of symptoms.[82] A European multicentre study has indicated eight bands suitable for diagnostic purposes.[83] Compared with IgG, the specificity of IgM components is lower, since rheumatoid factor, acute Epstein-Barr virus (EBV), cytomegalovirus (CMV) infection, multiple sclerosis and other autoimmune diseases can also give a false-positive test.[82,84,85] Newer serological tests include an ELISA detecting antibodies against C6, a 26-amino acid peptide that reproduces the sequence of the sixth invariable region (IR6) within the central domain of the VlsE protein of *B. burgdorferi* s.l.[86] Despite these new developments, two-tier testing is still considered to be necessary, since the immunoblot has a higher specificity than ELISA or EIA. However, in the very early stages of Lyme borreliosis, the immunoblot can be false negative.[86,87] Recently, Branda *et al.* proposed a new testing strategy, two-tiered IgG testing, which avoids the use of IgM blots. Compared with the standard two-tier testing, this method had significantly better sensitivity in early disseminated Lyme borreliosis, the same sensitivity in early and late Lyme borreliosis and a comparable specificity.[87] Other validated and widely accepted diagnostics include culture and polymerase chain reaction (PCR).[34] Clearly, a positive culture in the presence of ongoing specific symptoms indicates an active infection and should be considered as the 'gold standard'. Unfortunately, there are limitations to culture.[36] It is expensive, tissue samples should be incubated in special medium for weeks and there is limited availability in the Netherlands.[34] Sensitivity of culture and PCR to detect *Borrelia* in different tissues/fluids during the different stages of Lyme borreliosis is highly variable (*table 1* and see below). In theory, specificity for culture is 100%. Overall specificity for PCR is 93 to 100%,[88-90] provided certain measures are undertaken to avoid contamination, and amplified products are specified by an appropriate method, e.g. sequencing.[82]

Diagnostic tests for Lyme borreliosis manifestations

Early Lyme borreliosis. EM is a clinical diagnosis and serological tests are not necessary and not recommended *(table 1)*. In case of atypical EM and borrelial lymphocytoma, serological testing can be considered at least six to eight weeks after onset of symptoms *(table 1)*.[34] Alternatively, during these cutaneous manifestations, a skin biopsy at the margins of the EM could be considered for PCR or culture, for which sensitivities of 60 to 80%[90-92] and 40 to 88%,[93,94] respectively, have been reported. Culture for borrelial lymphocytoma has a sensitivity of 24%.[95] Antibiotic treatment during early phases of infection cause a decrease in antibody titres against *Borrelia*.[96]

Early disseminated Lyme borreliosis. Because clinical aspects of early disseminated Lyme borreliosis are not as visually clear as EM and have a broader differential diagnosis, laboratory evidence is necessary *(table 1)*. A guideline from Mygland *et al.* in 2010 provides recommendations for the diagnosis and treatment of neuroborreliosis in Europe.[97] To demonstrate intrathecal production of anti-*Borrelia* antibodies, the cerebral spine fluid (CSF)/serum antibody index (AI) should be performed. Early in the course of neuroborreliosis, absent pleocytosis in CSF has been described and sensitivity of AI is around 55 to 80%.[98,99] After six weeks of symptoms the sensitivity of AI approximates 100%.[100] The specificity of AI has not been the topic of extensive investigation, but ranges from 63[101] to 97%.[98] Several studies have shown a sensitivity of 10 to 50% for the PCR on CSF during early neuroborreliosis.[102-104] PCR on CSF could be useful when there is a strong suspicion of neuroborreliosis and the AI is negative or in patients with an immunodeficiency.[97] Indeed, in a recent patient, who presented to our medical department with a history of tick exposure and symptoms compatible with early neuroborreliosis, we demonstrated the presence of *Borrelia* DNA in CSF by PCR, without the presence of specific anti-*Borrelia* antibodies in serum or CSF. Importantly, this patient had previously been treated with chemotherapy for T-cell lymphoma and was receiving Rituximab, an anti-CD20 monoclonal depleting B-cells, which could explain the absent antibody response (unpublished data). More often, anti-*Borrelia* antibodies are found in CSF, but patients have other neurological diseases. This is illustrated by a study in which, of in total 123 patients with positive *Borrelia* serology (IgG) in CSF, 74 patients had another aetiological diagnosis.[98] Recently, the chemokine CXCL-13 in CSF has been shown to be a promising future diagnostic/treatment marker for neuroborreliosis.[105,106] Both early Lyme arthritis and myocarditis have a broad differential diagnosis and other causes need to be excluded. This, combined with a low a priori change when other Lyme borreliosis manifestations are absent, results in a low positive predictive value for positive antibodies. Therefore, especially for Lyme arthritis, other diagnostic tests such as PCR on synovial fluid should be considered *(table 1)*. PCR of synovial fluid has a sensitivity of 46 to 88%.[89,107] Notably, antibodies are present in 100% of Lyme arthritis and in 80% of Lyme myocarditis cases *(table 1)*.

Late disseminated Lyme borreliosis. Patients with ACA have detectable antibodies in 100% of the cases, the sensitivity of PCR on ACA skin biopsy ranges from 68 to 92%,[91,92,108,109] whereas culture has a lower sensitivity ranging from 22 to 60%.[88] For late neuroborreliosis, criteria include symptoms suggestive of late neuroborreliosis – no other obvious reason for the presenting symptoms, pleocytosis, and demonstration of intrathecal specific antibody synthesis (AI). In late neuroborreliosis, antibodies are present in 100% of the cases, but PCR and culture have a low sensitivity,[97,110] and are therefore not recommended (*table 1*). Late Lyme arthritis also has a 100% antibody detection rate. Routine screening of patients with idiopathic dilated cardiomyopathy for antibodies against *B. burgdorferi* s.l. is of limited utility and should be reserved for patients with a clear history of antecedent Lyme borreliosis symptoms or tick bite.[111]

Invalidated or not recommended diagnostics

Over the last few years Lyme borreliosis has attracted a lot of media attention. By some, an image is created of an insidious (almost) incurable disease, which is extremely difficult to diagnose and for which current diagnostic tests are totally useless. This has created a ground for commercial laboratories offering invalidated or not recommended diagnostic tests. By some, blood and urine are offered for the detection of *Borrelia* DNA. PCR on these body fluids is not validated and not recommended for microbiological diagnosis.[82] A meta-analysis showed a wide range in sensitivity of the urine *Borrelia* PCR of 13 to 100%.[112] A study showed that, after establishing an optimal PCR protocol with spiked urine, *Borrelia* DNA was detected in only one of 12 patients with an acute infection (EM).[113] PCR on blood has a poor sensitivity of only 10 to 18%[88,104,114,115] and there are no European studies performed with a good control group, thus the specificity remains unclear. Theoretically, the specificity should be 100%; however, as outlined before, it is of paramount importance that (commercial) laboratories avoid DNA contamination, perform the correct controls and validate their PCR amplicon. A prospective controlled, blinded study from the USA in 1995 reported a sensitivity of PCR on blood of 18.4% in patients with EM.[114] Blood microscopy should not be used for diagnosis.[37] In addition, in a study using healthy volunteers as a control group, elevated complement factors, such as C3a and C4a, have been associated with acute and chronic Lyme disease.[116,117] These factors will be elevated in many other medical conditions and should therefore not be used to diagnose Lyme disease. Finally, diminished expression of CD57 on mononuclear cells has been claimed to be associated with PLDS.[118] In this study HIV-infected individuals were used as a control group. In HIV infection it has been shown that CD57 expression is upregulated,[119] making it impossible to draw conclusions from this study, and others could not confirm this finding.[120] Finally, commercial laboratories offer PCR to determine if attached ticks are infected with *Borrelia*. This is not recommended by the guidelines, since a positive PCR is not predictable for infection.[121]

THERAPY FOR SPECIFIC LYME BORRELIOSIS MANIFESTATIONS

Antibiotics are effective in all manifestations of Lyme borreliosis *(table 1)*.[42] The difference between antibiotics and expectative policy for EM has never been studied, because the justified use of antibiotics has been shown by randomised double-masked trials in which different antibiotics were compared. However, in theory, in analogy with other manifestations of Lyme borreliosis and other spirochetal diseases such as syphilis, EM could spontaneously resolve. For early Lyme borreliosis manifestations, such as EM and borrelial lymphocytoma, oral treatment, i.e. ten to 14 days of doxycycline, is as effective as parental antibiotics,[122] but has lower risks and adverse events. Importantly, two randomised double-masked trials support a ten-day course of doxycycline 100 mg twice daily.[123,124] Finally, a recent European trial confirmed that oral treatment of early Lyme borreliosis is successful in almost 100% of the cases. Moreover, in the unlikely event of treatment failure (0.4 to 0.7%), objective symptoms of Lyme borreliosis occurred. Importantly, not only were the newly developed aspecific symptoms in the treated EM group comparable with those in the treated age- and sex-matched control group, also the frequency of these symptoms was identical in both groups.[78]

For early disseminated Lyme borreliosis doxycycline is also recommended, except for neuroborreliosis with CNS manifestations, for which ceftriaxone iv is the treatment of choice *(table 1)*. Notably, a multicentre double-blind randomised trial compared ceftriaxone iv with oral doxycycline for adults with (early) neuroborreliosis and concluded that both are equally effective.[125] Although older open studies have suggested that longer treatment, i.e. longer than the recommended 14 to 30 days, might be justified for early (and late) disseminated Lyme borreliosis, a multicentre placebo-controlled randomised trial has shown that prolonged treatment of both early and late disseminated Lyme borreliosis is not warranted,[126] which is in line with most, if not all, esteemed and peer-reviewed international guidelines.[37]

In late manifestations of Lyme borreliosis, the same antibiotics are recommended, but with a longer duration of treatment (*table 1*).[127] For selected individuals with aspecific symptoms in combination with positive Lyme serology antibiotic treatment could be considered. Although, to our knowledge, evidence-based guidelines for this are non-existent, treatment could be adjusted based on the duration of symptoms, e.g. short duration of symptoms (<3 months) could be treated with 10-14 days of doxycycline 100 mg twice a day and longer lasting symptoms with 30 days of doxycycline 100 mg twice a day.

In case of persistence of specific Lyme borreliosis symptoms, persisting *B. burgdorferi* s.l. infection, or re-infection, should be considered and additional or prolonged therapy could be indicated. In stark contrast, patients with PLDS, or individuals with false-positive Lyme serology and aspecific symptoms, such as fatigue, myalgia, headache and joint pain, should not receive antibiotic treatment. However, some of these patients are occasionally unjustly treated for months to years with (multiple) intravenously administered antibiotics, for which no credible scientific evidence exists. Such approaches pose a great risk for serious adverse effects.[68,69] As stated before, multiple placebo-controlled randomised trials have shown no substantial additional effect for additional antibiotic treatment in these individuals.[67-70]

PREVENTION

The best preventive method to prevent *Borrelia* infection is to attempt to avoid exposure to ticks by wearing protective clothing. In addition, a full body check within 24 hours after possible tick exposure could detect attached ticks, which should be promptly removed. This strategy is promoted by the Dutch National Institute for Public Health and Environment (RIVM) and accessible for the public at www.rivm.nl/cib/themas/teken-lyme. Calculations have indicated that a Lyme vaccine could be economically attractive when used in persons living in an area with an annual risk of more than 1% of contracting Lyme borreliosis.[128] Such regions are prevalent in North Eastern USA,[129] however are yet to be identified in the Netherlands. The only licensed Lyme vaccine was based on recombinant OspA, which showed a 70% efficacy in phase III human trial.[130] It became available in 1998, but was removed from the market in 2002 because of public perceptions on adverse events. We recently discussed the possibilities for vaccine strategies against Lyme borreliosis,[131] such as vaccines based on the combination of *Borrelia* and tick (saliva) proteins. Indeed, antibodies against the tick salivary gland protein Salp15 – by itself able to impair *B. burgdorferi* infection in tick-challenged mice – had synergistic effects in conjunction with a vaccine directed against *B. burgdorferi* antigens.[132] Also, combination vaccines, consisting of multiple *Borrelia* antigens, showed higher efficacy compared with vaccination based on single or double antigens, in mice.[133] Such novel approaches have yet to be tested in humans. Finally, to reduce the risk of human Lyme borreliosis, preventive approaches include decreasing tick densities, tick *B. burgdorferi* s.l. infection rates and wildlife control. The latter can be achieved by the use of acaricides.[134] However, resistance to acaricides in ticks occurs, and acaracides are harmful for humans, animals and the environment.[135] Novel strategies comprise wildlife Lyme vaccines[132] or prophylactic treatment of wildlife with doxycycline[136] (and personal communication Dr. Piesman, Center for Disease Control (CDC), Fort Collins, Colorado, USA).

CONCLUSIONS/SUMMARY

Lyme borreliosis is endemic in the Netherlands with a yearly incidence of EM of approximately 133 cases/100,000. It is a zoonotic disease, with well-defined symptoms, caused by *B. burgdorferi* s.l. and transmitted by ticks. Diagnosis of early Lyme borreliosis, i.e. EM, is made clinically and there is no need for serological tests. Diagnosis of later manifestations is based on the combination of specific clinical symptoms and positive serology and/or other diagnostic tests. In longer lasting manifestations of Lyme borreliosis sensitivity of serology approaches 100%. Specificity of serology is lower, since the seroprevalence of antibodies against *B. burgdorferi* s.l. is approximately 4 to 8% in the general population. This includes treated Lyme borreliosis patients, individuals who have spontaneously cleared asymptomatic *Borrelia* infection or have cross-reacting antibodies. Therefore, in individuals with aspecific symptoms it is not recommended to test for antibodies against *Borrelia*. Antibiotics are effective in all manifestations of Lyme borreliosis and prognosis is usually excellent. However, a minority of patients experience potentially severe, but aspecific symptoms after previous adequate treatment for Lyme borreliosis. In these individuals, additional antibiotics have no substantial beneficial effects compared with placebo.[67-70] A challenge for the future is to develop a test to detect, or rule out, persistent active *B. burgdorferi* s.l. infection. This could reassure individuals who experience aspecific symptoms after previous recommended therapy for Lyme borreliosis, prevent unnecessary treatment and pave the way for research on the true aetiologies of aspecific symptoms after recommended antibiotic treatment for Lyme borreliosis. Finally, preventing Lyme borreliosis, by the development of novel vaccination strategies or wildlife control, remains an important challenge for the future. Thus, bearing these developments in mind, we should definitely not allow ourselves to become tired of Lyme borreliosis.

REFERENCES

1. Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. J Clin Invest. 2004;113:1093-101.
2. Steere AC, Malawista SE, Snydman DR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum. 1977;20:7-17.
3. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? Science. 1982;216:1317-9.
4. Bannwarth A. Chronische lymphocytäre meningitis, entzündliche polyneuritis und rheumatismus. Arztl Wochensch. 1948;3:417-23.
5. Schaltenbrand G. Durch Arthropoden ubertragene Erkrankung der Haut und des Nervensystems. Verhandlungen der Deutschen Gesellschaft fur Innere Medizin. 1967;72:975-1006.

6. Steere AC. Lyme disease. N Engl J Med. 1989;321:586-96.

7. Fingerle V, Schulte-Spechtel UC, Ruzic-Sabljic E, et al. Epidemiological aspects and molecular characterization of Borrelia burgdorferi s.l. from southern Germany with special respect to the new species Borrelia spielmanii sp. nov. Int J Med Microbiol. 2008;298:279-90.

8. van Dam AP. Diversity of Ixodes-borne Borrelia species--clinical, pathogenetic, and diagnostic implications and impact on vaccine development. Vector Borne Zoonotic Dis. 2002;2:249-54.

9. Hofhuis A, Harms MG, van der Giessen JWB, Sprong H, Notermans DW, van Pelt W. Ziekte van Lyme in Nederland 1994-2009: Aantal huisarts-consulten blijft toenemen. Is voorlichting en curatief beleid genoeg? Infectieziekten Bulletin. 2010;21:84-7.

10. Hofhuis A, van der Giessen JW, Borgsteede FH, Wielinga PR, Notermans DW, van Pelt W. Lyme borreliosis in the Netherlands: strong increase in GP consultations and hospital admissions in past 10 years. Euro Surveill. 2006;11:E060622 2.

11. Anderson JF. Epizootiology of Borrelia in Ixodes tick vectors and reservoir hosts. Rev Infect Dis. 1989;11 Suppl 6:S1451-9.

12. Rauter C, Hartung T. Prevalence of Borrelia burgdorferi sensu lato genospecies in Ixodes ricinus ticks in Europe: a metaanalysis. Appl Environ Microbiol. 2005;71:7203-16.

13. Takken W, van Vliet AJH, van Overbeek L, et al. Teken, tekenbeten en Borrelia infecties in Nederland Deel II. Wageningen UR, Wageningen. 2008.

14. Hovius JW, van Dam AP, Fikrig E. Tick-host-pathogen interactions in Lyme borreliosis. Trends Parasitol. 2007;23:434-8.

15. Pal U, Li X, Wang T, et al. TROSPA, an Ixodes scapularis receptor for Borrelia burgdorferi. Cell. 2004;119:457-68.

16. Yang XF, Pal U, Alani SM, Fikrig E, Norgard MV. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. J Exp Med. 2004;199:641-8.

17. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on Borrelia burgdorferi during tick feeding. Proc Natl Acad Sci U S A. 1995;92:2909-13.

18. Ramamoorthi N, Narasimhan S, Pal U, et al. The Lyme disease agent exploits a tick protein to infect the mammalian host. Nature. 2005;436:573-7.

19. Schuijt TJ, Hovius JW, van Burgel ND, Ramamoorthi N, Fikrig E, van Dam AP. The tick salivary protein Salp15 inhibits the killing of serum-sensitive Borrelia burgdorferi sensu lato isolates. Infect Immun. 2008;76:2888-94.

20. Hovius JW, Schuijt TJ, de Groot KA, et al. Preferential protection of Borrelia burgdorferi sensu stricto by a Salp15 homologue in Ixodes ricinus saliva. J Infect Dis. 2008;198:1189-97.

21. Anguita J, Ramamoorthi N, Hovius JW, et al. Salp15, an ixodes scapularis salivary protein, inhibits CD4(+) T cell activation. Immunity. 2002;16:849-59.

22. Hovius JW, de Jong MA, den Dunnen J, et al. Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. PLoS Pathog. 2008;4:e31.

23. Hovius JW, Levi M, Fikrig E. Salivating for knowledge: potential pharmacological agents in tick saliva. PLoS Med. 2008;5:e43.

24. Coburn J, Fischer JR, Leong JM. Solving a sticky problem: new genetic approaches to host cell adhesion by the Lyme disease spirochete. Mol Microbiol. 2005;57:1182-95.

25. Fikrig E, Feng W, Aversa J, Schoen RT, Flavell RA. Differential expression of Borrelia burgdorferi genes during erythema migrans and Lyme arthritis. J Infect Dis. 1998;178:1198-201.

26. McDowell JV, Sung SY, Hu LT, Marconi RT. Evidence that the variable regions of the central domain of VlsE are antigenic during infection with lyme disease spirochetes. Infect Immun. 2002;70:4196-203.

27. Kraiczy P, Skerka C, Brade V, Zipfel PF. Further characterization of complement regulator-acquiring surface proteins of Borrelia burgdorferi. Infect Immun. 2001;69:7800-9.

28. Kraiczy P, Rossmann E, Brade V, et al. Binding of human complement regulators FHL-1 and factor H to CRASP-1 orthologs of Borrelia burgdorferi. Wien Klin Wochenschr. 2006;118:669-76.

29. Yang X, Lenhart TR, Kariu T, Anguita J, Akins DR, Pal U. Characterization of unique regions of Borrelia burgdorferi surface-located membrane protein 1. Infect Immun. 2010.

30. Strle F, Nadelman RB, Cimperman J, et al. Comparison of culture-confirmed erythema migrans caused by Borrelia burgdorferi sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann Intern Med. 1999;130:32-6.

31. Jacobs JJ, Noordhoek GT, Brouwers JM, Wielinga PR, Jacobs JP, Brandenburg AH. [Small risk of developing Lyme borreliosis following a tick bite on Ameland: research in a general practice]. Ned Tijdschr Geneeskd. 2008;152:2022-6.

32. Piesman J, Mather TN, Sinsky RJ, Spielman A. Duration of tick attachment and Borrelia burgdorferi transmission. J Clin Microbiol. 1987;25:557-8.

33. Piesman J, Maupin GO, Campos EG, Happ CM. Duration of adult female Ixodes dammini attachment and transmission of Borrelia burgdorferi, with description of a needle aspiration isolation method. J Infect Dis. 1991;163:895-7.

34. Speelman P, de Jongh BM, Wolfs TF, Wittenberg J. [Guideline 'Lyme borreliosis']. Ned Tijdschr Geneeskd. 2004;148:659-63.

35. Warshafsky S, Lee DH, Francois LK, Nowakowski J, Nadelman RB, Wormser GP. Efficacy of antibiotic prophylaxis for the prevention of Lyme disease: an updated systematic review and meta-analysis. J Antimicrob Chemother. 2010;65:1137-44.

36. Hovius JWR, van Dam AP, Fikrig E. Late Manifestations of Lyme Borreliosis. In: Fratamico PM, Smith JL, Brogden KA, editors. Sequela and Long-Term Consequences of Infectious Diseases. Washington DC: ASM Press; 2009.

37. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2006;43:1089-134.

38. Strle F, Pleterski-Rigler D, Stanek G, Pejovnik-Pustinek A, Ruzic E, Cimperman J. Solitary borrelial lymphocytoma: report of 36 cases. Infection. 1992;20:201-6.

39. Dattwyler RJ, Luft BJ, Kunkel MJ, et al. Ceftriaxone compared with doxycycline for the treatment of acute disseminated Lyme disease. N Engl J Med. 1997;337:289-94.

40. Berglund J, Eitrem R, Ornstein K, et al. An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med. 1995;333:1319-27.

41. Huppertz HI, Bohme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme borreliosis in the Wurzburg region of Germany. Eur J Clin Microbiol Infect Dis. 1999;18:697-703.

42. Steere AC. Lyme disease. N Engl J Med. 2001;345:115-25.

43. Stanek G, O'Connell S, Cimmino M, et al. European Union Concerted Action on Risk Assessment in Lyme Borreliosis: clinical case definitions for Lyme borreliosis. Wien Klin Wochenschr. 1996;108:741-7.

44. Hansen K, Lebech AM. The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985-1990. A prospective study of 187 patients with Borrelia burgdorferi specific intrathecal antibody production. Brain. 1992;115(Pt 2):399-423.

45. Kaiser R. Neuroborreliosis. J Neurol. 1998;245:247-55.

46. 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.

47. Brehmer-Andersson E, Hovmark A, Asbrink E. Acrodermatitis chronica atrophicans: histopathologic findings and clinical correlations in 111 cases. Acta Derm Venereol. 1998;78:207-13.

48. Fagrell B, Stiernstedt G, Ostergren J. Acrodermatitis chronica atrophicans Herxheimer can often mimic a peripheral vascular disorder. Acta Med Scand. 1986;220:485-8.

49. 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.

50. Muellegger RR, Schluepen EM, Millner MM, Soyer HP, Volkenandt M, Kerl H. Acrodermatitis chronica atrophicans in an 11-year-old girl. Br J Dermatol. 1996;135:609-12.

51. Oschmann P, Dorndorf W, Hornig C, Schafer C, Wellensiek HJ, Pflughaupt KW. Stages and syndromes of neuroborreliosis. J Neurol. 1998;245:262-72.

52. Halperin JJ, Krupp LB, Golightly MG, Volkman DJ. Lyme borreliosis-associated encephalopathy. Neurology. 1990;40:1340-3.

53. Mygland A, Skarpaas T, Ljostad U. Chronic polyneuropathy and Lyme disease. Eur J Neurol. 2006;13:1213-5.

54. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. Ann Intern Med. 1987;107:725-31.

55. Strle K, Shin JJ, Glickstein L, Steere AC. Toll-Like Receptor 1 Polymorphism (1805GG) Is a Risk Factor for Antibiotic-Refractory Lyme arthritis. ICAAC 2010. Abstract.

56. Drouin EE, Seward RJ, Yao C, Costello CE, Steere AC. A novel Human Autoantigen Endothelial Cell Growth Factor Induces Linked T and B Cell Responses in Patients with Antibiotic-Refractory Lyme Arthritis. ICAAC 2010. Abstract.

57. Gross DM, Forsthuber T, Tary-Lehmann M, et al. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. Science. 1998;281:703-6.

58. Jones KL, McHugh GA, Glickstein LJ, Steere AC. Analysis of Borrelia burgdorferi genotypes in patients with Lyme arthritis: High frequency of ribosomal RNA intergenic spacer type 1 strains in antibiotic-refractory arthritis. Arthritis Rheum. 2009;60:2174-82.

59. Codolo G, Amedei A, Steere AC, et al. Borrelia burgdorferi NapA-driven Th17 cell inflammation in lyme arthritis. Arthritis Rheum. 2008;58:3609-17.

60. Shen S, Shin JJ, Strle K, et al. Treg cell numbers and function in patients with antibiotic-refractory or antibiotic-responsive Lyme arthritis. Arthritis Rheum. 2010;62:2127-37.

61. Gasser R, Dusleag J, Reisinger E, et al. Reversal by ceftriaxone of dilated cardiomyopathy Borrelia burgdorferi infection. Lancet. 1992;339:1174-5.

62. Marques A. Chronic Lyme disease: a review. Infect Dis Clin North Am. 2008;22:341-60, vii-viii.

63. Feder HM Jr, Johnson BJ, O'Connell S, et al. A critical appraisal of "chronic Lyme disease". N Engl J Med. 2007;357:1422-30.

64. Luo N, Johnson JA, Shaw JW, Feeny D, Coons SJ. Self-reported health status of the general adult U.S. population as assessed by the EQ-5D and Health Utilities Index. Med Care. 2005;43:1078-86.

65. Gutierrez J, Guerrero M, Nunez F, Soto MJ, Piedrola G, Maroto MC. Antibodies to Borrelia burgdorferi in European populations. J Clin Lab Anal. 2000;14:20-6.

66. Carlsson SA, Granlund H, Nyman D, Wahlberg P. IgG seroprevalence of Lyme borreliosis in the population of the Aland Islands in Finland. Scand J Infect Dis. 1998;30:501-3.

67. Fallon BA, Keilp JG, Corbera KM, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology. 2008;70:992-1003.

68. Klempner MS, Hu LT, Evans J, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med. 2001;345:85-92.

69. Krupp LB, Hyman LG, Grimson R, et al. Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial. Neurology. 2003;60:1923-30.

70. Kaplan RF, Trevino RP, Johnson GM, et al. Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology. 2003;60:1916-22.

71. Moody KD, Adams RL, Barthold SW. Effectiveness of antimicrobial treatment against Borrelia burgdorferi infection in mice. Antimicrob Agents Chemother. 1994;38:1567-72.

72. Straubinger RK, Summers BA, Chang YF, Appel MJ. Persistence of Borrelia burgdorferi in experimentally infected dogs after antibiotic treatment. J Clin Microbiol. 1997;35:111-6.

73. Wormser GP, Schwartz I. Antibiotic treatment of animals infected with Borrelia burgdorferi. Clin Microbiol Rev. 2009;22:387-95.

74. Nowakowski J, McKenna D, Nadelman RB, et al. Failure of treatment with cephalexin for Lyme disease. Arch Fam Med. 2000;9:563-7.

75. 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;28:312-4.

76. Carlson D, Hernandez J, Bloom BJ, Coburn J, Aversa JM, Steere AC. Lack of Borrelia burgdorferi DNA in synovial samples from patients with antibiotic treatment-resistant Lyme arthritis. Arthritis Rheum. 1999;42:2705-9.

77. Dattwyler RJ, Halperin JJ. Failure of tetracycline therapy in early Lyme disease. Arthritis Rheum. 1987;30:448-50.

78. Cerar D, Cerar T, Ruzic-Sabljic E, Wormser GP, Strle F. Subjective symptoms after treatment of early Lyme disease. Am J Med. 2010;123:79-86.

79. Luger SW, Paparone P, Wormser GP, et al. Comparison of cefuroxime axetil and doxycycline in treatment of patients with early Lyme disease associated with erythema migrans. Antimicrob Agents Chemother. 1995;39:661-7.

80. Nohlmans MK, van den Bogaard AE, Blaauw AA, van Boven CP. [Prevalence of Lyme borreliosis in The Netherlands]. Ned Tijdschr Geneeskd. 1991;135:2288-92.

81. Lakos A, Reiczigel J, Solymosi N. The positive predictive value of Borrelia burgdorferi serology in the light of symptoms of patients sent to an outpatient service for tick-borne diseases. Inflamm Res. 2010.

82. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol. 2007;49:13-21.

83. Robertson J, Guy E, Andrews N, et al. A European multicenter study of immunoblotting in serodiagnosis of lyme borreliosis. J Clin Microbiol. 2000;38:2097-102.

84. Goossens HA, Nohlmans MK, van den Bogaard AE. Epstein-Barr virus and cytomegalovirus infections cause false-positive results in IgM two-test protocol for early Lyme borreliosis. Infection. 1999;27:231.

85. Bunikis J, Barbour AG. Laboratory testing for suspected Lyme disease. Med Clin North Am. 2002;86:311-40.

86. Wormser GP, Liveris D, Hanincova K, et al. Effect of Borrelia burgdorferi genotype on the sensitivity of C6 and 2-tier testing in North American patients with culture-confirmed Lyme disease. Clin Infect Dis. 2008;47:910-4.

87. Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJ, Wormser GP, Steere AC. 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test. Clin Infect Dis. 2010;50:20-6.

88. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin Microbiol Rev. 2005;18:484-509.

89. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med. 1994;330:229-34.

90. Cerar T, Ruzic-Sabljic E, Glinsek U, Zore A, Strle F. Comparison of PCR methods and culture for the detection of Borrelia spp. in patients with erythema migrans. Clin Microbiol Infect. 2008;14:653-8.

91. 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.

92. Rijpkema SG, Tazelaar DJ, Molkenboer MJ, et al. Detection of Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia garinii and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol Infect. 1997;3:109-16.

93. Wormser GP, Bittker S, Cooper D, Nowakowski J, Nadelman RB, Pavia C. Yield of large-volume blood cultures in patients with early Lyme disease. J Infect Dis. 2001;184:1070-2.

94. Ruzic-Sabljic E, Lotric-Furlan S, Maraspin V, et al. Comparison of isolation rate of Borrelia burgdorferi sensu lato in MKP and BSK-II medium. Int J Med Microbiol. 2006;296 Suppl 40:267-73.

95. Maraspin V, Cimperman J, Lotric-Furlan S, et al. Solitary borrelial lymphocytoma in adult patients. Wien Klin Wochenschr. 2002;114:515-23.

96. Kannian P, McHugh G, Johnson BJ, Bacon RM, Glickstein LJ, Steere AC. Antibody responses to Borrelia burgdorferi in patients with antibiotic-refractory, antibiotic-responsive, or non-antibiotic-treated Lyme arthritis. Arthritis Rheum. 2007;56:4216-25.

97. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol. 2010;17:8-16, e1-4.

98. Blanc F, Jaulhac B, Fleury M, et al. Relevance of the antibody index to diagnose Lyme neuroborreliosis among seropositive patients. Neurology. 2007;69:953-8.

99. Hansen K, Hindersson P, Pedersen NS. Measurement of antibodies to the Borrelia burgdorferi flagellum improves serodiagnosis in Lyme disease. J Clin Microbiol. 1988;26:338-46.

100. Ljostad U, Skarpaas T, Mygland A. Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis. Eur J Neurol. 2007;14:873-6.

101. Ljostad U, Mygland A. CSF B--lymphocyte chemoattractant (CXCL13) in the early diagnosis of acute Lyme neuroborreliosis. J Neurol. 2008;255:732-7.

102. Gooskens J, Templeton KE, Claas EC, van Dam AP. Evaluation of an internally controlled real-time PCR targeting the ospA gene for detection of Borrelia burgdorferi sensu lato DNA in cerebrospinal fluid. Clin Microbiol Infect. 2006;12:894-900.

103. Roux F, Boyer E, Jaulhac B, Dernis E, Closs-Prophette F, Puechal X. Lyme meningoradiculitis: prospective evaluation of biological diagnosis methods. Eur J Clin Microbiol Infect Dis. 2007;26:685-93.

104. Cerar T, Ogrinc K, Cimperman J, Lotric-Furlan S, Strle F, Ruzic-Sabljic E. Validation of cultivation and PCR methods for diagnosis of Lyme neuroborreliosis. J Clin Microbiol. 2008;46:3375-9.

105. Senel M, Rupprecht TA, Tumani H, Pfister HW, Ludolph AC, Brettschneider J. The chemokine CXCL13 in acute neuroborreliosis. J Neurol Neurosurg Psychiatry. 2010;81:929-33.

106. van Burgel ND, Kroes ACM, van Dam AP. CXCL13 in CSF as a biomarker for diagnosing Lyme neuroborreliosis. ICAAC 2010. Abstract.

107. Priem S, Rittig MG, Kamradt T, Burmester GR, Krause A. An optimized PCR leads to rapid and highly sensitive detection of Borrelia burgdorferi in patients with Lyme borreliosis. J Clin Microbiol. 1997;35:685-90.

108. Moter SE, Hofmann H, Wallich R, Simon MM, Kramer MD. Detection of Borrelia burgdorferi sensu lato in lesional skin of patients with erythema migrans and acrodermatitis chronica atrophicans by ospA-specific PCR. J Clin Microbiol. 1994;32:2980-8.

109. 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.

110. Karlsson M, Hovind-Hougen K, Svenungsson B, Stiernstedt G. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. J Clin Microbiol. 1990;28:473-9.

111. Pinto DS. Cardiac manifestations of Lyme disease. Med Clin North Am. 2002;86:285-96.

112. Dumler JS. Molecular diagnosis of Lyme disease: review and meta-analysis. Mol Diagn. 2001;6:1-11.

113. Rauter C, Mueller M, Diterich I, et al. Critical evaluation of urine-based PCR assay for diagnosis of Lyme borreliosis. Clin Diagn Lab Immunol. 2005;12:910-7.

114. Goodman JL, Bradley JF, Ross AE, et al. Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using the polymerase chain reaction. Am J Med. 1995;99:6-12.

115. Oksi J, Marttila H, Soini H, Aho H, Uksila J, Viljanen MK. Early dissemination of Borrelia burgdorferi without generalized symptoms in patients with erythema migrans. APMIS. 2001;109:581-8.

116. Stricker RB, Savely VR, Motanya NC, Giclas PC. Complement split products c3a and c4a in chronic lyme disease. Scand J Immunol. 2009;69:64-9.

117. Shoemaker RC, Giclas PC, Crowder C, House D, Glovsky MM. Complement split products C3a and C4a are early markers of acute lyme disease in tick bite patients in the United States. Int Arch Allergy Immunol. 2008;146:255-61.

118. Stricker RB, Winger EE. Decreased CD57 lymphocyte subset in patients with chronic Lyme disease. Immunol Lett. 2001;76:43-8.

119. Bogner JR, Goebel FD. Lymphocyte subsets as surrogate markers in antiretroviral therapy. Infection. 1991;19 Suppl 2:S103-8.

120. Marques A, Brown MR, Fleisher TA. Natural killer cell counts are not different between patients with post-Lyme disease syndrome and controls. Clin Vaccine Immunol. 2009;16:1249-50.

121. Sood SK, Salzman MB, Johnson BJ, et al. Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. J Infect Dis. 1997;175:996-9.

122. Weber K, Preac-Mursic V, Wilske B, Thurmayr R, Neubert U, Scherwitz C. A randomized trial of ceftriaxone versus oral penicillin for the treatment of early European Lyme borreliosis. Infection. 1990;18:91-6.

123. Wormser GP, Ramanathan R, Nowakowski J, et al. Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2003;138:697-704.

124. Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. Antibiotic treatment duration and long-term outcomes of patients with early lyme disease from a lyme disease-hyperendemic area. Clin Infect Dis. 2010;50:512-20.

125. Ljostad U, Skogvoll E, Eikeland R, et al. Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. Lancet Neurol. 2008;7:690-5.

126. Oksi J, Nikoskelainen J, Hiekkanen H, et al. Duration of antibiotic treatment in disseminated Lyme borreliosis: a double-blind, randomized, placebo-controlled, multicenter clinical study. Eur J Clin Microbiol Infect Dis. 2007;26:571-81.

127. Mullegger RR. Dermatological manifestations of Lyme borreliosis. Eur J Dermatol. 2004;14:296-309.

128. Shadick NA, Liang MH, Phillips CB, Fossel K, Kuntz KM. The cost-effectiveness of vaccination against Lyme disease. Arch Intern Med. 2001;161:554-61.

129. Bacon RM, Kugeler KJ, Mead PS. Surveillance for Lyme disease--United States, 1992-2006. MMWR Surveill Summ. 2008;57:1-9.

130. Abbott A. Lyme disease: uphill struggle. Nature. 2006;439:524-5.

131. Schuijt TJ, Hovius JW, van der Poll T, van Dam AP, Fikrig E. Lyme borreliosis vaccination: the facts, the challenge and the future. Trends Parasitol. 2010.

132. Dai J, Wang P, Adusumilli S, et al. Antibodies against a tick protein, Salp15, protect mice from the Lyme disease agent. Cell Host Microbe. 2009;6:482-92.

133. Brown EL, Kim JH, Reisenbichler ES, Hook M. Multicomponent Lyme vaccine: three is not a crowd. Vaccine. 2005;23:3687-96.

134. Curran KL, Fish D, Piesman J. Reduction of nymphal Ixodes dammini (Acari: Ixodidae) in a residential suburban landscape by area application of insecticides. J Med Entomol. 1993;30:107-13.

135. Graf JF, Gogolewski R, Leach-Bing N, et al. Tick control: an industry point of view. Parasitology. 2004;129 Suppl:S427-42.

136. Dolan MC, Zeidner NS, Gabitzsch E, et al. A doxycycline hyclate rodent bait formulation for prophylaxis and treatment of tick-transmitted Borrelia burgdorferi. Am J Trop Med Hyg. 2008;78:803-5.

137. Bacon RM, Biggerstaff BJ, Schriefer ME, et al. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of Borrelia burgdorferi compared with 2-tiered testing using whole-cell lysates. J Infect Dis. 2003;187:1187-99.

138. Tumani H, Nolker G, Reiber H. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. Neurology. 1995;45:1663-70.

139. Logigian EL, Steere AC. Clinical and electrophysiologic findings in chronic neuropathy of Lyme disease. Neurology. 1992;42:303-11.