Lyme borreliosis: diagnosis and management

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ABSTRACT

Lyme borreliosis is the most common vectorborne disease in the northern hemisphere. It usually begins with erythema migrans; early disseminated infection particularly causes multiple erythema migrans or neurologic disease, and late manifestations predominantly include arthritis in North America, and acrodermatitis chronica atrophicans (ACA) in Europe. Diagnosis of Lyme borreliosis is based on characteristic clinical signs and symptoms, complemented by serological confirmation of infection once an antibody response has been mounted. Manifestations usually respond to appropriate antibiotic regimens, but the disease can be followed by sequelae, such as immune arthritis or residual damage to affected tissues. A subset of individuals reports persistent symptoms, including fatigue, pain, arthralgia, and neurocognitive symptoms, which in some people are severe enough to fulfil the criteria for post-treatment Lyme disease syndrome. The reported prevalence of such persistent symptoms following antimicrobial treatment varies considerably, and its pathophysiology is unclear. Persistent active infection in humans has not been identified as a cause of this syndrome, and randomized treatment trials have invariably failed to show any benefit of prolonged antibiotic treatment. For prevention of Lyme borreliosis, post-exposure prophylaxis may be indicated in specific cases, and novel vaccine strategies are under development.

Introduction

Lyme borreliosis, or Lyme disease, is an emerging tickborne disease, primarily caused by the bacterium *Borrelia burgdorferi* sensu stricto (ss) in North America and predominantly *Borrelia afzelii* or *Borrelia garinii* in Europe. Reported incidence has been increasing, and the clinical manifestations of Lyme borreliosis are diverse. Establishing the diagnosis may be complex, particularly for early manifestations, before a serological response has developed, and where there is disseminated infection in the joints, the heart, or the central nervous system. Another limitation of serological testing is that antibodies can remain for years after infection, and serology can therefore not be used to assess the efficacy of antibiotic therapy. Thus, the diagnosis and evaluation of Lyme borreliosis mainly depends on clinical evaluation, as is discussed in this review. Most people respond well to antibiotic therapy as recommended by treatment guidelines. However, some report post-infectious signs or symptoms, despite recommended antibiotic therapy and putative clearance of infection. These symptoms, which include fatigue, pain, and neurocognitive symptoms, may be persistent and highly disabling. Common uncertainties among patients and physicians include the reliability of diagnostic tests for Lyme borreliosis, and the pathogenesis and therapy of persistent symptoms. In this review, we assess the diagnostic and therapeutic approach to Lyme borreliosis, and the evidence related to pathogenesis and management of sequelae and post-infectious symptoms attributed to Lyme borreliosis.

Sources and selection criteria

We searched PubMed for publications between January 2009 and January 2019, using the search terms “Borrelia Infections”, “Borrelia”, “borrelia”, “b. afzelii”, “b. burgdorferi”, “b. garinii”, “Borrelia afzelii,,” “Borrelia burgdorferi”, “Borrelia garinii”, “borreliosis”, “Erythema Chronicum Migrans”, “Erythema Migrans”, “lyme”, “Lymes”, “Lyme’s”, “Neuroborreliosis”

We categorized human, animal, and in vitro studies based on title and abstract into the topics of this review, and favored randomized trials, systematic reviews, and guidelines in English. Observational patient studies, in vitro, and animal studies with adequate study design and statistical methods were also reviewed. Because of the clinical focus of this review, we also included case series.

We also reviewed clinical trials of limited quality and correspondence to describe actual controversies. Studies published before 2009 were included if they were referred to by selected papers, guidelines, or reviews. We included articles of sufficient quality published after January 2019 (during the writing

process of this review) if relevant to the scope of this review.

Incidence and epidemiology

Borrelia burgdorferi sensu lato and their vectors

Lyme borreliosis is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato (sl) complex, which consists of ~20 genospecies with a complex genomic structure.[1] Not all *B burgdorferi* sl species are pathogenic, and in North America, *Borrelia burgdorferi* ss is the dominant genospecies associated with Lyme borreliosis,[2] although a novel genospecies, *Borrelia mayonii*, was recently identified.[3] In Eurasia, *B afzelii* and *B garinii* are the most common *B burgdorferi* sl genospecies in ticks and humans.[4 5]*B burgdorferi* sl genospecies are genetically distinct from other species within the genus *Borrelia*—ie, those causing relapsing fever. Recently, it was suggested to divide the genus *Borrelia* into two, with the new genus name for the Lyme borreliosis group of spirochetes being *Borreliella*,[6] although it is debatable whether such a split is justified.[7 8]

B burgdorferi sl spirochetes are transmitted through the bite of tick species belonging to the genus *Ixodes*, which are largely confined to temperate climate zones of the northern hemisphere.[2] In North America, the *Ixodes* species that transmits the causative agent of Lyme borreliosis is primarily *Ixodes scapularis*; however, *Ixodes pacificus* also acts as a vector in the western coastal regions.[9] In Europe, *Ixodes ricinus* is the tick species primarily responsible for transmitting *B burgdorferi* sl, whereas *Ixodes persulcatus* is predominant in large parts of Russia and Asia.[2] On average, in Europe 12% of nymphal and 15% of adult *I ricinus* ticks are infected with *B burgdorferi* sl,[5] and 2-3% of humans develop Lyme borreliosis after a tick bite,[10-12] which is similar to the incidence in the US.[13]

Incidence of Lyme borreliosis in the northern hemisphere

In 2006 roughly 85 000 cases of Lyme borreliosis were reported annually in Europe,[14] and more recently, this was estimated to be approximately 230 000 in Western Europe, although this is thought to be an underestimate.[15] Incidences in some countries peak as high as 350 per 100 000 population and have increased in the past two decades.[15-17] Lyme borreliosis is also highly prevalent in North America; the number of reported cases has gradually increased over time in the US,[18] and the Centers for Disease Control and Prevention (CDC) has estimated that there are more than 300 000 new cases each year.[19] The causes for this increase include greater abundance of wildlife hosts on which ticks feed and propagate, and climatic changes, which result in expansion of the latitude, altitude, and seasonality at which ticks are found.[20 21] This increase has led to a substantial disease burden and economic costs,[22] and has brought societal and political concerns in both the US and Europe.[23 24]

Clinical manifestations and diagnosis

Below we describe the most common clinical disease manifestations in Europe and North America in children and adults. The clinical spectrum is more diverse,[25] but rare disease manifestations are beyond the scope of this review. The diagnostic strategies for Lyme borreliosis are reviewed in box 1 and table 1.

Cutaneous manifestations

Erythema migrans

Erythema migrans, the most common manifestation of Lyme borreliosis,[77] is characterized by a red or bluish-red macular skin lesion expanding over the course of days to weeks (fig 1).[78] In contrast, a tick bite rash may develop within hours to days and generally fades after several days. Only half of people diagnosed with erythema migrans recall a tick bite.[78] Historically, a typical case of erythema migrans has been characterized by a bright red outer border with central clearing. However, *B burgdorferi* ss and *B garinii* usually lead to homogeneous erythema migrans, as opposed to *B afzelii* erythema migrans, which is characterized by central clearing in 60% of cases.[79 80] In 2-18% of cases, erythema migrans is multiple.[77 78 80] Untreated erythema migrans may persist for several weeks and occasionally months.[81]

Borrelial lymphocytoma

Borrelial lymphocytoma is a rare skin manifestation characterized by a painless bluish-red nodule, which is predominantly reported in children.[82] Typically, borrelial lymphocytoma is localized on the ear lobe, nipple, or scrotum. Often, there is a preceding or concomitant erythema migrans.[54 77] With antibiotics, borrelial lymphocytoma is usually cleared within several weeks.

Acrodermatitis chronica atrophicans

Acrodermatitis chronica atrophicans (ACA) is reported in 1-3% of Lyme borreliosis cases in Europe, and is predominantly caused by *B afzelii*.[77 83] ACA manifests as a chronic, slowly progressive red or bluish skin lesion, which eventually may become atrophic.[57 84] ACA is a late manifestation of Lyme borreliosis, and may present several months to years after an untreated erythema migrans.[57 84 85] It has been postulated that ACA does not resolve spontaneously, in contrast to most other manifestations of Lyme borreliosis.[57] Indeed, even lesions present for 10 years may reveal active infection by culture or PCR positivity, and respond to antimicrobial therapy.[86 87] Concurrent peripheral neuropathy is common, and local joint involvement may occur.[25]

Nervous system manifestations

Dissemination of *Borrelia* spp primarily involves the skin, nervous system, joints, or, more rarely, the heart. Neurologic manifestations, henceforth called Lyme neuroborreliosis, are reported in ~10% of all cases of Lyme borreliosis.[77] Early Lyme neuroborreliosis usually presents days to weeks

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Box 1: Laboratory support for diagnosis of Lyme borreliosis

Lyme borreliosis has a diverse clinical presentation, and, with the exception of erythema migrans, laboratory support for evidence of infection should be sought

Serology
- Most readily available form of laboratory support. Should be interpreted in combination with the clinical symptoms and signs
- Two tier testing is typically performed, where equivocal or positive ELISA based screening test results are technically confirmed using a second test, which can be another ELISA, western blot, or immunoblot[26]
- Clinical signs often precede an antibody response. Therefore, in early phases of the disease, antibody responses may be absent, but sensitivity increases over time*
- Absence of antibody response has been reported in cases confirmed by polymerase chain reaction (PCR) or culture[27]
- Antibiotic therapy may abort serologic response or prevent seroconversion[28]
- Background seroprevalence exists, from 5% in the general population in endemic regions to 50% in hunters[29 30]
- Serum Borrelia IgG may persist for decades. Therefore, serology cannot be used to monitor disease activity or eradication[31]

Polymerase chain reaction
- Reasonable sensitivity on skin and synovial samples, low sensitivity on cerebrospinal fluid*
- In other materials, such as blood or urine, PCR has no or limited diagnostic value[32 33]
- Limited utility in monitoring treatment response, as Borrelia DNA may be detected after successful antibiotic treatment[34 35]

Cerebrospinal fluid (CSF) analysis
- Similar to PCR, CSF cultures for Borrelia spp have a low yield*
- Diagnosis relies on indirect measures of meningeal inflammation: pleocytosis, intrathecal antibody production[36 37]
- Intrathecal Borrelia antibody is measured by calculating the CSF:serum antibody index and has been shown to persist for years after successful treatment, and thus cannot be used to monitor treatment[38]
- Chemokine C-X-C motif ligand 13 (CXCL13) is an early biomarker and its concentration falls rapidly after initiation of antibiotic therapy. Elevated CXCL13 concentrations in CSF may also be detected in other disorders, particularly neurosyphilis and central nervous system lymphoma[39]

Cellular immune response tests and other non-recommended tests
- Based on assessing T cell mediated immune responses following in vitro stimulation by a specific pathogen. Interferon gamma based cellular tests are well established for tuberculosis, but experimental for Lyme borreliosis[40]
- In Lyme borreliosis, results are inconsistent because of small patient cohorts, no clear case definitions, no or poorly defined control groups, and lack of independent academic validation[41]
- In a small cohort study, the sensitivity of a cellular assay measuring IFN-γ release was suggested to exceed that of serology during early infection[42]
- Validation of four cellular assays in larger populations with early Lyme borreliosis is ongoing[43]
- A variety of commercially available testing methods, including urine antigen testing,[44] quantitative CD57 assay,[45] or dark field microscopy on blood,[46] lack a solid scientific basis as well as independent, reproducible validation and should be avoided for clinical use[47]

*Sensitivity and specificity are reported in table 1.

after a tick bite, as a lymphocytic meningitis, cranial neuritis, or radiculoneuritis.[36 88] Cranial neuritis most commonly affects the facial nerve, which may be bilateral in up to 25% of individuals.[88 89] Involvement of other cranial nerves may occur, leading to diplopia, pain, hearing loss, or vertigo. Lyme neuroborreliosis is more frequent in children, commonly manifesting as facial nerve palsy,[77 90] in contrast to adults who typically present with radiculoneuritis and lymphocytic CSF pleocytosis.[37] Lyme radiculitis may present with signs resembling disc herniation. Pain is neuropathic, and dermatomal in distribution, while sensory defects and paresis may occur.[36 37] Rarely, *Borrelia* spp may cause a wide variety of peripheral nerve disorders, characterized by a mononeuropathia multiplex.[91 92] Parenchymal brain involvement is extremely rare. Only sporadic cases of chronic encephalitis or encephalomyelitis owing to Lyme borreliosis have been reported.[37 93] Additionally, cerebrovascular events resulting from CNS vasculitis have been associated with Lyme borreliosis, based on brain biopsy or intrathecal synthesis of anti-*Borrelia* antibodies, responding to antibiotic therapy.[94] Most of the individuals who presented with these conditions lived in an endemic area and had a history of erythema migrans, cranial neuritis, or radiculoneuritis.[95]

Lyme arthritis

Lyme arthritis typically presents as an oligo- or monoarthritis, often involving the knee joint, three to six months after infection.[58 81] In contrast to other causes of septic arthritis, Lyme arthritis usually is less painful and not accompanied by fever. Most patients do not report a preceding tick bite or

Table 1 | Diagnostic tests for Lyme borreliosis

Manifestations		Serology[α]	PCR[β]	Culture[γ]	Comments
Erythema migrans (EM)	Sensitivity (%)	Summary sensitivity 50 (95% CI 40 to 61)[48]	Range 30-89[49]	Range 40-90[47]	Serology: IgM was slightly more sensitive than IgG.[48] For microbiologically confirmed, solitary EM, positivity <20% by two tier testing within 1 week of presentation,[50] increasing to 86% in 4th week of symptoms.[50] Sensitivity of two tier testing is generally lower than one tier testing. Specificity is approximately 95% (95% CI 75 to 99%) in case-control studies and approximately 80% (95% CI 40 to 95%) in cross sectional studies.[48] Background seroprevalence is an important confounder in the latter study design. Specificity of two tier testing is generally higher than one tier testing PCR assay: In a recent large study sensitivity was 77.7% (n=121).[51] Median sensitivity was higher in European than in US studies.[49] EM is a clinical diagnosis. PCR is mostly used in research or in atypical cases. In such cases, histological findings may also support the diagnosis[52] Culture: In a European study, 55.1% of biopsies were culture positive, of which 96.8% *B afzelii*, and 3.2% *B garinii*.[51] Sensitivity of large volume blood cultures from EM patients in the US, of whom 30% had multiple EM, was 44%[53]
	Specificity (%)	Summary specificity 95 (95% CI 92 to 97)[48]	Range 98-100[49]	100	
Borrelial lymphocytoma	Sensitivity (%)	Range 35-95[52 54 55]	67[56]	Range 24-32[54 55]	Serology: Most cases had serum anti-*Bburgdorferi* IgG antibodies (with or without IgM antibodies).[52] Higher seropositivity rates were observed in more recent studies (2001-14 *v* 1986-2000), most likely owing to more sensitive diagnostic tests.[54 55] †Specificity as described for erythema migrans PCR assay: In this study, the diagnosis was based on clinical and pathological criteria and samples were formalin fixed and paraffin embedded, which could have impaired sensitivity.[57] Histological findings may support the diagnosis.[57] †Specificity as described for erythema migrans Culture: In a European study, 31.8% were culture positive, predominantly *B afzelii*[54]
	Specificity (%)	†	†	100	
Acrodermatitis chronica atrophicans (ACA)	Sensitivity (%)	Summary sensitivity 98 (95% CI 84 to 100)[48]	Range 20-100[49]	Range 20-60[47]	Serology: Mostly IgG antibodies, occasionally IgM antibodies, were found. High quality case-control studies reported average sensitivity of 98%[48] PCR assay: median sensitivity 75%.[49] Studies restricted to Europe, as ACA is associated with *B afzelii*. Histological findings may support the diagnosis[52]
	Specificity (%)	Summary specificity 94 (95% CI 90 to 97)[48]	100[49]	100	
Lyme arthritis Specificity (%)	Sensitivity (%)	Median sensitivity* 96 (IQR 93 to 100)[48]	Range 40-96[58]	NA	Serology: In European studies IgG had a higher sensitivity than IgM.[48] Similar sensitivities for Lyme arthritis were reported by studies from North America.[59] *Because of limited number of studies, median sensitivity and specificity is reported PCR assay (synovial fluid/tissue): for Lyme arthritis, PCR is an important tool. Sensitivity in synovial tissue was higher than in synovial fluid.[60] PCR did not discriminate between residual DNA and viable organisms[34 60] Culture (synovial fluid/tissue): Cultivation of *B burgdorferi* from synovial fluid is generally unsuccessful, but may reveal non-motile spirochetes[61]
	Median specificity 94* (IQR 91 to 97)[48]	100[49]	NA		
Lyme neuroborreliosis Specificity (%)	Sensitivity (%)	Summary sensitivity 78 (95% CI 53 to 92)[48]	Range 5-17[62 63]	Range 10-26[47 64]	For a definite diagnosis of Lyme neuroborreliosis, three of the following criteria should apply: 1. Neurologic signs compatible with Lyme neuroborreliosis; 2. CSF pleocytosis, defined as >5 cells $\times 10^9$L[3 37 47 65] although pleocytosis may be absent in case of peripheral Lyme neuroborreliosis; 3. Intrathecal production of *B burgdorferi* antibodies.[66] For a probable diagnosis, two criteria should be met[66] Serology: Sensitivity 95% CI was 41 to 92%.[48] †Specificity as described for erythema migrans Intrathecal antibody synthesis (CSF): Average sensitivity was ~80% (95% CI 34 to 97%).[48] Sensitivity in US patients 87%,[67] compared with European patients 56-79%.[65 68 69] In early cases, intrathecal antibodies may still be absent; at 6-8 weeks after onset of symptoms, specific IgG production is expected to be detectable in all patients.[70] Other non-specific signs of inflammation in CSF, such as elevated total protein level or intrathecal synthesis of total IgM, IgG, or IgA, may also be present[25] PCR assay (CSF): Median sensitivity was 22.5%, and lower in European than in US studies[49] CXCL13 (CSF): In a European meta-analysis, a pooled sensitivity of 85-93% and pooled specificity of 92-98% was found using an optimal cut-off value of 162 pg/mL.[39] Elevated CXCL13 may also be a result of CNS infection or malignancy.[71 72] CXCL13 correlates with intrathecal *B burgdorferi* antibody response in acute Lyme meningitis[71 73]
	Summary specificity 78 (95% CI 40 to 95)[48]	99-100[49]	100		

NA=not available, IQR=interquartile range, PCR=polymerase chain reaction, CI=confidence interval, CSF=cerebrospinal fluid, CXCL13=C-X-C motif ligand 13.
α.Serology results mainly based on a meta-analysis from Europe.[48] A systematic review and meta-analysis of North American data showed the same trend—ie, higher sensitivity in later stages of Lyme borreliosis; however, the classification was different from that in the European meta-analysis.[59] General limitations of *B burgdorferi* sl serology include differences between commercials assays;[74] cross reactivity[75];9 false negative results in early phase of disease[75]; and antibodies—even IgM—can remain detectable in blood for many years, thus, seropositivity does not indicate disease.[31]
β.PCR results are mainly based on a review.[49]
γ.Culture sensitivities are mainly based on a review.[47] Culture of *B burgdorferi* sl requires special media. The organism grows slowly, requiring up to 8-12 weeks before being detectable,[76] and culture techniques are beyond the capabilities of most clinical laboratories. Therefore, *B burgdorferi* sl culture mainly is a research tool.

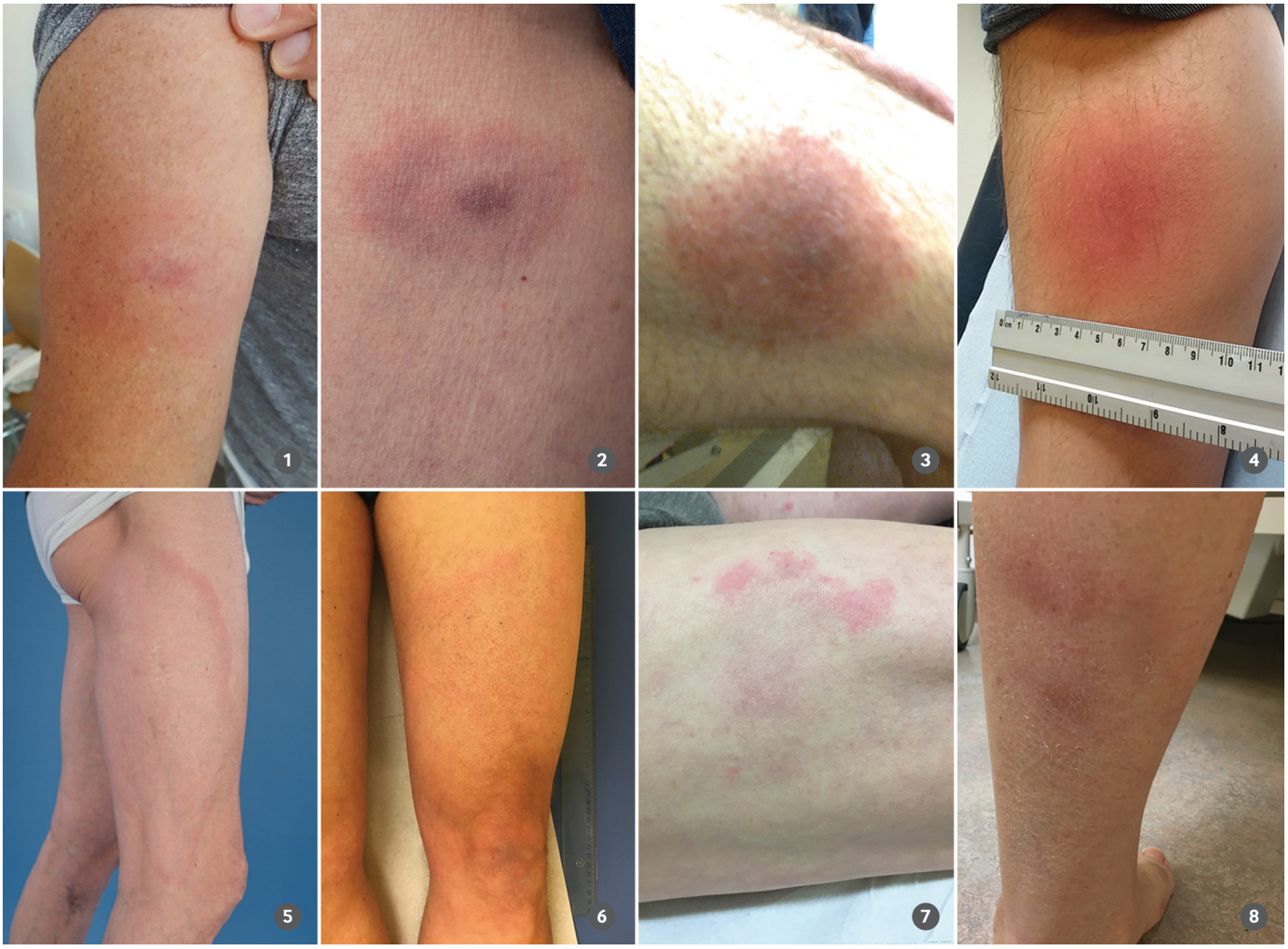

Fig 1 | Divergent characteristics of erythema migrans. Patients with proven erythema migrans, presenting with classic lesions with central clearing (panels 1, 2), homogeneous lesions (panels 3, 4), large lesion with sharply defined borders (panels 5, 6), or patchy skin discolorations (panels 7, 8). *B burgdorferi* skin infection was proven by positive culture and PCR (panels 1, 2, 4, 5, 7) or positive PCR only (panels 3, 6, 8)

erythema migrans. If untreated, symptoms include intermittent or persistent joint swelling and pain during a period of months to several years. With appropriate antimicrobial therapy, the arthritis is cured and *Borrelia* is eradicated in most patients, but in some cases proliferative synovitis may persist for months or several years, requiring anti-inflammatory therapy.[58 96]

Lyme carditis

During early disseminated infection, acute cardiac involvement may occur, characterized by atrioventricular conduction defects in varying degrees.[97] Less common cardiac manifestations include acute myopericarditis or, rarely, cardiomyopathy. Lyme carditis usually is self-limited, but is potentially fatal if untreated.[98] Most case series on the relation between carditis and Lyme borreliosis lack causality; however, selected studies have identified *B burgdorferi* sl in endomyocardial biopsy samples from patients with dilated cardiomyopathy.[99 100]

Differences between clinical manifestations in North America and Europe

The differences in *Borrelia* genospecies between the continents[101] result in differences in clinical presentation.[102] In North America, central clearing of erythema migrans is uncommon: up to 18% of erythema migrans cases are multiple, and Lyme borreliosis is more often associated with constitutional symptoms, such as fever and malaise.[80 103] *B burgdorferi* ss in North America is more arthritogenic, and Lyme arthritis is more frequently encountered in North America (28% of Lyme borreliosis cases)[104] than in Europe (3-7%).[77 105] Lyme neuroborreliosis in North America mostly presents as subacute meningitis with or without cranial neuropathy (usually facial palsy), and less frequently as painful radiculoneuritis.[88] In Europe, *B garinii* is particularly neurotropic, and typically associated with painful radiculoneuritis and lymphocytic meningitis (originally described as Bannwarth syndrome).[36] *B afzelii* primarily causes skin infections, including ACA and borrelial

lymphocytoma, both of which are virtually absent in North America.[52 101 105 106]

Pregnancy and congenital infection

Adverse outcomes of infection on offspring during pregnancy have been known for other spirochetal diseases, such as syphilis.[107] During spirochetemia in the acute phase of infection, *B burgdorferi* sl may spread transplacentally, and evidence for congenital infection has indeed been reported in a few cases where *Borrelia* species were cultured from the newborn post mortem.[108-111] A meta-analysis of nine studies suggested fewer adverse birth outcomes in women who were treated for gestational Lyme borreliosis compared with untreated cases, suggesting indirect evidence for adverse birth outcomes.[112] Conversely, untreated ACA during pregnancy, an active chronic infection, was not associated with adverse outcomes in a retrospective survey,[113] and in a systematic review, eight epidemiological studies reporting on potential associations between *Borrelia* sl exposure and adverse birth outcomes did not suggest any relation.[114]

Treatment

Early localized/disseminated disease

For treatment of erythema migrans, doxycycline, amoxicillin, and oral cephalosporins were equally effective in randomized clinical trials, with complete response rates >90%.[115] Azithromycin was as effective as doxycycline or amoxicillin in European open label trials,[116-119] but not in a randomized double blind controlled study in 246 patients in North America.[120] As a result, doxycycline generally is regarded first choice therapy for erythema migrans.[115] Randomized trials have shown that doxycycline for a duration of 10 days is as effective as 15 or 21 days.[121 122] Persistent symptoms after treatment were no more frequent in patients treated for ≤10 days as compared with longer courses in a retrospective cohort study with a mean follow-up duration of 2.9 years.[123] For multiple erythema migrans, oral doxycycline for 14 days has been shown to be as effective as intravenous ceftriaxone in an open label alternate treatment trial among 200 patients.[106]

Lyme neuroborreliosis

Lyme neuroborreliosis is typically treated with intravenous ceftriaxone for at least 14 days.[124] After meningoradiculitis, clinical recovery often is slow, and neurologic sequelae or subjective symptoms may persist in up to 40-50% of patients after 30 months.[36 125] While no studies have assessed the optimal duration of ceftriaxone therapy, the slow clinical resolution and long term neurologic sequelae have led some clinicians to extend the duration of treatment to up to 28 days.[95] In a prospective, double blind study in Europe of 102 adults with early Lyme neuroborreliosis, oral doxycycline was found to be as effective as ceftriaxone.[126] Long term outcomes (neurologic sequelae, quality of life, fatigue, cognition) were similar after either treatment.[125] Based on this study, various treatment guidelines now consider doxycycline as a reasonable choice for Lyme neuroborreliosis.

Lyme encephalomyelitis, involving the brain parenchyma as evidenced by focal neurologic defects or magnetic resonance imaging findings, is extremely rare, and is typically treated with two to four weeks of intravenous ceftriaxone.[127]

Lyme arthritis

After treatment for Lyme arthritis with antibiotics, clinical resolution may take six to 12 months in some patients.[96 128] A 30 day course of either oral doxycycline or amoxicillin led to resolution of arthritis in ~90% of 38 patients in a randomized trial,[128] while 10-14 days of intravenous ceftriaxone led to lower success rates (19/40; 48%) in a small randomized, placebo controlled, double blind trial.[129] Patients with Lyme arthritis typically are treated with 30 days of doxycycline, while those who continue to have symptoms of arthritis after oral antibiotics subsequently are re-treated with either doxycycline or intravenous ceftriaxone.[128 130] Between 10% and 20% of patients may develop "antibiotic refractory" Lyme arthritis, a proliferative synovitis that no longer responds to antimicrobial therapy and requires therapy with intra-articular corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), hydroxychloroquine, methotrexate, biologic response modifiers, or even synovectomy.[130]

Acrodermatitis chronica atrophicans

For ACA, observational studies have shown disappointing results of shorter term therapies—eg, intravenous ceftriaxone for two weeks or doxycycline for 20 days, while treatment success was 85% to 100% with doxycycline for up to four weeks. Hence, a treatment duration of four weeks is recommended.[131 132] Resolution may take many months after antibiotic therapy, while skin atrophy and neuropathy often are irreversible.[132]

Chronic symptoms attributed to Lyme borreliosis

Most people with Lyme borreliosis respond well to antimicrobial treatment. Despite antibiotic therapy, some patients with Lyme borreliosis develop disabling persistent symptoms, including fatigue, pain, and neurocognitive disturbances.[2 133] Their exact incidence, pathogenesis, and prognosis are not well known and are an ongoing source of debate.[134] Longlasting signs and symptoms attributed to Lyme borreliosis often are referred to as "chronic Lyme." It is essential to discriminate antibiotic therapy failure, which results in progressive infection or persistent signs at the primary sites of infection, from new subjective symptoms developing after resolution of the initial disease manifestations. Other patients seek medical attention for longlasting symptoms which are usually medically unexplained, questioning whether these may be attributable to an unnoticed episode of Lyme borreliosis, even when there is little or no evidence of previous *B burgdorferi* sl infection (fig 2).

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Persistent infection

In patients with ACA, diagnosis is often delayed, and duration of signs and symptoms for many years have been reported.[86 87] Even skin biopsy samples from untreated lesions present for ≥10 years reveal positive PCR and culture results,[36 86 87] indicating an active infection that can persist for years. After antibiotic treatment, culture results become negative, and skin lesions and accompanying signs may resolve completely, whereas local atrophy may persist in others.[25 57] Whereas a primate model has suggested that the persistence of bacteria after antibiotic therapy may drive other Lyme borreliosis manifestations,[135 136] ACA is the only manifestation of Lyme borreliosis in humans where chronic infection has been unequivocally demonstrated. Reported antimicrobial treatment failure rates, defined as development of disseminated Lyme borreliosis after treatment of early Lyme borreliosis, are low (≤1%).[121 123] In most studies where *B burgdorferi* sl was cultured from skin biopsy specimens of erythema migrans lesions before antibiotic therapy, post-treatment cultures yielded negative results.[51 122 137] Recurrences of erythema migrans are not uncommon in endemic areas, and molecular typing showed that repeat episodes of erythema migrans in 17 appropriately treated patients were caused by re-infection and not relapse.[138]

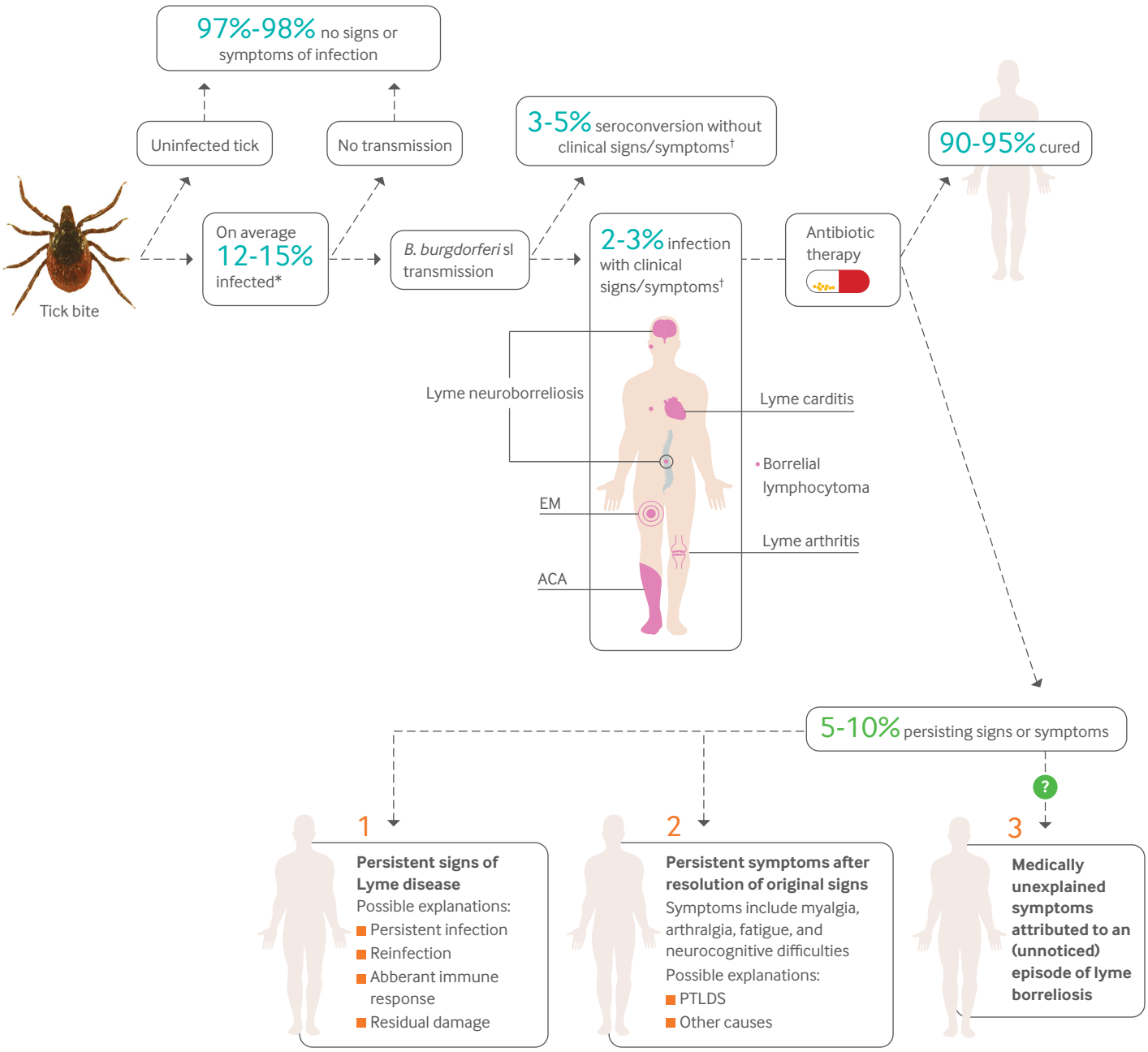

Fig 2 | Potential courses of disease after a tick bite. PTLDS=post-treatment Lyme disease syndrome; EM=erythema migrans; ACA=acrodermatitis chronica atrophicans. *Based on Strnad et al 2017[5]; based on Wilhelmsson et al 2016[11]; and Hofhuis et al 2017[10]

Immune response

Ongoing infection has not been shown in patients who have been treated for Lyme arthritis. However, persistent or recurrent synovial inflammation is observed in up to one third of patients after first antibiotic treatment, and in 10-17% after repeated courses, the latter being referred to as antibiotic refractory Lyme arthritis.[96 128] In these cases, there is no clinical benefit from prolonged or recurrent antibiotic courses, but most patients do respond to immunosuppressive therapy.[58] Hypotheses on the underlying mechanisms include persistence of non-viable spirochetal components or debris in the joint resulting in recurrent synovitis,[61 114] and potential immunological mechanisms, such as auto-antibodies, autoinflammatory processes, and dysregulated T cell responses.[139 140 141] Likewise, association with HLA-DR alleles,[142] upregulated expression of specific microRNA,[143] and a Toll-like receptor 1 polymorphism[144] have been suggested. Together with the observation that synovial PCR and culture results are negative or show non-viable spirochetes after antibiotic treatment, antibiotic refractory Lyme arthritis is likely based on immunological mechanisms, and persistence of *B burgdorferi* sl infection as a cause is highly unlikely.[34 130]

Residual damage

In patients with Lyme neuroborreliosis, longlasting signs may be attributable to irreversible neurological injury caused by the infection. Persistent symptoms have been reported by 12-48% of 77 and 85 patients in two prospective follow-up studies.[36 145] Specific neurologic findings have been observed in 30% of patients included in a randomized treatment trial at four months,[126] and 25-28% at two to five years in prospective and retrospective cohorts, including radiculopathy, paresis, hyposensibility, or hearing loss, without evidence for microbiological persistence.[146-148] In a prospective case-control study including 50 patients with Lyme neuroborreliosis and matched controls, patients had a statistically significantly lower quality of life after 30 months, measured by the Medical Outcomes Study 36-Item Short-Form General Health Survey (SF-36) physical component summary.[125] Delayed start of treatment, symptoms before treatment, and non-complete recovery after four months were possible predictors of a poorer quality of life and severe fatigue.[146 147] The association of unfavorable outcomes with longer duration or severity of symptoms before the start of treatment supports the concept of irreversible neurological damage as the underlying mechanism.[36 146 148] Similar long term neurological sequelae after microbial eradication have been observed in bacterial meningitis caused by other bacteria.[149]

Functional disability and neurocognitive symptoms

The reported prevalence in observational studies of persistent symptoms, such as musculoskeletal pain, fatigue, and cognitive complaints varies considerably, between 0% and 48%.[145 150-152] Case definition, Lyme borreliosis manifestation, follow-up, geographic location, and use of self-reported symptoms rather than validated measures might explain the divergence in prevalence. In a prospective study, 26/71 (36%) patients had self-reported ongoing symptoms at six months after treatment of erythema migrans.[151] In 11%, subjective symptoms were associated with functional disability as measured with the SF-36 questionnaire, while none had microbiological or clinical evidence for ongoing infection. Another prospective study among 128 US patients with culture confirmed erythema migrans followed for >10 years found a 10.9% incidence of self-reported ongoing symptoms, predominantly memory or concentration difficulties, fatigue, and joint pain.[152] In most patients, one or more symptoms persisted for >10 years.

The appreciation of long term outcomes of Lyme borreliosis is hampered by the lack of proper control groups in most studies. In a controlled prospective study from Europe in 285 patients with erythema migrans, the prevalence of symptoms was highest at baseline (33.3%), decreasing to 4.6% at six months and 2.2% at 12 months. In 259 matched controls without Lyme borreliosis, these rates were similar (3.0% at 12 months). Microbiological failure requiring retreatment was documented in two cases only.[150] This particular study did not include patients with multiple erythema migrans or manifestations such as Lyme neuroborreliosis, who might have a greater likelihood of developing persistent symptoms. Also, *B burgdorferi* ss infected patients in the US are more likely to be symptomatic at baseline, and therefore might have a greater likelihood of developing persistent symptoms.[152] While many patients report cognitive problems such as memory loss, word-finding difficulties, and lack of concentration, subjective memory complaints were not associated with impaired objective test performances in a prospective study on 279 patients with persistent symptoms attributed to Lyme borreliosis.[153] Only 3% of patients included in that study were classified as having clinically impaired cognitive performance compared with normative data.[154]

It has been hypothesized that chronic pain and fatigue syndromes may be part of a central sensitization syndrome that follows non-infectious or infectious diseases.[155] Central sensitization is thought to involve activation of central neurons, leading to synaptic and neurotransmitter changes, and an increased sensitization to pain signals that may last for months or years.[155] After Lyme borreliosis, such a mechanism might be elicited by past infection, persistence of remnant bacterial proinflammatory triggers,[61 156] or other physiological or behavioral mechanisms.[155]

Post-treatment Lyme disease syndrome

The complex of persistent or recurrent symptoms despite antibiotic treatment is often referred to as

post-treatment Lyme disease syndrome (PTLDS), for which a case definition was proposed by the Infectious Diseases Society of America (IDSA) (box 2).[157] This definition includes a clinical syndrome of patient reported symptoms in the context of a previously treated, physician diagnosed case of Lyme borreliosis meeting the CDC surveillance criteria.[151 157] Additional criteria exclude patients with untreated Lyme borreliosis or other tickborne infections, and those with objective persistent signs of the initial episode of Lyme borreliosis, such as recurrent arthritis, ACA, or neurologic sequelae of Lyme meningoradiculitis. Others have proposed to better define the "functional impact" component of the case definition, using an SF-36 questionnaire threshold.[151] By definition, patients with PTLDS have no compelling clinical or laboratory support for the diagnosis of ongoing *B burgdorferi* sl infection, and neither have they signs suggesting immunological phenomena, such as recurrent synovitis, or irreversible nerve damage after Lyme neuroborreliosis. Several hypotheses on the causes of PTLDS exist. First, microbiological mechanisms have been considered, including tickborne co-infections. Whereas ticks that transmit *B burgdorferi* sl are known vectors of other human pathogens, none of these pathogens are known to cause chronic symptoms.[159] Likewise, round morphologic forms of *B burgdorferi* sl have been suggested to cause persistent disease. While morphologic variants have been reported in human tissue specimens in a small number of cases, none of the patients had symptoms resembling those of PTLDS, and conversely, morphologic variants have never been identified in patients with chronic Lyme attributed symptoms.[160] Second, immunogenetic mechanisms have been proposed, but prospective observational studies on dysregulated immune responses[161-163] and case-control studies on autoimmune processes[164 165] have been inconclusive. Third, associations of PTDLS have been described with demographic, clinical, and epidemiological patient characteristics, such as advanced age, female sex,[166 167] comorbidity, and duration of pre-treatment symptoms.[168 169] Finally, cognitive behavioral characteristics, including depression, anxiety, negative affect, and catastrophizing have been associated with the risk of developing persistent symptoms.[170]

Trials of prolonged antimicrobial therapy

Empirical antibiotic treatment studies have targeted the possibility of concealed infection in patients with persistent symptoms, despite the weight of evidence against persistent infection as the explanation for PTLDS. Initial open cohort studies have claimed successful antimicrobial therapy in patients with "chronic Lyme."[171 172] Oral tetracycline for a median of four months was reportedly associated with a 90% success rate in a case series of 277 patients. Likewise, the combination of clarithromycin and hydroxychloroquine reportedly was as effective as prolonged tetracycline in another series of 235 cases. Most patients improved within two weeks after initiation of therapy, and all patients had improved after three months.[172] However, in both studies, inclusion criteria were not clearly defined, and serologic reactivity against *B burgdorferi* sl, but no documented Lyme borreliosis, was required. The studies were non-randomized and uncontrolled, and did not use standardized questionnaire outcomes.

Five randomized controlled clinical trials have been performed in patients with persistent symptoms attributed to Lyme borreliosis (table 2).[173-177] One trial did not find any beneficial effects on quality of life in 115 patients randomized to prolonged therapy compared with the matching placebo group.[173] While the study found improvement in self-reported cognitive functioning in both randomization arms, no improvement in objective neurocognitive functioning was found.[17 31 82]

In a small study, 37 patients with memory impairment were randomized to ceftriaxone versus placebo.[175] At 12 weeks, the ceftriaxone group showed a statistically significantly greater effect on objective neurocognitive functioning, but this was not sustained to week 24, whereas the effect on self-reported fatigue and physical functioning was only sustained among a subgroup more severely affected at baseline.[175] How this short term cognitive improvement relates to other patients with PTLDS is uncertain, as participants were selected from a cohort of >3000 patients, and were required to have objectified memory impairment on neuropsychological testing, which is rare among patients with PTLDS.[153 182]

Another trial randomized 55 patients with severe fatigue to four weeks of ceftriaxone versus placebo.[174] The primary endpoint failed to show improvement in neuropsychological performance, but self-reported fatigue improved in the group taking ceftriaxone. Despite the statistically significant effect reported, the authors themselves conclude that the study does not support the use of additional antibiotic therapy, because fatigue, a non-specific symptom, was the only outcome that improved.[174]

A trial that randomized 86 patients with "a recurrence of Lyme borreliosis symptoms" to oral amoxicillin for three months or placebo was hampered by several shortcomings. It was prematurely terminated because of slow recruitment, the publication did not provide details on the intention-to-treat population, and a large proportion of patients were excluded from the analysis "because of persistent symptoms."

In another trial, the PLEASE trial, 281 patients were randomized to receive 14 days of ceftriaxone, followed by 12 weeks of either doxycycline, clarithromycin plus hydroxychloroquine, or placebo.[177] Neither regimen of 12 weeks of therapy yielded benefit over placebo with respect to serial mental and physical health related quality of life measures during follow-up until 52 months. This study has built upon lessons learnt from earlier studies. Choice and duration of the treatment regimens were based

Box 2: Proposed definitions of post-treatment Lyme disease syndrome (PTLDS)

IDSA guidelines criteria[157]

Inclusion criteria

a. Documented episode of early or late Lyme disease fulfilling the case definition of the CDC.[158] If based on erythema migrans, the diagnosis must be made and documented by an experienced healthcare practitioner
b. Resolution or stabilization of the objective manifestation(s) of Lyme disease after treatment of the episode of Lyme disease with a generally accepted treatment regimen
c. Onset of any of the following subjective symptoms within six months of the diagnosis of Lyme disease and persistence for at least a six month period after completion of antibiotic therapy:
   - fatigue
   - widespread musculoskeletal pain
   - complaints of cognitive difficulties
d. Subjective symptoms of such severity that they result in substantial reduction in previous levels of occupational, educational, social, or personal activities

Exclusion criteria

a. Active *Borrelia burgdorferi* infection diagnosed by a reliable method based on either culture or PCR
b. Active, untreated, well documented co-infection, such as babesiosis
c. Presence of objective abnormalities on physical examination or on neuropsychologic testing that may explain the patient's complaints. For example, a patient with antibiotic refractory Lyme arthritis or with late neuroborreliosis associated with objective cognitive dysfunction would be excluded
d. Diagnosis of fibromyalgia or chronic fatigue syndrome or a prolonged history of undiagnosed or unexplained somatic complaints, such as musculoskeletal pains or fatigue, before the onset of Lyme disease
e. Underlying disease or condition that might explain the patient's symptoms, or laboratory or imaging abnormalities that might suggest an undiagnosed process distinct from post-Lyme disease syndrome

on uncontrolled studies which reported that almost 75% of patients improved within one month and 92% to 100% within three months of treatment, which suggested that a three month regimen should be optimal to assess whether those observations are sustained when placebo controlled.[171 172] Whereas earlier randomized trials might have been influenced by baseline differences, the PLEASE trial analysis was corrected for baseline health related quality of life. Finally, the endpoint was based on a minimal clinically relevant treatment effect on the SF-36 summary score specific for patients with PTLDS, as prospectively assessed in a pilot study.[183] Three aspects of the study design have been subject of debate. First, patients received two weeks of open label antibiotics preceding the randomized treatment phase, to standardize and synchronize pretreatment, while previous trials allowed for a wide variance of antibiotic pretreatments. Consequently, the study was designed as a randomized, placebo controlled trial to compare longer term with standardized shorter term therapy. Sensitivity analyses showed that excluding patients without prior oral pretreatment did not affect the outcomes.[177] Second, all three study groups slightly improved during the 52 weeks of follow-up, irrespective of randomization arm, and some have attributed this to the standardized pretreatment with ceftriaxone, rather than placebo effects or regression to the mean. However, regression of symptoms, including fatigue severity, has consistently been reported in the placebo arm of other studies, and was of similar magnitude (table 2).[174 175 177] This is in agreement with the finding that positive pretreatment expectancies and higher self-efficacy were the major predictors of outcome, regardless of randomization arm.[184] Third, it has been argued by some that 14 weeks of treatment was insufficient to show a beneficial effect on alleviation of symptoms, in contrast to the earlier uncontrolled studies.[171 172] While prolonged antimicrobial treatment is not uncommon for various infectious diseases, such as tuberculosis, in the case of TB it is aimed at preventing microbiological relapse, and not at a delayed onset of clinical alleviation, as no infectious diseases have been described in which the initial effect on signs, symptoms, and laboratory findings is delayed beyond the first three months of effective therapy.

In summary, five randomized clinical trials have provided little support for prolonged antibiotic treatment in patients with persistent symptoms attributed to Lyme borreliosis. While smaller studies reported limited, or non-sustained effects on selected outcomes,[174 175] the two largest trials did not find beneficial effects of prolonged treatment on any of the domains studied (table 2).[15 41 73 177 182]

Management of patients with persistent Lyme attributed symptoms

Attributing a cause to medically unexplained symptoms is challenging, and the lack of abnormal test results or objective findings can be frustrating for both patients and physicians. A stepwise approach can help assess whether persistent symptoms attributed to Lyme borreliosis are indeed related to previous *B burgdorferi* sl infection, and may be caused by active infection (thus, potentially amenable to antimicrobial treatment), or caused by immune mediated mechanisms or residual damage (fig 3, box

Table 2 | Overview of randomized controlled treatment trials in patients with symptoms attributed to previously documented Lyme borreliosis

	Klempner, 2001[173]	Krupp, 2003[174]	Fallon, 2008[175]	Cameron, 2008[176]	Berende, 2016[177]
Study design	Randomized, placebo controlled, double blind trial				
Inclusion criteria	Adults with physician documented Lyme borreliosis. Persistent symptoms that had begun <6 months after initial infection and persisted for >6 months	Adults with physician documented Lyme borreliosis, with serologic confirmation.[c] Severe fatigue (severity score ≥4.0 on the fatigue severity scale, FSS-11), that had begun coincident with initial infection	Adults with physician documented Lyme borreliosis, with serologic confirmation,[c] current positive IgG western blot, and subjective and objective memory impairment (Wechsler Memory Scale-III)	Adults with "recurrence of Lyme disease symptoms after previous successful treatment"	Adults with persistent symptoms >6 months attributed to Lyme borreliosis (temporally related to proven Lyme borreliosis manifestation or current positive IgG western blot[c])
Pretreatment	≥1 course of recommended antibiotic regimen	≥3 weeks of doxycycline or intravenous ceftriaxone	≥3 weeks of intravenous ceftriaxone	"Successful treatment"	Intravenous ceftriaxone 2 g once daily for 14 days per study protocol
Randomization	1:1	1:1	2:1	2:1	1:1:1
Intervention arm	Intravenous ceftriaxone 2 g once daily for 30 days followed by oral doxycycline 100 mg twice daily for 60 days	Intravenous ceftriaxone 2 g once daily for 28 days	Intravenous ceftriaxone 2 g once daily for 70 days	Oral amoxicillin 3 g once daily for 90 days	Oral doxycycline 100 mg twice daily for 56 days, or oral clarithromycin 500 mg twice daily+hydroxychloroquine 200 mg three times for 56 days
Control arm	Intravenous placebo for 30 days followed by oral placebo twice daily for 60 days	Intravenous placebo for 28 days	Intravenous placebo for 70 days	Oral placebo for 90 days	Oral placebo for 56 days
Follow-up	180 days	6 months	24 weeks	6 months	1 year
Primary endpoint	SF-36 score at 180 days[a]	Fatigue (FSS-11 score) and mental speed (α-arithmetic test) at 6 months	Neurocognitive performance (6 domains tested) at 12 weeks	SF-36 score at 6 month[s]f	SF-36 score (PCS) at 14 weeks
Number of subjects (ITT analysis)	115[b]	55	37	86	280
Primary outcome (intervention group/ placebo group)	SF-36 (total score): improved in 37% *v* 40%, Δ −3%; 95% confidence interval −26 to 20 (ns)	Fatigue assessed by FSS-11 improved in 64% *v* 19%; ratio 3.5; 95% confidence interval 1.50 to 8.03; P=0.001 Mental speed (A-A test) improved in 8% *v* 9% (ns)	Neurocognitive performance (longitudinal mixed effects model) drug *v* placebo at week 12, 0.28; 95% confidence interval −0.01 to 0.56; P=0.053 at week 24, 0.04; 95% confidence interval −0.24 to 0.33; P=0.76	SF-36 (total score): improved in 46% *v* 18% (P=0.007)	SF-36 score (PCS)[g] Mean 35.0 *v* 35.6 *v* 34.8; P=0.69; Δ 0.2 [95% confidence interval −2.4 to 2.8] for doxycycline *v* placebo; Δ 0.9 [95% confidence interval −1.6 to 3.3] for clarithromycin- hydroxychloroquine *v* placebo)
Secondary outcomes (intervention group/ placebo group)	PCS improved in 35% *v* 26%, Δ 9%; 95% confidence interval −8,26 (ns) MCS improved in 33% *v* 38%, Δ −5%, 95% confidence interval −22 to 13 (ns)	Fatigue assessed by VAS at primary endpoint, improved in 29% *v* 10% (ns). No significant difference in pain, mood, and perceived health changes between groups. CSF OspA antigen status positive to negative, 4/4 *v* 3/4 (ns)[d]	Mean PCS score at 12 weeks, 40.4 *v* 36.0[e]; MCS, 43.0 *v* 51.6 (ns). Mean PCS at 24 weeks 42.0 *v* 36.8[e]; MCS, 42.1 *v* 50.7 (ns)	Average improvement in 48 evaluable patients, PCS, 8.5 *v* 7 (ns) MCS, 14.4 *v* 6.2 (P=0.04)	CIS fatigue at 14 weeks, 39.4 *v* 38.6 *v* 38.3 (ns) Mean MCS at 14 weeks, 40.2 *v* 40.5 *v* 40.1 (ns) PCS at 26, 40, and 52 weeks, no significant difference between treatment arms. Neurocognitive performance (5 domains tested) at 26, 40, and 52 weeks, no significant difference between treatment arm[s17]8

(*continued*)

Table 2 | Continued

	Klempner, 2001[173]	Krupp, 2003[174]	Fallon, 2008[175]	Cameron, 2008[176]	Berende, 2016[177]
Course of symptoms in placebo group	26% of patients improved >6.5 points on PCS; 38% of patients improved >7.9 points on MCS	Mean decrease in fatigue score, 9%; 23% of patients improved >0.7 points on FSS-11 fatigue scale at 6 months	Mean decrease in fatigue score, 14%	Mean increase in PCS, 7 points; MCS, 6.2 points	Mean decrease in fatigue score, 11%; mean increase in PCS, 3 points
Comments	Two separate trials for *Borrelia* IgG seropositive[c] and seronegative patients, with combined analysis. This study has been subject of divergent opinions, for not adjusting the analysis for baseline levels of impairment, and it was suggested to be underpowered as a result of an optimistic estimate of treatment effect size[179 180]	No significant effect on 2 of 3 co-primary endpoints. This study has been subject to criticism as 9 of 27 patients in the treatment arm dropped out prematurely, patients still fulfilled the entry criteria for severe fatigue at completion, and there was no significant difference in fatigue as measured by an alternate (VAS) scale[181]	Trend toward improvement in primary outcome (cognition), not sustained to week 24. Authors' conclusion: "not an effective strategy for sustained cognitive improvement"	Inclusion criteria for prior Lyme borreliosis and current symptoms not specified. 38 of 86 patients were excluded from analysis, of whom 17 were "because of treatment failure." No comprehensive analysis on full cohort reported. No significant difference between groups in PCS score at primary endpoint	See main text

CIS = Checklist individual strength; CSF=cerebrospinal fluid; ITT=intention to treat; MCS=SF-36 mental component summary score; ns=not significant; OspA=borrelia outer surface protein A; PCS=SF-36 physical component summary; SF-36=36 item short-form general health survey; QoL=quality of life; VAS=visual analog scale.
SF-36 scores are generally transformed into a norm based score, with score 50 ±10 being the population average. Higher scores correspond to better self-reported health related quality of life.
[a]Participants were categorized based on SF-36 scores after 180 days compared with baseline: unchanged, improved (≥2 SE higher), worsened (≥2 SE lower). Two SE correspond to 6.5 points for PCS and 7.9 points for MCS.
[b]This study was discontinued after an interim analysis indicated that significant differences in treatment efficacy would unlikely be observed. Intended sample size was 260 patients.
[c]Positive serology had to be confirmed by western blot.
[d]Course of CSF OspA positivity in four patients in each arm; designated as co-primary endpoint.
[e4]Significant results in PCS scores in drug versus placebo treated patients with greater severity of symptoms at baseline; sustained at 24 weeks.
[f]Total SF-36 score: % patients improved (better scores at both skills, or one better and one unchanged), worsened (worse score on one or both skills), unchanged.
[g]Values for doxycycline, clarithromycin-hydroxychloroquine, and placebo group, respectively.

3). A temporal relation of persistent constitutional symptoms with primary Lyme borreliosis may suggest PTLDS (box 2). For a large group of patients who have little or no evidence of previous *B burgdorferi* sl infection, who understandably seek explanation for chronic fatigue, pain, and other incapacitating symptoms, Lyme borreliosis has become a common consideration. Anchoring bias to ascribe symptoms to Lyme borreliosis or even tick bites is a potential pitfall. Alternative explanations, such as chronic fatigue syndrome or fibromyalgia, may be considered but may be less acceptable for some patients. As described, the weight of evidence is strongly against persistent infection as the explanation for persistent symptoms in such patients, and treatment trials have consistently provided evidence against prolonged antibiotic treatment.

Clinicians caring for patients with poorly understood syndromes of fatigue, pain, and impaired mental acuity often have the difficult task of explaining that there is no straightforward diagnosis or treatment, such as an active infection to be cured with antibiotics. Understandably, this often appears to be removing hope for seriously ill patients, who are indisputably suffering, regardless of the cause of their symptoms. Good medical care for this group includes listening to patients, understanding their concerns and suffering, and discussing the pitfalls and misconceptions surrounding the diagnosis and management of Lyme borreliosis, while remaining aware of potential alternative diagnoses. In close consultation with the patient, the optimal management strategy should be determined, without reverting to unsubstantiated, irrational, or even potentially harmful therapies.

Co-infections

Other microorganisms present in ticks

Numerous bacteria, parasites, and viruses have been detected in *Ixodes* ticks[178 185] and *Ixodes* ticks are well known vectors for other human diseases, including anaplasmosis,[186] babesiosis,[187] *Borrelia miyamotoi* disease,[188] ehrlichiosis,[186] rickettsioses, and disease caused by tickborne flaviviruses.[189 190] In the US, the number of reported cases of these *Ixodes* tickborne diseases has increased over time.[191 192] Multiple new tickborne pathogens have been described in *Ixodes* species in Europe, the US, and Asia; for example, *Ehrlichia muris eauclairensis*,[193] *Neoehrlichia mikurensis*,[194] [195] *Borrelia miyamotoi*,[196] *Borrelia mayonii*,[3] and Alongshan virus.[197] Notably, the mere presence of a microbe—let alone its DNA—does not render it a tickborne pathogen. Indeed, ticks have many commensal bacteria and endosymbionts,[198] and can acquire bacteria, parasites, and viruses from an infected host through a blood meal, which they are unable to transmit, or which, if transmitted, do not lead to disease.[185]

Lyme borreliosis: evidence for role of co-infections

Co-infection of *Ixodes* ticks with multiple tickborne pathogens is well established,[178 185] although even

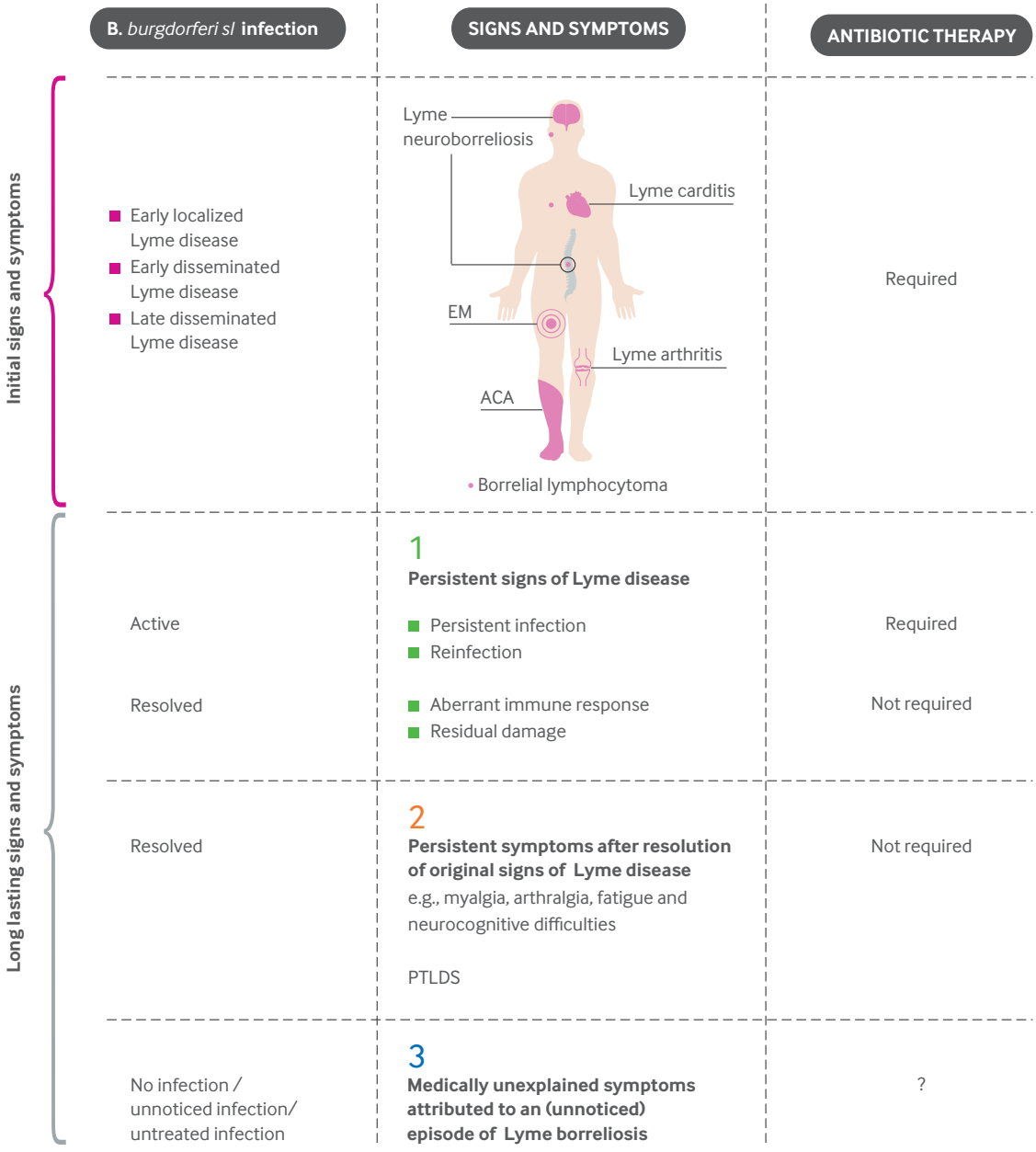

Fig 3 | Presentation of persisting symptoms attributed to Lyme borreliosis. PTLDS=post-treatment Lyme disease syndrome; EM=erythema migrans; ACA=acrodermatitis chronica atrophicans

in regions where multiple tickborne diseases are endemic, human co-infections appear to be relatively rare.[199 200] In the US, *B burgdorferi* ss-*Anaplasma phagocytophilum* and *B burgdorferi* ss-*Babesia microti* co-infections are relatively common.[178] Experimental evidence suggests that *Anaplasma phagocytophilum* may alter the course of acute Lyme borreliosis[201] but clinical data are inconclusive.[157] In contrast, human co-infection with *Babesia microti* can increase the duration and severity of symptoms caused by acute Lyme borreliosis.[202] Of note, patients with acute Lyme borreliosis accompanied by persisting high grade fever in the US, any fever in Europe, or abnormal blood counts (anemia, thrombocytopenia, or leukopenia) should raise a suspicion of co-infection.

In contrast, there is little evidence for the notion that "chronic Lyme" can be attributed to Lyme borreliosis and a wide range of co-infections. These include the pathogens mentioned above, but also other microorganisms, such as *Chlamydia*, *Brucella*, or *Mycoplasma* species, *Toxoplasma gondii*, Epstein-Barr virus, cytomegalovirus, or human herpes virus-6. However, these patients frequently lack clinical symptoms compatible with such infections.[203] A systematic review of patients diagnosed with "chronic Lyme" did not find evidence for chronic anaplasmosis or babesiosis in humans, tick transmission of *Bartonella* species, or *B burgdorferi* sl-*Bartonella* co-infections.[159] In Europe, a large prospective clinical study is currently ongoing, assessing the role of several known *Ixodes*

Box 3: Stepwise approach to management of patients with persisting symptoms attributed to Lyme borreliosis

- **Goal:** to assess whether persisting symptoms are related to previous *B burgdorferi* infection, and may be due to active infection and, thus, potentially amenable to antimicrobial treatment
- A careful medical history should show whether there may have been a *B burgdorferi* infection and clinical signs of localized or disseminated Lyme borreliosis
- Physical examination should focus on persistent signs at the primary sites of infection or new, localized signs of disseminated infection that may indicate persistent infection. If persisting infection is deemed unlikely, immune mediated arthritis or neuroborreliosis induced residual damage should be ruled out
- A temporal relation of subjective symptoms and primary Lyme borreliosis may suggest PTLDS
- Consider other underlying diseases or conditions that may explain the patient's symptoms, or laboratory or imaging abnormalities that might suggest an undiagnosed process
- In patients with constitutional symptoms and little or no evidence of previous *B burgdorferi* infection, the evidence is against persistent *B burgdorferi* infection as the explanation for persistent symptoms, and treatment trials have provided evidence against prolonged antibiotic therapy
- Listen to the patient's concerns and acknowledge their suffering, and discuss the pitfalls and misconceptions surrounding the diagnosis and management of Lyme borreliosis

Ricinus-borne pathogens in the development of longlasting symptoms.[134]

Post-exposure prophylaxis

A meta-analysis of four placebo controlled clinical trials (totaling 1082 patients) in the US showed a risk of developing Lyme borreliosis of 2.2% (95% confidence interval 1.2% to 3.9%) in the placebo group, compared with 0.2% (95% confidence interval 0.0% to 1.0%) in the prophylaxis group,[13] indicating that prophylaxis to ~50 individuals prevents one case of Lyme borreliosis.[204] A trial with topical azithromycin was stopped prematurely because of a lack of effect.[205] In current guidelines from the US and Europe, watchful waiting is primarily recommended, while a single dose of doxycycline (200 mg) within 72 hours after a tick bite[204] may be offered in highly endemic settings, when the tick has been attached for longer periods.[157 206 207]

Vaccination

The OspA vaccine

In the late 1990s, two vaccines, LYMErix[208] and ImuLyme,[209] were assessed in large phase III clinical, double blind, randomized, placebo controlled trials. Both vaccines were based on recombinant OspA of *B burgdorferi* ss. The vaccines were well tolerated, efficacy ranged from 76% to 92% after three immunizations, and they were shown to be cost effective.[208-211] LYMErix was commercially launched in the US in 1998, and in 2002, manufacturer GSK voluntarily withdrew the vaccine, citing poor sales on lack of demand.[212] However, the reasons were multifactorial and extensively discussed previously,[213 214] cumulating in class action lawsuits and final withdrawal of the vaccine. Several OspA based veterinary vaccines are still available,[215] but a commercial vaccine to prevent Lyme borreliosis in humans does not exist.

Modified OspA vaccines

As multiple *B burgdorferi* sl genospecies can cause Lyme borreliosis, second generation OspA vaccines targeting multiple *B burgdorferi* sl serotypes are being developed. Baxter BioScience has developed a chimeric recombinant vaccine that contained six OspA serotypes,[216] lacking the alleged "auto-reactive" *B burgdorferi* ss epitope. Phase I/II vaccine trials have shown safety and immunogenicity in naive and previously *B burgdorferi* sl exposed individuals.[217 218] Another novel multivalent OspA vaccine, VLA15, consists of three heterodimers linking C-termini of two OspA serotypes and covering six clinically relevant *B burgdorferi* sl serotypes,[219] while the alleged "auto-reactive" epitope was replaced with the corresponding sequence of *B afzelii*. A phase I trial indicated seroconversion of 71.4% to 96.4% for multiple OspA serotypes after three doses of VLA15, which were well tolerated.[220] VLA15 is currently assessed in phase II clinical trials with higher dosages and alternative vaccination schedules (NCT03769194/NCT03970733).

Emerging treatments

Other spirochetal recombinant protein based vaccines, including a vaccine that targets a *B burgdorferi* sl protein of unknown function, BB0405,[221] and novel delivery strategies are under development.[222 223] Alternatively, vaccination against tick proteins is considered,[222] based on the phenomenon known as "tick immunity": guinea pigs, rabbits, and possibly humans, develop immune responses against tick proteins after repeated tick infestation, resulting in impaired tick feeding and protection against *B burgdorferi* ss infection.[223] Regardless of the approach, a future human vaccine would need to be sufficiently safe, efficacious, and cost effective, to achieve acceptance from the medical community and public and to prevent a repetition of the past.[223 224]

Guidelines

Guidelines on management of Lyme borreliosis are available in the US[157 225 226] and Europe.[66 206 207 227 228] For the antibiotic treatment of Lyme borreliosis, the recommended agents, doses, and durations are

highly consistent through different guidelines, and are predominantly based on studies described in table 3. Alternative recommendations, provided in a position paper by the International Lyme and Associated Diseases Society (ILADS), have not provided any credible clinical or scientific evidence to support prolonged antibiotic therapy. Their designation as "evidence based guidelines" belies their anecdotal nature and lack of coherent and evidence based guidance.[226]

Conclusion

The manifestations of Lyme borreliosis are diverse, the diagnosis is not always straightforward, diagnostic tests may have limitations, and their results should be interpreted in the context of the clinical symptoms. However, the disease generally responds well to antibiotic treatment. Despite antibiotic therapy, patients may develop disabling persistent symptoms, sometimes referred to as "chronic Lyme." For proper patient management, it is of critical importance to discriminate antibiotic therapy failure from immune driven post-infectious phenomena such as relapsing Lyme arthritis, residual tissue damage, such as post-neuroborreliosis neuropathy, or new subjective symptoms developing after resolution of the initial disease manifestations. The latter may be classified as PTLDS, of which the pathogenesis has not fully been elucidated.[134] Finally, "chronic Lyme" remains a popular consideration for patients, often with little or no evidence of previous *B burgdorferi* infection, who understandably seek explanation for fatigue, pain, and other incapacitating symptoms. These patients, who are indisputably suffering regardless of the cause of their symptoms, deserve a thorough analysis whether there may have been an undiagnosed *B burgdorferi* infection, or other post-infectious sequelae. Often, the lack of objective

Table 3 | Overview of treatment guidelines for Lyme borreliosis*

Manifestation	Recommendations
Prophylaxis	• Not addressed in all guidelines • Routine use of antibiotic prophylaxis is not recommended in two guidelines[16 227] • Specific conditions in which a single dose of doxycycline (200 mg for adults if not contraindicated) may be offered are considered by IDSA: bite by tick species known to potentially transmit *B burgdorferi* sl with a local infection rate of ≥20%, tick attached for ≥36 hours (estimated), and start of prophylaxis within 72 hours after removal of the tick[157] • ILADS recommends treatment with doxycycline for 20 days in all patients with evidence of tick feedin[8226]
Erythema migrans	• Oral doxycycline therapy for 10-21 days as first line therapy[157 206 207 227 228] • Second line includes oral amoxicillin (mostly preferred), cefuroxime axetil, or phenoxymethylpenicillin for 14-21 days[157 206 207 227 228] • Azithromycin (or other macrolides) for 5-17 days as third line option[157 207 227] • Longer duration in case of concomitant non-focal symptoms or multiple erythema migrans is specifically not recommended in some guidelines,[206 207] whereas in others it is recommended[227] • ILADS recommends oral antibiotics for 4-6 weeks (amoxicillin, cefuroxime, or doxycycline) or 21 days for azithromycin, with continuation of therapy if full recovery has not been achieved[226]
Lyme neuroborreliosis	• Intravenous ceftriaxone for 10-28 days favored for meningitis/radiculopathy by several guidelines[66 157 207 225] with oral doxycycline as reasonable alternative Other guidelines favor doxycycline as first choice,[206] or consider both equivalent.[59] For cranial neuritis, doxycycline is generally favored
Lyme arthritis	• Doxycycline for 28-30 days as first line treatment,[206 207 228] in absence of neurologic disease[157] • If no improvement after first line therapy: intravenous ceftriaxone for 28 days[157 206] • National Institute for Health and Care Excellence (NICE) guidelines recommend oral amoxicillin for 28 days as first alternative to doxycycline, and intravenous ceftriaxone as second alternative[207] • If persistent after repeated antibiotic regimens, reactive/inflammatory arthritis is considered and anti-inflammatory therapy may be offered[157 206]
ACA	• Doxycycline for 28-30 days as first line treatment,[206 207 227] whereas shorter duration of oral treatment (14-21 days) is recommended in two guidelines[157 228] • Intravenous ceftriaxone for 28 days as second line [206] or third line[207] or in case of concomitant neurological symptoms as first choice for 14-21 days[227] • Irreversible skin damage (skin atrophy, sensory deficits) may persist[157 206 227]
Borrelial lymphocytoma	• Therapy similar to that of erythema migrans, but for a minimal duration of 14[157 228] to 21 days[206 227] • NICE guidelines withhold from general recommendations because of its low incidence and lack of evidence[207]
Lyme carditis	• Oral doxycycline (or equivalent) for 14-30 days[157 206 207 228] • Intravenous ceftriaxone in case of symptomatic carditis,[207] switch to oral antibiotics based on clinical response, for a total duration of 14-21 days[157 206]
Ongoing symptoms after treatment for Lyme borreliosis	• If there is no suspicion of re-infection or failure of antibiotic therapy after careful review of a patient's history and symptoms[206 207] and in patients with PTLDS,[66 157 225] prolonged antibiotic treatment or re-treatment is not recommended • ILADS guidelines suggest antibiotic treatment may be considered in the heterogeneous population of "patients with persistent manifestations of Lyme disease" for a duration of 4-6 weeks or longer, as "the evidence regarding persistent infection is at hand and the potential benefits of retreatment are adequate to support those who wish to treat, but is not overwhelming enough to mandate treatment." For patients who partially respond after 4-6 weeks, "the decision to continue treatment may depend on the length of time between the initial and subsequent re-treatment, the strength of the patient's response to retreatment, the severity of the patient's current impairments"[226]

*Two guidelines specifically focus on neurologic Lyme borreliosis manifestations,[66 225] one on dermatologic manifestations,[227] and one on antibiotic prophylaxis after atick bite, erythema migrans, and persistent symptoms.[226]
Treatment recommendations for children are largely similar to adults, although for children aged under 8 or 9 doxycycline is relatively contraindicated[157 206 207 226 227]

findings and abnormal test results are frustrating for physicians as well as for patients. Good medical care for these patients includes listening, understanding, and discussing personalized treatment options, including non-pharmacological options such as rehabilitation. Future research may reveal additional underlying causal mechanisms, indicating how to best diagnose and treat these patients.

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RESEARCH QUESTIONS

- What are the main factors responsible for the rise in the abundance of *Ixodes* ticks that vector the causative agents of Lyme borreliosis; can we find effective and interdisciplinary countermeasures to halt this upsurge?
- Can we discover and develop novel diagnostics markers or tests with increased sensitivity for localized disease and early disseminated Lyme borreliosis, and tests that can differentiate between an active *B burgdorferi* sl infection and past infection, in particular for patients with unexplained signs and symptoms after completing antibiotic treatment?
- What are effective strategies for the prevention of antibiotic refractory Lyme arthritis, and can we develop evidence based guidelines for its treatment?
- What are the underlying causal mechanisms in patients with longlasting symptoms after antibiotic treatment for Lyme borreliosis? Can we identify microbiological determinants, co-infections, immunological or genetic mechanisms, epidemiological determinants, or cognitive behavioral factors that are associated with developing persistent post-treatment symptoms?
- What is the long term outcome in patients with PTLDS? Which factors may influence their prognosis, and can we define optimal treatment strategies for these patients?
- Can we find a safe, efficacious, and cost effective vaccine that is able to protect both children and adults against Lyme borreliosis in Europe and in North America? What is needed for the general public to accept such a vaccine?

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1 Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. *Nature* 1997;390:580-6.
2 Steere AC, Strle F, Wormser GP, et al. Lyme borreliosis. *Nat Rev Dis Primers* 2016;2:16090.
3 Pritt BS, Mead PS, Johnson DKH, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis* 2016;16:556-64.
4 Estrada-Pena A, Cutler S, Potkonjak A, et al. An updated meta-analysis of the distribution and prevalence of Borrelia burgdorferi sl in ticks in Europe. *Int J Health Geogr* 2018;17:41.
5 Strnad M, Honig V, Ruzek D, Grubhoffer L, Rego ROM. Europe-wide meta-analysis of borrelia burgdorferi sensu lato prevalence in questing Ixodes ricinus ticks. *Appl Environ Microbiol* 2017;83. doi:10.1128/aem.00609-17.
6 Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). *Antonie van Leeuwenhoek* 2014;105:1049-72.
7 Margos G, Gofton A, Wibberg D, et al. The genus Borrelia reloaded. *PLoS One* 2018;13:e0208432.
8 Gupta RS. Distinction between Borrelia and Borreliella is more robustly supported by molecular and phenotypic characteristics than all other neighbouring prokaryotic genera: Response to Margos' et al. "The genus Borrelia reloaded. *PLoS One* 2019;14:e0221397.
9 Xu G, Pearson P, Dykstra E, Andrews ES, Rich SM. Human-biting Ixodes ticks and pathogen prevalence from California, Oregon, and Washington. *Vector Borne Zoonotic Dis* 2019;19:106-14.
10 Hofhuis A, van de Kassteele J, Sprong H, et al. Predicting the risk of Lyme borreliosis after a tick bite, using a structural equation model. *PLoS One* 2017;12:e0181807.
11 Wilhelmsson P, Fryland L, Lindblom P, et al. A prospective study on the incidence of Borrelia burgdorferi sensu lato infection after a tick bite in Sweden and on the Aland Islands, Finland (2008-2009). *Ticks Tick Borne Dis* 2016;7:71-9.
12 Huegli D, Moret J, Rais O, et al. Prospective study on the incidence of infection by Borrelia burgdorferi sensu lato after a tick bite in a highly endemic area of Switzerland. *Ticks Tick Borne Dis* 2011;2:129-36.
13 Warshafsky S, Lee DH, Francois LK, et al. Efficacy of antibiotic prophylaxis for the prevention of Lyme disease: an updated systematic review and meta-analysis. *J Antimicrob Chemother* 2010;65:1137-44.
14 Lindgren E, Jaenson TGT. *Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures.* WHO Regional Office for Europe, 2006, http://www.euro.who.int/__data/assets/pdf_file/0006/96819/E89522.pdf.
15 Sykes RA, Makiello P. An estimate of Lyme borreliosis incidence in Western Europe. *J Public Health (Oxf)* 2017;39:74-81.
16 Rizzoli A, Hauffe H, Carpi G, et al. Lyme borreliosis in Europe. *Euro Surveill* 2011;16:19906.
17 van den Wijngaard CC, Hofhuis A, Simoes M, et al. Surveillance perspective on Lyme borreliosis across the European Union and European Economic Area. *Euro Surveill* 2017;22. doi:10.2807/1560-7917.Es.2017.22.27.30569.
18 Rosenberg R, Lindsey NP, Fischer M, et al. Vital signs: trends in reported vectorborne disease cases—United States and Territories, 2004-2016. *MMWR Morb Mortal Wkly Rep* 2018;67:496-501.
19 Kuehn BM. CDC estimates 300 000 US cases of Lyme disease annually. *JAMA* 2013;310:1110.
20 Sprong H, Azagi T, Hoornstra D, et al. Control of Lyme borreliosis and other Ixodes ricinus-borne diseases. *Parasit Vectors* 2018;11:145.
21 Lindgren E, Talleklint L, Polfeldt T. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick Ixodes ricinus. *Environ Health Perspect* 2000;108:119-23.
22 Mac S, da Silva SR, Sander B. The economic burden of Lyme disease and the cost-effectiveness of Lyme disease interventions: A scoping review. *PLoS One* 2019;14:e0210280.
23 European Parliament. Parliament calls for "alarming" spread of Lyme disease to be tackled 2018. https://www.europarl.europa.eu/news/en/press-room/20181106IPR18328/parliament-calls-for-alarming-spread-of-lyme-disease-to-be-tackled

24 NIH. Strategic plan for tickborne disease research, 2019. https://www.niaid.nih.gov/sites/default/files/NIH-Strategic-Plan-Tickborne-Disease-Research-2019.pdf
25 Stanek G, Fingerle V, Hunfeld KP, et al. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* 2011;17:69-79.
26 Dessau RB, van Dam AP, Fingerle V, et al. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. *Clin Microbiol Infect* 2018;24:118-24.
27 Holl-Wieden A, Suerbaum S, Girschick HJ. Seronegative Lyme arthritis. *Rheumatol Int* 2007;27:1091-3.
28 Hammers-Berggen SH, Lebech AM, Karlsson M, et al. Serological follow-up after treatment of patients with erythema migrans and neuroborreliosis. *J Clin Microbiol* 1994;32:1519-25.
29 Hilton E, DeVoti J, Benach JL, et al. Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the northeast United States. *Am J Med* 1999;106:404-9.
30 Cetin E, Sotoudeh M, Auer H, Stanek G. Paradigm Burgenland: risk of Borrelia burgdorferi sensu lato infection indicated by variable seroprevalence rates in hunters. *Wien Klin Wochenschr* 2006;118:677-81.
31 Kalish RA, McHugh G, Granquist J, et al. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease. *Clin Infect Dis* 2001;33:780-5.
32 Babady NE, Sloan LM, Vetter EA, Patel R, Binnicker MJ. Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue. *Diagn Microbiol Infect Dis* 2008;62:464-6.
33 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.
34 Li X, McHugh GA, Damle N, et al. Burden and viability of Borrelia burgdorferi in skin and joints of patients with erythema migrans or lyme arthritis. *Arthritis Rheum* 2011;63:2238-47.
35 Hunfeld KP, Ruzic-Sabljic E, Norris DE, Kraiczy P, Strle F. In vitro susceptibility testing of Borrelia burgdorferi sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial chemotherapy. *Antimicrob Agents Chemother* 2005;49:1294-301.
36 Ogrinc K, Lusa L, Lotric-Furlan S, et al. Course and outcome of early European Lyme neuroborreliosis (Bannwarth Syndrome): clinical and laboratory findings. *Clin Infect Dis* 2016;63:346-53.
37 Hansen K, Lebech AM. The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985-1990. *Brain* 1992;115:399-423.
38 Ljostad U, Skarpaas T, Mygland A. Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis. *Eur J Neurol* 2007;14:873-6.
39 Rupprecht TA, Manz KM, Fingerle V, et al. Diagnostic value of cerebrospinal fluid CXCL13 for acute Lyme neuroborreliosis. A systematic review and meta-analysis. *Clin Microbiol Infect* 2018;24:1234-40.
40 Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. *Clin Infect Dis* 2017;64:e1-33.
41 Dessau RB, Fingerle V, Gray J, et al. The lymphocyte transformation test for the diagnosis of Lyme borreliosis has currently not been shown to be clinically useful. *Clin Microbiol Infect* 2014;20:0786-7.
42 Callister SM, Jobe DA, Stuparic-Stancic A, et al. Detection of IFN-gamma secretion by T cells collected before and after successful treatment of early Lyme disease. *Clin Infect Dis* 2016;62:1235-41.
43 van de Schoor FR, Baarsma ME, Gauw SA, et al. Validation of cellular tests for Lyme borreliosis (VICTORY) study. *BMC Infect Dis* 2019;19:732.
44 Magni R, Espina BH, Shah K, et al. Application of Nanotrap technology for high sensitivity measurement of urinary outer surface protein A carboxyl-terminus domain in early stage Lyme borreliosis. *J Transl Med* 2015;13:346.
45 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.
46 Aase A, Hajdusek O, Oines O, et al. Validate or falsify: lessons learned from a microscopy method claimed to be useful for detecting Borrelia and Babesia organisms in human blood. *Infect Dis (Lond)* 2016;48:411-9.
47 Lohr B, Fingerle V, Norris DE, Hunfeld KP. Laboratory diagnosis of Lyme borreliosis: current state of the art and future perspectives. *Crit Rev Clin Lab Sci* 2018;55:219-45.
48 Leeflang MM, Ang CW, Berkhout J, et al. The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: a systematic review and meta-analysis. *BMC Infect Dis* 2016;16:140.
49 Ruzic-Sabljic E, Cerar T. Progress in the molecular diagnosis of Lyme disease. *Expert Rev Mol Diagn* 2017;17:19-30.
50 Wormser GP, Nowakowski J, Nadelman RB, et al. Impact of clinical variables on Borrelia burgdorferi-specific antibody seropositivity in acute-phase sera from patients in North America with culture-confirmed early Lyme disease. *Clin Vaccine Immunol* 2008;15:1519-22.
51 Stupica D, Lusa L, Maraspin V, et al. Correlation of culture positivity, PCR positivity, and burden of Borrelia burgdorferi sensu lato in skin samples of erythema migrans patients with clinical findings. *PLoS One* 2015;10:e0136600.
52 Mullegger RR, Glatz M. Skin manifestations of Lyme borreliosis: diagnosis and management. *Am J Clin Dermatol* 2008;9:355-68.
53 Wormser GP, Bittker S, Cooper D, et al. Yield of large-volume blood cultures in patients with early Lyme disease. *J Infect Dis* 2001;184:1070-2.
54 Maraspin V, Nahtigal Klevisar M, Ruzic-Sabljic E, Lusa L, Strle F. Borrelial lymphocytoma in adult patients. *Clin Infect Dis* 2016;63:914-21.
55 Maraspin V, Cimperman J, Lotric-Furlan S, et al. Solitary borrelial lymphocytoma in adult patients. *Wien Klin Wochenschr* 2002;114:515-23.
56 Colli C, Leinweber B, Müllegger R, et al. Borrelia burgdorferi-associated lymphocytoma cutis: clinicopathologic, immunophenotypic, and molecular study of 106 cases. *J Cutan Pathol* 2004;31:232-40.
57 Asbrink E, Hovmark A. Early and late cutaneous manifestations in Ixodes-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). *Ann N Y Acad Sci* 1988;539:4-15.
58 Arvikar SL, Steere AC. Diagnosis and treatment of Lyme arthritis. *Infect Dis Clin North Am* 2015;29:269-80.
59 Waddell LA, Greig J, Mascarenhas M, et al. The accuracy of diagnostic tests for Lyme disease in humans, a systematic review and meta-analysis of North American research. *PLoS One* 2016;11:e0168613.
60 Jaulhac B, Chary-Valckenaere I, Sibilia J, et al. Detection of Borrelia burgdorferi by DNA amplification in synovial tissue samples from patients with Lyme arthritis. *Arthritis Rheum* 1996;39:736-45.
61 Wormser GP, Nadelman RB, Schwartz I. The amber theory of Lyme arthritis: initial description and clinical implications. *Clin Rheumatol* 2012;31:989-94.
62 Lebech AM, Hansen K, Brandrup F, Clemmensen O, Halkier-Sorensen L. Diagnostic value of PCR for detection of Borrelia burgdorferi DNA in clinical specimens from patients with erythema migrans and Lyme neuroborreliosis. *Mol Diagn* 2000;5:139-50.
63 Avery RA, Frank G, Eppes SC. Diagnostic utility of Borrelia burgdorferi cerebrospinal fluid polymerase chain reaction in children with Lyme meningitis. *Pediatr Infect Dis J* 2005;24:705-8.
64 Cerar T, Ogrinc K, Cimperman J, et al. Validation of cultivation and PCR methods for diagnosis of Lyme neuroborreliosis. *J Clin Microbiol* 2008;46:3375-9.
65 Tumani H, Nolker G, Reiber H. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. *Neurology* 1995;45:1663-70.
66 Mygland A, Ljostad U, Fingerle V, et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol* 2010;17:8-16.
67 Halperin JJ, Volkman DJ, Wu P. Central nervous system abnormalities in Lyme neuroborreliosis. *Neurology* 1991;41:1571-82.
68 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.
69 Cerar T, Ogrinc K, Strle F, Ruzic-Sabljic E. Humoral immune responses in patients with Lyme neuroborreliosis. *Clin Vaccine Immunol* 2010;17:645-50.
70 Hansen K, Lebech AM. Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal synthesis of Borrelia burgdorferi—specific immunoglobulin G, A, and M. *Ann Neurol* 1991;30:197-205.
71 Hytonen J, Kortela E, Waris M, et al. CXCL13 and neopterin concentrations in cerebrospinal fluid of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammation. *J Neuroinflammation* 2014;11:103.
72 van Burgel ND, Bakels F, Kroes AC, van Dam AP. Discriminating Lyme neuroborreliosis from other neuroinflammatory diseases by levels of CXCL13 in cerebrospinal fluid. *J Clin Microbiol* 2011;49:2027-30.
73 Cerar T, Ogrinc K, Lotric-Furlan S, et al. Diagnostic value of cytokines and chemokines in lyme neuroborreliosis. *Clin Vaccine Immunol* 2013;20:1578-84.
74 Ang CW, Notermans DW, Hommes M, Simoons-Smit AM, Herremans T. Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. *Eur J Clin Microbiol Infect Dis* 2011;30:1027-32.
75 Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. *Clin Microbiol Rev* 2005;18:484-509.

76 Coulter P, Lema C, Flayhart D, et al. Two-year evaluation of Borrelia burgdorferi culture and supplemental tests for definitive diagnosis of Lyme disease. *J Clin Microbiol* 2005;43:5080-4.
77 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.
78 Strle F, Nelson JA, Ruzic-Sabljic E, et al. European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. *Clin Infect Dis* 1996;23:61-5.
79 Bennet L, Fraenkel CJ, Garpmo U, et al. Clinical appearance of erythema migrans caused by Borrelia afzelii and Borrelia garinii—effect of the patient's sex. *Wien Klin Wochenschr* 2006;118:531-7.
80 Nadelman RB, Nowakowski J, Forseter G, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. *Am J Med* 1996;100:502-8.
81 Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. *Ann Intern Med* 1987;107:725-31.
82 Arnez M, Ruzic-Sabljic E. Borrelial lymphocytoma in children. *Pediatr Infect Dis J* 2015;34:1319-22.
83 Bennet L, Halling A, Berglund J. Increased incidence of Lyme borreliosis in southern Sweden following mild winters and during warm, humid summers. *Eur J Clin Microbiol Infect Dis* 2006;25:426-32.
84 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.
85 Ogrinc K, Wormser GP, Visintainer P, et al. Pathogenetic implications of the age at time of diagnosis and skin location for acrodermatitis chronica atrophicans. *Ticks Tick Borne Dis* 2017;8:266-9.
86 Lenormand C, Jaulhac B, Debarbieux S, et al. Expanding the clinicopathological spectrum of late cutaneous Lyme borreliosis (acrodermatitis chronica atrophicans [ACA]): A prospective study of 20 culture- and/or polymerase chain reaction (PCR)-documented cases. *J Am Acad Dermatol* 2016;74:685-92.
87 Agterof MJ, ter Borg EJ. Erythematous pigmentation of the arm for more than ten years. *Neth J Med* 2008;66:176-9.
88 Halperin JJ. Nervous system Lyme disease: diagnosis and treatment. *Curr Treat Options Neurol* 2013;15:454-64.
89 Rojko T, Bogovic P, Lotric-Furlan S, et al. Borrelia burgdorferi sensu lato infection in patients with peripheral facial palsy. *Ticks Tick Borne Dis* 2018;. doi:10.1016/j.ttbdis.2018.11.019.
90 Sodermark L, Sigurdsson V, Nas W, Wall P, Trollfors B. Neuroborreliosis in Swedish children: a population-based study on incidence and clinical characteristics. *Pediatr Infect Dis J* 2017;36:1052-6.
91 Halperin JJ. Lyme disease and the peripheral nervous system. *Muscle Nerve* 2003;28:133-43.
92 Wormser GP, Strle F, Shapiro ED, Dattwyler RJ, Auwaerter PG. A critical appraisal of the mild axonal peripheral neuropathy of late neurologic Lyme disease. *Diagn Microbiol Infect Dis* 2017;87:163-7.
93 Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. *N Engl J Med* 1990;323:1438-44.
94 Garkowski A, Zajkowska J, Zajkowska A, et al. Cerebrovascular manifestations of Lyme neuroborreliosis—a systematic review of published cases. *Front Neurol* 2017;8:146.
95 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.
96 Grillon A, Scherlinger M, Boyer PH, et al. Characteristics and clinical outcomes after treatment of a national cohort of PCR-positive Lyme arthritis. *Semin Arthritis Rheum* 2018. doi:10.1016/j.semarthrit.2018.09.007.
97 Lelovas P, Dontas I, Bassiakou E, Xanthos T. Cardiac implications of Lyme disease, diagnosis and therapeutic approach. *Int J Cardiol* 2008;129:15-21.
98 Scheffold N, Herkommer B, Kandolf R, May AE. Lyme carditis—diagnosis, treatment and prognosis. *Dtsch Arztebl Int* 2015;112:202-8.
99 Stanek GKJ, Bittner R, Glogar D. Isolation of borrelia burgdorferi from the myocardium of a patient with long-standing cardiomyopathy. *N Engl J Med* 1990;322:249-52.
100 Kubanek M, Sramko M, Berenova D, et al. Detection of Borrelia burgdorferi sensu lato in endomyocardial biopsy specimens in individuals with recent-onset dilated cardiomyopathy. *Eur J Heart Fail* 2012;14:588-96.
101 Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet* 2012;379:461-73.
102 van Dam AP, Kuiper H, Vos K, et al. Different genospecies of Borrelia burgdorferi are associated with distinct clinical manifestations of Lyme borreliosis. *Clin Infect Dis* 1993;17:708-17.
103 Strle F, Ruzic-Sabljic E, Logar M, et al. Comparison of erythema migrans caused by Borrelia burgdorferi and Borrelia garinii. *Vector Borne Zoonotic Dis* 2011;11:1253-8.
104 Centers for Disease Control and Prevention. Lyme disease: data and Surveillance. 2019. https://www.cdc.gov/lyme/datasurveillance/index.html.
105 Stanek G, Strle F. Lyme borreliosis-from tick bite to diagnosis and treatment. *FEMS Microbiol Rev* 2018;42:233-58.
106 Stupica D, Veluscek M, Blagus R, et al. Oral doxycycline versus intravenous ceftriaxone for treatment of multiple erythema migrans: an open-label alternate-treatment observational trial. *J Antimicrob Chemother* 2018;73:1352-8.
107 Taber LH, Feigin RD. Spirochetal infections. *Pediatr Clin North Am* 1979;26:377-413.
108 Schlesinger PA, Duray PH, Burke BA, Steere AC, Stillman MT. Maternal-fetal transmission of the Lyme disease spirochete, Borrelia burgdorferi. *Ann Intern Med* 1985;103:67-8.
109 Markowitz LE, Steere AC, Benach JL, Slade JD, Broome CV. Lyme disease during pregnancy. *JAMA* 1986;255:3394-6.
110 Weber K, Bratzke HJ, Neubert U, Wilske B, Duray PH. Borrelia burgdorferi in a newborn despite oral penicillin for Lyme borreliosis during pregnancy. *Pediatr Infect Dis J* 1988;7:286-9.
111 Shirts SR, Brown MS, Bobitt JR. Listeriosis and borreliosis as causes of antepartum fever. *Obstet Gynecol* 1983;62:256-61.
112 Waddell LA, Greig J, Lindsay LR, Hinckley AF, Ogden NH. A systematic review on the impact of gestational Lyme disease in humans on the fetus and newborn. *PLoS One* 2018;13:e0207067.
113 Lakos A, Solymosi N. Maternal Lyme borreliosis and pregnancy outcome. *Int J Infect Dis* 2010;14:e494-8.
114 Jutras BL, Lochhead RB, Kloos ZA, et al. Borrelia burgdorferi peptidoglycan is a persistent antigen in patients with Lyme arthritis. *Proc Natl Acad Sci USA* 2019;116:13498-507.
115 Torbahn G, Hofmann H, Rucker G, et al. Efficacy and safety of antibiotic therapy in early cutaneous Lyme borreliosis: a network meta-analysis. *JAMA Dermatol* 2018;154:1292-303.
116 Arnez M, Ruzic-Sabljic E. Azithromycin is equally effective as amoxicillin in children with solitary erythema migrans. *Pediatr Infect Dis J* 2015;34:1045-8.
117 Strle F, Preac-Mursic V, Cimperman J, et al. Azithromycin versus doxycycline for treatment of erythema migrans: clinical and microbiological findings. *Infection* 1993;21:83-8.
118 Strle F, Maraspin V, Lotric-Furlan S, Ruzic-Sabljic E, Cimperman J. Azithromycin and doxycycline for treatment of Borrelia culture-positive erythema migrans. *Infection* 1996;24:64-8.
119 Barsic B, Maretic T, Majerus L, Strugar J. Comparison of azithromycin and doxycycline in the treatment of erythema migrans. *Infection* 2000;28:153-6.
120 Luft BJ, Dattwyler RJ, Johnson RC, et al. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. *Ann Intern Med* 1996;124:785-91.
121 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.
122 Stupica D, Lusa L, Ruzic-Sabljic E, Cerar T, Strle F. Treatment of erythema migrans with doxycycline for 10 days versus 15 days. *Clin Infect Dis* 2012;55:343-50.
123 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.
124 Dersch R, Freitag MH, Schmidt S, et al. Efficacy and safety of pharmacological treatments for acute Lyme neuroborreliosis—a systematic review. *Eur J Neurol* 2015;22:1249-59.
125 Eikeland R, Mygland A, Herlofson K, Ljostad U. European neuroborreliosis: quality of life 30 months after treatment. *Acta Neurol Scand* 2011;124:349-54.
126 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.
127 Bremell D, Dotevall L. Oral doxycycline for Lyme neuroborreliosis with symptoms of encephalitis, myelitis, vasculitis or intracranial hypertension. *Eur J Neurol* 2014;21:1162-7.
128 Steere AC, Levin RE, Molloy PJ, et al. Treatment of Lyme arthritis. *Arthritis Rheum* 1994;37:878-88.
129 Caperton EM, Heim-Duthoy KL, Matzke GR, Peterson PK, Johnson RC. Ceftriaxone therapy of chronic inflammatory arthritis. A double-blind placebo controlled trial. *Arch Intern Med* 1990;150:1677-82.
130 Steere AC, Angelis SM. Therapy for Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. *Arthritis Rheum* 2006;54:3079-86.
131 Aberer E, Breier F, Stanek G, Schmidt B. Success and failure in the treatment of acrodermatitis chronica atrophicans. *Infection* 1996;24:85-7.
132 Kindstrand E, Nilsson BY, Hovmark A, Pirskanen R, Asbrink E. Peripheral neuropathy in acrodermatitis chronica atrophicans - effect of treatment. *Acta Neurol Scand* 2002;106:253-7.
133 Cairns V, Godwin J. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. *Int J Epidemiol* 2005;34:1340-5.
134 Vrijmoeth HD, Ursinus J, Harms MG, et al. Prevalence and determinants of persistent symptoms after treatment for Lyme borreliosis: study protocol for an observational, prospective cohort study (LymeProspect). *BMC Infect Dis* 2019;19:324.

135 Embers ME, Barthold SW, Borda JT, et al. Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. *PLoS One* 2012;7:e29914.
136 Wormser GP, O'Connell S, Pachner AR, et al. Critical analysis of a doxycycline treatment trial of rhesus macaques infected with Borrelia burgdorferi. *Diagn Microbiol Infect Dis* 2018;92:183-8.
137 Nadelman RB, Nowakowski J, Forseter G, et al. Failure to isolate Borrelia burgdorferi after antimicrobial therapy in culture-documented Lyme borreliosis associated with erythema migrans: report of a prospective study. *Am J Med* 1993;94:583-8.
138 Nadelman RB, Hanincova K, Mukherjee P, et al. Differentiation of reinfection from relapse in recurrent Lyme disease. *N Engl J Med* 2012;367:1883-90.
139 Lochhead RB, Arvikar SL, Aversa JM, et al. Robust interferon signature and suppressed tissue repair gene expression in synovial tissue from patients with postinfectious, Borrelia burgdorferi-induced Lyme arthritis. *Cell Microbiol* 2018e12954.
140 Strle K, Sulka KB, Pianta A, et al. T-Helper 17 cell cytokine responses in Lyme disease correlate with Borrelia burgdorferi antibodies during early infection and with autoantibodies late in the illness in patients with antibiotic-refractory Lyme arthritis. *Clin Infect Dis* 2017;64:930-8.
141 Crowley JT, Strle K, Drouin EE, et al. Matrix metalloproteinase-10 is a target of T and B cell responses that correlate with synovial pathology in patients with antibiotic-refractory Lyme arthritis. *J Autoimmun* 2016;69:24-37.
142 Steere AC, Dwyer E, Winchester R. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. *N Engl J Med* 1990;323:219-23.
143 Lochhead RB, Strle K, Kim ND, et al. MicroRNA expression shows inflammatory dysregulation and tumor-like proliferative responses in joints of patients with postinfectious Lyme arthritis. *Arthritis Rheumatol* 2017;69:1100-10.
144 Strle K, Shin JJ, Glickstein LJ, Steere AC. Association of a toll-like receptor 1 polymorphism with heightened Th1 inflammatory responses and antibiotic-refractory Lyme arthritis. *Arthritis Rheum* 2012;64:1497-507.
145 Ljostad U, Mygland A. Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. *Eur J Neurol* 2010;17:118-23.
146 Eikeland R, Mygland A, Herlofson K, Ljostad U. Risk factors for a non-favorable outcome after treated European neuroborreliosis. *Acta Neurol Scand* 2013;127:154-60.
147 Berglund J, Stjernberg L, Ornstein K, Tykesson-Joelsson K, Walter H. 5-y Follow-up study of patients with neuroborreliosis. *Scand J Infect Dis* 2002;34:421-5.
148 Knudtzen FC, Andersen NS, Jensen TG, Skarphedinsson S. Characteristics and clinical outcome of Lyme neuroborreliosis in a high endemic area, 1995-2014: a retrospective cohort study in Denmark. *Clin Infect Dis* 2017;65:1489-95.
149 Chiang SS, Khan FA, Milstein MB, et al. Treatment outcomes of childhood tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2014;14:947-57.
150 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.
151 Aucott JN, Crowder LA, Kortte KB. Development of a foundation for a case definition of post-treatment Lyme disease syndrome. *Int J Infect Dis* 2013;17:e443-9.
152 Weitzner E, McKenna D, Nowakowski J, et al. Long-term assessment of post-treatment symptoms in patients with culture-confirmed early Lyme disease. *Clin Infect Dis* 2015;61:1800-6.
153 Berende A, Agelink van Rentergem J, Evers AWM, et al. Cognitive impairments in patients with persistent symptoms attributed to Lyme disease. *BMC Infect Dis* 2019;19:833.
154 Berende A, Ter Hofstede HJM, Vos FJ, et al. Effect of prolonged antibiotic treatment on cognition in patients with Lyme borreliosis. *Neurology* 2019;92:e1447-55.
155 Batheja S, Nields JA, Landa A, Fallon BA. Post-treatment Lyme syndrome and central sensitization. *J Neuropsychiatry Clin Neurosci* 2013;25:176-86.
156 Barbour A. Remains of infection. *J Clin Invest* 2012;122:2344-6.
157 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.
158 Centers for Disease Control and Prevention. Lyme disease (Borrelia burgdorferi) 2017 case definition. 2017
159 Lantos PM, Wormser GP. Chronic coinfections in patients diagnosed with chronic Lyme disease: a systematic review. *Am J Med* 2014;127:1105-10.
160 Lantos PM, Auwaerter PG, Wormser GP. A systematic review of Borrelia burgdorferi morphologic variants does not support a role in chronic Lyme disease. *Clin Infect Dis* 2014;58:663-71.
161 Aucott JN, Soloski MJ, Rebman AW, et al. CCL19 as a chemokine risk factor for posttreatment Lyme disease syndrome: a prospective clinical cohort study. *Clin Vaccine Immunol* 2016;23:757-66.
162 Strle K, Stupica D, Drouin EE, Steere AC, Strle F. Elevated levels of IL-23 in a subset of patients with post-Lyme disease symptoms following erythema migrans. *Clin Infect Dis* 2014;58:372-80.
163 Sjowall J, Fryland L, Nordberg M, et al. Decreased Th1-type inflammatory cytokine expression in the skin is associated with persisting symptoms after treatment of erythema migrans. *PLoS One* 2011;6:e18220.
164 Chandra A, Wormser GP, Klempner MS, et al. Anti-neural antibody reactivity in patients with a history of Lyme borreliosis and persistent symptoms. *Brain Behav Immun* 2010;24:1018-24.
165 Klempner MS, Wormser GH, Wade K, et al. A case-control study to examine HLA haplotype associations in patients with posttreatment chronic Lyme disease. *J Infect Dis* 2005;192:1010-3.
166 Borsic K, Blagus R, Cerar T, Strle F, Stupica D. Clinical course, serologic response, and long-term outcome in elderly patients with early Lyme Borreliosis. *J Clin Med* 2018;7:506.
167 Wormser GP, Shapiro ED. Implications of gender in chronic Lyme disease. *J Womens Health (Larchmt)* 2009;18:831-4.
168 Wills AB, Spaulding AB, Adjemian J, et al. Long-term follow-up of patients with Lyme disease: longitudinal analysis of clinical and quality-of-life measures. *Clin Infect Dis* 2016;62:1546-51.
169 Shadick NA, Phillips CB, Logigian EL, et al. The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. *Ann Intern Med* 1994;121:560-7.
170 Hassett AL, Radvanski DC, Buyske S, Savage SV, Sigal LH. Psychiatric comorbidity and other psychological factors in patients with "chronic Lyme disease.". *Am J Med* 2009;122:843-50.
171 Donta ST. Tetracycline therapy for chronic Lyme disease. *Clin Infect Dis* 1997;25(suppl 1):S52-6.
172 Donta ST. Macrolide therapy of chronic Lyme disease. *Med Sci Monit* 2003;9:PI136-42.
173 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.
174 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.
175 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.
176 Cameron D. Severity of Lyme disease with persistent symptoms. Insights from a double-blind placebo-controlled clinical trial. *Minerva Med* 2008;99:489-96.
177 Berende A, ter Hofstede HJ, Vos FJ, et al. Randomized trial of longer-term therapy for symptoms attributed to Lyme disease. *N Engl J Med* 2016;374:1209-20.
178 Eisen RJ, Eisen L. The blacklegged tick, Ixodes scapularis: an Increasing public health concern. *Trends Parasitol* 2018;34:295-309.
179 Fallon BA, Petkova E, Keilp JG, Britton CB. A reappraisal of the U.S. Clinical trials of post-treatment lyme disease syndrome. *Open Neurol J* 2012;6:79-87.
180 Delong AK, Blossom B, Maloney EL, Phillips SE. Antibiotic retreatment of Lyme disease in patients with persistent symptoms: a biostatistical review of randomized, placebo-controlled, clinical trials. *Contemp Clin Trials* 2012;33:1132-42.
181 Klempner MS, Baker PJ, Shapiro ED, et al. Treatment trials for post-Lyme disease symptoms revisited. *Am J Med* 2013;126:665-9.
182 Kaplan RF, Trevino RP, Johnson GM, et al. Cognitive function in post-treatment Lyme disease: do additional antibiotics help?*Neurology* 2003;60:1916-22.
183 Berende A, ter Hofstede HJ, Donders AR, et al. Persistent Lyme empiric Antibiotic Study Europe (PLEASE)--design of a randomized controlled trial of prolonged antibiotic treatment in patients with persistent symptoms attributed to Lyme borreliosis. *BMC Infect Dis* 2014;14:543.
184 Van Middendorp H, Berende A, Vos F, et al. Expectancies as predictors of symptom improvement after antimicrobial therapy for persistent symptoms attributed to Lyme disease. Submitted 2020
185 Jahfari S, Hofhuis A, Fonville M, et al. Molecular detection of tick-borne pathogens in humans with tick bites and erythema migrans, in the Netherlands. *PLoS Negl Trop Dis* 2016;10:e0005042.
186 Dumler JS. Anaplasma and Ehrlichia infection. *Ann N Y Acad Sci* 2005;1063:361-73.
187 Vannier E, Krause PJ. Human babesiosis. *N Engl J Med* 2012;366:2397-407.
188 Wagemakers A, Staarink PJ, Sprong H, Hovius JW. Borrelia miyamotoi: a widespread tick-borne relapsing fever spirochete. *Trends Parasitol* 2015;31:260-9.
189 Charrel RN, Attoui H, Butenko AM, et al. Tick-borne virus diseases of human interest in Europe. *Clin Microbiol Infect* 2004;10:1040-55.

190 Telford SR3rd, Armstrong PM, Katavolos P, et al. A new tick-borne encephalitis-like virus infecting New England deer ticks, Ixodes dammini. *Emerg Infect Dis* 1997;3:165-70.
191 Paules CI, Marston HD, Bloom ME, Fauci AS. Tickborne diseases—confronting a growing threat. *N Engl J Med* 2018;379:701-3.
192 Stein E, Elbadawi LI, Kazmierczak J, Davis JP. Babesiosis surveillance—Wisconsin, 2001-2015. *MMWR Morb Mortal Wkly Rep* 2017;66:687-91.
193 Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic Ehrlichia species, Wisconsin and Minnesota, 2009. *N Engl J Med* 2011;365:422-9.
194 Wenneras C. Infections with the tick-borne bacterium Candidatus Neoehrlichia mikurensis. *Clin Microbiol Infect* 2015;21:621-30.
195 Wass L, Grankvist A, Bell-Sakyi L, et al. Cultivation of the causative agent of human neoehrlichiosis from clinical isolates identifies vascular endothelium as a target of infection. *Emerg Microbes Infect* 2019;8:413-25.
196 Krause PJ, Narasimhan S, Wormser GP, et al. Human Borrelia miyamotoi infection in the United States. *N Engl J Med* 2013;368:291-3.
197 Wang ZD, Wang B, Wei F, et al. A new segmented virus associated with human febrile illness in China. *N Engl J Med* 2019;380:2116-25.
198 Narasimhan S, Rajeevan N, Liu L, et al. Gut microbiota of the tick vector Ixodes scapularis modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* 2014;15:58-71.
199 Strle F, Bogovic P, Cimperman J, et al. Are patients with erythema migrans who have leukopenia and/or thrombocytopenia coinfected with Anaplasma phagocytophilum or tick-borne encephalitis virus?*PLoS One* 2014;9:e103188.
200 Steere AC, McHugh G, Suarez C, et al. Prospective study of coinfection in patients with erythema migrans. *Clin Infect Dis* 2003;36:1078-81.
201 Thomas V, Anguita J, Barthold SW, Fikrig E. Coinfection with Borrelia burgdorferi and the agent of human granulocytic ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. *Infect Immun* 2001;69:3359-71.
202 Krause PJ, Telford SR3rd, Spielman A, et al. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA* 1996;275:1657-60.
203 Kobayashi T, Higgins Y, Samuels R, et al. Misdiagnosis of Lyme disease with unnecessary antimicrobial treatment characterizes patients referred to an academic infectious diseases clinic. *Open Forum Infect Dis* 2019;6:ofz299.
204 Nadelman RB, Nowakowski J, Fish D, et al. Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an Ixodes scapularis tick bite. *N Engl J Med* 2001;345:79-84.
205 Schwameis M, Kundig T, Huber G, et al. Topical azithromycin for the prevention of Lyme borreliosis: a randomised, placebo-controlled, phase 3 efficacy trial. *Lancet Infect Dis* 2017;17:322-9.
206 Jaulhac B, Saunier A, Caumes E, et al. Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies (II). Biological diagnosis, treatment, persistent symptoms after documented or suspected Lyme borreliosis. *Med Mal Infect* 2019;49:335-46.
207 Cruickshank M. O 'Flynn N, Faust SN. Lyme disease: summary of NICE guidance. *BMJ* 2018;361:k1261.
208 Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant Borrelia burgdorferi outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. *N Engl J Med* 1998;339:209-15.
209 Sigal LH, Zahradnik JM, Lavin P, et al. A vaccine consisting of recombinant Borrelia burgdorferi outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. *N Engl J Med* 1998;339:216-22.
210 Meltzer MI, Dennis DT, Orloski KA. The cost effectiveness of vaccinating against Lyme disease. *Emerg Infect Dis* 1999;5:321-8.
211 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.
212 Hitt E. Poor sales trigger vaccine withdrawal. *Nat Med* 2002;8:311-2.
213 Aronowitz RA. The rise and fall of the lyme disease vaccines: a cautionary tale for risk interventions in American medicine and public health. *Milbank Q* 2012;90:250-77.
214 Nigrovic LE, Thompson KM. The Lyme vaccine: a cautionary tale. *Epidemiol Infect* 2007;135:1-8.
215 Vogt NA, Sargeant JM, MacKinnon MC, Versluis AM. Efficacy of Borrelia burgdorferi vaccine in dogs in North America: A systematic review and meta-analysis. *J Vet Intern Med* 2018;33:23-36.
216 Livey I, O'Rourke M, Traweger A, et al. A new approach to a Lyme disease vaccine. *Clin Infect Dis* 2011;52(suppl 3):s266-70.
217 Wressnigg N, Barrett PN, Pollabauer EM, et al. A novel multivalent OspA vaccine against Lyme borreliosis is safe and immunogenic in an adult population previously infected with Borrelia burgdorferi sensu lato. *Clin Vaccine Immunol* 2014;21:1490-9.
218 Wressnigg N, Pollabauer EM, Aichinger G, et al. Safety and immunogenicity of a novel multivalent OspA vaccine against Lyme borreliosis in healthy adults: a double-blind, randomised, dose-escalation phase 1/2 trial. *Lancet Infect Dis* 2013;13:680-9.
219 Comstedt P, Hanner M, Schuler W, et al. Characterization and optimization of a novel vaccine for protection against Lyme borreliosis. *Vaccine* 2015;33:5982-8.
220 Valneva. Valneva reports positive initial booster data and final phase 1 data for its Lyme disease vaccine candidate. 2019. https://valneva.com/press-release/valneva-reports-positive-initial-booster-data-and-final-phase-1-data-for-its-lyme-disease-vaccine-candidate/
221 Kung F, Kaur S, Smith AA, et al. A Borrelia burgdorferi surface-exposed transmembrane protein lacking detectable immune responses supports pathogen persistence and constitutes a vaccine target. *J Infect Dis* 2016;213:1786-95.
222 Nassal M, Skamel C, Vogel M, et al. Development of hepatitis B virus capsids into a whole-chain protein antigen display platform: new particulate Lyme disease vaccines. *Int J Med Microbiol* 2008;298:135-42.
223 Gomes-Solecki M, Arnaboldi PM, Backenson PB, et al. Protective immunity and new vaccines for Lyme disease. *Clin Infect Dis* 2019;70:1768-73.
224 Plotkin SA. Need for a new Lyme disease vaccine. *N Engl J Med* 2016;375:911-3.
225 Halperin JJ, Shapiro ED, Logigian E, et al. Practice parameter: treatment of nervous system Lyme disease (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2007;69:91-102.
226 Cameron DJ, Johnson LB, Maloney EL. Evidence assessments and guideline recommendations in Lyme disease: the clinical management of known tick bites, erythema migrans rashes and persistent disease. *Expert Rev Anti Infect Ther* 2014;12:1103-35.
227 Hofmann H, Fingerle V, Hunfeld KP, et al. Cutaneous Lyme borreliosis: guideline of the German Dermatology Society. *Ger Med Sci* 2017;15:Doc 14.
228 Pancewicz SA, Garlicki AM, Moniuszko-Malinowska A, et al. Diagnosis and treatment of tick-borne diseases recommendations of the Polish Society of Epidemiology and Infectious Diseases. *Przegl Epidemiol* 2015;69:309-16.