TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

LYME BORRELIOSIS: DIAGNOSTICS, TREATMENT AND THE ROLE OF HEALTHCARE SERVICES

Elisa Kortela

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1562 | MEDICA – ODONTOLOGICA | TURKU 2021

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

LYME BORRELIOSIS: DIAGNOSTICS, TREATMENT AND THE ROLE OF HEALTHCARE SERVICES

Elisa Kortela

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1562 | MEDICA – ODONTOLOGICA | TURKU 2021

University of Turku

Faculty of Medicine
Department of Clinical Medicine
Infectious Diseases
Doctoral Program in Clinical Research

Supervised by

Professor Jarmo Oksi, MD, PhD
Department of Clinical Medicine
University of Turku,
Turku, Finland

Assoc. prof. Jukka Hytönen, MD, PhD
Institute of Biomedicine
University of Turku,
Turku, Finland

Reviewed by

Docent Heikki Kauma, MD, PhD
Department of Clinical Medicine
University of Oulu
Oulu, Finland

Docent Sari Hämäläinen, MD, PhD
Department of Clinical Medicine
University of Eastern Finland
Kuopio, Finland

Opponent

Docent Reetta Huttunen, MD, PhD
Department of Internal Medicine
Tampere University Hospital
University of Tampere
Tampere, Finland

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin Originality Check service.

ISBN 978-951-29-8503-6 (PRINT)
ISBN 978-951-29-8504-3 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2021

To my family

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Clinical Medicine
Infectious Diseases
ELISA KORTELA: Lyme borreliosis: diagnostics, treatment and the role of healthcare services
Doctoral Dissertation, 210 pp.
Doctoral Program in Clinical Research
August 2021

ABSTRACT

Lyme borreliosis (LB) is the most common tick-borne disease in Europe, caused by spirochete *Borrelia burgdorferi* sensu lato. Approximately 3–16% of patients with *B. burgdorferi* infection develop neurological symptoms, leading to a disease called Lyme neuroborreliosis (LNB). Serological response in LB may take several weeks, which complicates the diagnostics in early LNB. The chemokine CXCL13 is a promising biomarker, the concentration of which starts to rise in acute LNB before intrathecal production of *B. burgdorferi*–specific antibodies begins. In Finland, LNB has been treated with a 3-week course of intravenous ceftriaxone.

The aims of this study were to specify the manifestations of LNB in Finland, investigate the role of CXCL13 as a tool in the LNB diagnostics, compare oral doxycycline to intravenous ceftriaxone in the treatment of LNB, study the diagnostic accuracy of suspected LB with the most typical differential diagnoses, and evaluate the use of antibiotics and healthcare services in patients with suspected LB.

The most typical signs and symptoms of LNB were painful meningoradiculitis, facial nerve palsy, Garin-Bujadoux-Bannwarth syndrome, headache and unspecific neck-shoulder pain. Cerebrospinal fluid CXCL13 concentration was elevated in the acute phase of infection and decreased rapidly after treatment. LNB patients who received doxycycline improved equally well as patients with ceftriaxone treatment. LB was unlikely in half of the patients with suspected LB. The most typical conditions causing the symptoms in patients with unlikely LB were of musculoskeletal, neurological, psychological and functional origin. Patients with unlikely LB used more healthcare services than patients with definite, probable or possible LB. Antimicrobial utilization did not differ among the groups of differing LB certainty.

In conclusion, our results support the clinical use of CXCL13 in the diagnostics of LNB, and oral doxycycline in the treatment of LNB. Additionally, because various conditions may be confused with LB, physicians must pay attention to differential diagnostics of LB to avoid unnecessary antibiotic treatments and treatment delays.

KEYWORDS: Lyme borreliosis, Lyme neuroborreliosis, *Borrelia burgdorferi*, CXCL13, cerebrospinal fluid, doxycycline, ceftriaxone, differential diagnostics, antimicrobial utilization, healthcare services

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Infektiotautioppi
ELISA KORTELA: Lymen borrelioosi: diagnostiikka, hoito ja terveydenhuollon palvelujen käyttö
Väitöskirja, 210 s.
Turun kliininen tohtoriohjelma
Elokuu 2021

TIIVISTELMÄ

Lymen borrelioosi (LB) on tavallisin puutiaisvälitteinen sairaus Euroopassa. Sen aiheuttaa spirokeetta *Borrelia burgdorferi* sensu lato. Noin 3–16 %:lle potilaista kehittyy neurologisia oireita, jolloin sairautta kutsutaan Lymen neuroborrelioosiksi (LNB). Serologisen vasteen kehittyminen voi kestää viikkoja, mikä vaikeuttaa alkuvaiheen LNB:n diagnostiikkaa. Kemokiini CXCL13 on lupaava merkkiaine, jonka pitoisuus alkaa nousta akuutissa LNB:ssä ennen aivokalvojen sisäisen vasta-ainemuodostuksen alkamista. Suomessa LNB on hoidettu kolmen viikon kestoisella suonensisäisellä keftriaksonihoidolla.

Tämän tutkimuksen tavoitteina oli täsmentää LNB:n ilmenemismuotoja Suomessa, tutkia CXCL13 hyödyllisyyttä LNB diagnostiikassa, verrata suun kautta otettavaa doksisykliiniä keftriaksoniin LNB:n hoidossa, tutkia epäillyn LB:n todennäköisyyttä ja tyypillisimpiä erotusdiagnooseja, sekä arvioida antibioottien ja terveyspalvelujen käyttöä potilailla, joilla epäillään LB:a.

LNB-potilaiden yleisimmät oireet ja löydökset olivat kivulias hermojuurten tulehdus, kasvohermohalvaus, Garin-Bujadoux-Bannwarthin oireyhtymä, päänsärky ja epäspesifinen niska-hartiaseudun kipu. Aivo-selkäydinnesteen CXCL13 pitoisuus oli kohonnut akuutissa infektiossa ja väheni nopeasti hoidon jälkeen. Doksisykliinillä hoidetut LNB-potilaat paranivat yhtä hyvin kuin keftriaksoni-hoidon saaneet potilaat. LB oli epätodennäköinen puolella potilaista, joilla sairautta epäiltiin. Näillä potilailla oireiden taustalla oli useimmiten tuki- ja liikuntaelimistön ongelmia tai neurologisia, psykiatrisia tai toiminnallisia sairauksia. Potilaat, joilla LB osoittautui epätodennäköiseksi, käyttivät terveydenhuollon palveluja enemmän kuin potilaat, joiden LB-diagnoosi oli varma, todennäköinen tai mahdollinen. Mikrobilääkkeiden kulutuksessa ei ollut eroa näiden ryhmien välillä.

Yhteenvetona voidaan todeta, että tulokset tukevat CXCL13 käyttöä akuutin LNB:n diagnostiikassa sekä suun kautta otettavan doksisykliinin käyttöä LNB:n hoidossa. Lisäksi, koska useat sairaudet voidaan sekoittaa LB:in, lääkäreiden tulee kiinnittää huomiota LB:n erotusdiagnostiikkaan tarpeettomien antibioottikuurien sekä hoitoviiveiden välttämiseksi.

AVAINSANAT: Lymen borrelioosi, Lymen neuroborrelioosi, *Borrelia burgdorferi*, CXCL13, aivo-selkäydinneste, doksisykliini, keftriaksoni, erotusdiagnostiikka, antimikrobien käyttö, terveydenhuollon palvelut

Table of Contents

Abbreviations

AAN	American Academy of Neurology
ACA	acrodermatitis chronica atrophicans
ACR	American College of Rheumatology
AI	antibody index
AMS	antimicrobial stewardship
Avohilmo	the Register for Primary Health Care Visits
CAM	complementary and alternative medicine
CCL-19	C-C motif chemokine ligand 19
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CLIA	chemiluminescence immunoassay
CNS	central nervous system
CRO	ceftriaxone
CRP	C-reactive protein
CSF	cerebrospinal fluid
CXCL13	C-X-C motif chemokine ligand 13
DOX	doxycycline
ECLIA	electrochemiluminescence immunoassay
EEG	electroencephalogram
EFNS	European Federation of Neurological Societies
EIA	enzyme immunoassay
ELFA	enzyme-linked fluorescence assay
ELISA	enzyme-linked immunosorbent assay
EM	erythema migrans
ENMG	electroneuromyography
EUCALB	the European Union Concerted Action on Lyme Borreliosis
Hilmo	the National Hospital Discharge Register
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IDSA	The Infectious Disease Society of America
IQR	interquartile range

LA	Lyme arthritis
LB	Lyme borreliosis
LNB	Lyme neuroborreliosis
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
MRI	magnetic resonance imaging
NA	not available
NIDR	the National Infectious Disease Register
OspA	Outer surface protein A
PCR	polymerase chain reaction
PNS	peripheral nervous system
PTLDS	Post-treatment Lyme disease syndrome
RDT	rapid diagnostic test
SD	standard deviation
s.l.	sensu lato
s.s.	sensu stricto
TBE	Tick-borne encephalitis
VAS	visual analogue scale

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hytönen J, Kortela E, Waris M, Puustinen J, Salo J, Oksi J. CXCL13 and neopterin concentrations in cerebrospinal fluid of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammation. *J Neuroinflammation*, 2014; 11: 103-114.

II Kortela E, Kanerva M, Puustinen J, Hurme S, Airas L, Lauhio A, Hohenthal U, Jalava-Karvinen P, Nieminen T, Finnilä T, Häggblom T, Pietikäinen A, Koivisto M, Vilhonen J, Marttila-Vaara M, Hytönen J*, Oksi J*. Oral doxycycline compared to intravenous ceftriaxone in the treatment of Lyme neuroborreliosis: a multicenter, equivalence, randomized, open-label trial. *Clin Infect Dis*, 2021; 72(8):1323-1331.

III Kortela E, Kanerva M, Kurkela S, Oksi J, Järvinen A. Suspicion of Lyme borreliosis in patients referred to an infectious diseases clinic: what did the patients really have? *Clin Microbiol Infect*, 2020; S1198-743X(20)30574-7. doi: 10.1016/j.cmi.2020.09.022. Epub ahead of print.

IV Kortela E, Kanerva M, Kurkela S, Oksi J, Koivisto M, Järvinen A. Consumption of healthcare services and antibiotics in patients with presumed disseminated Lyme borreliosis before and after evaluation of an infectious disease specialist. *Submitted.*

*These authors contributed equally to this manuscript.

1 Introduction

Lyme borreliosis (LB), also called Lyme disease, is caused by *Borrelia burgdorferi* sensu lato complex transmitted through the tick bite of genus *Ixodes* hard ticks. In Europe, LB is mainly caused by genospecies *B. burgdorferi* sensu stricto (s.s.), *B. garinii* and *B. afzelii,* while in North America the main causative agent is *B. burgdorferi* s.s. LB has been an emerging disease during the last two decades in the Northern Hemisphere. In Finland, the incidence of microbiologically confirmed disseminated LB cases has increased from 7/100,000 population in 1995 to 31/100,000 population in 2014 (Sajanti et al. 2017).

The most common manifestation of LB is erythema migrans (EM), an expanding skin lesion developing at the site of a tick bite, which is induced by migrating spirochetes. If not noticed or left untreated, spirochetes may disseminate via blood circulation or lymphatics to other organs, mainly the skin, nervous system, joints, and, more rarely, to the heart or eyes. Lyme neuroborreliosis (LNB) usually presents as an acute disease a few weeks after the tick bite, with typical symptoms of lymphocytic meningitis, painful meningoradiculitis, or peripheral nerve palsy.

B. burgdorferi–specific antibodies in serum and intrathecally develop within 2–8 weeks and may therefore be undetectable in the early phase of the infection. This complicates diagnostics because signs and symptoms of LNB are not pathognomonic to the disease, and laboratory proof is mandatory for the diagnosis. C-X-C motif chemokine ligand 13 (CXCL13) is a novel biomarker that is very sensitive and quite specific to LNB. Cerebrospinal fluid (CSF) CXCL13 concentration begins to rise in early infection before intrathecal antibody production starts. CSF CXCL13 may also be useful in the evaluation of treatment response because the concentration decreases quickly when spirochetes are eradicated. In study I, the utility of CXCL13 concentration is evaluated in the diagnostics of LNB as well as the pattern of CXCL13 concentration during the period of three weeks of antibiotic therapy.

In Finland, LNB has been traditionally treated with intravenous ceftriaxone for three weeks, even if oral doxycycline is proposed to be non-inferior to ceftriaxone (Ljøstad et al. 2008). Study II compares oral doxycycline to intravenous ceftriaxone in the treatment of LNB. Our hypothesis is that LNB patients improve equally well with oral doxycycline compared to intravenous ceftriaxone.

The diagnosis of LB may be challenging because many LB symptoms are not specific to LB. The overdiagnosis and overtreatment of LB are well-known phenomena, which cause both delay in appropriate treatment for the underlying condition and adverse events because of unnecessary antibiotic treatments (Haddad et al. 2019). In study III, we define the certainty of suspected LB and report the various conditions causing the symptoms. In study IV, the antimicrobial purchases and healthcare service utilization is evaluated in the groups of different LB certainty.

The aims of this thesis were to specify the manifestations of LNB in Finland, evaluate the role of CXCL13 in LNB diagnostics, compare oral doxycycline to intravenous ceftriaxone in the treatment of LNB, study the diagnostic accuracy of suspected LB with the most typical differential diagnoses, and investigate the utilization of antibiotics and healthcare services in patients with suspected LB.

2 Review of the Literature

2.1 Etiology of Lyme borreliosis

2.1.1 History of Lyme borreliosis

Lyme borreliosis (LB) is named after the town of Old Lyme in Connecticut, USA where an epidemic form of arthritis with preceding skin lesion was shown to be associated with tick bites in the 1970s (Steere et al. 1977b). Further studies revealed that the causative organism was an extracellular spirochete, named *Borrelia burgdorferi*, from the deer tick currently known as *Ixodes scapularis* (Burgdorfer et al. 1982, Oliver et al. 1993, Steere et al. 1978). It was subsequently found that this pathogen can cause a multi-system disease involving joints, skin, the nervous system and heart (Steere et al. 1977a). The term Lyme arthritis (LA) began to be used for joint manifestations, and the term Lyme disease was adopted for the whole spectrum of LB manifestations in the USA, while in Europe the disease was commonly referred to as Lyme borreliosis (Stanek G. et al. 2011). In Europe, many manifestations of LB had been known previously with the names of erythema chronicum migrans, acrodermatitis chronica atrophicans, meningopolyneuritis or Garin-Bujadoux-Bannwarth syndrome, and lymphadenosis benigna cutis (Stanek S. et al. 2002). Actually, the first documentation of a tick bite–associated erythema migrans (EM) is from a dermatologic meeting in Sweden in 1909 (Afzelius 1920, Sternbach and Dibble 1996).

2.1.2 Ticks

The main vectors of LB are *Ixodes* ticks, a genus of hard-bodied ticks, of which four species commonly bite humans. Of these, *Ixodes ricinus* (Figure 1) occurs throughout Europe, while *I. scapularis* and *I. pacificus* are abundant in the USA, and *I. persulcatus*, or taiga tick is the main vector in Asia (Estrada-Pena 2001, Piesman and Gern 2004). *I. persulcatus* has previously been prevalent mainly in eastern Europe and northern Asia but it has been detected also in Finland and in the Bothnian Bay of northern Sweden in the current century (Jaaskelainen et al. 2006, Jaenson et

al. 2016). Because of northeast location in Europe and proximity of Asia, both *I. ricinus* and *I. persulcatus* species exist in Finland (Laaksonen et al. 2017).

Ixodes species evolves through four life stages: egg, larva, nymph and adult (Boulanger et al. 2019, Estrada-Pena and de la Fuente 2014). Tick feeds once in every active developmental stage and drops off the host after a few days of bloodmeal. Tick needs a relative humidity of 80% to survive on the ground and develop to the next life stage, which takes several months. Many different animals, from small rodents and insectivores to deer, serve as a host reservoir for ticks. (Matuschka et al. 1992, Talleklint and Jaenson 1993) Eggs and larvae are not considered to be infected with pathogens before first bloodmeal. Naive vector can be infected by vector-borne pathogen either by systemic transmission from the host or co-feeding transmission from the other vector feeding in proximity at the same time. (Randolph 2011, Randolph et al. 1996, Voordouw 2015)

Ticks are capable of transmitting several pathogens to humans, including viruses, bacteria and parasites. Infections caused by these pathogens are mostly zoonoses, in which human represents accidental and dead-end host to the infectious agent (Boulanger et al. 2019). In Europe, endemic tick-borne infections transmitted by genus *Ixodes* ticks include tick-borne encephalitis (TBE), LB, rickettsioses, tularemia, *Borrelia miyamotoi* relapsing fever, ehrlichiosis, bartonellosis, human granulocytic anaplasmosis and babesiosis (Figoni et al. 2019, Gray et al. 2010, Matei et al. 2019, Parola et al. 2013, Socolovschi et al. 2009). The prevalence of *B. burgdorferi* sensu lato (s.l.) in ticks varies geographically and, also between the different developmental stages. In Europe, excluding the Nordic countries, the highest prevalence of 19.3% is detected in Central Europe, while the lowest prevalence of 3.6% is reported in the British Isles (Strnad et al. 2017). In Finland, the overall prevalence of *B. burgdorferi* s.l. in *I. ricinus* and *I. persulcatus* was 17% in 2015 (Laaksonen et al. 2018). Prevalence of *B. burgdorferi* s.l in all genus *Ixodes* ticks was 20% in *I. ricinus* -dominated south Finland, 15% in sympatric area of both species in middle Finland and 21% in *I. persulcatus* -dominated north Finland. In the Åland Islands, 23% of ticks contained *B. burgdorferi* s.l. in 2008-2009 (Wilhelmsson et al. 2013).

2.1.3 *Borrelia burgdorferi* sensu lato complex

LB is caused by gram-negative spirochetes of *B. burgdorferi* s.l. complex (later *B. burgdorferi*), a heterogeneous group which contains at least 18 named genospecies (Holt 1978, Mead 2015). Three of these, *B. burgdorferi* sensu stricto (s.s.), *B. afzelii* and *B. garinii* are responsible for the most of human infections (Baranton et al. 1992). *B. burgdorferi* s.s. is the major cause of LB in North America, while all three genospecies are present in Europe. *B. burgdorferi* s.s. has a tendency to cause

arthritis, while *B. garinii* is particularly neurotropic and *B. afzelii* is found typically behind skin infections, which leads to slightly different clinical pictures of LB in different continents (Steere 2001). In addition to these three *B. burgdorferi* genospecies, six other species are potentially pathogenic to humans: *B. bavariensis*, *B. bissettii*, *B. kurtenbachii*, *B. lusitaniae*, *B. spielmanii* and *B. valaisiana* (Collares-Pereira et al. 2004, Girard et al. 2011, Picken et al. 1996, Rijpkema et al. 1997, Rudenko et al. 2008, Ryffel et al. 1999, Strle et al. 1997, Wang et al. 1999). Of these rarities, *B. valaisiana* and *B. spielmanii* has been found from the Åland Islands and *B. valaisiana* also from the main Finland (Sormunen et al. 2016, Wilhelmsson et al. 2013).

B. burgdorferi spirochetes are motile and capable of invading through vertebrate host tissues and fluids (Nakamura 2020). Chemotactic signals attract spirochetes to the tick feeding site, where the tick ingests them during the feeding (Murfin et al. 2019). Most of the *B. burgdorferi* spirochetes remain colonized in a lumen of tick midgut while a tick moults to the next life stage. Spirochetes migrate to the salivary glands only during following feeding, where they are transmitted with the tick saliva to the new host (De Silva and Fikrig 1995). The minimum feeding time for *B. burgdorferi* to transmit to human is estimated to be 12–24 hour. Nevertheless, in studies in Slovenia and Austria, 14–18 % of patients with EM had removed the tick within 6 hours. (Stanek G. and Kahl 1999, Strle et al. 1996a)

Figure 1. *Ixodes ricinus* male and female. Photo Kari Kaunisto, University of Turku.

2.2 Epidemiology of Lyme borreliosis

LB is the most common vector-borne disease in the Northern Hemisphere (Lindgren and Jaenson 2006). It occurs especially in the Northeastern, mid-Atlantic and the upper Midwestern states in the USA, and throughout Europe, Russia and Asia (Schotthoefer and Frost 2015). In the USA, approximately 30000 cases of LB are reported annually to the Centers for Disease Control and Prevention (CDC) by state health departments and the District of Columbia. This number does not include every diagnosed LB case, and in fact the estimated number of new LB cases is approximately 300000 each year (Hinckley et al. 2014, Nelson C. A. et al. 2015b). In Europe, the highest reported incidences of the disease are in central Europe and Scandinavia (Lindgren and Jaenson. 2006, Rizzoli et al. 2011).

In 2019, laboratories in Finland reported 2228 serological or polymerase chain reaction (PCR) findings of *B. burgdorferi* to the National Infectious Disease Registry (NIDR). LB cases were reported from all over the country, but the highest incidence (1084/100000) was in the Åland Islands (THL 2020). Excluding the Åland Islands, the highest incidence of LB cases is in the coastal areas of Finland (Figure 2). The incidence of reported *B. burgdorferi* findings in Hospital District of Southwest Finland was 83/100000 and in Hospital District of Helsinki and Uusimaa 52/100000 in 2019 (THL 2019).

The incidence of LB has increased in North America and Europe in past two to three decades (Schotthoefer and Frost 2015). In Finland, the incidence of microbiologically confirmed cases increased from 7/100000 to 31/100000 during the years 1995–2014 and incidence of primary LB cases, mainly EM, based on the Register for Primary Health Care Visits (Avohilmo) increased from 44/100000 to 61/100000 in 2011–2014 (Sajanti et al. 2017). The reasons behind this increasing trend are diverse. It is suggested that climate change favors tick reproduction and spreading to new areas (Bennet et al. 2006, Lindgren et al. 2000, Ogden et al. 2006). The public awareness of LB has increased, possibly leading to more frequent patient contacts to healthcare. In addition, by training of healthcare professionals, the recognition and diagnostics of LB may have become more accurate.

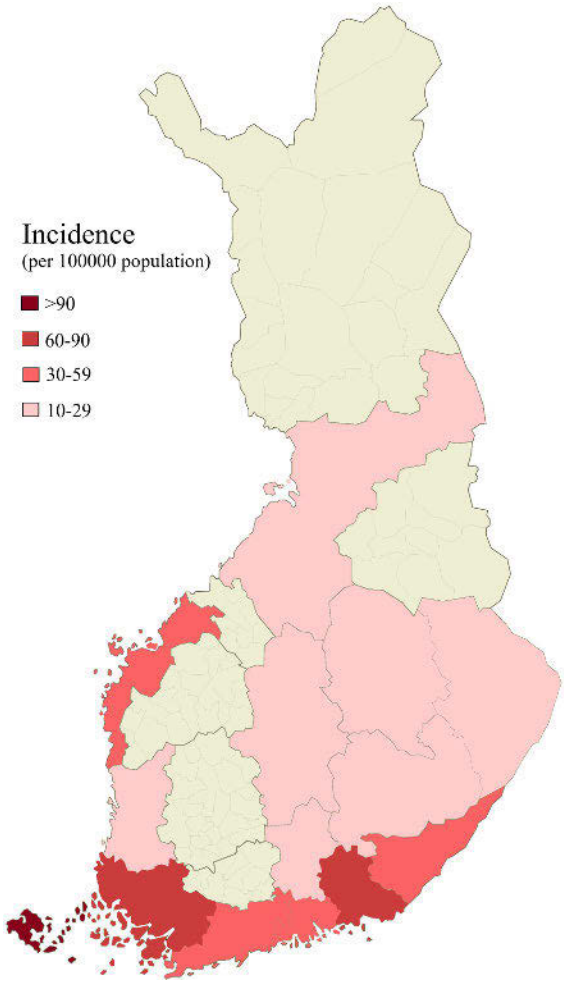

Figure 2. The incidence of microbiologically confirmed LB cases reported by laboratories in different hospital districts in Finland in 2019 (THL 2019).

2.3 Manifestations of Lyme borreliosis

2.3.1 Skin manifestations

LB can appear as a variety of different clinical manifestations in several organs. Three typical disease manifestations are presented on the skin: EM, *B. burgdorferi*–associated lymphocytoma, and acrodermatitis chronica atrophicans (ACA) (Mullegger and Glatz 2008). All three most important human pathogens of *B. burgdorferi* genospecies can cause all these skin lesions. EM (Figure 3) is mainly caused by *B. afzelii*, though *B. garinii* and *B. burgdorferi* s.s. account for about 30%

of the cases. On the contrary, ACA is almost invariably caused by *B. afzelii* (Mullegger 2004).

The most common sign of LB is EM, which is present in circa 80–90% of infections caused by *B. burgdorferi* (Aucott J. et al. 2009, Huppertz et al. 1999, Steere and Sikand 2003). EM presents as a circular expanding skin rash with or without central clearing and appears in the location of a tick bite. Tick bite leads often to a localized and transient inflammatory reaction, which might be caused by components of tick saliva or mechanical irritation and emerges shortly after tick bite resolving spontaneously in a few days (Feder and Whitaker 1995, Oksi J. and Koulu, L. 2017, Wormser 2006). On the other hand, EM is the result of inoculated migrant spirochetes, and can be observed typically 7 to 14 (range 1–36) days after the tick bite (Berger 1989, Nadelman et al. 1996, Nadelman and Wormser 1995, Steere et al. 1983). To separate EM from a hypersensitivity reaction to the tick bite, a size limitation of 5 centimeters or more is recommended for the diagnosis of EM (Hofmann et al. 2017). Only roughly 23–58% of patients with EM have noticed or remember a tick bite (Arnez et al. 2001, Nadelman et al. 1996, Strle et al. 1996b). EM is typically located in or around the bend of a large joint, but it may appear anywhere on the body except for the soles and palms (Mullegger and Glatz 2008, Stanek G. et al. 1996, Strle et al. 1996a, Strle et al. 2002). About 50% of patients with EM reports other local, usually mild symptoms, like itching or burning, but also severe pain caused by a local neuritis is possible (Strle et al. 1996b, Strle et al. 2002).

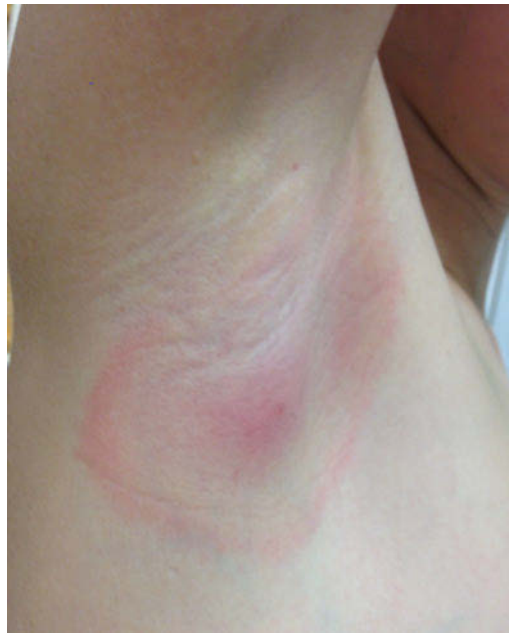

Figure 3. Erythema migrans in the armpit. Photo Jarmo Oksi, Turku University Hospital.

EM may be associated with nonspecific, extracutaneous symptoms approximately in half of the patients and the symptoms in many cases resemble a viral infection (Nadelman et al. 1996). These associated symptoms appear more often in the USA than in Europe (60–80% vs 30–40%), which is probably caused by the occurrence of different *B. burgdorferi* species on these continents (Jones et al. 2008, Strle et al. 1999). The most typical extracutaneous symptoms are arthralgias, myalgias, nausea, headache, fatigue, fever and local lymphadenopathy (Nadelman et al. 1996, Strle et al. 1996b). Diarrhea or respiratory symptoms do not associate to LB, and should lead the diagnostic reasoning to an alternative disease (Nadelman 2015). It has been shown in the USA, that 19–44% of patients with EM have concurrent spirochetemia and these patients were more likely to have systemic symptoms (Nowakowski et al. 2009, Wormser et al. 2005b). Other reasons for these nonspecific symptoms with solitary EM caused by less invasive *B. burgdorferi* subtypes might be derived from host factors, e.g. from the human leukocyte antigen (HLA) system (Wormser et al. 2005a). It is unclear how HLA system is associated with the clinical presentation of LB.

Multiple EM is caused by hematogenous dissemination of *B. burgdorferi* (Asbrink and Hovmark 1988, Wormser et al. 2005b). In multiple EM, several lesions appear in various locations on the skin of the infected individual usually a few days after the primary EM has emerged (Nadelman et al. 1996). Multiple EM is detected in 4–20% of patients with EM being more common in the USA than in Europe (Berger 1989, Nadelman et al. 1996, Strle et al. 2002). Extracutaneous symptoms or additional organ manifestations are reported in 40–70% patients with multiple EM, again with more frequent appearance in the USA compared to Europe (Arnez et al. 2001, Maraspin et al. 2002b, Stupica et al. 2018).

B. burgdorferi–associated lymphocytoma, a single red or bluish nodule in size of 1–5 cm, is an infrequent cutaneous manifestation of LB (Cardenas-de la Garza et al. 2019). Lymphocytoma covers about 5% of all skin manifestations of LB in Europe with an obvious predominance in children (Weber and Neubert 1986). A typical location for a *B. burgdorferi*–associated lymphocytoma is an ear lobe, a nipple, scrotum or an axillary fold, and it may develop weeks or even months after a tick bite as a subacute manifestation (Hovmark et al. 1986, Picken et al. 1997, Stanek G. and Strle 2008). Simultaneous extracutaneous symptoms and signs are very rare (Maraspin et al. 2002a).

ACA is a late-stage cutaneous manifestation of LB in Europe representing 1–3% of LB cases with the annual incidence of 4/100000 (Bennet et al. 2006, Berglund et al. 1995, O'Connell et al. 1998). It may be manifested months to years after an untreated EM (Asbrink et al. 1986, Ogrinc et al. 2017). ACA appears as an atrophic, slowly progressive bluish or red skin lesion predominantly in elderly females (Asbrink and Hovmark 1988, Asbrink et al. 1986). Typical locations are extensor

surfaces of hands and feet, although it can also be located on other skin areas, such as the face or abdomen (Asbrink et al. 1986, Moniuszko-Malinowska et al. 2018, Muller D. E. et al. 1994). Approximately two-thirds of patients have concurrent peripheral neuropathy in the same extremity, though patients may present also with disseminated polyneuropathy (Kindstrand et al. 1997). A representative feature of ACA is allodynia, an exaggerated nociceptive pain reaction to light touch (Brehmer-Andersson et al. 1998, Kindstrand et al. 1997).

2.3.2 Lyme neuroborreliosis

Approximately 3–16% of LB patients both in Europe and the USA have neurological symptoms (Aucott J. et al. 2009, Berglund et al. 1995, Huppertz et al. 1999, Sajanti et al. 2017). Early Lyme neuroborreliosis (LNB) generally develops within a few weeks after initial infection, which results in a peak in the incidence from July to December (Hansen and Lebech 1992). In early LNB, signs and symptoms have lasted less than six months (Mygland et al. 2010). More than 95% of LNB cases are classified as early LNB (Oschmann et al. 1998). LNB clinical picture can be divided into peripheral nervous system including meninges (PNS) and central nervous system (CNS) manifestations (Mygland et al. 2010).

In Europe, the most common neurological manifestations of early LNB are painful meningoradiculitis and facial nerve palsy (Hansen and Lebech 1992, Kaiser 1994, Kruger et al. 1989, Oschmann et al. 1998, Pfister et al. 1984). A term Garin-Bujadoux-Bannwarth syndrome is used, when a patient has intense radicular pain, lymphocytic meningitis and peripheral motor nerve palsy (Ackermann et al. 1984). Typical feature of meningoradiculitis is intensification of pain at night, but the intensity and localization may vary daily (Aarli et al. 1998). Most commonly affected peripheral nerves are cranial nerves, particularly facial nerve, but occasionally peripheral nerves in abdominal wall or the limbs may be involved (Halperin 2015). Parenchymal CNS involvement is very uncommon, but acute or subacute focal encephalitis, myelitis, apraxia, hemiparesis or cerebellar ataxia have been described (Kohlhepp et al. 1989, Mygland et al. 2010, Schwenkenbecher et al. 2017, Sokolov et al. 2015).

Late LNB, with duration of symptoms and signs for more than six months, is rare, involving less than 5% of LNB patients (Oschmann et al. 1998). LNB manifestations in late disease differ from those of early LNB. PNS manifestations of late LNB include radiculopathy, polyneuropathy and mononeuropathy (Logigian et al. 1990, Pfister and Rupprecht 2006). In Europe, polyneuropathy is almost always associated with ACA (Kindstrand et al. 1997). Late CNS manifestations of LNB may be encephalomyelitis with tetraspastic syndrome, progressive encephalitis, cerebral vasculitis or disturbed micturition (Ackermann et al. 1985, Beuchat et al. 2018,

Garkowski et al. 2017, Kohler et al. 1986, Pfister and Rupprecht 2006, Shamim et al. 2005).

2.3.3 Other disseminated forms of Lyme borreliosis

Besides cutaneous and neurologic manifestations, LB may be presented as Lyme arthritis (LA) or rarely carditis (Bateman and Sigal 2000, Verdon and Sigal 1997). Also, ocular manifestations are described (Raja et al. 2016). Patients with early LB in the USA had hepatomegaly (5%), splenomegaly (6%), and symptoms suggestive of hepatitis (10%) (Steere et al. 1983). As a rarity, few case reports of Lyme myositis or osteomyelitis, very unusual manifestations of LB, have been published (Holmgren and Matteson 2006, Horowitz et al. 1994, Oksi J. et al. 1994, Reimers et al. 1993). A timeline in conventional manifestations from initial infection of *B. burgdorferi* is shown in Figure 4.

Circa 60% of untreated EM patients in the USA developed LA before the use of antibiotics in the treatment of LB (Steere et al. 1987). In the first decade of 2000, about 10 000 cases of LA were reported to CDC in the USA (Bacon et al. 2008). In Finland, the incidence of LA remained below 1/100000 during the years 1996-2014, and LA accounted for 4–13% of all LB cases in the National Hospital Discharge Register (Hilmo) (Sajanti et al. 2017). LA may be either monoarthritis or oligoarthritis. LA most commonly affects knees, but other large or small joints may also be involved (Arvikar and Steere 2015). In an European study, 90% of LA cases had only one affected joint and in 97% of the cases the knee was involved (Grillon et al. 2019). LA is easily distinguished from purulent arthritis because the patient typically lacks generalized symptoms. The affected joint is recurrently or persistently warm and swollen, but pain is usually mild to moderate and mean synovial white blood cell count ranges from 10000 to 25000 leukocytes/µl (Puius and Kalish 2008).

Cardiac manifestations of LB affect only a minority of patients (Stanek G. et al. 2012). Surveillance data from the USA indicate, that 1% of LB patients have second- or third-degree atrioventricular conduction blockade (Forrester et al. 2014). In Europe, the reported proportion of cardiac manifestations of all LB cases has ranged from 1% to 2% (Cimmino 1998, Oschmann et al. 1998). More than half of the patients with Lyme carditis are men (Bacon et al. 2008, Forrester and Mead 2014, Forrester et al. 2014). The majority of Lyme carditis involve a quite narrow spectrum of manifestations. The cardiac conduction delays with varying degrees of conduction block are the main findings, but also fast arrhythmias, like atrial fibrillation, supraventricular tachycardia, and ventricular tachycardia are seldomly described (Fish et al. 2008, Konopka et al. 2013, Robinson et al. 2015, Vlay et al. 1991, Zainal et al. 2019). Myocarditis and pericarditis are infrequent manifestations and acute heart failure is a very rare event (Gilson et al. 2017, Koene et al. 2012, Robinson et

al. 2015). A typical time interval for cardiac involvement to occur is between 2 to 5 weeks after the appearance of EM, but the time window can be as wide as from 2 days to 7 months after the infection (Forrester and Mead 2014, McAlister et al. 1989, Steere et al. 1980).

In early LB, 11% of patients are reported to suffer from transient follicular conjunctivitis (Bergloff et al. 1994). Except for conjunctivitis, all the other ocular manifestations are uncommon and described only in case reports. In later stages of LB, inflammatory lesions in multiple sites of the eye have been documented, including uveitis and keratitis (Bergloff et al. 1994, Zaidman 1997), and even blindness due to severe involvement has been described (Arnold and Schriever 1993, Steere et al. 1985). A few case reports concerning LB–associated orbital myositis have been published (Carvounis et al. 2004, Holak et al. 2006, Nieto et al. 2008). Other symptoms related to late LB may be photophobia and severe periodic ocular pain, and additionally LB has to be taken into account in the differential diagnostics of retinal vasculitis (Mikkila et al. 2000). Generally, orbital/adnexal inflammation due to LB and secondary findings, like conjunctival hyperemia, mild exophthalmos, eyelid swelling, and discomfort in eye movement, have been occasionally described (Raja et al. 2016, Xu et al. 2012).

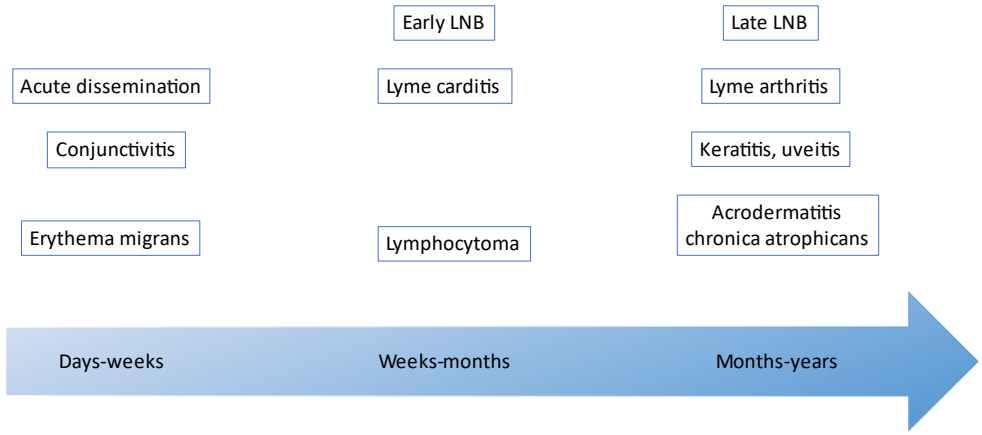

Figure 4. A timeline from the initial infection to different manifestations of LB, modified from (Steere et al. 2016).

2.3.4 Post-treatment Lyme disease syndrome

Even if most patients with acute LB improve satisfyingly after treatment, others may have residual symptoms that last many months or even years (Cerar et al. 2010, Ljøstad et al. 2008, Ogrinc et al. 2016). Often these sequelae are subjective symptoms such as fatigue, musculoskeletal pain, and neurocognitive difficulties

without objective findings (Cairns and Godwin 2005). These symptoms can be very disabling and lead to functional impairment and loss of ability to work (Batheja et al. 2013).

LNB may cause irreversible neurological damage. In one study, 30% of patients with LNB had neurological findings after four months of treatment (Ljøstad et al. 2008). In another study, 28% of LNB (defined as intrathecal *B. burgdorferi* antibody production) patients reported residual symptoms after treatment (Knudtzen et al. 2017). The most common symptoms were radicular pain, paresis and cognitive impairment. Conclusions of a systematic review of 44 clinical trials were similar: LNB patients may suffer from residual symptoms after treatment with prevalence of approximately 28% (Dersch et al. 2016). After treatment of EM, a proportion of patients with symptoms attributed to LB was smaller and further decreased over time, so that 94% of patients were asymptomatic after one year (Borsic et al. 2018). On the other hand, also apparently healthy population has subjective symptoms without any identified condition or LB. In one study, 52 patients with EM were compared to 104 matched control subjects (Wormser et al. 2020). The finding was, that LB patients were more likely symptomatic at baseline, but after six months there were no significant differences in frequency or severity of the evaluated symptoms.

The Infectious Disease Society of America (IDSA) proposed detailed diagnostic criteria for post-treatment Lyme disease syndrome (PTLDS) in 2006 (Wormser et al. 2006). Inclusion criteria included: 1) a documented episode of LB fulfilling a case definition by the CDC (CDC 2017); 2) and resolution or stabilization of the objective manifestations of LB after proper treatment; 3) and onset of subjective symptoms (for example fatigue, musculoskeletal pain, cognitive difficulties) within six months of the diagnosis and persistence of these symptoms for at least six months after therapy; 4) and the severity of these subjective symptoms leading to significant decline in occupational, educational, social, or personal performance. Exclusion criteria are: 1) an untreated coinfection; 2) a diagnosis of fibromyalgia or chronic fatigue syndrome before LB; 3) the presence of objective abnormalities on physical examination or neuropsychologic testing that explains symptoms of the patient; 4) a diagnosis of an underlying disease that might explain the symptoms; 5) laboratory or imaging abnormalities; 6) and a history of prolonged somatic complaints before the onset of LB.

The reason for persistent subjective symptoms after adequate antimicrobial treatment of LB remains unclear. Dysregulated immune response has been proposed to be one potential factor in some patients (Strle and Strle 2020). Several studies have attempted to identify objective biomarkers for PTLDS (Aucott J. N. et al. 2016, Chandra et al. 2010, Fitzgerald et al. 2020, Strle et al. 2014). In these studies, elevated levels of anti-neuronal antibodies, C-C motif chemokine ligand 19 (CCL-19) or interleukin 23 were associated with post-Lyme symptoms, and PTLDS and

non-PTLDS patients had differences in glycerophospholipid, bile acid, and acylcarnitine metabolism. Unfortunately, these studies did not investigate the immune mediators in the general population with similar subjective symptoms. Because it is unclear when long-term symptoms are related to previous LB, an objective biomarker would be very beneficial. Importantly, the evidence does not support the hypothesis of persistent infection of *B. burgdorferi* causing the chronic symptoms after adequate antibiotic therapy, and the misleading term "chronic Lyme disease" should be avoided (Feder et al. 2007, Wormser et al. 2006).

2.4 Laboratory diagnostics of Lyme borreliosis

2.4.1 Serology

EM diagnosis is clinical and no laboratory support is usually required nor recommended. Diagnosis of other forms of LB should include medical history with clinical presentation suggestive of LB, possible tick exposure, laboratory confirmation, and exclusion of other conditions (Dessau et al. 2018, Stanek G. et al. 2011). Serologic response to bacteria may take some weeks to develop after infection. Antibodies against *B. burgdorferi* are assumed to develop in more than 99% patients within 6–8 weeks (Hansen 1994, Hansen and Asbrink 1989, Hansen et al. 1988, Hansen and Lebech 1991, Stanek G. et al. 2011, Wilske et al. 1993, Wilske et al. 2007, Wormser et al. 2006). Detection of IgM antibodies may be relevant in early infection but isolated IgM response in late stages of LB has no diagnostic implication (Stanek G. et al. 2011). Therefore, the detection of IgG antibodies to *B. burgdorferi* is mandatory in diagnosis of LB with clinical duration of more than 6–8 weeks (Dessau et al. 2018). LB incidence at population level is relatively low leading to low pre-test probability and high negative predictive value of negative test result (Dessau et al. 2018). As an exception, some immunocompromised patients may lack detectable antibody response (Harrer et al. 2007, Ryan and Thorn 2013, van Dop et al. 2013). Importantly, seronegative LB is extremely rare in immunocompetent individuals after symptom duration of several weeks (Lohr et al. 2018).

One challenge in serological diagnostics of LB is, that IgM and IgG antibodies to *B. burgdorferi* may persist for months to several years after successful antimicrobial treatment (Hammers-Berggren et al. 1993, Kalish et al. 2001b). Serology is of minor value in evaluation of treatment response and complicated in diagnosing reinfection. If reinfection is suspected, other diagnostic methods (e.g. cerebrospinal fluid [CSF] analysis, PCR, culture) should be utilized. In addition, an overall seroprevalence of Finnish population is 3.9%, which has to be taken into account in diagnostic decision making (van Beek et al. 2018).

The laboratory diagnostics leans on two-tier serologic testing (Figure 5). First a screening test with high analytical sensitivity is performed, and when positive, a confirmatory test of high specificity verifies the result (Lohr et al. 2018). Assays available for screening tests include enzyme-linked immunosorbent assays (ELISAs), enzyme-linked fluorescence assays (ELFAs), electrochemiluminescence immunoassay (ECLIA) and chemiluminescence immunoassays (CLIAs). Confirmatory tests consist of whole cell lysate immunoblots (Western blots), line immunoblots, or different variations of similar test formats using recombinant antigens (Kullberg et al. 2020, Lohr et al. 2018). In addition, a combination of two ELISAs is possible. In summary, it remains ambiguous which combinations of first-line and second-line tests are optimal (Ang et al. 2011).

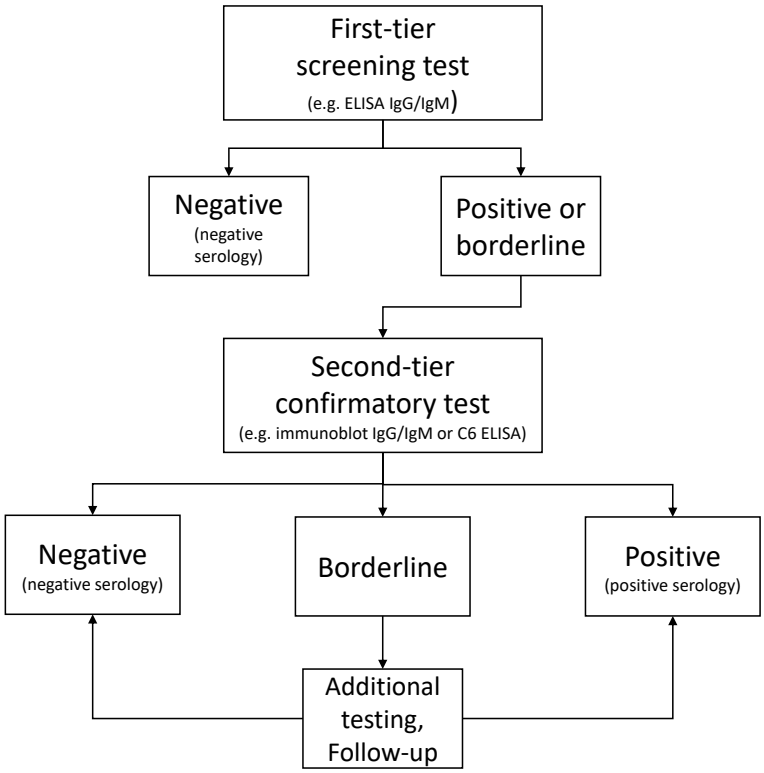

Figure 5. The diagnostic algorithm for two-tier serological testing, adapted from (Hunfeld and Kraiczy 2009, Lohr et al. 2018).

2.4.2 Cerebrospinal fluid analysis

CSF sampling is strongly recommended in suspicion of LNB. Diagnostic criteria for definite LNB are neurological symptoms suggestive of LNB without other obvious

reasons, CSF pleocytosis and intrathecal *B. burgdorferi* antibody production (Mygland et al. 2010). LNB is associated with lymphocytic pleocytosis, typically 10–1000 leucocytes/µl (Oschmann et al. 1998). Neutrophil–dominated pleocytosis should guide to consider an acute purulent meningitis. Other ordinary findings in LNB are elevated CSF protein level and oligoclonal IgG bands (Ogrinc et al. 2016, Oschmann et al. 1998). These findings, lymphocytic pleocytosis, CSF elevated protein level and oligoclonal IgG bands, may be present in many neuroinflammatory conditions such as herpes simplex encephalitis, other viral meningitis or multiple sclerosis (Benninger and Steiner 2017, Jarius et al. 2019).

CSF should be examined for intrathecal *B. burgdorferi* antibody production (antibody index, AI) in LNB suspicion by analysing paired serum and CSF specimens obtained on the same (or subsequent) days (Dessau et al. 2018). Determination of the AI is more specific than measuring antibodies in either CSF or serum alone (Hansen and Lebech 1991, Mygland et al. 2010, Stanek G. et al. 2011). Like antibodies in serum, also intrathecally produced antibodies may persist for several years after treated infection, which underlines the importance of including other inflammatory markers (e.g. CSF cell count and CXCL13 concentration) in interpretation of the results (Dessau et al. 2018). Also, induction of intrathecal antibody synthesis takes several weeks, which leads to diagnostic sensitivity of the AI of about 80% during the first weeks of LNB symptoms, but almost of 100% when symptoms have lasted for at least 8 weeks (Blanc et al. 2007, Hansen and Lebech 1991, Ljostad et al. 2007, Mygland et al. 2010, Wilske et al. 2007).

2.4.3 CXCL13 and neopterin

CXCL13, a member of the CXC chemokine family, attracts mainly B lymphocytes but also helper T cells to peripheral lymphoid organs and sites of infection (Lalor and Segal 2010, Moser and Ebert 2003). High concentrations of CXCL13 in the CSF of acute LNB patients were noticed in 2005 (Rupprecht et al. 2005). The role of CXCL13 in the pathogenesis of LNB is significant. Mononuclear cells release CXCL13 into the CSF after *B. burgdorferi* has penetrated through the blood-brain barrier, leading to B cell recruitment and lymphocytic pleocytosis typical of LNB (Cepok et al. 2003, Rupprecht et al. 2007, Rupprecht et al. 2009). CXCL13 concentration starts to rise in the early stage of LNB, even days to weeks before intrathecal antibody production begins (Rupprecht et al. 2006). Additionally, CXCL13 concentration is known to decline quickly after antimicrobial therapy, which may enable CXCL13 concentration determination as a tool for estimating the treatment efficacy (Bremell et al. 2013, Senel et al. 2010).

CXCL13 is not totally specific to LNB, since CXCL13 level may be elevated also in other neuroinflammatory conditions or CNS infections, such as neurosyphilis,

CNS lymphoma, cryptococcosis, trypanosomiasis, or human immunodeficiency virus (HIV) infection (Fischer et al. 2009, Rubenstein et al. 2013, Rupprecht et al. 2018, van Burgel et al. 2011). Neurosyphilis is a CNS infection affecting meninges, brain and spinal cord and caused by a sexually transmitting spirochete *Treponema pallidum* (Tuddenham and Ghanem 2018). Reports from several studies indicate, that CXCL13 could be useful also in diagnostics of neurosyphilis and follow-up of therapy response (Hu et al. 2016, Marra et al. 2010, Zeng et al. 2016). In a small study, including five patients with neurosyphilis, five patients with LNB and ten patients with multiple sclerosis, median CFS CXCL13 concentrations were 972, 8000 and 7.8 pg/ml, respectively (Dersch et al. 2015b). Like *B. burgdorferi* and *T. pallidum*, also HIV has neurotropic properties, causing a chronic, low-grade inflammatory reaction in the CNS (Chiodi et al. 1988). Both CSF and serum CXCL13 concentrations have been shown to be elevated in neurologically asymptomatic patients with HIV (Bremell et al. 2013, Widney et al. 2005). Even if CXCL13 levels have overlapped between asymptomatic untreated HIV (median CXCL13 concentration of 10 pg/ml, range 0–498) and LNB patients (median 500 pg/ml, range 34–11678), they have still been lower in HIV than in most of the acute LNB patients (Bremell et al. 2013).

An optimal CSF CXCL13 cut-off value has to be determined locally depending on analytic test used and target population. Previous studies have applied cut-off values from 61 pg/ml to 1229 pg/ml (Bremell et al. 2013, Schmidt et al. 2011, Senel et al. 2010, Tjernberg et al. 2011, van Burgel et al. 2011). A systematic review and meta-analysis including 18 studies with 618 acute LNB patients and 2326 individuals with other neurologic conditions reported that a pooled sensitivity for CSF CXCL13 was 89% and a pooled specificity was 96% using the cut-off value of 162 pg/ml (Rupprecht et al. 2018).

Neopterin is a proinflammatory factor of the type 1 T-helper cell -related cellular immune response and produced mainly by monocytes and activated macrophages (Hagberg et al. 2010, Viaccoz et al. 2015). Elevated CSF neopterin concentrations have been reported in several neuroinflammatory disorders (Di Stefano et al. 2020, Hagberg et al. 1993). Moderately increased levels have been reported in traumatic brain injuries and Parkinson disease (Lenzlinger et al. 2001, Widner et al. 2002). Neopterin has also shown to be elevated in LNB (Dotevall et al. 1990). As CSF neopterin is a nonspecific marker of CNS inflammation, it may not have marked impact in differential diagnostics of LNB.

2.4.4 PCR testing and culture

B. burgdorferi DNA can be detected by PCR from various body fluids or tissues, including skin biopsy specimen, synovial fluid, blood, and CSF (Aguero-Rosenfeld

et al. 2005, Babady et al. 2008). The density of spirochetes in various tissues is low, leading to low sensitivity, particularly in the case of blood and CSF samples (Aguero-Rosenfeld et al. 2005). The highest sensitivity is observed with skin biopsy samples from EM with median sensitivity of 69% in eleven different studies (Dessau et al. 2018). Sensitivity of PCR in synovial fluid is estimated to be over 75% (Moore et al. 2016, Nocton et al. 1994). The median sensitivity of PCR from CSF samples in 17 studies was 40%, and from serum or plasma samples in 5–6 studies 30%, which limits the clinical utility of these body fluids in PCR diagnostics (Dessau et al. 2018, van Dam 2011). Thus, negative PCR result does not exclude LB. False positive PCR results may originate from contamination, the reason why PCR result must always be interpreted together with signs, symptoms and serology (Molloy et al. 2001). Furthermore, *B. burgdorferi* DNA might persist for months in skin or synovial fluid after appropriate antimicrobial treatment without evidence of ongoing infection (Li et al. 2011).

Culture of slow-growing *B. burgdorferi* is challenging and not available in every clinical laboratory. Cultivation of *B. burgdorferi* from skin biopsy specimens obtained from skin manifestations of LB is in many cases successful, and occasionally direct detection by culture is successful also from synovial fluid or biopsies and blood (Hofmann et al. 2017). Spirochetes require culture in appropriate environment for 8 to 12 weeks before the culture can be considered negative, which is too slow for clinical purposes (Waddell et al. 2016). The sensitivity is improved, if verification of bacterial isolation is done with PCR instead of visual confirmation by dark-field or fluorescent microscopy (Liveris et al. 2011). In conclusion, sensitivity of culture is poor, and cultivation is recommended only for research purposes (Moore et al. 2016). Guidelines of LB diagnostics consider PCR and culture as supplementary diagnostic methods for special indications, such as diagnostic confirmation in patients with atypical cutaneous manifestations or suspected LA (Stanek G. et al. 2011).

2.5 Clinical case definitions for Lyme borreliosis

To overcome problems in LB diagnostics and also for surveillance and clinical management purposes, the European Union Concerted Action on Lyme Borreliosis (EUCALB) and CDC published separately case definitions for LB in the 1990s. Afterwards, case definitions have been updated (CDC 2017, Stanek G. et al. 2011). Case definitions consist of basic clinical features and serological test results either as a supporting or an essential proof of infection (Table 1). Laboratory methods have to be reliable, standardized and quality-controlled. (Stanek G. et al. 2011) These case definitions standardize reporting in epidemiological studies and guide in decision making in clinical practice.

Table 1. Clinical case definitions for Lyme borreliosis. Modified from (Stanek G. et al. 2011).

Manifestation	Clinical case definition	Essential laboratory evidence	Supporting clinical/laboratory evidence
Erythema migrans	Expanding red or bluish-red skin lesion for at least 5 cm in diameter with or without central clearing	Unnecessary	Detection of *B. burgdorferi* by PCR or culture from skin biopsy.
Borrelial lymphocytoma	Painless bluish-red nodule or plaque	Positive serology or seroconversion and histology in unclear cases	Detection of *B. burgdorferi* by PCR or culture from skin biopsy. Histology. Concomitant or recent EM.
Acrodermatitis chronica atrophicans	Long-term red or bluish-red lesions, which eventually become atrophic, usually on the extensor surfaces of extremities	High level of *B. burgdorferi*–specific IgG antibodies in serum.	Detection of *B. burgdorferi* by PCR or culture from skin biopsy. Histology.
Lyme neuroborreliosis	Meningoradiculitis, meningitis, rarely encephalitis, myelitis, or cerebral vasculitis	Pleocytosis and intrathecal *B. burgdorferi*–specific antibody synthesis. In early cases intrathecally produced antibodies may be absent.	Detection of *B. burgdorferi* by PCR or culture from CSF. *B. burgdorferi*–specific serum IgG antibodies. Concomitant or recent EM.
Lyme arthritis	Recurrent or persisting objective joint swelling in one or a few large joints without an alternative explanation	*B. burgdorferi*–specific IgG antibodies	Detection of *B. burgdorferi* by PCR or culture from synovial fluid or tissue.
Lyme carditis	Acute onset of atrioventricular (I–III) conduction disturbances, rhythm disturbances, myocarditis or pancarditis without an alternative explanation	*B. burgdorferi*–specific IgG antibodies	Detection of *B. burgdorferi* by PCR or culture from endomyocardial biopsy. Concomitant or recent EM or neurologic disorders.
Ocular manifestations	Conjunctivitis, uveitis, keratitis, episcleritis, papillitis	*B. burgdorferi*–specific IgG antibodies	Concomitant or recent LB manifestations. Detection of *B. burgdorferi* by PCR or culture from ocular fluid.

PCR = polymerase chain reaction; EM = erythema migrans; CSF = cerebrospinal fluid; LB= Lyme borreliosis.

2.6 Management of Lyme borreliosis

2.6.1 *In vitro* antimicrobial susceptibility and acquired resistance of *Borrelia burgdorferi*

There is no standardized methodology for *in vitro* antimicrobial susceptibility testing of *B. burgdorferi*. There are several studies concerning *in vitro* antimicrobial susceptibility of *B. burgdorferi*. These studies have significant differences in test conditions and the definitions for correct determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Boerner et al. 1995, Dever et al. 1992, Hunfeld and Brade 2006, Hunfeld et al. 2002). Due to these challenges, the reported MICs and MBCs for antimicrobial agents vary widely (Dever et al. 1992, Hunfeld et al. 2000a, Levin et al. 1993, Ruzic-Sabljic et al. 2005, Sicklinger et al. 2003, Veinovic et al. 2013). Also, the evaluation in MICs and/or MBCs has revealed slight differences in the *in vitro* susceptibilities of the various genospecies to penicillin, amoxicillin, aztreonam and fluoroquinolones (Boerner et al. 1995, Hunfeld et al. 2000a, b, Hunfeld et al. 2001, Kraiczy et al. 2001). *B. garinii* strains seem to be more sensitive to antimicrobial agents than isolates of *B. burgdorferi* s.s. and *B. afzelii* (Hunfeld et al. 2000a, b, Kraiczy et al. 2001, Sicklinger et al. 2003). Generally, *B. burgdorferi* has been highly susceptible to several antibiotics, e.g. most penicillins, tetracyclines, and many second- or third-generation cephalosporines in *in vitro* studies (Agger et al. 1992, Dever et al. 1992, Hunfeld et al. 2002, Johnson R. C. et al. 1987, Johnson S. E. et al. 1984, Mursic et al. 1987). Macrolide susceptibility fluctuates depending on the *B. burgdorferi* strain (Dever et al. 1993, Hunfeld et al. 2004, Terekhova et al. 2002), but rifampin and first-generation cephalosporines are ineffective (Agger et al. 1992, Johnson S. E. et al. 1984). Table 2 presents *in vitro* activity of selected antimicrobial agents against *B. burgdorferi.*

Treatment failures in LB patients, even if very exceptional, have been reported using almost every generally used antimicrobial agent (Hunfeld et al. 2005, Lomholt et al. 2000, Oksi J. et al. 1999, Preac-Mursic et al. 1989, Viljanen et al. 1992). In one study, the proportion of persistent *B. burgdorferi* infection after appropriate antimicrobial treatment in 1148 patients with culture-confirmed EM was 1.7% (Hunfeld et al. 2005). Nevertheless, there were no significant changes in MICs or MBCs in cultured isolates before and after antimicrobial treatment meaning that the isolates had not developed resistance to the used antimicrobial agents (Hunfeld et al. 2005). *B. burgdorferi* has developed resistance to some antimicrobial agents, such as moxifloxacin and aminoglycosides, after prolonged exposure of subinhibitory antibiotic concentrations *in vitro* by generating specific genetic mutations (Criswell et al. 2006, Galbraith et al. 2005). In summary, there is no evidence for acquired

resistance against antibiotics commonly used in LB treatment (Hunfeld and Brade 2006). Deficient absorption of a drug or poor treatment compliance are more likely explanations for treatment failure than antibiotic resistance.

Table 2. Antibiotic susceptibility of *B. burgdorferi*. Data are collected from different studies with different test conditions, so results are not standardized. Modified from (Ates et al. 2010, Hunfeld and Brade 2006, Kraiczy et al. 2001, Rodel et al. 2007, Sicklinger et al. 2003, Veinovic et al. 2013).

Antimicrobial agent	MIC	MBC
Penicillin G	0.03–8	0.05–50
Amoxicillin	0.03–2	<0.03–32
Piperacillin	<0.06–1	1.3–2.6
Cefuroxime	0.06–0.5	0.25–2
Cefotaxime	0.01–1	0.02–16
Ceftriaxone	<0.01–0.25	0.02–3.81
Aztreonam	2–64	64–256
Doxycycline	0.06–2	0.25–32
Minocycline	0.03–1	3–8
Tigecycline	<0.016	0.25–1
Tetracycline	0.01–20	0.8–4.1
Erythromycin	<0.007–1	0.05–2.17
Roxithromycin	0.015–0.125	0.125–1.8
Azithromycin	0.003–0.22	0.007–0.5
Ertapenem	0.031–0.125	0.5–4
Meropenem	0.015–0.5	0.5–32
Imipenem	0.062–0.5	16–32
Levofloxacin	0.5–8	4–16
Ciprofloxacin	0.25–8	4–16
Moxifloxacin	0.25–2	4–16
Norfloxacin	1–16	8–64
Tobramycin	8–64	>64
Vancomycin	0.25–2	2–32

2.6.2 Pharmacokinetics and pharmacodynamics of amoxicillin, doxycycline, ceftriaxone, cefuroxime axetil and azithromycin

Amoxicillin is a beta-lactam antibiotic with a broad antibacterial spectrum and a short half-life of about 1–2 hours in humans (Staniforth et al. 1983). Bioavailability of oral amoxicillin is 77% (Arancibia et al. 1980). Amoxicillin is rapidly eliminated

due to active tubular secretion. According to most distribution studies, amoxicillin reaches effective antibacterial concentrations in bone tissues, synovial fluid and sputum, but the penetration to CSF is sub-optimal even in patients with severely inflamed meninges (Bakken et al. 1986, Decazes et al. 1987, Lovering et al. 1990, Reed 1996, Weismeier et al. 1989). This important finding indicates that amoxicillin is inadequate in the treatment of LNB, though in other manifestations it is effective. Amoxicillin inhibits synthesis of bacterial cell wall and activates bacterial autolysis (Ruskoaho et al. 2014). The effect of amoxicillin in bacterial killing is time-dependent, which means, that the time of the drug level exceeding the MIC is the best parameter predicting the treatment efficacy (Sanchez Navarro 2005).With the dosages of amoxicillin 875 mg twice daily, 500 mg three times daily and 1000 mg three times daily, an unbound plasma concentration was at least 0.25 mg/l, 0.5 mg/l and 1 mg/l for at least 40% of time, respectively (de Velde et al. 2016). Amoxicillin administration three times daily is more favorable than twice a day. Clinical trials, animal models and *in vitro* studies have demonstrated, that maximum killing of bacteria is achieved at concentrations 3- to 4-fold higher than the MIC, and increasing the concentration above this does not lead to better eradication of the bacteria (Hyatt et al. 1995, Mouton and Vinks 1996, Turnidge 1998).

Doxycycline is a second-generation tetracycline with oral bioavailability of approximately 93%, a long half-live of 18–24h and high lipid solubility (Cunha et al. 1982, Klein and Cunha 1995). A lipophilicity of the agent increases its ability to penetrate through CNS lipid membranes (Nau et al. 2010). Doxycycline inhibits protein synthesis and is mainly bacteriostatic (Ruskoaho et al. 2014). Doxycycline has many applications besides LB: for instance brucellosis, plague, tularemia, rickettsioses and in certain cases of infections caused by *Streptococcus pneumoniae* or *Staphylococcus aureus* (Blanton 2019, Cunha et al. 2018, Franco et al. 2007, Ulu-Kilic and Doganay 2014, Yang 2018). Regardless of absence or presence of meningeal inflammation, CSF concentration of doxycycline is near 20% of that found in serum (Karlsson et al. 1996, Yim et al. 1985). Doxycycline dosing of 200 mg once a day and 100 mg twice a day has led to the median CSF concentration of 0.6 µg/ml, which is close to the MICs for *B. burgdorferi* (Dotevall and Hagberg 1989, Karlsson et al. 1996). With a dose of 200 mg doxycycline twice a day, the mean drug level in CSF was 1.1–1.3 µg/ml (Dotevall and Hagberg 1989, Yim et al. 1985). Since the doxycycline concentration in CSF may remain under MIC for *B. burgdorferi* with 100 mg twice a day administration, some authorities have recommended the use of 200 mg twice a day dosing in LNB (Dotevall and Hagberg 1989). Nevertheless, the outcome of LNB patients with daily dosing of 200 mg doxycycline have been satisfying, leading to adoption of this dosing to the clinical practice (Ljøstad et al. 2008, Mygland et al. 2010). In addition, the higher dosing

causes more side-effects with gastrointestinal irritation being the most prevalent (Klein and Cunha 1995).

Ceftriaxone is a third-generation cephalosporin that has a broad spectrum of *in vitro* activity to gram-positive and gram-negative bacteria, and also to a few anaerobic bacteria (Perry T. R. and Schentag 2001). Antibacterial function of ceftriaxone, like of other cephalosporines, is similar to the function of penicillins and it is bactericidal (Ruskoaho et al. 2014). Ceftriaxone is highly bound to plasma proteins, mainly albumin, and unbound compound is the active form (Perry T. R. and Schentag 2001). It has an elimination half-life from 6 to 9 hours, which enables once daily administration (Richards et al. 1984). Another benefit of ceftriaxone is excellent tissue penetration and ability to diffuse to CSF both via inflamed and uninflamed meninges (Dankner et al. 1988, Martin et al. 1984, Nau et al. 1993).

Cefuroxime axetil is an oral second-generation cephalosporin. It has antibacterial activity against several gram-positive and gram-negative bacteria by preventing bacterial growth (Perry C. M. and Brogden 1996). Cefuroxime axetil is well absorbed from the gastrointestinal tract and the bioavailability is 68% (Lang et al. 1990). An elimination half-life of 2.2–3 hours allows the administration of twice daily (Finn et al. 1987). It penetrates well to sinuses and tonsil tissues, bronchial mucosa, middle ear effusion and joint fluid (Scott et al. 2001). There are no data on ability of cefuroxime axetil to penetrate through blood-brain barrier. Approximately 50% of cefuroxime axetil is eliminated unchanged through kidneys in 12 hours, which has to be taken into account in renal insufficiency (Perry C. M. and Brogden 1996). In Finland, cefuroxime axetil is used only in exceptional cases.

Azithromycin, like other macrolides, inhibits bacterial protein synthesis. They also reduce biofilm formation and production of mucus (Amsden 2005, Kobayashi H. 1995, Tateda et al. 2001). Approximately 30% of azithromycin binds to plasma proteins, and the bioavailability with oral administration is 17–37% (Foulds et al. 1990, Luke and Foulds 1997). A plasma half-life is very long, approximately 50–70 hours, both with oral and intravenous formulations (Lode et al. 1996). Azithromycin has a high cellular accumulation with tissue concentrations up to 100-fold higher than in plasma (Foulds et al. 1990, Shepard and Falkner 1990). Azithromycin concentrations are higher in lungs, middle ear fluids, conjunctiva, inflammatory skin lesions, prostate and seminal fluids than in plasma (Parnham et al. 2014). In one study, the concentration in brain tissue has been shown to be high after single 500 mg oral dose, while simultaneous CSF concentration was undetectable (Jaruratanasirikul et al. 1996).

2.6.3 Treatment of various Lyme borreliosis manifestations

Oral amoxicillin, doxycycline and cefuroxime axetil have equivalent efficacy in the treatment of early LB without neurological manifestations (Dattwyler et al. 1990, Luger et al. 1995, Nadelman et al. 1992). In the USA, where *B. burgdorferi* s.s. is the prevalent genospecies, amoxicillin is a better agent than azithromycin in patients with EM (Luft et al. 1996). In randomized, double-blinded, double-dummy, multicenter study, 246 adults with EM received either amoxicillin 500 mg three times daily for 20 days or azithromycin 500 mg once daily for 7 days. Patients treated with amoxicillin were more likely to achieve completely resolution of disease at day 20 than patients with azithromycin (88% vs 76%, $p = 0.024$). A relapse was more common in patients with azithromycin compared to patients with amoxicillin (16% vs 4%, $p = 0.043$) (Luft et al. 1996). Because of treatment failures, azithromycin and other macrolides are not recommended as first-line therapy for EM (Wormser et al. 2000). Recommended duration of antibiotic course for EM is 14 days (Stanek G. et al. 2012, Wormser 2006), with the exception 7–10 days with azithromycin (Wormser 2006). A 10-day course with doxycycline is proven to be effective for EM in the USA (Kowalski et al. 2010, Wormser et al. 2003). In Europe, some authorities suggest 21-day antibiotic course for EM with symptoms of early dissemination, e.g flu-like symptoms and multiple EM (Hofmann et al. 2017). In the USA, doxycycline is often used as the first-line therapy for EM, because it is effective also against potential co-infections like anaplasmosis (Sanchez et al. 2016).

The European Federation of Neurological Societies (EFNS) composed guidelines on the diagnosis and management of European Lyme neuroborreliosis in 2009, and recently published guidelines from North America adheres to these previous recommendations fairly consistently (Lantos et al. 2021, Mygland et al. 2010). Therapy recommendations are based on several studies with different quality (Borg et al. 2005, Dattwyler et al. 1988, Dattwyler et al. 1990, Hassler et al. 1990, Karlsson et al. 1994, Kohlhepp et al. 1989, Ljøstad et al. 2008, Oksi J. et al. 2007, Pfister et al. 1989). High dose intravenous penicillin, ceftriaxone and cefotaxime have been shown to have similar efficacy in early LNB (Dattwyler et al. 1988, Pfister et al. 1989, Pfister et al. 1991). Studies comparing doxycycline to ceftriaxone in the treatment of LNB or possible neurological involvement of LB are shown in Table 3. Oral doxycycline was non-inferior to ceftriaxone in the randomized, double-blinded study (Ljøstad et al. 2008). The question, what is the optimal treatment for LNB with CNS manifestations (e.g. encephalitis, myelitis, vasculitis), and whether it is different from the treatment of LNB with only PNS manifestations, is unsolved (Mygland et al. 2010). Case studies and a small prospective, non-randomized study have shown, that LNB patients with CNS manifestations improve both with ceftriaxone and doxycycline (Borg et al. 2005, Bremell and Dotevall 2014, Charles et al. 2007, May and Jabbari 1990, Peter et al. 2006). However, both EFNS and IDSA

Table 3. Previous studies comparing doxycycline to ceftriaxone in the treatment of Lyme neuroborreliosis or Lyme borreliosis with possible neurological involvement.

First author	Study design	Study center	Inclusion criteria	Treatment	Outcome	Results
Borg et al. 2005	Prospective, multicenter, open-label, non-randomized	Ljubljana, Slovenia and Göteborg, Sweden	Clinical signs and symptoms compatible with LNB with mononuclear pleocytosis and: 1) isolation of *B. burgdorferi*, or 2) intrathecal antibody production, or 3) EM within 4 months prior to neurological involvement or seroconversion	Oral DOX 200mg x 2 for 10–14 days (Göteborg, n=36) or Intravenous CRO 2g x 1 for 10–14 days (Ljubljana, n=29)	Clinical improvement 6–8 weeks and 6 months after the treatment initiation. CSF mononuclear cell count 6–8 weeks after the treatment initiation.	After 6 months follow-up, 26 (72%) of the DOX- and 23 (79%) of the CRO-treated patients were completely recovered. No significant difference between the two antibiotic treatments in CSF pleocytosis after 6–8 weeks.
Ogrinc et al. 2006	Prospective, open-label, non-randomized	Ljubljana, Slovenia	Symptoms compatible with LB possibly indicating neurological involvement for at least 6 months without CSF pleocytosis and: 1) positive serum *B. burgdorferi* –specific IgG antibodies and/or 2) documented EM prior to the onset of symptoms and 3) no previous treatment for LB	Oral DOX 100mg x 2 for 28 days (n=23) or CRO 2g x 1 for 14 days and then oral DOX 100mg x 2 for 14 days (n=23)	Clinical improvement 6 and 12 months after treatment.	The differences between the two groups after treatment were not significant.
Ljøstad et al. 2008	Multicenter, non-inferiority, double-blind, randomized	Nine hospitals from Norway	Neurological symptoms suggestive of LNB without other obvious reasons and: 1) CSF pleocytosis or 2) Intrathecal *B. burgdorferi* antibody production or 3) *B. burgdorferi* antibodies in serum or 4) EM during past 4 months	Oral DOX 200mg x 1 for 14 days (n=59) or CRO 2g x 1 for 14 days (n=59)	Composite clinical score based on standard neurological interview and clinical examination at the inclusion, and at 13 days and at 4 months after the start of treatment.	Improvement in the two groups was similar without statistically significant difference.

LNB = Lyme neuroborreliosis; EM = erythema migrans; DOX = doxycycline; CRO = ceftriaxone; CSF = cerebrospinal fluid; LB = Lyme borreliosis

guidelines recommend intravenous therapy with ceftriaxone for patients with CNS involvement (Lantos et al. 2021, Mygland et al. 2010). Additionally, the optimal duration of LNB treatment is unclear because of the lack of randomized, controlled trials concerning the issue (Mygland et al. 2010). Usually, the treatment duration has ranged from 8 to 28 days in different studies with LNB patients (Borg et al. 2005, Dattwyler et al. 1988, Hassler et al. 1990, Karkkonen et al. 2001, Kohlhepp et al. 1989, Ogrinc et al. 2006, Pfister et al. 1989). In the Finnish study, 152 patients with disseminated LB, including 62 LNB patients, were randomized to receive oral amoxicillin or placebo for 100 days after three weeks therapy with ceftriaxone (Oksi J. et al. 2007). In that study, both groups improved similarly after one-year follow-up period. Because symptoms may persist several months, no antimicrobial re-treatments are recommended, even if a patient is symptomatic at the end of the first-line treatment. Treatment failures (specified as loss of recovery) and relapses are very rare. (Mygland et al. 2010)

Treatment with 30 days of doxycycline or amoxicillin or 2–4 weeks of ceftriaxone is reported to lead to the resolution of LA in 90% patients (Dattwyler et al. 1988, Steere et al. 1994). Guidelines and expert opinions suggest a 28–30-day oral treatment for LA, and for patients with unresponsive persistent or recurrent joint swelling re-treatment with 2–4-week course of intravenous ceftriaxone (Arvikar and Steere 2015, Jaulhac et al. 2019, Lantos et al. 2021, Wormser et al. 2006). The aimed duration of intravenous therapy is 14 days, but it can be extended to 28 days if inflammation continues. Because a rate of adverse events increases along with treatment duration, the patient has to be monitored closely and discontinue the treatment if necessary. If residual joint swelling is mild, also treatment with oral regimen for another 28–30 days may be sufficient. After intravenous treatment, and especially if synovial fluid *B. burgdorferi* PCR is negative, symptomatic treatment with non-steroidal anti-inflammatory agents, anti-rheumatic drugs (e.g. hydroxychloroquine) or intra-articular corticosteroid injections are the other options in patients with persistent joint symptoms. (Arvikar and Steere 2015, Lantos et al. 2021, Wormser et al. 2006)

In Table 4, there are listed treatment recommendations of different LB manifestations based on guidelines and review articles emphasizing European perspective (Hofmann et al. 2017, Jaulhac et al. 2019, Mygland et al. 2010, Yeung and Baranchuk 2019). Guidelines in Europe and the USA are rather concordant, although first-line antibiotic regimen and preferred treatment duration may differ slightly (Kullberg et al. 2020). In Finland, treatment durations of different manifestations have usually been slightly longer (HUS 2020). Our recommended treatment duration for EM has been 14–21 days and for other manifestations 21–28 days depending on the antibiotic used.

Table 4. Treatment of different manifestations of LB in Europe. Adapted from (Hofmann et al. 2017, Jaulhac et al. 2019, Mygland et al. 2010, Wormser et al. 2006, Yeung and Baranchuk 2019)

Manifestation	Drug	Dose (adult)	Duration	Comment
EM without symptoms of early dissemination	Amoxicillin	500 mg x 3 p.o.	14 days	
	Doxycycline	100 mg x 2 p.o.	10–14 days	
	Cefuroxime axetil	500 mg x 2 p.o.	14 days	Second-line treatment.
	Azithromycin	250 mg x 2 or 500 mg x 1 p.o.	5–10 days	If other options are contraindicated. Treatment failures described.
EM with symptoms of early dissemination or lymphocytoma	Doxycycline	100 mg x 2 p.o.	14 (–21) days	First-line treatment
	Amoxicillin	500 mg x 3 p.o.	14 (–21) days	Second-line treatment.
	Cefuroxime axetil	500 mg x 2 p.o.	14 (–21) days	Second-line treatment.
ACA without neurological symptoms	Doxycycline	100 mg x 2 p.o.	21–30 days	
	Amoxicillin	500 mg x 3 p.o.	21–30 days	
	Cefuroxime axetil	500 mg x 2 p.o.	21–30 days	Second-line treatment.
Early LNB (symptom onset < 6 months) with symptoms of PNS and meninges	Doxycycline	100 mg x 2 p.o. or 200 mg x 1 p.o.	14 days	
	Ceftriaxone	2 g x 1 i.v.	14 days	
	Penicillin	5 million units x 4 i.v.	14 days	
	Cefotaxime	2 g x 3 i.v	14 days	
Early LNB (symptom onset < 6 months) with CNS symptoms	Ceftriaxone	2 g x 1 i.v.	14 days	
Late LNB (symptom onset > 6 months) with peripheral neuropathy or ACA	Doxycycline	100 mg x 2 p.o. or 200 mg x 1 p.o.	21 days	
	Ceftriaxone	2 g x 1 i.v.	21 days	
Late LNB (symptom onset > 6 months) with CNS manifestations	Ceftriaxone	2 g x 1 i.v.	21 days	

Manifestation	Drug	Dose (adult)	Duration	Comment
Lyme arthritis	Doxycycline	100 mg x 2 p.o.	28–30 days	First-line treatment
	Amoxicillin	500–1000 mg x 3 p.o.	28–30 days	If a patient does not have neurological symptoms.
	Ceftriaxone	2 g x 1 i.v.	28 days	Second-line treatment. Recommended for a 14–28-day course if moderate to severe joint swelling continues after 28–30 days oral treatment.
Lyme carditis with high-degree AV block, PR interval ≥ 300ms or other cardiac symptoms	Ceftriaxone	2 g x 1 i.v.	10–14 (up to 28) days with ceftriaxone, total 14–21 (up to 28) days	Switch to oral antibiotics based on clinical response (until high-degree AV block has resolved and the PR interval becomes < 300 ms.)
Lyme carditis, mild presentation	Doxycycline	100 mg x 2 p.o.	14–21 days	
	Amoxicillin	500 mg x 3 p.o.	14–21 days	
	Cefuroxime axetil	500 mg x 2 p.o.	14–21 days	
Ophthalmologic surface lesions (except keratitis): conjunctivitis, episcleritis	Doxycycline	100 mg x 2 p.o.	14 days	
	Ceftriaxone	2 g x 1 i.v.	14 days	
Other ophthalmologic manifestations: keratitis, intraocular, orbital, neuro-ophthalmologic presentations	Ceftriaxone	2 g x 1 i.v.	21 days	Intraocular penetration of doxycycline is poor.
	Doxycycline	100 mg x 2 p.o.	21 days	Second-line treatment

EM = erythema migrans; ACA = acrodermatitis chronica atrophicans; LNB = Lyme neuroborreliosis; PNS = peripheral nervous system; CNS = central nervous system; i.v. = intravenous; p.o. = oral; AV = atrioventricular

2.6.4 Typical adverse events of antibiotics used in the treatment of Lyme borreliosis

According to a meta-analysis, a reported adverse event to antibiotics (including doxycycline, minocycline, amoxicillin, penicillin V, azithromycin, cefuroxime axetil, ceftriaxone) occurred in 31% of patients with early cutaneous LB (Torbahn et al. 2018). Adverse events were commonly classified as mild and sometimes as moderate. Irrespective of dosage or treatment duration, penicillin V had the lowest risk of any adverse event (Torbahn et al. 2018). In western countries, circa 10% of adults assume that they suffer from penicillin allergy (Phillips et al. 2019, Zhou et al. 2016). Actual penicillin allergy is rare, and 95% of inpatients and 98% of outpatients with reported penicillin allergy tolerated penicillin during exposure (Macy and Ngor 2013, Sacco et al. 2017, Tucker et al. 2017). However, penicillin allergy is a common reason to avoid amoxicillin in the treatment of LB. In addition to hypersensitivity problems, gastrointestinal side effects are common. Diarrhea is reported with frequency of over 5% and abnormal hepatic function in 1% of patients (Lode et al. 2004). Also, as a rare adverse event in prolonged use, the use of amoxicillin has shown increased risk for seizures (Meropol et al. 2008).

Doxycycline has traditionally been contraindicated in pregnancy, though cumulative evidence indicates, that the use of doxycycline also during the first trimester is not associated with any increased risk (e.g. teratogenic effects, permanent inhibitory bone growth effects or dental staining) to the fetus (Briggs 2002, Cross et al. 2016, Czeizel and Rockenbauer 1997, Horne and Kundsin 1980). However, doxycycline is not recommended during pregnancy in Finland. Breastfeeding is not a contraindication to use of doxycycline. Estimated average doxycycline intake of an infant via milk after normal dosing to mother was about 0.8% of the maternal weight-adjusted dosage (Drugline 1992). Tetracyclines are associated with liver problems especially in high doses, but the use of doxycycline has not shown increased risk of hepatotoxicity even during pregnancy (Cross et al. 2016, Heaton et al. 2007, Smith and Leyden 2005). The incidence of photosensitivity reactions ranging from mild sunburn-like reactions to widespread photodermatitis associated with doxycycline is reported to vary from 1.9% to as high as 16% (Lim and Murphy 2003, Veluscek et al. 2018). Gastrointestinal side effects are the most common adverse events for doxycycline, e.g. nausea, vomiting, diarrhea, gastritis and heartburn, ranging from 0.5% to 52% in different studies (Casado 1975, Smith and Leyden 2005, Wormser et al. 2003).

The most common adverse events associated with ceftriaxone treatment are diarrhea, nausea, abdominal pain, rash, pruritus, candidiasis, thrombophlebitis, neutropenia and elevated liver enzymes (Francioli et al. 1995, Lamb et al. 2002, Sexton et al. 1998). Diarrhea is the most often reported side effect in 1% to 15% of adult patients. Reversible biliary pseudolithiasis or gallbladder sludge are unique

adverse events when using ceftriaxone with the true incidence of less than 0.1% (Roche 2000). Nevertheless, sonographic abnormalities indicative of biliary pseudolithiasis are detected in 21–25% of ceftriaxone-treated adults (Heim-Duthoy et al. 1990, Pigrau et al. 1989). Ceftriaxone, like actually all antibiotics, alters for an example the intestinal and vaginal normal microbiota. This causes overgrowth and sometimes treatment-related infections of ceftriaxone-resistant organisms, like *Pseudomonas aeruginosa*, *Enterococcus faecalis, Clostridioides difficile* and Candida spp. (Fekety 1990) Neutropenia has been developed to 6% of patients treated with ceftriaxone (median duration 41 days), and median onset of neutropenia has been 22 (15–28) days (Veve et al. 2019). In general, adverse events caused by ceftriaxone are usually mild or moderate in severity and withdraw spontaneously (Lamb et al. 2002). Ceftriaxone is not contraindicated in pregnancy or lactation (Pharmaca Fennica 2008).

Adverse events occur in up to 6% of patients treated with cefuroxime axetil for 5–10 days (Scott et al. 2001). Most adverse events were gastrointestinal, and also hypersensitivity reactions and elevated live enzymes have been noticed (Perry C. M. and Brogden 1996). The proportion of patients with diarrhea has ranged from 2 to 10% of patients. Tolerability profile of cefuroxime axetil has been usually similar to other commonly used oral antibacterial drugs, including cephalosporins, fluoroquinolones, and phenoxymethylpenicillin. (Scott et al. 2001) Surprisingly, cefuroxime axetil has been better tolerated than the combination of amoxicillin with clavulanic acid (Gooch et al. 1996, Henry et al. 1999, Pessey et al. 1999).

Like with all antibiotics used for LB treatment, also azithromycin causes gastrointestinal side effects. According to a previous study, diarrhea was reported by almost 20% of patients (Uzun et al. 2014, Wong et al. 2012). A well-known adverse event of azithromycin therapy is increased risk for QT-interval prolongation, proarrhythmic effects and, to a minor extent, cardiovascular death, especially in patients with a cardiovascular co-morbidity (Ray et al. 2012, Russo et al. 2006).

When antibiotics are used without an acceptable indication, the patient is exposed to adverse events without benefit. The misuse of antimicrobials is also associated with antimicrobial resistance, which is a serious, worldwide threat to public health (Huttner et al. 2013). World Health Organization has raised antimicrobial resistance as one of the top 10 global public health threats facing the mankind. Misuse and overuse of antimicrobials are the main reasons for the evolution of drug-resistant microbes. (WHO 2020) Multidrug-resistant microbes spread in population, and also to animals and environment. Antimicrobial stewardship (AMS) programs in different countries are established to perform interventions and create guidance for various control measures against antimicrobial resistance (Hakanen et al. 2017).

2.6.5 Corticosteroid treatment and Lyme neuroborreliosis–associated facial nerve palsy

Facial nerve palsy occurs approximately in 10% of untreated LB cases (Clark et al. 1985, Halperin 2015, Kalish et al. 2001a). LB–associated facial nerve palsy presents as a promptly developing loss of motor function across all facial areas of the affected side, followed by progressive recovery after a few weeks to months (Clark et al. 1985). Majority of patients obtain normal facial nerve function over time, but circa 20% maintain residual deficits (Clark et al. 1985, Kalish et al. 2001a). The reason for this dysfunction, either hypo- or hyperactivity of facial muscles, is thought to be caused by aberrant neuronal regeneration (Sumner 1990, Wetzig 1957).

A short-term course of oral corticosteroids is recommended for all patients with recent (less than 72 hours) Bell's palsy, also referred to as idiopathic facial nerve palsy (Baugh et al. 2013, de Almeida et al. 2014, Gronseth et al. 2012). On the contrary, patients with LB–associated facial nerve palsy do not seem to benefit from corticosteroids, and the effect may be even harmful (Clark et al. 1985, Jowett et al. 2017). There are three retrospective cohort studies concerning corticosteroid use in LB–associated facial nerve palsy. In a previous study, no difference was demonstrated in facial outcomes among patients, who received corticosteroids alone or with antibiotics (n=44), antibiotics alone (n=37), or no treatment at all (n=26), but the follow-up time was described inadequately (Clark et al. 1985). The other small retrospective study included 31 LB patients with facial nerve palsy, of which only 15 received antibiotics. The sample size of nine patients with simultaneous antibiotic and corticosteroid treatment compared to six patients with antibiotics alone did not demonstrate statistically significant difference in outcomes. In contrast, 14 patients without antibiotic treatment developed LA (Kalish et al. 2001a). Jowett et al. demonstrated that patients had significantly worse outcome in parallel corticosteroid and antibiotic therapy compared to antibiotic monotherapy among 51 patients with LB–associated facial nerve palsy (Jowett et al. 2017). In a prospective cohort study with 11 patients with LB–associated facial nerve palsy and corticosteroid treatment, 6 patients had residual dysfunction after 10–20 months follow-up (Wormser et al. 2018). As a conclusion, there is a need for a well-designed, prospective clinical trial to confirm benefits and harms of corticosteroid use to outcome of LB–associated facial nerve palsy.

2.6.6 Treatment of post-treatment Lyme disease syndrome

PTLDS may be problematic to treat. Randomized, controlled, clinical trials of antimicrobial therapy in PTLDS patients have not proven any benefit of 70–90 days antibiotic treatment compared to placebo (Fallon et al. 2008, Kaplan et al. 2003, Klempner et al. 2001). In a double-blinded trial, 281 patients with persistent

symptoms attributed to LB were randomized to receive either doxycycline, clarithromycin-hydroxychloroquine or placebo for 12 weeks (Berende et al. 2016). According to the results of this study, the SF-36 physical-component summary score (Hays 1998) did not differ significantly among the groups, and patients did not have any additional benefit from long-term antibiotics. In addition to antibiotics, nor hyperbaric oxygen, psychotropic medication or immunomodulators have been helpful (Nemeth et al. 2016, Price et al. 2008, Steere and Arvikar 2015).

Re-evaluation and differential diagnostics are the recommended approach, when a patient has persistent symptoms after appropriate initial therapy. Fibromyalgia, depression, or some other clinical entity may cause similar symptoms than PTLDS. Also, permanent tissue damage is possible after LNB. Unfortunately, there is no specific treatment for PTLDS. Painkillers or medication for coexistent mood disorder may be beneficial. (Nemeth et al. 2016) Nonpharmacological interventions with counselling, regular aerobic exercise and cognitive behavioral therapy are the recommended approaches (Dinerman and Steere 1992, Price et al. 2008).

2.6.7 Prevention

Primary prevention strategy of LB constitutes of personal protection measures against tick vectors, including avoidance of tick-infested environments, protective clothing and repellents. Repellents are recommended only occasionally, because tolerability data with long-term exposure do not exist. Repellents are not suitable for pregnant women or children under 2 years old. (Figoni et al. 2019) Most repellents cannot kill the ticks, but they prevent ticks from detecting hosts (Pages et al. 2014). Secondary prevention contains checking for ticks on skin daily after exposure, and prompt tick removal as soon as possible, or at least within 24 hours of an attachment. It is not recommended to perform any tests of the removed tick with the purpose of finding infectious agents, but instead, the skin area around the tick bite should be observed regularly for four weeks after tick removal to detect possible EM (Figoni et al. 2019).

In the USA, a single 200 mg dose of doxycycline has been shown to be effective for preventing LB in certain circumstances (Nadelman et al. 2001). In this randomized, double-blind, placebo-controlled trial, 1/235 (0.4%) of patients with doxycycline prophylaxis and 8/247 (3.2%) of patients with placebo developed EM after tick removal ($p<0.04$). The treatment efficacy was 87%. Randomized, placebo-controlled trials with amoxicillin prophylaxis after tick bite have failed to detect any protection against LB (Agre and Schwartz 1993, Costello et al. 1989, Shapiro et al. 1992, Warshafsky et al. 1996). Novel clinical practice guidelines for the prevention, diagnosis and treatment of Lyme disease by IDSA, American Academy of Neurology (AAN), and American College of Rheumatology (ACR) recommend

prophylactic single dose of 200 mg oral doxycycline to adults and children within 72 hours of removal of a high-risk tick bite (Lantos et al. 2021). A tick bite is considered to be high-risk if following three criteria are met: 1) the tick is identified to be a genus *Ixodes* tick, 2) it occurred in a highly endemic area, and 3) the tick was attached for more than 36 hours. Highly endemic area is defined as an area with average incidence of at least 10 confirmed cases per 100000 for previous three years (CDC 2019). In patients with contraindication to doxycycline, no alternate antibiotic prophylaxis is recommended, since there is no evidence supporting short courses of other antibiotics, and longer courses may cause more harm than benefit to patients. (Wormser et al. 2006) Also in a recent European study, a single dose of 200 mg doxycycline within 72 hours after *I. ricinus* tick bite reduced a relative risk of LB with 67%, and the number-needed-to-treat was 51 (Harms et al. 2021). For the present, European guidelines do not recommend antimicrobial prophylaxis after a tick bite.

Human vaccine to prevent LB has been under development for more than 20 years, but still commercial vaccine for humans does not exist (Kullberg et al. 2020). The first LB vaccine, LYMErix®, was launched for human use in the USA in 1998, but withdrawn four years later because of poor sales (Hitt 2002). It was well-tolerated with overall efficacy of 76% in definite LB cases (Thanassi and Schoen 2000). In addition to low demand, there were concerns among the general population of the suspected immunogenic arthritis due to the vaccine (Embers and Narasimhan 2013). The blamed antigen of LYMErix® was recombinant Outer surface protein A (OspA) of *B. burgdorferi* s.s. Since then, second generation OspA vaccines without the concerned epitope of LYMErix® are in the development. These new multivalent OspA vaccines are targeted against several *B. burgdorferi* serotypes and are currently in phase I or II trials (Kullberg et al. 2020). Additionally, different kinds of spirochetal recombinant protein based vaccines are under development, and vaccination using tick proteins are under investigation (Nassal et al. 2008).

2.7 Consequences of Lyme borreliosis diagnoses to public health

2.7.1 Overdiagnostics and overtreatment

Many individuals with medically unexplained prolonged symptoms like myalgia, arthralgia, headache, fatigue, and memory or concentration problems, receive the diagnosis of LB without the fulfillment of appropriate diagnostic criteria (Haddad et al. 2019). These patients are often subjected to repeated laboratory, imaging and physiological examinations in addition to recurrent antimicrobial treatments. The phenomenon of misdiagnosis and mistreatment of LB has drawn attention during the

recent two decades (Haddad et al. 2019, Reid et al. 1998, Steere et al. 1993). In some countries, dedicated multidisciplinary centers for patients with LB suspicion have been established to provide better care for the patients (Cottle et al. 2012, Coumou et al. 2015, Jacquet et al. 2019).

In Table 5, the results from different studies concerning the accuracy of LB diagnosis are described. Patients in these studies have received a wide spectrum of different diagnoses after the exclusion of LB. Symptoms of presumed LB have most commonly been due to psychological disorders, or musculoskeletal, rheumatologic or neurological diseases (Haddad et al. 2019, Reid et al. 1998). Also, autoimmune or other infectious diseases, or functional disorders are usual (Jacquet et al. 2019). Sometimes, diagnosis and treatment of malignancies have been delayed when symptoms have falsely been attributed to be caused by chronic LB (Nelson C. et al. 2015a).

One reason for overdiagnostics of LB are certain features of the laboratory methods used for LB diagnostics and the complexity of interpretation of the results. Some commercially available serological assays demonstrate insufficient specificity especially in IgM testing (Ang et al. 2015). In a study conducted in the USA, the proportion of false positive IgM immunoblot test results at Air Force healthcare facilities was 53%, and 81% of these patients with false positive test results received antibiotics (Webber et al. 2019). A significant number of tests are requested against recommendations of guidelines, for instance from the patients with atypical symptoms and a low pre-test probability of LB (Coumou et al. 2014). In a Dutch study, only 9% of performed serological tests were supported by guideline's recommendations (Coumou et al. 2014). The experts suggest, that AMS should begin by avoiding inappropriate laboratory testing among individuals without tick exposure or LB–specific symptoms (Webber et al. 2019, Wormser et al. 2006). Although it is possible to improve the performance of laboratory assays, it is easier and more beneficial to reduce unnecessary testing (Webber et al. 2019).

In addition to the use of appropriate tests for incorrect situations, some laboratories in Europe offers unreliable and clinically irrelevant tests for diagnostics of LB including antigen detection, lymphocyte transformation test, and level of CD57+/CD3- lymphocyte subpopulation (Mygland et al. 2010). Pharmacies offer rapid diagnostic tests (RDT) for patient self-use, which require a drop of patient blood. In Finland, the performance of two commercially available RDTs was compared to laboratory-based serological assays. The result of RDT sensitivities being only 26–32% and specificities 85–88% does not encourage the use of RDTs for LB diagnostics (Smit et al. 2015). False positive results from tests that should not been used for diagnosis of LB may lead to difficulties in interaction between patient and physician and mistrust to conventional healthcare.

Use of intravenous and oral antibiotics for PTLDS is noticed to cause adverse events and increased patient morbidity (Goodlet and Fairman 2018). In a commercially insured sample of patients with PTLDS, intravenous, and to a lesser extent oral, antibiotic therapy was related to higher incidence rates of all-cause hospitalisation and visits to emergency department (Goodlet and Fairman 2018). In the same study, electrolyte imbalance and infection were more prevalent in patients treated with intravenous and oral antibiotics compared to patients without antibiotic treatment. Additionally, case reports have described serious bacterial infections, such as paraspinal abscess, *C. difficile*–colitis, septic shock, and osteomyelitis, during intravenous antibiotics in patients with the diagnosis of "chronic LB". At worst, these complications have been fatal (Holzbauer et al. 2010, Marzec et al. 2017).

The use of unconventional therapies, also called complementary and alternative medicine (CAM) therapies, is scarcely investigated. In the USA, a qualitative, phenomenological study was conducted by interviewing 12 adults, who presented themselves as chronic Lyme disease patients (Ali et al. 2014). Participants reported decline in health condition and notable limitations in their daily activities. They had experienced a dismissive and patronizing attitude from conventional healthcare providers, therefore the use of consultations and therapies provided by CAM practitioners was usual. Reported CAM therapies were diverse, including vitamins and minerals, colloidal silver, herbal products and other natural products, acupuncture, reiki, Rife machine and even thujone-containing extracts of *Artemisia absinthium*. Some of the CAM therapies may be even dangerous, for example colloidal silver can cause neurological deficits, and thujone is associated with seizures, rhabdomyolysis, and acute renal failure (Burkhard et al. 1999, Hadrup and Lam 2014, Weisbord et al. 1997).

2.7.2 Financial issues

LB causes loss of everyday health and productivity and leads to travel and informal care costs in addition to direct healthcare costs including diagnostics and treatment (Mattingly and Shere-Wolfe 2020). These factors have been studied both in Europe and the USA, but some of the non-health sector costs, such as social services, consumption, education, environment or housing, have not been evaluated in any study. Comparing the results between different countries or continents is difficult because different healthcare systems and methods to specify costs. (Mattingly and Shere-Wolfe 2020)

In the Netherlands, tick bites and EM led to 495 and 132 general practitioner consultations per 100000 inhabitants in 2010 (Hofhuis et al. 2015). In Finland, 3628 and 1420 patients with LB diagnosis visited public primary healthcare centers and

hospitals, respectively, in 2014 (Feuth et al. 2020). LB testing is noticed to be frequent and costly. In Germany, with the population of 82 million, it was estimated that 1,4 million individuals are tested for suspected LB annually, and the annual costs for diagnostics and treatment was 51 million (Muller I. et al. 2012). In the USA, seven commercial laboratories in four states in the endemic areas (Connecticut, Maryland, Minnesota, and New York) performed 3.4 million LB tests at an estimated cost of 492 million USD in 2008 (Hinckley et al. 2014).

Besides diagnostics, treatment leads to costs as well. In the USA, patients with LB were associated with circa 3000 USD higher healthcare costs and 87% more outpatient visits over a 1-year period as compared to individuals without LB (Adrion et al. 2015). In the same study, patients with PTLDS-related diagnosis were associated with circa 3800 € higher total healthcare costs per patient and 66% more outpatient visits over 1-year period compared to LB patients without PTLDS-related diagnoses. According to the authors, healthcare costs due to LB were clearly higher than could be expected for an infection, which is rather easy to be treated. Similar results are revealed also in Europe, for example in Sweden, where the total costs of healthcare and social benefits were estimated to be 3300 € and 2000 € per patient with LNB, respectively, in 2000–2005 (Henningsson et al. 2010). In the Netherlands, tick bites and LB led to societal costs of 19 million € in 2010, of which healthcare costs, patient costs, and costs of production loss constituted 48%, 4%, and 48%, respectively (van den Wijngaard et al. 2017). In the same study, the mean annual cost, including both direct and indirect costs per patient was estimated to be circa 5700 € in 2014. Hospitalization because of LB is estimated to cost 2800 € for a German adult in 2008–2011 (Lohr et al. 2015).

Table 5. Studies on patients referred to consultation because of suspected Lyme borreliosis (Cottle et al. 2012, Coumou et al. 2015, Haddad et al. 2019, Jacquet et al. 2019, Kobayashi T. et al. 2019, Reid et al. 1998).

	Reid et al.	**Cottle et al.**	**Coumou et al.**	**Kobayashi et al.**	**Jacquet et al.**	**Haddad et al.**
Study design	Prospective, observational study	Retrospective, descriptive casenotes review	Retrospective case series	Retrospective, observational cohort study	Retrospective, observational cohort study	Retrospective, descriptive cohort study
Study Center	The Yale University Lyme Disease Clinic, Connecticut, USA	The Tropical and Infectious Disease Unit at the Royal Liverpool University Hospital, UK	Amsterdam Multidisciplinary Lyme borreliosis Center (tertiary care center), Netherlands	Johns Hopkins University School of Medicine, Maryland, USA	The teaching hospital of Nancy, France	Infectious Disease Department, university hospital in Paris, France
Number of included patients	209	115	200	1261	468	301
Timeframe	4/1994–5/1995	1/2006–12/2010	1/2011–4/2013	1/2000–12/2013	11/2016–10/2017	1/2014–12/2017
Antibiotic treatment before referral	80%	40%	52%	84%	85%	50%
LB diagnosis (definite or possible, active, or previous)	40%	23%	23%	28%	15%	13%
Alternative diagnosis achieved	47%	44%	22%	-	49%	81%
No identified diagnosis for the symptoms	13%	33%	39%	-	26%	7%

3 Aims

1. To study the role of CXCL13 as a diagnostic tool in LNB
2. To specify the manifestations of LNB
3. To compare doxycycline to ceftriaxone in the treatment of LNB
4. To evaluate the common differential diagnostic pitfalls in patients with suspicion of LB
5. To study the utilization of healthcare services and the number of antibiotic treatment days in patients with suspicion of LB

4 Materials and Methods

4.1 Randomized, controlled, open-label Lyme neuroborreliosis treatment trial, and evaluation of CXCL13 as a biomarker of Lyme neuroborreliosis (I and II)

4.1.1 Study design, participants and randomization

The study was conducted in two centers in Southern Finland, in Turku University Hospital and Helsinki University Hospital. Study design was open-label, controlled and randomized. Patients were recruited from Infectious Diseases Outpatient Clinic and Inpatient ward, Neurocenter and Emergency Department of Turku University Hospital and from Infectious Diseases Outpatient Clinic and Inpatient wards of Helsinki University Hospital. Eligible patients were adults with neurological symptoms suggestive of LNB without other obvious reason. The aim was to recruit 150 patients. Inclusion and exclusion criteria are shown in Tables 6–7.

Eligible patients were informed about the trial. After a signed informed consent was obtained, patients were randomly assigned in a 1:1 ratio to receive either doxycycline or ceftriaxone. No stratification factors were used. Patients with ceftriaxone were considered as a control group.

Table 6. Inclusion criteria for possible and definite LNB. Eligible patients were adults with neurological symptoms suggestive of LNB without other obvious reason.

Inclusion criteria for possible LNB (criteria 1 or 2 fulfilled)	
1.	Erythema migrans during the previous three months
2.	*B. burgdorferi*–specific antibodies in serum

Inclusion criteria for definite LNB (criteria 1 or 2 fulfilled)	
1.	Intrathecal production of *B. burgdorferi*–specific antibodies and cerebrospinal fluid pleocytosis (≥ 5 leukocytes / µl)
2.	Detection of *B. burgdorferi* DNA in cerebrospinal fluid

Table 7. Exclusion criteria.

Exclusion criteria	
1.	Any antibiotic treatment within two weeks before recruitment
2.	Allergy for tetracyclines or cephalosporins
3.	Age under 18
4.	Pregnancy and breastfeeding
5.	Women planning to become pregnant within the following two months
6.	Handicapped persons
7.	Prisoners

4.1.2 Procedures

The patients were evaluated by an infectious disease specialist or a neurologist at the beginning of the treatment. Blood sample was taken at first visit and analysed regarding to *B. burgdorferi* serology, blood cell count, C-reactive protein (CRP), liver function tests (alanine aminotransferase, alkaline phosphatase), and TBE serology. CSF specimen was drawn before antibiotic treatment, and following parameters were analysed from CSF: leukocyte count, levels of protein, glucose, and lactate, and CXCL13 concentration, intrathecal production of *B. burgdorferi*–specific antibodies and *B. burgdorferi* PCR. When necessary, additional laboratory tests and imaging procedures were performed. The patients defined their subjective condition by a visual analogue scale (VAS) on a scale of 0–10 (0 = normal, 10 = worst) before antibiotic treatment.

Antibiotic treatment was initiated without delay after randomization. Doxycycline was administered 100 mg twice a day orally, while the dose of intravenous ceftriaxone was 2 g once a day. Length of doxycycline treatment was 28 days and ceftriaxone 21 days. Doxycycline was self-administered by the participants and ceftriaxone was usually administered as outpatient parenteral therapy.

All patients were contacted after three weeks. Patients with more than 50 leukocytes/µl in the first CSF specimen were called for a visit and new CSF and serum specimens were collected. If the CSF cell count had not decreased by at least 80% from the initial value, the antibiotic treatment was prolonged. Doxycycline course was extended to 56 days, and ceftriaxone to 28 days. The patients without need for additional CSF collection at 3 weeks were contacted by phone to evaluate the condition of the patient and potential side effects of the therapy.

If the patient developed a new objective symptom (such as a new facial nerve palsy) during the antibiotic treatment, the patients in the doxycycline group were treated with ceftriaxone for 21 days simultaneously with doxycycline, and the patients in the ceftriaxone group were treated concurrently with doxycycline for 28 days (meaning doxycycline in total 28 days and ceftriaxone in total 21 days). This procedure is called a treatment modification.

Patients were called for a follow-up visits 4 months and 12 months after randomization. At every visit, a new blood sample for *B. burgdorferi* serology was collected and the patient defined his/her subjective condition with VAS on a scale 0 to 10, and possible sequelae were recorded.

4.1.3 Outcome measures

The primary outcomes were VAS score at 12 months and the change in VAS score defined as the difference between the VAS score at the beginning of the treatment and at 12 months. The secondary outcomes included CXCL13 concentration, leukocyte count, and protein, lactate and glucose level in the CSF specimens collected after 3 weeks of treatment.

4.1.4 Cerebrospinal fluid samples of control patients

CSF samples of control patients for evaluation of CXCL13 and neopterin as biomarkers of LNB (I) were identified retrospectively from the laboratory information-management system of the diagnostic laboratory of University of Turku. All CSF samples were collected from patients with suspected neurologic disease as a part of routine diagnostics, and the diagnoses were obtained from patient records. CSF samples from 328 patients were utilized for the study including 10 patients with TBE, 19 patients with VZV, 14 patients with HSV, 3 patients with HHV6, 8 patients with enterovirus, 34 patients with multiple sclerosis, 1 patient with neurosyphilis and 239 non-LNB patients. The non-LNB group consisted of patients with samples that were sent to laboratory for *B. burgdorferi* antibody analysis, and that were found to be *B. burgdorferi* antibody negative without other identified diagnosis.

4.2 Retrospective, observational, population-based Lyme borreliosis -study (III and IV)

4.2.1 Patients

Helsinki University Hospital provided special healthcare for 1.58 million residents in the District of Helsinki and Uusimaa in 2013. Local guidelines recommend to refer all adult patients with suspected disseminated LB to the Department of Infectious Diseases at Helsinki University Hospital.

We included all adult (≥ 16 years) patients referred to Helsinki University Hospital due to presumed LB between 1.1.2013, and 31.12.2013. We evaluated patients both whose referral led to an appointment with an infectious diseases specialist and whose referral was returned with a written consultation reply. No

exclusion criteria were used. Additionally, we searched patient database of Helsinki University Hospital for ICD-10 code A69.2 (Lyme borreliosis) to find patients treated at another clinics (for example, rheumatology and neurology).

4.2.2 Data collection

Medical records of included patients were reviewed from 1.1.2012 to 31.12.2017. Medical records were reviewed from databases of Helsinki University Hospital, public and private healthcare centers, occupational health service of a patient, and the Finnish Student Health Service in southern Finland. The information collected included age, gender, comorbidities, history of tick bites and EM, symptoms and signs, symptom duration, laboratory and imaging results, physiological and neurophysiological examinations, number of phone calls and visit to any physician due to symptoms leading to suspicion of LB, number and duration of antibiotic treatments, and novel diagnoses after the referral.

4.2.3 Categorization of patients according to certainty of Lyme borreliosis

After all collected data were evaluated, the patients were categorized into four groups according to the certainty of LB. The criteria used for categorization were developed for this study (Table 8). In the case of some patients, a previous condition or a novel diagnosis may have explained some symptoms among the patients with definite, probable, or possible LB. Previous conditions were comorbidities that were diagnosed before the referral, but the patient or the treating physician might have speculated on the role of LB as an explanatory condition for the overlapping symptoms of the previous condition and asked for the opinion of an infectious disease specialist. The groups of different LB certainty were compared concerning contacts to healthcare and antibiotic treatments.

Table 8. Categorization of the patients into groups according to certainty of LB.

Criteria for definite LB (criteria 1, 2, or 3 fulfilled)	
1.	Symptoms suggestive of LNB[a] without other obvious reason together with intrathecal production of *B. burgdorferi*–specific antibodies and CSF pleocytosis (≥ 5 leukocytes/µl).
2.	Symptoms suggestive of LB[a] without other obvious reason and seroconversion[b] of *B. burgdorferi*
3.	Positive *B. burgdorferi* PCR of CSF, synovial fluid, or skin biopsy sample
Criteria for probable LB (criteria 1 and 2 or 3 fulfilled)	
1.	Symptoms suggestive of LB[a] without other obvious reasons and improvement after antimicrobial therapy[c]
2.	Markedly positive[d] *B. burgdorferi* antibody levels in serum
3.	Typical EM during the previous three months
Criteria for possible LB (criteria 1 or 2 fulfilled)	
1.	Symptoms suggestive of LB[a] without other obvious reasons and *B. burgdorferi*–specific IgG antibodies in serum[e]
2.	In the absence of *B. burgdorferi*–specific antibodies, the duration of symptoms less than two months, specificity of symptoms for LB, and response to antimicrobial treatment[c]
Criteria for unlikely LB (criteria 1, 2, or 3 fulfilled)	
1.	Atypical symptoms of LB and failure to respond to antimicrobial treatment[c]
2.	Absence of *B. burgdorferi* IgG antibodies in the serum or CSF with symptom duration of more than two months
3.	Other obvious reasons for symptoms

LB = Lyme borreliosis; LNB = Lyme neuroborreliosis; PCR = polymerase chain reaction; CSF = cerebrospinal fluid; EM = erythema migrans

[a]A symptom was considered as suggestive of LB or LNB, if it is mentioned in selected review articles or guidelines (Halperin 2003, Mygland et al. 2010, Stanek G. and Strle 2003, Steere et al. 2016). In addition, disappearing of subjective symptoms associated with acute dissemination were considered suggestive of LB, unless they continued despite antimicrobial therapy (Nadelman et al. 1996).

[b]Increase in IgG antibodies between concurrently analysed paired serum samples: IgG CLIAs ≥ 30 units and ≥ 50% (DiaSorin) together with an increase in Borrelia afzelii + VlsE IgG ELISA (Sekisui Virotech).

[c]Reported by the patient.

[d]Presented in section 4.3.1 Serological methods and *B. burgdorferi* PCR. Intermediate and high antibody levels were regarded as markedly positive.

[e]Sum of numeric values from two EIA tests (Sekisui Virotech and DiaSorin) was ≥ 25.

4.3 Laboratory analyses

4.3.1 Serological methods and *B. burgdorferi* PCR

B. burgdorferi serology was performed using the two-tier approach both in Turku and Helsinki. In Turku, serum and CSF samples were screened for *B. burgdorferi*–specific antibodies by an in-house whole cell *B. burgdorferi* ELISA (Viljanen and Punnonen 1989). As the second-tier test, a C6-peptide based assay (C6 Lyme ELISA; Immunetics, Boston, Massachusetts, USA) was used. RecomLine Borrelia

or RecomBead Borrelia (Mikrogen GmbH, Neuried, Germany) assays were performed as additional tests when the results of the screening test and C6 ELISA were inconclusive. Intrathecal *B. burgdorferi* antibody production was determined using IDEIA Lyme Neuroborreliosis kit (Oxoid, Basingstoke, UK).

In Helsinki, serum samples and CSF samples were screened for *B. burgdorferi*–specific antibodies by a Borrelia afzelii + VlsE IgG and Borrelia afzelii IgM ELISA tests (Sekisui Virotech, Rüsselsheim, Germany). As the second-tier test, IgG and IgM CLIAs (Liaison® Borrelia IgG and IgM DiaSorin, Saluggia, Italy) were used. Borrelia Europe Plus TpN17 Line IgG and Borrelia Europe Line IgM line immunoassays were performed as additional tests when requested by a treating physician. CSF samples were analysed for *B. burgdorferi*–specific antibodies and intrathecal antibody production was determined using Borrelia afzelii + VlsE IgG Europe ELISA Testkit and Borrelia afzelii IgM ELISA Testkit (Sekisui Virotech, Rüsselsheim, Germany).

In Turku, *B. burgdorferi* PCR was performed using the method described by Ivacic et al. (Ivacic et al. 2007). In Helsinki, *B. burgdorferi* PCR was performed using the method accredited by the Helsinki University Hospital Laboratory.

In the retrospective, observational, population-based study, the sum of numeric values from the two EIA tests for IgG (Sekisui Virotech and DiaSorin) was used for antibody level categorization. The sum < 25 was considered negative, 25–59 low concentration, 60–179 intermediate concentration and ≥ 180 together with a positive immunoblot for IgG high concentration. Antibody level categorization for high required additionally positive immunoblot for IgG. The sum of the IgM concentration more than 25 without IgG antibodies was categorized as low total antibody concentration. Intermediate and high antibody levels were regarded as markedly positive in the patient classification according to the certainty of LB. Seroconversion was defined as increase in IgG antibodies (IgG CLIAs ≥ 30 units and ≥ 50% measured by Diasorin together with an increase in Borrelia afzelii + VlsE IgG ELISA measured by Sekisui Virotech) between simultaneously analyses paired serum specimens.

4.3.2 CXCL13 and neopterin

The CSF samples were stored at −20°C and defrosted at room temperature before analysing. The analyses were performed according to the instructions of the manufacturer. CXCL13 levels were measured by using a human CXCL13 kit (Quantikine; R&D Systems, Minneapolis, MN, USA). Samples with concentrations over the standard curve of the assay (>500 pg/ml) were further diluted 10 to 100 times with the calibration diluent buffer of the kit, and reanalysed. CSF neopterin concentrations were analysed by using Neopterin ELISA kit (IBL International GmbH, Hamburg, Germany) according to the instructions of the manufacturer.

4.4 Statistical analyses

Sample size calculations were performed for a change in the VAS score over 12 months using equivalence design. Calculations were based on a test of the supposed mean difference 0 between doxycycline and ceftriaxone groups. Standard deviation was assumed to be 2.0. Pre-specified equivalence margins of -1 to 1 units were used. An α-level of 0.05 and a power of 80% were used in calculations. Based on these calculations, 70 patients per group were needed, and to take into account possible drop-outs, it was planned that a total of 150 study patients should be recruited in the study. The number of drop-outs was calculated every six months, and the number of patients needed to recruit was increased to reach the necessary amount of participants.

Categorical variables were characterised using counts and percentages. In the case of continuous variables, means and standard deviations (SD) for normally distributed variables and medians and interquartile range (IQR) for non-normally distributed variables were used. Normality of the residuals of the models were estimated visually with histograms and by using Kolmogorov-Smirnov-test.

Differences between the study groups in categorical variables were evaluated using Pearson's chi squared or Fisher's exact test (if $n<7$ in any group). Differences between the groups in the normally distributed continuous variables were studied using One-Way ANOVA and non-normally distributed variables were evaluated first by using Kruskall Wallis test and further with Mann-Whitney U-test. Bonferroni correction was used in multiple analyses. The difference between the groups was considered significant if $p < 0.05$.

The equivalence of change in the VAS score over 12 months follow-up between the doxycycline and ceftriaxone groups was calculated with the 95% confidence interval (95% CI) for the difference between the groups. The groups were considered equivalent, if the 95% CI of difference was within predefined equivalence margins of -1 to 1.

Sample size calculations were performed using SAS system for Windows, Version 9.4 (SAS Institute Inc, Cary, NC, USA) and statistical analyses were carried out using IBM SPSS Statistics software, version 25.

4.5 Ethics

Written informed consent was obtained from all study participants of the randomized, controlled LNB treatment trial. Ethical approval was given by the National Committee on Medical Research Ethics. The trial was registered with ClinicalTrials.gov (NCT01635530) and EudraCT (2012-000313-37).

Retrospective, observational, population-based LB-study was approved by the research board of the Inflammation Center at the Helsinki University Hospital. No

ethical approval was obtained due to retrospective nature of this study. The usage of the patient records of the municipal health centres, occupational healthcare centers, The Finnish Student Health Service and private healthcare clinics was approved by the Finnish Institute for Health and Welfare (Dnro THL/1174/5.05.00/2016). The Social Insurance Institution provided information on all antimicrobial purchases from pharmacies of the patients included in this study.

5 Results

5.1 Randomized, controlled Lyme neuroborreliosis treatment trial (I and II)

5.1.1 Baseline characteristics of the study population

Roughly 300 adults with a suspicion of LNB were screened for eligibility and 210 of them were randomly assigned to receive either doxycycline (n=104) or ceftriaxone (n=106) between 14.9.2012 and 28.12.2017 (Figure 6). Most of them were males (n=135, 61%), and mean age was 59 years (SD 15). Among all randomized patients, 99 (47%) had definite LNB, 88 (42%) had possible LNB, and 23 (11%) patients did not have LNB. The baseline characteristics are presented in Table 9.

Only 22 patients from all recruited participants reported a labour or a hobby which expose to tick contacts: 4 patients worked with forestry and 2 with farming, 7 patients were into sailing, 3 gardening, 2 fishing, 2 mushroom/berry picking and 2 golf. All participants lived in LB endemic area.

Altogether, 83 (39.5%) patients recalled a tick bite during the previous year, and 47 of them had had several tick bites. EM was noticed by 62 (29.5%) patients during the past year, and eight of them recalled multiple EM lesions. The mean time from EM to the beginning of the symptoms was 2.7 weeks (SD 5.4 weeks). Fourteen patients had received treatment for EM before recruitment. In the group of definite LNB, two patients had been prescribed a three weeks course of amoxicillin for EM, 11 weeks and 22 weeks before recruitment to the study. Both patients suffered from neurological symptoms in addition to EM before amoxicillin was prescribed. One patient with definite LNB had been prescribed a cephalexin course for seven days because of suspected erysipelas. In the group of possible LNB, six patients had been previously prescribed 2–3 weeks course of amoxicillin for EM and one patient had received 7 days course of cephalexin. In the group of unlikely LB, four patients had been prescribed 2–3 weeks course of amoxicillin.

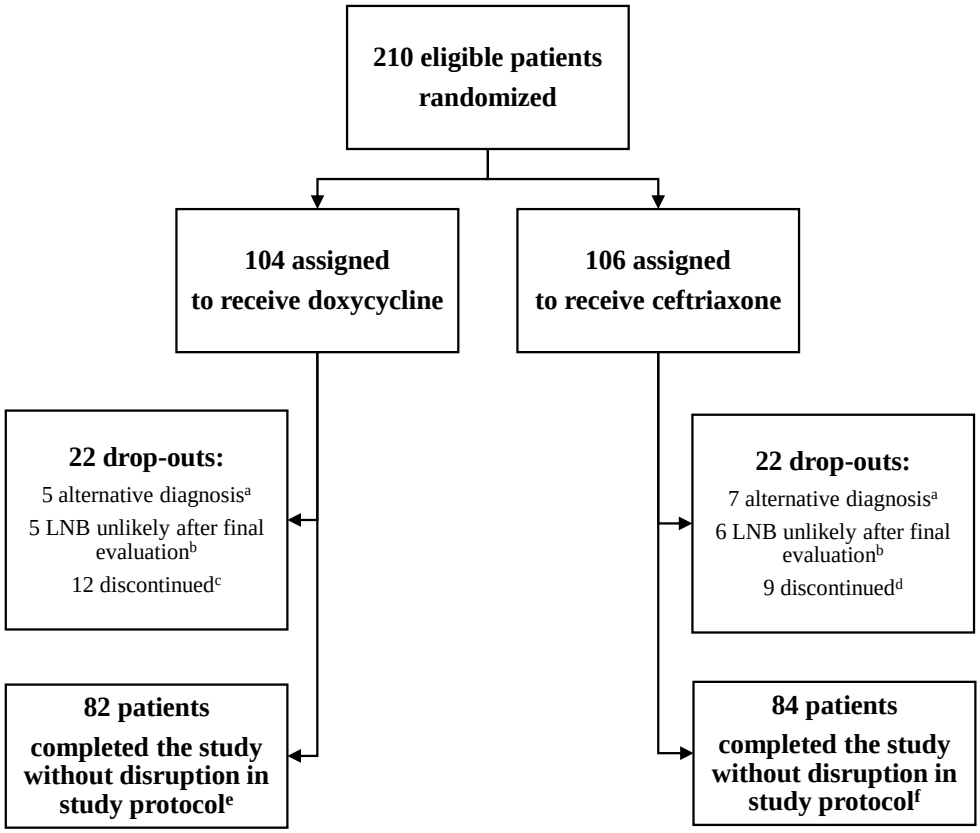

Figure 6. The flow chart of the study. [a]Patients were diagnosed with mononucleosis, cytomegaloviremia, viral meningitis, two cases of TBE, two cases of multiple sclerosis, polymyalgia rheumatica, chronic headache, mechanical cervical radiculopathy, osteoarthritis, and seronegative rheumatoid arthritis instead of LNB. [b]Patients whose symptoms evaluated with laboratory findings did not suggest LNB, but no other alternative condition was diagnosed either. [c]Reasons for discontinuing: 4 lost to follow-up or refused, 1 nausea, 1 eczema, 1 with severe other diseases after follow-up period, 1 with antibiotic treatment during two weeks before recruitment (exclusion criteria), 1 with simultaneous antibiotic treatment for diverticulitis during the LNB treatment, 3 with simultaneous ceftriaxone treatment (treatment modification). [d]Reasons for discontinuing: 2 lost to follow-up or refused, 4 allergic reactions, 1 difficulty in intravenous administration, 2 with simultaneous doxycycline treatment (treatment modification). [e]46 patients with definite LNB and 36 patients with possible LNB. [f]44 patients with definite LNB and 40 patients with possible LNB.

Table 9. Baseline demographics of all randomized patients (n=210).

	Doxycycline (n=104)	Ceftriaxone (n=106)
Male gender, n (%)	64 (62%)	71 (67%)
Age in years, mean (SD)	54 (15)	58 (15)
Number of comorbidities, median (IQR)	1 (0–3)	1 (0–3)
Cardiovascular disease[a], n (%)	27 (26%)	43 (41%)
Diabetes, n (%)	8 (8%)	10 (9%)
Lung disease[b], n (%)	15 (14%)	10 (9%)
Psychiatric illness[c], n (n%)	7 (7%)	4 (4%)
Neurologic illness[d], n (%)	14 (13%)	3 (3%)
Musculoskeletal problems, n (%)	10 (10%)	18 (17%)
No comorbidities, n (%)	45 (43%)	35 (33%)
Immunosuppression[e], n (%)	5 (5%)	3 (3%)
Constant tick exposure or several tick bites in previous years, n (%)	29 (28%)	28 (26%)
Tick bites during the previous year, n (%)	42 (40%)	41 (39%)
Previous LB, n (%)	14 (13%)	15 (14%)
EM during previous year, n (%)	26 (25%)	36 (34%)
Diameter of EM in cm, mean (SD)	14 (8)	12 (7)
Duration of symptoms in weeks, median (IQR)	4 (3–12)	5 (3–11)
Duration of symptoms for more than 6 months, n (%)	12 (12%)	10 (9%)
Improvement of symptoms before recruitment, n (%)	28 (27%)	31 (29%)
Certainty of LNB, n (%)		
Definite LNB	51 (49%)	48 (45%)
Possible LNB	43 (41%)	45 (42%)
LNB unlikely after final evaluation	10 (10%)	13 (12%)

LB = Lyme borreliosis; EM = erythema migrans; LNB = Lyme neuroborreliosis
[a]hypertension, coronary artery disease, cardiomyopathy, atrial fibrillation
[b]chronic obstructive pulmonary disease, asthma, bronchiectasis, pulmonary asbestosis
[c]depression, bipolar disorder, panic disorder, attention deficit hyperactivity disorder, obsessive compulsive disorder
[d]epilepsy, migraine, Alzheimer`s disease, mixed dementia, previous polio, previous stroke
[e]alcoholism (n=3), sulfasalazine (n=1), malignancy (n=1), hypogammaglobulinemia (n=1), methotrexate (n=1), mercaptopurine (n=1)

When the information of the appearance of the EM lesion was available, EM lesions with central clearing were more common (n=16/21, 76%) than homogenic lesions (n=5/21, 24%). Information of EM location was available in 61 patients. Most often EM was located on trunk (n=27, 44%). EM was in lower limb in 26 (43%) patients

and in upper limb in 6 (10%) patients. Two patients (3%) reported EM in multiple locations.

The median duration of symptoms before initiation of antibiotic treatment, which can also be considered as a treatment delay, was 4 (IQR 3–8), 8 (IQR 3–20), and 5 (IQR 2–23) weeks in the definite, possible, and unlikely LNB patients. The symptoms had partly relieved before initiation of treatment in 33 (33%), 22 (25%) and 4 (17%) patients in definite, possible, and unlikely LNB patients. The median duration of symptoms in patients, whose symptoms had partly relieved, was 6 (IQR 4–10), 8 (IQR 5–11) and 7 (IQR 4–27) weeks in the groups of definite, possible, and unlikely LNB. All patients with positive CSF AI had also *B. burgdorferi* antibodies in serum, except two patients of over 70 years old. Of these patients, the other seronegative patient with 10-year symptom duration had otherwise normal CSF findings except positive IgG AI. The other seronegative patient had pleocytosis, high CXCL13 concentration and only IgM antibodies in CSF after 7 weeks of symptom onset.

5.1.2 Manifestations of definite and possible Lyme neuroborreliosis

The most typical signs or symptoms were radiculitis and facial nerve palsy in definite LNB patients and headache and facial nerve palsy in possible LNB patients (Table 10). There was statistically significant difference in occurrence of facial nerve palsy ($p=0.001$), radiculitis ($p < 0.001$) Garin-Bujadoux-Bannwarth syndrome ($p < 0.001$), vertigo ($p=0.040$) and nonspecific neck-shoulder pain ($p=0.018$) between patients with definite and possible LNB, but otherwise occurrence of symptoms did not differ statistically significantly between patients. No patients with carditis, cerebral vasculitis or ACA were found in this study.

5.1.3 Primary outcome measures

Mean VAS score before treatment was 5.0 (SD 2.4) in patients assigned to receive doxycycline (n=100) and 5.1 (SD 2.2) in patients assigned to receive ceftriaxone (n=104). In the case of six patients, the VAS score at the beginning was not documented. After a follow-up of 12 months, the median VAS score was 0 (IQR 0–1.9) in patients with doxycycline (n=85) and 1 (IQR 0–2.1) in patients with ceftriaxone (n=94) among all patients attended at last follow-up visit. No statistically significant difference in VAS score after the follow-up of 12 months was noticed among these patient groups (n=0.160).

Table 10. Signs and symptoms of randomized participants with definite or possible LNB (n=187).

	Definite LNB (n=99)	Possible LNB (n=88)
Facial nerve palsy, n (%)	57 (58%)	29 (33%)
Radiculitis, n (%)	65 (66%)	24 (27%)
Garin-Bujadoux-Bannwarth syndrome[a], n (%)	34 (34%)	4 (5%)
Peripheral nerve palsy or neuritis[b], n (%)	5 (5%)	6 (7%)
Abducens or recurrens nerve palsy, n (%)	1 (1%)	2 (2%)
Diplopia[c], n (%)	6 (6%)	4 (5%)
Guillain-Barre syndrome, n (%)	1 (1%)	0
Hypacusis, n (%)	3 (3%)	7 (8%)
Altered sense of taste, n (%)	5 (5%)	4 (5%)
Polyneuropathy, n (%)	1 (1%)	3 (3%)
Peripheral muscle weakness[d], n (%)	12 (12%)	7 (8%)
Cognition difficulty, n (%)	5 (5%)	6 (7%)
Memory difficulty, n (%)	4 (4%)	9 (10%)
Convulsions, n (%)	1 (1%)	0
Vertigo, n (%)	14 (14%)	23 (26%)
Weight loss > 3 kg[e], n (%)	5 (5%)	3 (3%)
Lymphocytoma, n (%)	2 (2%)	1 (1%)
Mono- or polyarthritis, n (%)	1 (1%)	1 (1%)
Iritis, n (%)	0	1 (1%)
Headache, n (%)	48 (48%)	42 (48%)
Nonspecific neck-shoulder pain, n (%)	41 (41%)	22 (25%)
Neck stiffness, n (%)	11 (11%)	9 (10%)
Myalgia, n (%)	29 (29%)	24 (27%)
Arthralgia, n (%)	23 (23%)	18 (20%)
Fatigue, n (%)	38 (38%)	25 (28%)
Fever > 38°C, n (%)	14 (14%)	9 (10%)

[a]A triad of lymphocytic meningitis, radiculitis and peripheral nerve palsy is reported as Garin-Bujadoux-Bannwarth syndrome. The patients with Garin-Bujadoux-Bannwarth syndrome are additionally included in the lines of facial nerve palsy (n=37) and radiculitis (n=38) or abducens nerve palsy (n=1).

[b]Cranial nerve palsies and other peripheral nerve palsies are reported separately.

[c]Symptom was temporary, and clinical examination did not reveal a cranial nerve palsy.

[d]Nonspecific weakness without findings in electroneuromyography.

[e]Median time range 7 (IQR 3.6–11.0) weeks.

The mean change of the VAS score was -3.9 (SD 2.5) in doxycycline group and -3.8 (SD 2.5) in ceftriaxone group, without statistically significant difference (p=0.839). The mean difference between the groups was 0.07 and with a 95% confidence interval (CI) difference from -0.67 to 0.82, the patients in doxycycline and ceftriaxone groups improved equally. To eliminate the sources of any bias, all randomized patients, who defined their VAS score after 12 months, are included in this analysis, regardless of their possible baseline conditions or alternative diagnoses or change of their antibiotic treatment after randomization (intention-to-treat - analysis). The VAS scores of patients with ceftriaxone and doxycycline at different time points are presented in Figure 7. The changes of the VAS scores in different subgroups (definite LNB with doxycycline, definite LNB with ceftriaxone, possible LNB with doxycycline and possible LNB with ceftriaxone) are presented in Table 11.

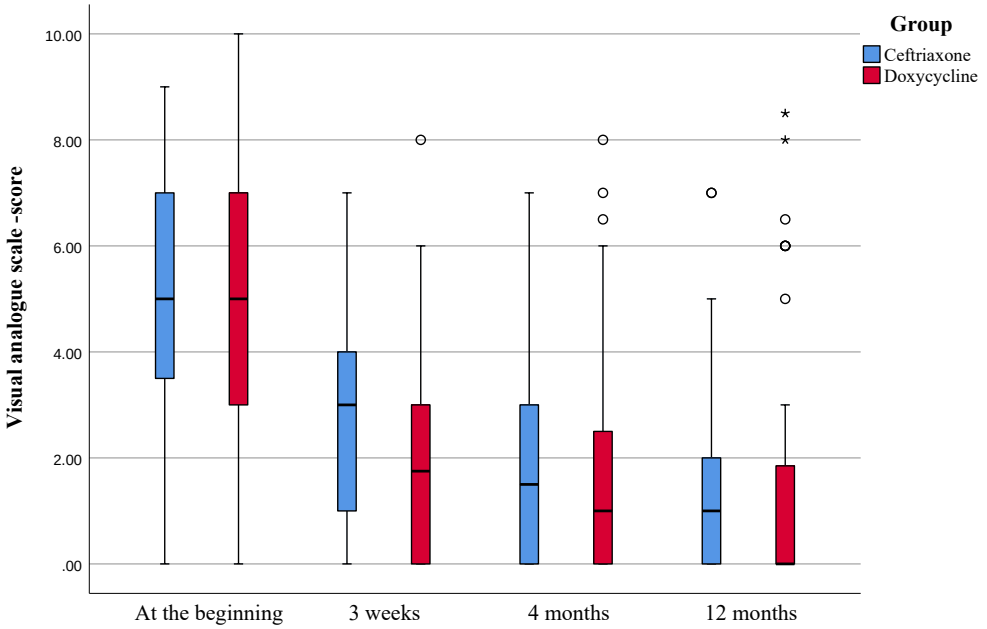

Figure 7. The VAS scores of patients with ceftriaxone and doxycycline at the beginning, after 3 weeks, 4 months and 12 months. The box stands for interquartile range (IQR), and the black line across the box designates the median. The whiskers represent the minimum and maximum values less than 1.5 times the IQR. Outliers (o) are values between 1.5 and 3 times the IQR and extremes (*) are values more than 3 times the IQR. The number of patients who defined VAS score was 204 in the beginning (ceftriaxone n=104, doxycycline n=100), 69 after 3 weeks (ceftriaxone n=38, doxycycline n=31), 179 after 4 months (ceftriaxone n=93, doxycycline n=86), and 179 after 12 months (ceftriaxone n=94, doxycycline n=85).

Table 11. VAS scores at the initiation of the treatment, at 4 months, and at 12 months after the treatment, and the mean change of the VAS score presented in different subgroups (definite LNB with doxycycline, definite LNB with ceftriaxone, possible LNB with doxycycline and possible LNB with ceftriaxone).

	Definite LNB with doxycycline (n=51)	**Definite LNB with ceftriaxone (n=48)**	**Possible LNB with doxycycline (n=43)**	**Possible LNB with ceftriaxone (n=45)**	**p value**
VAS at the beginning, median (SD)	5.0 (2.4) n=51	5.4 (2.3) n=48	5.0 (2.5) n=41	4.8 (2.2) n=45	
VAS after 4 months, median (IQR)	0.5 (0–2.0) n=47	2.0 (0–2.6) n=48	1.0 (0–3.0) n=36	1.4 (0–3.0) n=39	0.424*
VAS after 12 months, median (IQR)	0 (0–1.2) n=48	0.8 (0–2.4) n=48	0.5 (0–2.5) n=37	1.0 (0–2.0) n=41	0.490*
The change of the VAS score, mean (SD)	-4.2 (2.6) n=48	-4.3 (2.4) n=48	-3.4 (2.5) n=37	-3.5 (2.4) n=41	0.281**

LNB = Lyme neuroborreliosis; VAS = Visual analogue scale

*Difference between the groups in the VAS score at 4 months and 12 months was evaluated using Kruskall Wallis test and **the change of the VAS score with One-way ANOVA.

Residual complaints of the 166 patients without any protocol violation were collected after 12 months follow-up. In doxycycline group, 35/82 (43%) had residual complaints, while 36/84 (43%) patients with ceftriaxone reported sequelae. No statistically significant difference was noticed (p=0.982). 22 (27%) patients treated with doxycycline and 22 (26%) with ceftriaxone had only subjective complaints. Objective sequelae were radiculopathy (n=6), facial nerve palsy (n=5), polyneuropathy (n=1), and neuropathy (n=1) in doxycycline group and radiculopathy (n=9), facial palsy (n=3), polyneuropathy (n=1), and objective cognition and memory difficulties (n=1) in ceftriaxone group.

5.1.4 Performance of CXCL13, other secondary outcomes, and neopterin

Lumbar puncture was performed in the case of 206/210 (98%) patients before treatment. Two patients refused, one patient had contraindication (thrombocytopenia) and in one case procedure was unsuccessful despite several attempts. Initial CSF findings in treatment groups are presented in Table 12 and in different groups of LNB certainty in Table 13.

Table 12. Initial CSF findings in all randomized patients.

	Doxycycline (n=102)	**Ceftriaxone (n=104)**
CSF leukocyte count / µl, median (IQR)	28 (2–91)	16 (1–81)
CSF protein level, g/l, median (IQR)	602 (421–1092)	532 (386–914)
CSF glucose level, mmol/l, mean (SD)	3.7 (1.2)	3.5 (0.7)
CSF lactate level, mmol/l, median (IQR)	1.9 (1.5–2.2)	1.8 (1.6–2.2)
CSF CXCL13 concentration, pg/ml, median (IQR)	104 (<7.8–2895)	192 (<7.8–3384)
Positive *B. burgdorferi* PCR, n (%)	12/92 (13%)	12/97 (12%)
CSF IgG-Oc, abnormal finding, n (%)	38/87 (44%)	40/87 (46%)

CSF = cerebrospinal fluid; IgG-Oc = oligoclonal bands of immunoglobulin G

Table 13. Initial CSF findings presented in groups of different LNB certainty.

	Definite LNB n=99	**Possible LNB n=84**	**Unlikely LNB n=23**
CSF leukocyte count / µl, median (IQR)	85 (39–204)	2 (0,3–14)	1 (0–2)
CSF protein level, g/l, median (IQR)	1005 (654–1497)	431 (335–552) n=81*	412 (324–508)
CSF glucose level, mmol/l, median (IQR)	3.3 (2.9–3.6) n=90*	3.5 (3.2–3.8) n=72*	3.6 (3.4–3.9) n=18*
CSF lactate level, mmol/l, median (IQR)	2.2 (1.8–2.6) n=77*	1.7 (1.5–1.9) n=72*	1.7 (1.4–1.9) n=18*
CSF CXCL13 concentration, pg/ml, median (IQR)	3120 (498–12200) n=91*	<7.8 (<7.8–81) n=81*	<7.8 (<7.8–<7.8) n=21*
CSF IgG-Oc, abnormal finding, n (%)	63 (78%) n=79*	13 (17%) n=76*	2 (11%) n=19*

LNB = Lyme neuroborreliosis; CSF = cerebrospinal fluid; IgG-Oc = oligoclonal bands of immunoglobulin G
*Number of specimens analysed, if less than presented in the top row.

CXCL13 concentration was analysed from the initial CSF specimens of 91 definite, 81 possible and 21 unlikely LNB patients (Figure 8). CSF CXCL13 concentration ranged from < 7.8 to 675000 pg/ml in definite LNB patients and was elevated in 86/91 (95%) patients. CXCL13 concentration was above the previously identified optimal cut-off value of 162 pg/ml (Rupprecht et al. 2018) in 80/91 definite LNB patients leading to sensitivity of 88% in this study. Table 14 presents clinical characteristics and CSF findings in patients with definite LNB with CSF CXCL13 concentration under cut-off value 162 pg/ml. The number of possible LNB patients with normal CXCL13 concentration was 50 (62%). All recruited patients (n=21) with unlikely LNB had CSF CXCL13 concentration ≤ 18 pg/ml. Two of them had TBE and one had nonspecific viral meningitis. The rest of the patients with unlikely LNB did not have pleocytosis.

In study I, CSF CXCL13 concentration among untreated LNB patients ranged from 424 to 158000 pg/ml (median 6480 pg/ml), among retrospectively identified control patients with viral CNS infection from <7.8 to 406 pg/ml and among patients with noninfectious neuroinflammatory conditions from < 7.8 to 280 pg/ml. CXCL13 concentration in a patient with neurosyphilis was 37000 pg/ml. CSF CXCL13 concentration ranged from <7.8 to 153 pg/ml with median of < 7.8 pg/ml in 239 retrospectively identified non-LNB patients. There were statistically significant differences in CSF CXCL13 concentrations between the untreated LNB patients and the non-LNB group ($p<0.001$), and various viral CNS infections ($p=0.0013$ or less), and noninfectious neuroinflammatory conditions ($p<0.001$). Based on the analysed patient samples in study I, the optimal cut-off value was determined to be 415 pg/ml with specificity of 99.7% for the diagnosis of LNB.

CSF neopterin level was measured from 38 LNB patients. CSF neopterin concentrations were high among patients with LNB (median 26.6 nmol/l) and neurosyphilis (92.2 nmol/l). Among retrospectively identified patients with non-LNB, median CSF neopterin concentration was 6.3 nmol/l. CSF neopterin concentrations were significantly higher in patients with HSV- ($p<0.01$) and VZV-infection ($p<0.05$) than in definite LNB patients. The use of neopterin concentration 10.6 nmol/l as the cut-off led to sensitivity of 89% and specificity of 65% for the diagnosis of LNB.

A control CSF specimen was collected from 58 participants with definite LNB, 6 patients with possible LNB and 2 patients with unlikely LNB after 3 weeks from the beginning of the treatment. There were no statistically significant differences between the ceftriaxone and doxycycline group in CSF leukocyte count, protein, glucose, and lactate level or CXCL13 concentration after antimicrobial treatment of three weeks (Table 15). Decrease in CXCL13 concentration along with the treatment is shown in Figure 9.

Table 14. Clinical characteristics and cerebrospinal fluid (CSF) findings in definite LNB patients with CXCL13 < 162 pg/ml. Each row represents one patient.

Previous antimicrobial treatment	Duration of symptoms in weeks	Symptoms relieved before recruitment	Symptoms	CSF CXCL13	CSF leukocyte count	CSF BorrNhO
-	20	yes	radiculitis, headache, myalgia, fatigue, decline in general condition	<7.8	7	neg
-	18	yes	diplopia, myalgia, neck-shoulder pain, headache	<7.8	21	neg
Amoxicillin, 1 g three times a day, 21 days	13	yes	radiculitis, headache, tinnitus	<7.8	8	neg
Amoxicillin, 1 g three times a day,30 days	23	no	radiculitis, paraesthesia, fever, fatigue, arthralgia, polyarthritis	<7.8	5	neg
-	5	no	facial nerve palsy, neck-shoulder pain, arthralgia, headache, hypoacusis	<7.8	3	pos
Doxycycline 150 mg twice a day, 14 days	8	no	facial nerve palsy, radiculitis, neck-shoulder pain, headache, arthralgia, myalgia	18	39	neg
-	8	yes	fever, headache, myalgia	54	30	neg
-	10	yes	radiculitis, arthralgia, myalgia, neck-shoulder pain, fatigue	54	13	neg
-	12	yes	radiculitis, headache	70	27	neg
-	12	no	radiculitis, vertigo, tremor, fatigue, diplopia, cognition disturbance	77	93	neg
-	18	no	facial nerve palsy, headache, radiculitis, neck-shoulder pain, arthralgia, vertigo, fatigue, nausea, paraesthesia	104	292	pos

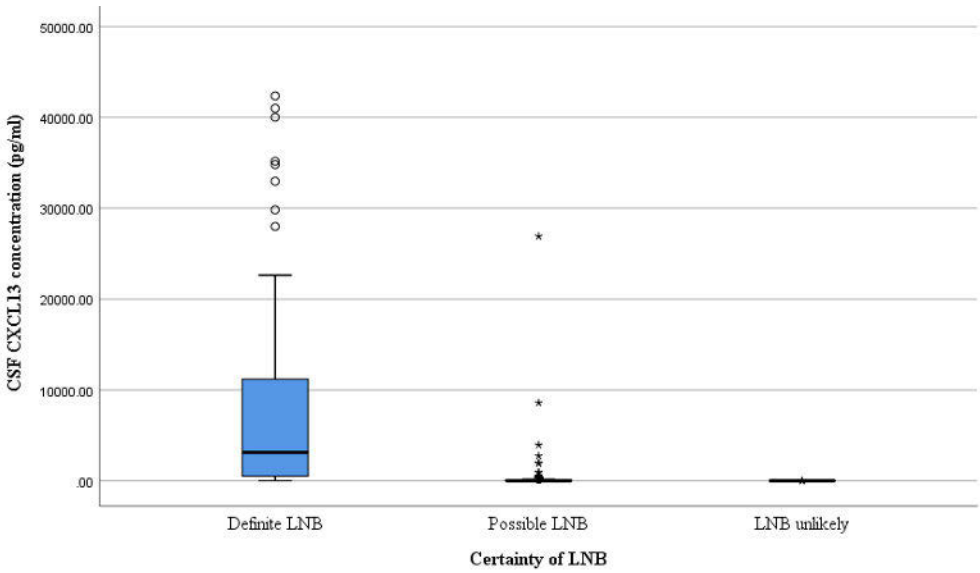

Figure 8. Initial CXCL13 concentrations in patients with definite, possible, and unlikely LNB. The box indicates the interquartile range (IQR), which contains the middle 50% of the values. The line across the box represents the median. The whiskers show the minimum and maximum values not greater than 1.5 times the IQR. Outliers (o) are values between 1.5 and 3 times the IQR, and extremes (*) are values with >3 times the IQR.

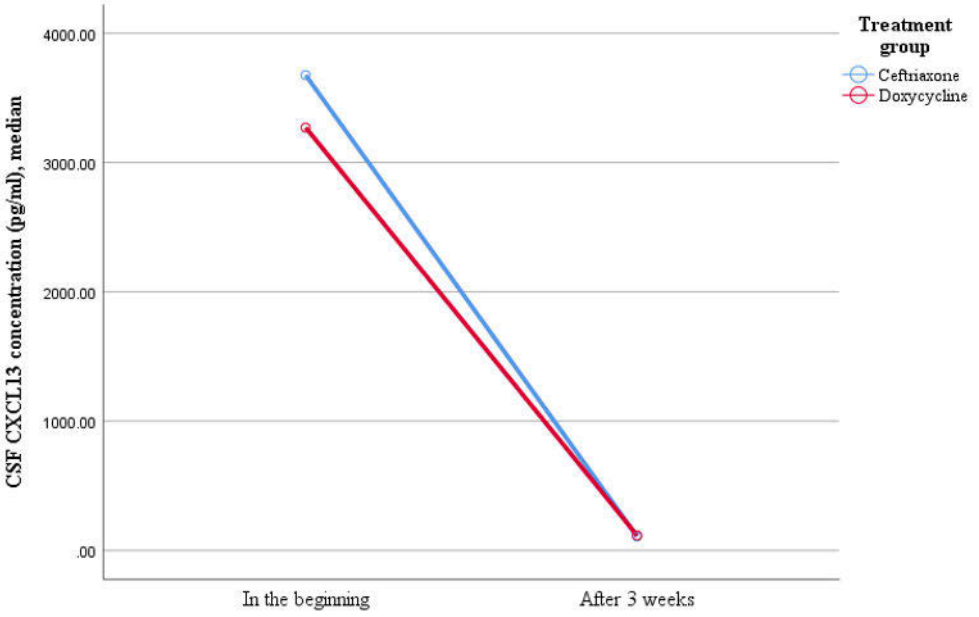

Figure 9. Decrease in median values of CXCL13 concentration during the antibiotic treatment in all patients with control lumbar puncture.

Table 15. Secondary outcomes presented only in patients with control lumbar puncture.

	Doxycycline n=32	Ceftriaxone n=34	P value
CSF leukocyte count / µl, median (IQR)			
At the beginning	115 (80–285)	128 (74–232)	0.778
After 3 weeks	31 (20–51)	36 (22–57)	0.314
CSF protein level, g/l, median (IQR)			
At the beginning	1186 (882–1672)	1167 (754–1697)	0.827
After 3 weeks, nDOX=31, nCRO=33	569 (442–758)	599 (505–760)	0.819
CSF glucose level, mmol/l, median (IQR)			
At the beginning, nDOX=26, nCRO=33	3.3 (3.0–3.7)	3.2 (2.9–3.8)	0.603
After 3 weeks, nDOX=24, nCRO=33	3.3 (3.0–3.4)	3.3 (3.0–3.6)	0.845
CSF lactate level, mmol/l, median (IQR)			
At the beginning, nDOX=28, nCRO=26	2.3 (1.9–3.1)	2.3 (1.8–2.5)	0.461
After 3 weeks, nDOX=28, nCRO=32	1.8 (1.5–2.2)	1.8 (1.6–2.0)	0.882
CSF CXCL13 concentration, pg/ml, median (IQR)			
At the beginning, nDOX=31, nCRO=32	3270 (984–10177)	3675 (694–16450)	0.747
After 3 weeks	117 (38–180)	109 (48–278)	0.710

CSF = cerebrospinal fluid; nDOX = number of specimens analysed in doxycycline group, unless n=32; nCRO = number of specimens analysed in ceftriaxone group, unless n=34

5.1.5 Impact of corticosteroid use in Lyme neuroborreliosis–associated facial nerve palsy

Of all recruited patients, 92/210 (44%) had facial nerve palsy. Of all patients with facial nerve palsy, 57 (62%) patients had definite LNB, 29 (32%) patients had possible LNB, and 6 (7%) patients did not have LNB. Unilateral facial function impairment was experienced by 78/92 (85%) patients, of which left-sided were 38/92 (41%) and right-sided 40/92 (43%). Facial nerve palsy was bilateral in the case of 14/92 (15%) patients, which all had definite LNB.

Of all patients with facial nerve palsy, 70 (76%) received corticosteroid therapy. Almost in every case, this was ten days' course with prednisolone, starting at dose 60 mg once a day for five days, after which the dose was reduced by 10 mg daily. Six patients did not have LNB, and they were excluded from further analysis (Figure 10). Additionally, five patients were lost to follow up. After the follow-up of 12 months, 73/81 (90%) patients had normal facial nerve function, whereas 6/81 (7%) had mild and 2/81 (2%) had significant residual function defects. Seven of these patients with sequelae had received corticosteroid therapy. Only one patient without corticosteroid therapy had mild residual symptoms. However, patients with or without corticosteroid therapy did not have statistically significant difference in improvement ($p = 0.673$).

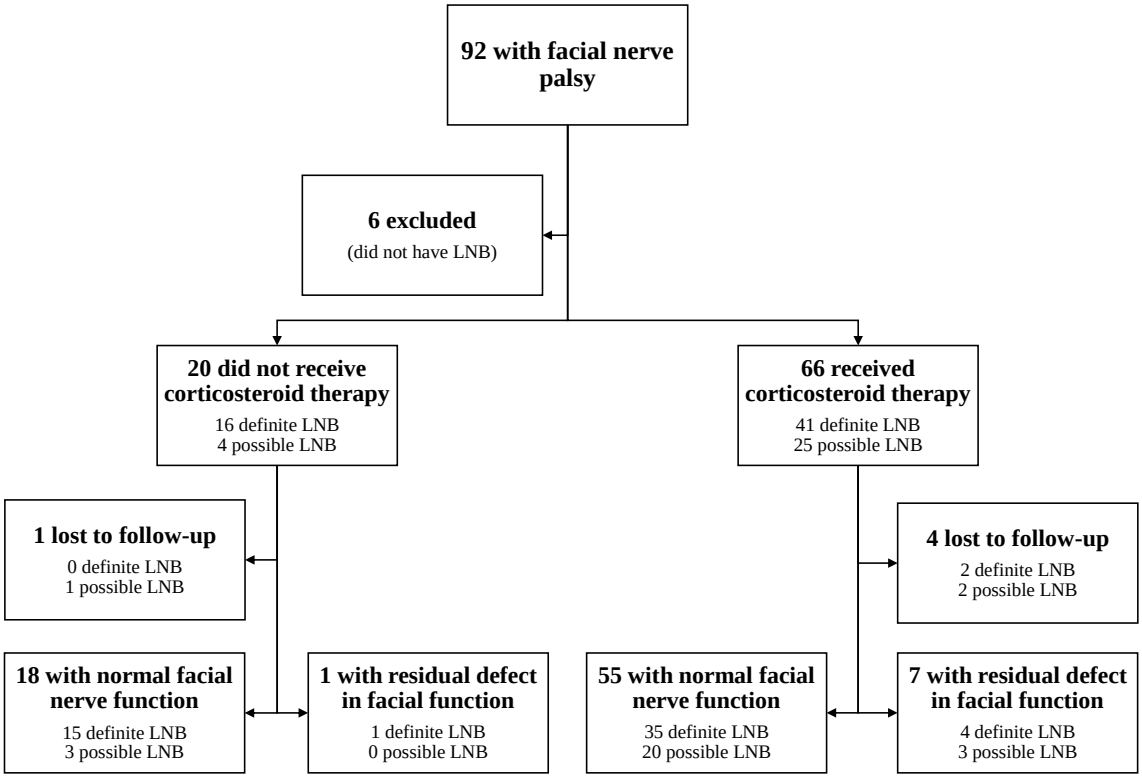

Figure 10. Flowchart of randomized patients with facial nerve palsy distributed according to received corticosteroid therapy.

5.2 Retrospective, observational, population-based Lyme borreliosis -study (III and IV)

5.2.1 Baseline characteristics and diagnostic procedures of the patient cohort

The number of patients referred to the Department of Infectious Diseases in Helsinki University Hospital due to suspected LB in 2013 was 256. The number of patients called for a visit at the Department of Infectious Diseases was 167 (65%), and the referral was responded with a written consultation reply in the case of 89 (35%) patients. Patients were categorized as having definite LB (n=30, 12%), probable LB (n=36, 14%), possible LB (n=65, 25%) or unlikely LB (n=121, 47%). The certainty of LB could not be determined in the case of 4 (2%) patients because of the deficient information on the symptoms and serologic tests. These 4 patients are excluded from the comparisons between the groups.

The mean age of the patients was 53 years (SD 15, range 16–85) and 92 (36%) were males. There were more males in the group of definite LB than unlikely LB (p=0.009). Patients with unlikely LB had statistically significantly longer duration of symptoms than patients with probable (p=0.012) or definite LB (p < 0.001), but the symptom duration of patients with possible LB did not differ statistically significantly from unlikely LB patients (p=0.234). Additionally, definite LB patients had a statistically significantly shorter duration of symptoms than possible LB patients (p=0.002) or probable LB patients (p=0.006). Comparisons between baseline demographic characteristics and signs and symptoms are shown in Table 16.

Table 16. Statistical comparison of baseline characteristics and symptoms and signs.

	Unlikely LB (n=121)	Possible LB (n=65)	Probable LB (n=36)	Definite LB (n=30)	P value
Male gender, n (%)	32 (26%)	26 (40%)	15 (42%)	17 (57%)	**0.010***
Age in years, mean (SD)	51 (15)	56 (15)	57 (14)	52 (16)	0.104
Number of comorbidities, median (IQR)	2 (1–3)	1 (1–3)	1 (0–3)	1 (1–3)	0.058
Tick bite during the past year, n (%)	35 (29%)	29 (47%)	15 (42%)	9 (30%)	0.052
EM during the past year, n (%)	16 (13%)	16 (25%)	21 (58%)	6 (20%)	**<0.001***
Duration of symptoms in months, median (IQR)	6 (2–24)	3 (1–10)	3 (1–5)	1 (1–2)	**<0.001***
Number of symptoms, median (IQR)	3 (2–5)	3 (2–4)	3 (2–4)	3 (2–6)	0.295
Arthralgia, n (%)	52 (43%)	36 (55%)	20 (56%)	3 (10%)	**<0.001***
Myalgia, n (%)	48 (40%)	23 (35%)	17 (47%)	7 (23%)	0.227
Fatigue, n (%)	47 (39%)	26 (40%)	18 (50%)	9 (30%)	0.427
Headache, n (%)	43 (36%)	17 (26%)	10 (28%)	9 (30%)	0.575
Vertigo, n (%)	24 (20%)	12 (19%)	4 (11%)	6 (20%)	0.721
Fever > 38°, n (%)	10 (8%)	4 (6%)	6 (17%)	6 (20%)	0.092
Paraesthesia, n (%)	38 (31%)	20 (31%)	5 (14%)	10 (33%)	0.174
Facial nerve palsy, n (%)	3 (3%)	3 (5%)	1 (3%)	18 (60%)	**<0.001***
Other peripheral nerve palsy, n (%)	5 (4%)	2 (3%)	1 (3%)	2 (7%)	0.862
Radiculitis, n (%)	1 (1%)	5 (8%)	2 (6%)	11 (37%)	**<0.001***
Monoarthritis, n (%)	6 (5%)	4 (6%)	3 (8%)	0	0.487
Oligoarthritis, n (%)	4 (3%)	1 (2%)	1 (3%)	0	0.875
Iritis or conjunctivitis, n (%)	2 (2%)	4 (6%)	2 (6%)	0	0.190
Atrioventricular conduction blockade, n (%)	0	0	2 (6%)	0	**0.034***
Arrhythmia (atrial fibrillation or palpitations), n (%)	4 (3%)	2 (3%)	0	0	0.762
Patient was evaluated at the Department of Infectious Diseases, n (%)	62 (51%)	51 (79%)	30 (83%)	24 (80%)	**<0.001***

LB = Lyme borreliosis; EM = erythema migrans; SD = standard deviation; IQR = interquartile range
*Pairwise comparison between the groups is shown in Tables 17A-H.

Proper serologic two-tier testing was performed to 232/256 (92%) patients (Table 18). In unlikely LB patients, 92/121 (76%) had negative or low positive antibody levels in serum. In the groups of definite, probable, possible, and unlikely LB, 24 (80%), 29 (81%), 34 (52%), and 16 (13%) patients had intermediate or high antibody levels, respectively. Seroconversion was detected in five patients, four of them categorized to definite LB group and one asymptomatic patient classified as an unlikely LB. CSF sampling was performed to 115 (45%) patients, with normal findings in 77/115 (67%) cases. CSF findings revealed definite LB with pleocytosis and positive antibody index (AI) in 27/115 (23%) patients. Additionally, 8 patients had pleocytosis without positive AI, and 3 patients had positive AI without pleocytosis. Other diagnostic procedures performed were electroneuromyography (ENMG; n=33), electroencephalogram (EEG; n=7), brain magnetic resonance imaging (MRI; n=45), MRI from other part of the body (n=15), and neuropsychological tests (n=15).

Table 17A. Pairwise comparison between the groups of different LB certainty in male gender.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.343		
Probable LB	0.480	0.999	
Definite LB	**0.009**	0.776	0.999

Numbers are p-values from pairwise comparison with Pearson's chi squared test. Bonferroni correction is used.

Table 17B. Pairwise comparison between the groups of different LB certainty in erythema migrans during the past year.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.298		
Probable LB	**<0.001**	**0.005**	
Definite LB	0.999	0.999	**0.014**

Numbers are p-values from pairwise comparison with Pearson's chi squared or Fisher's exact test. Bonferroni correction is used.

Table 17C. Pairwise comparison between the groups of different LB certainty in duration of symptoms.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.234		
Probable LB	**0.012**	0.999	
Definite LB	**<0.001**	**0.002**	**0.006**

Numbers are p-values from pairwise comparison with Mann-Whitney U-Test. Bonferroni correction is used.

Table 17D. Pairwise comparison between the groups of different LB certainty in arthralgia.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.636		
Probable LB	0.999	0.999	
Definite LB	**0.003**	**<0.001**	**<0.001**

Numbers are p-values from pairwise comparison with Pearson's chi squared or Fisher's exact test. Bonferroni correction is used.

Table 17E. Pairwise comparison between the groups of different LB certainty in facial nerve palsy.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.999		
Probable LB	0.999	0.999	
Definite LB	**<0.001**	**<0.001**	**<0.001**

Numbers are p-values from pairwise comparison with Fisher's exact test. Bonferroni correction is used.

Table 17F. Pairwise comparison between the groups of different LB certainty in radiculitis.

	Unlikely LB	Possible LB	Probable LB
Possible LB	0.122		
Probable LB	0.791	0.999	
Definite LB	**<0.001**	**0.006**	**0.012**

Numbers are p-values from pairwise comparison with Fisher's exact test. Bonferroni correction is used.

Table 17G. Pairwise comparison between the groups of different LB certainty in atrioventricular conduction blockade.

	Unlikely LB	Possible LB	Probable LB
Possible LB	-		
Probable LB	0.309	0.748	
Definite LB	**-**	**-**	0.999

Numbers are p-values from pairwise comparison with Fisher's exact test. Bonferroni correction is used.

Table 17H. Pairwise comparison between the groups of different LB certainty in patients evaluated at the Department of Infectious Diseases.

	Unlikely LB	Possible LB	Probable LB
Possible LB	**0.002**		
Probable LB	**0.004**	0.999	
Definite LB	0.026	0.999	0.999

Numbers are p-values from pairwise comparison with Pearson's chi squared test. Bonferroni correction is used.

Table 18. Diagnostic procedures of the patients consulted for suspicion of LB. Patients with unknown certainty of LB (n=4) are excluded.

	Unlikely LB n = 121	**Possible LB n = 65**	**Probable LB n = 36**	**Definite LB n = 30**
B.b antibody levels in serum*				
Not enough information	12 (9.9%)	6 (9.2%)	1 (2.8%)	1 (3.3%)
Negative	36 (29.8%)	3 (4.6%)	0	0
Low positive	56 (46.3%)	22 (33.8%)	6 (16.7%)	1 (3.3%)
Intermediate	12 (9.9%)	26 (40.0%)	14 (38.9%)	19 (63.3%)
High positive	4 (3.3%)	8 (12.3%)	15 (41.7%)	5 (16.7%)
Seroconversion	1 (0.8%)	0	0	4 (13.3%)
CSF sampling	n = 44 (36.4%)	n = 33 (50.8%)	n = 9 (25.0%)	n = 29 (96.7%)
Normal	41 (93.2%)	27 (81.8%)	7 (77.8%)	2 (6.9%)
Pleocytosis, normal AI	3 (6.8%)	4 (12.1%)	1 (11.1%)	0
No pleocytosis, positive AI	0	2 (6.1%)	1 (11.1%)	0
Pleocytosis and positive AI	0	0	0	27 (93.1%)
ENMG	n = 18 (14.9%)	n = 8 (12.3%)	n = 2 (5.6%)	n = 5 (16.7%)
No information	1 (5.6%)			
Normal	10 (55.6%)	2 (25.0%)	1 (50.0%)	1 (20.0%)
Abnormal**	7 (38.9%)	6 (75.0%)	1 (50.0%)	4 (80.0%)
EEG	n = 5 (4.1%)	n = 1 (1.5%)		n = 1 (3.3%)
Normal	3 (60.0%)	0	0	0
Abnormal**	2 (40.0%)	1 (100%)	0	1 (100%)
Brain MRI	n = 22 (18.2%)	n = 7 (10.8%)	n = 2 (5.6%)	n = 11 (36.7%)
Unknown result	1 (4.5%)	0	0	1 (9.1%)
Normal	19 (86.4%)	6 (85.7%)	2 (100%)	3 (27.3%)
Abnormal**	2 (9.1%)	1 (14.3%)	0	7 (63.6%)
Brain MRI + MRA (with normal findings)	2 (1.7%)	1 (1.5%)	0	0
Cervical spine MRI	n = 4 (3.3%)			n = 1 (3.3%)
Normal	0	0	0	0
Abnormal**	4 (100%)	0	0	1 (100%)
Lumbar spine MRI	n = 3 (2.5%)	n = 1 (1.5%)		n = 1 (3.3%)
Normal	2 (66.7%)	1 (100%)	0	1 (100%)
Abnormal**	1 (33.3%)	0	0	0
Whole spinal column MRI	n = 2 (1.7%)			n = 2 (6.7%)
Normal	2 (100%)	0	0	0
Abnormal**	0	0	0	2 (100%)
Foot MRI with abnormal result	1 (0.8%)	0	0	0
Neuropsychological tests	n = 11 (9.1%)	n = 2 (3.1%)	n = 1 (2.8%)	n = 1 (3.3%)
Normal	3 (27.3%)	0	0	0
Slightly abnormal**	6 (54.5%)	1 (50.0%)	0	1 (100%)
Abnormal**	2 (18.2%)	1 (50.0%)	1 (100%)	0

LB = Lyme borreliosis; *B.b.* = *Borrelia burgdorferi*; CSF = cerebrospinal fluid; AI = (anti-*Borrelia*) antibody index; ENMG = electroneuromyography; EEG = electroencephalogram; MRI = magnetic resonance imaging; MRA = magnetic resonance angiography.
Data are number of patients (%).
*Presented in methods.
**Including all kinds of abnormal changes not specific to LB.

5.2.2 Differential diagnoses of the study population

Diagnostic conclusions based on the evaluation of all gathered information after follow-up period are presented in Figure 11. Symptoms were at least partly explained by previous conditions or novel diagnoses in 107 (42%) patients. In the case of 121 patients with unlikely LB, 81 (67%) had either a previous condition or a novel diagnosis explaining the symptoms behind LB suspicion. Only 31/256 (12%) patients did not have any obvious reasons that could explain their symptoms.

Novel diagnoses, that explained some or even most of the symptoms mentioned in the referral, were discovered in 73 (29%) of the patients. Of these, 43 patients had one new diagnosis, 18 patients had two new diagnoses, and 12 patients received three new diagnoses. In 19 patients, a novel diagnosis explained the symptoms completely.

Previous disease was causing at least part of the symptoms in the case of 48 (19%) patients. Of these, 38 patients had one previous condition, six patients had two, and four patients had three previous conditions behind the symptoms. The reason for the symptoms was both previous condition and a novel disease in 14 patients. The underlying causes of the symptoms, both previous conditions and novel diagnoses, are listed in Table 19.

Table 19. Reasons behind the symptoms, both previously diagnosed conditions and novel diagnoses made after referral.

Reason	Diagnosis (n=177)
Musculoskeletal disorders	60 (34%)
Neurological disorders	23 (13%)
Psychiatric disorders	18 (10%)
Functional disorders	15 (9%)
Rheumatologic diseases	14 (8%)
Other infectious diseases	9 (5%)
Dermatological conditions	9 (5%)
Otorhinolaryngologic diseases	8 (5%)
Gastroenterologic diseases	5 (3%)
Ophthalmologic diseases	4 (2%)
Cardiovascular diseases	4 (2%)
Malignancy	3 (2%)
Other	5 (3%)

Data are the number of diagnoses (%).

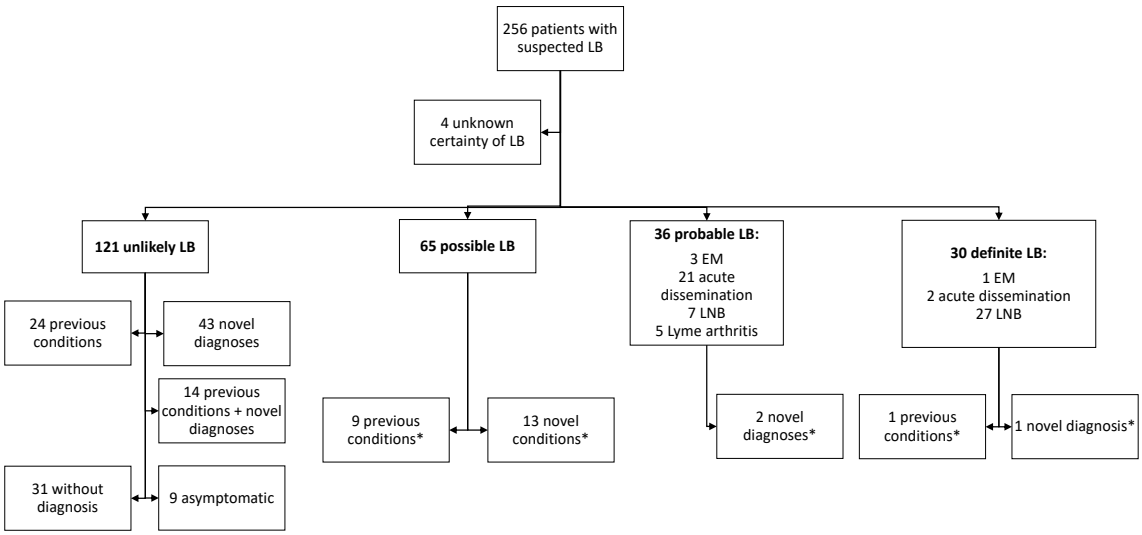

Figure 11. Diagnostic conclusion of patients with a referral to the Department of Infectious Diseases because of suspected LB. * Previous condition or novel diagnosis explains part of the symptoms. LB = Lyme borreliosis; EM = erythema migrans; LNB = Lyme neuroborreliosis.

5.2.3 Utilization of healthcare services

The symptoms leading to LB suspicion and onwards referral to the infectious diseases specialist consultation led to the median of 5 (IQR 3–8) and 1 (IQR 0–4) visits or phone calls to the general practitioners during the year before the referral and the year after the consultation or treatment at the Department of Infectious Diseases, respectively (Table 20). Patients with unlikely LB had more healthcare contacts than other groups of LB certainty (Table 21A–B). The contacts divided into different healthcare providers are presented in Figures 12–13.

5.2.4 Utilization of antibiotics

Before the referral, 50/252 (20%) patients received an effective treatment for LB. During two months or one year before referral, 83/252 (33%) or 151/252 (60%) patients, respectively, purchased antibiotics. The median duration of antimicrobial treatments was 18 (IQR 10–29) or 20 (IQR 10–32) days 2 months or one year before referral, respectively. Doxycycline, amoxicillin and cephalexin were the most commonly prescribed antibiotics (Figure 14). There were no statistically significant differences in general utilization of antibiotics between the groups of different LB certainty (Table 22).

Among patients who were evaluated at the Department of Infectious Diseases, 153/167 (92%) were treated with antimicrobials, even though 26 (16%) had already

been prescribed an effective treatment for LB. Ceftriaxone, with a median duration of 21 days (IQR 21–21), was the most commonly used first line antibiotic prescribed to 108 patients. Doxycycline (median duration 35 days, IQR 28–90) was prescribed to 102 patients, of which 30 were first treated with ceftriaxone. The treatment response was successful or complete in 92% patients with probable and definite LB, but 33% of patients with unlikely LB did not have any benefit from antibiotics. Adverse events were reported by 37/153 (24%) of patients treated with antimicrobials.

During the year after consultation or treatment by infectious diseases specialist, 127 (50%) patients received antimicrobial treatment. The median duration of treatment was 25 (IQR 8–32) or 18 (IQR 7–33) days during 2 months or one year after consultation reply or treatment by infectious disease specialist. Doxycycline, azithromycin and cephalexin were the most purchased antibiotics (Figure 15). The patients received median 74 (IQR 41–128) days of antibiotics in years 2012–2017.

Table 20. All contacts one year before referral and one year after consultation reply or treatment at the Department of Infectious Diseases in patients with different LB certainty.

	Unlikely LB n=121 n (%)	Number of contacts, median (IQR)	Possible LB n=65 n (%)	Number of contacts, median (IQR)	Probable LB n=36 n (%)	Number of contacts, median (IQR)	Definite LB n=30 n (%)	Number of contacts, median (IQR)	p value Kruskall Wallis
All contacts to healthcare 1 year before referral	121 (100%)	6 (3–10)	65 (100%)	4 (3–7)	36 (100%)	5 (3–7)	30 (100%)	4 (2–6)	**0.007***
All contacts to healthcare 1 year after consultation reply or treatment in DID	91 (75%)	3 (1–7)	37 (57 %)	1 (0–3)	20 (56%)	1 (0–3)	13 (43%)	1 (0–2)	**<0.001****

LB=Lyme borreliosis; DID=Department of Infectious Diseases

***Table 21A.** Pairwise comparison between the groups of different LB certainty in contacts the healthcare 1 year before referral.

	Unlikely LB	Possible LB	Probable LB
Possible LB	**0.036**		
Probable LB	0.227	0.999	
Definite LB	0.090	0.999	0.999

Numbers are p-values from pairwise comparison with Mann-Whitney U-test. Bonferroni correction is used.

****Table 21B.** Pairwise comparison between the groups of different LB certainty in contacts to healthcare 1 year after consultation reply or treatment at the Department of Infectious Diseases.

	Unlikely LB	Possible LB	Probable LB
Possible LB	**0.002**		
Probable LB	**0.004**	0.999	
Definite LB	**0.001**	0.999	0.999

Numbers are p-values from pairwise comparison with Mann-Whitney U-test. Bonferroni correction is used.

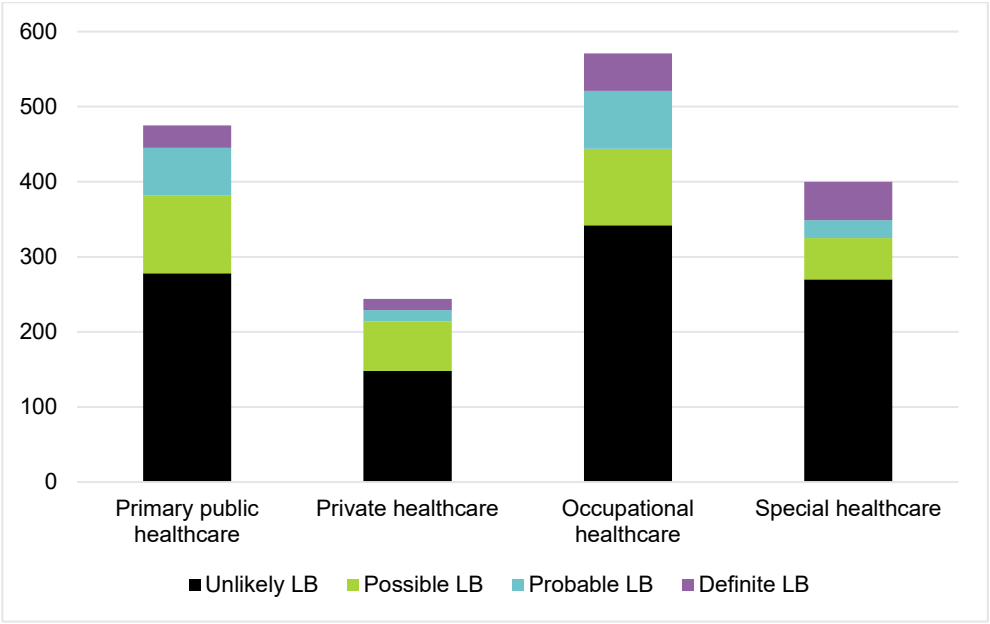

Figure 12. The sum of contacts to different healthcare providers one year before referral to the Department of Infectious Diseases in patients with suspected LB.

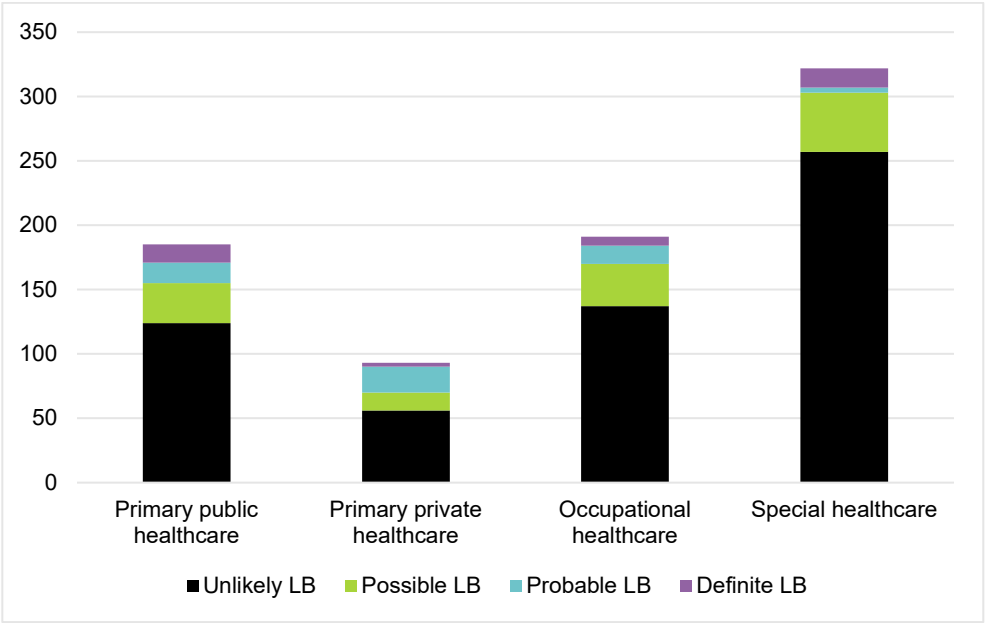

Figure 13. The sum of contacts to different healthcare providers one year after consultation reply or treatment at the Department of Infectious Diseases in patients with suspected LB.

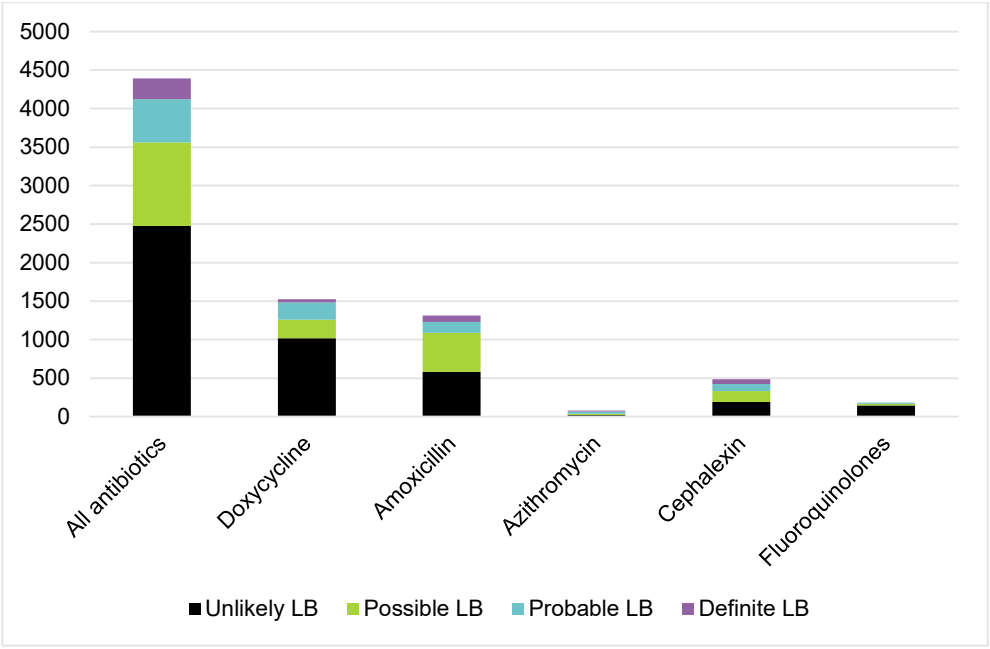

Figure 14. The sum of antibiotic treatment days in patients one year before referral to the Department of Infectious Diseases.

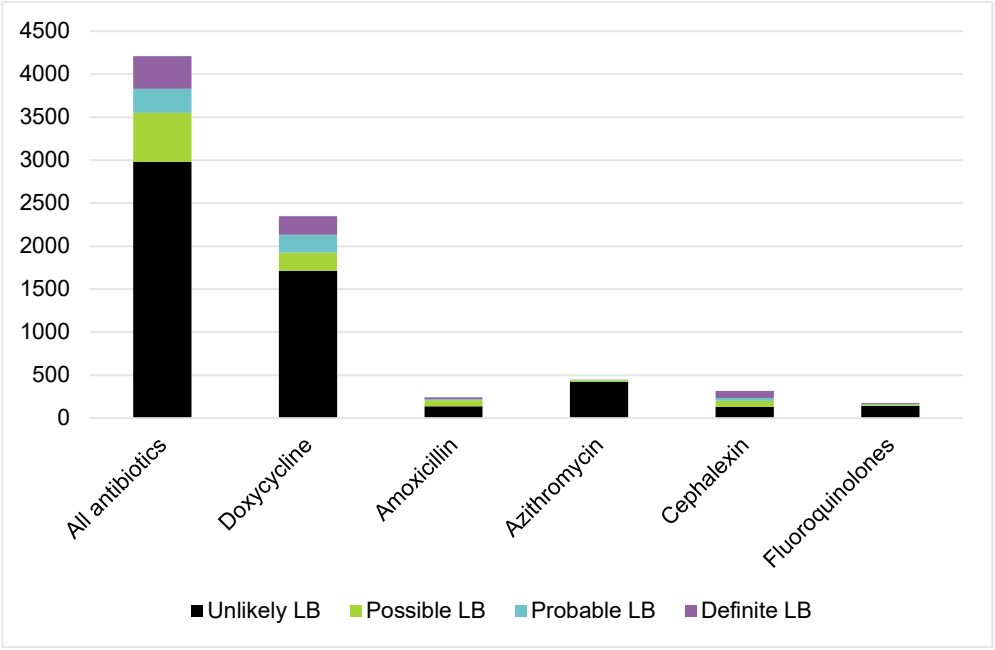

Figure 15. The sum of antibiotic treatment days one year after consultation reply or treatment at the Department of Infectious Diseases in patients with suspected LB.

Table 22. Antibiotic purchases of patients with the referral to the Department of Infectious Diseases because of LB suspicion.

	Unlikely LB n=121	Possible LB n=65	Probable LB n=36	Definite LB n=30	P value
Before*					
2 months	40 (33%)	22 (34%)	12 (33%)	9 (30%)	0.996
1 year	71 (59%)	43 (66%)	20 (56%)	17 (57%)	0.458
effective for LB	31 (26%)	10 (15%)	6 (17%)	3 (10%)	0.146
After**					
2 months	28 (23%)	11 (17%)	5 (14%)	6 (20%)	0.533
1 year	66 (55%)	36 (55%)	13 (36%)	12 (40%)	0.123
Antibiotics in 2012–2017	115 (95%)	65 (100%)	36 (100%)	30 (100%)	0.662

LB = Lyme borreliosis

Data are number (%) of treated patients.

*Antibiotics before the referral

**Antibiotics after the consultation reply or treatment at the Department of Infectious Diseases.

6 Discussion

6.1 The role of CXCL13 in the diagnostics of Lyme neuroborreliosis

In our study (I), CSF CXCL13 concentrations of untreated LNB patients were significantly higher than the CXCL13 concentrations of the non-LNB patients or patients with viral CNS infection or non-infectious neuroinflammatory condition. The results indicate that high CXCL13 concentrations are associated with untreated LNB. In line with our results, other studies have reported similar findings (Picha et al. 2016, van Burgel et al. 2011, Wagner et al. 2018). Additionally, these studies compared CSF CXCL13 concentrations of LNB patients with other aseptic CNS infections, CNS inflammatory conditions, or negative controls. Levels of intrathecal CXCL13 were higher among LNB patients when compared to other groups, with a few exceptions of patients with *T. pallidum* or *Cryptococcus neoformans* CNS infections or patients with intrathecal inflammation associated with HIV infection (van Burgel et al. 2011). In our study, a single patient with neurosyphilis had very high CSF CXCL13 concentration. The data suggest that CXCL13 cannot be used for differentiating LNB from neurosyphilis. Because some other neuroinflammatory conditions can also cause high CSF CXCL13 concentrations, CXCL13 should be interpreted with other diagnostic tests and the complete clinical picture of the patient. Another evaluated CSF marker, neopterin, was not useful in the differential diagnostics of LNB because the median neopterin concentrations were increased in every CNS infection with different causative microbes.

Intrathecal antibody synthesis or serum antibody concentration are not suitable for the follow-up of LNB patients because antibodies can persist months to years after an effective treatment (Hammers-Berggren et al. 1993, Kalish et al. 2001b). CSF CXCL13 concentration of all our patients decreased rapidly during the antibiotic treatment. A previous study with control CSF sampling in LNB patients reported similar results with median CXCL13 concentration decline from 3727 pg/ml before treatment to 38 pg/ml after treatment (Bremell et al. 2013). The median time between CSF samplings was 45 days. In another study, CXCL13 concentration decreased significantly 3–4 weeks after treatment initiation in LNB patients but remained elevated when compared to the control group (Moniuszko et al. 2014).

Based on these findings, it has been proposed that CXCL13 might be a valuable marker for treatment response in LNB patients (Gudowska-Sawczuk and Mroczko 2020).

CXCL13 performance in late LNB has not been demonstrated (Raffetin et al. 2020). Our study did not provide further clarity to this issue because none of our definite LNB patients had late LNB with symptom durations of more than six months. In a previous study, LNB patients with less than nine days duration of the disease showed significantly higher CSF CXCL13 concentrations as compared with patients with longer durations of the disease (Senel et al. 2010). According to another study, the highest CXCL13 concentrations were at the beginning of LNB, and CXCL13 correlated better with CSF pleocytosis than intrathecal antibody production (Picha et al. 2016).

A conclusion from a previous meta-analysis was that CSF CXCL13 has the potential to become a useful additional marker in the diagnostics of acute LNB (Rupprecht et al. 2018). Another recent meta-analysis states that CXCL13 is proven to diagnose early LNB accurately and is already in use but needs to be standardized (Raffetin et al. 2020). Novel clinical practice guidelines by IDSA, AAN, and ACR do not recommend the measurement of CXCL13, due to lack of adequate standardization and sufficient studies, and call for additional research (Lantos et al. 2021). However, CSF CXCL13 concentration has already been introduced for clinical use at different hospitals in Finland.

6.2 Doxycycline and ceftriaxone in the treatment of Lyme neuroborreliosis

Our study (II) confirmed that oral doxycycline is equally effective as intravenous ceftriaxone in the treatment of LNB. Patients with doxycycline improved equally well as patients with ceftriaxone, regardless of whether the improvement was evaluated based on the condition of the patient or CSF findings. These results are consistent with a previous study which compared doxycycline to ceftriaxone in LNB patients and concluded that doxycycline is non-inferior to ceftriaxone in the treatment of LNB (Ljøstad et al. 2008). In addition, previous non-randomized studies or patient cohorts have suggested that LNB patients improve with oral doxycycline (Borg et al. 2005, Dotevall and Hagberg 1999, Karkkonen et al. 2001, Karlsson et al. 1994).

The optimal duration of antimicrobial treatment in LNB is unknown. In our study (II), the duration of ceftriaxone and doxycycline treatments were 3 and 4 weeks, respectively. Generally, the recommended duration of treatment in LNB, regardless of antimicrobial choice, is from 14 to 21 days (Jaulhac et al. 2019, Lantos et al. 2021, Mygland et al. 2010, Rauer et al. 2020). In Finland, the duration of ceftriaxone

treatment in LNB has traditionally been 3 weeks. In a previous study, LNB patients received a 14-day treatment of doxycycline or ceftriaxone, and more than half of the patients in both groups had residual symptoms at 4 months after treatment (Ljøstad et al. 2008). Our choice to use longer treatments was based on the experience that Finnish patients have had fewer residual symptoms after treatment, and also because patients with late LNB were eligible. After 12 months follow-up, 43% of patients in our study reported LNB-related residual complaints. In the end, it proved difficult to compare the number of residual symptoms between patients in our trial and the study of Ljøstad et al. Some patients recover slowly and therefore it can be assumed that the amount of residual complaints at 12 months is less than at 4 months. In a systematic review including eight randomized clinical trials and eight non-randomized studies, the authors concluded that there is no evidence suggesting benefits of extended antibiotic treatments in LNB (Dersch et al. 2015a). A randomized clinical trial comparing different treatment durations (six versus two weeks) has recently been initiated, and the results are expected to be published in the near future (Solheim et al. 2019).

6.3 Conditions behind the presumed Lyme borreliosis

In study III, only 26% of patients with a referral to the Department of Infectious Diseases had definite or probable LB. Every second patient with presumed LB was categorized to the group of unlikely LB, and 67% of those had another reason for the symptoms. Musculoskeletal problems were the most common reasons for the symptoms. Other common disorders causing the symptoms of presumed LB were diverse, ranging from neurological pathologies to rheumatologic diseases, which underlines the diagnostic challenges.

Similar results have been reported before, as Haddad et al. reported that less than 10% of presumed LB were confirmed in their patient cohort, and the most common differential diagnoses were psychological, musculoskeletal, and neurological in origin, which is in line with other studies with slightly distinct disease classifications (Haddad et al. 2019, Jacquet et al. 2019, Reid et al. 1998). In another study, 15% of LB diagnoses were confirmed, and 49% of patients received a differential diagnosis (Jacquet et al. 2019). In all studies, underlying alternative diseases have been very diverse. In our patient cohort, cardiovascular diseases and malignancies were infrequent causes for the symptoms. Various conditions behind the presumed LB, occasionally severe or even fatal without appropriate treatment, emphasize the importance of differential diagnostics. In addition, a persistent suspicion of LB without confirmation may result in a delay in adequate therapy.

6.4 Utilization of healthcare services and antibiotics

In study IV, patients with unlikely LB had statistically significantly more contacts to healthcare because of symptoms leading to LB suspicion compared to possible LB patients one year before referral, and more contacts than any other group one year after the referral. The utilization of healthcare services in different groups of LB certainty had not been studied before. Our findings are easy to understand. Patients with unlikely LB often had symptoms that did not suggest LB or negative or low antibody levels, which do not directly lead one to suspect LB. We were unable to identify whether the LB suspicion originated from a patient or a primary healthcare physician. Patients with unlikely LB continued to use healthcare services after infectious disease consultation, which is also understandable. The problem was not solved, and the patient continued to seek relief for the symptoms.

Among all patients, 60% and 33% purchased antibiotics one year and two months before referral, respectively. Almost 20% of patients had received a treatment effective for the LB manifestation in question before referral. Further, 92% of patients evaluated at the Department of Infectious Diseases received antibiotics, irrespective of the certainty of LB. One year after the consultation reply or treatment at the Department of Infectious Diseases, 50% of patients purchased antibiotics. Almost every patient purchased at least one antimicrobial treatment between 2012 and 2017. There were no statistically significant differences between the groups of different LB certainties in antimicrobial utilization.

The utilization of antibiotics in our study can be considered surprisingly wide. Additionally, certainty of LB did not have an effect on antimicrobial purchases, and patients with unlikely LB purchased antibiotics as much as patients with definite LB. A previous Finnish study concluded that primary healthcare physicians have good compliance with the recommended treatment guidelines of clinically diagnosed LB (Feuth et al. 2020). That study evaluated LB cases from three national healthcare registers — Hilmo, Avohilmo, and NIDR — but was unable to extend to private healthcare. In addition, that study did not evaluate the accuracy of LB diagnoses. In other studies on presumed LB, 40–85% of patients were treated with antibiotics before referral to special healthcare (Cottle et al. 2012, Coumou et al. 2015, Haddad et al. 2019, Jacquet et al. 2019, Kobayashi T. et al. 2019, Reid et al. 1998).

6.5 Difficulties in the diagnostics of Lyme borreliosis

Most clinical presentations are not pathognomonic to LB. This poses problems in the recognition of a patient's signs and symptoms as manifestations of LB. The easiest sign to identify as a cause of LB after EM may be facial nerve palsy, especially if it

is associated with meningoradiculitis. In coastal Finland, physicians are advised to examine *B. burgdorferi* antibodies from every person with facial nerve palsy. In addition to LNB, facial nerve palsy may originate from *Varicella zoster* infection, otitis media, a cancer or a benign lesion compressing the nerve, iatrogenic injuries, or some other unidentified causes (Hohman and Hadlock 2014). In most cases, facial nerve palsy is idiopathic without a known cause. Meningoradiculitis without obvious peripheral palsy is far more difficult to identify, and may be confused with musculoskeletal disorders (Knudtzen et al. 2017). Additionally, vice versa, musculoskeletal problems were the most common conditions behind the symptoms in our patients with unlikely LB.

As observed in our patient material, nonspecific subjective symptoms, such as myalgia, fatigue, and headache, were common in all patient groups with different LB certainties (III), and should not be regarded as suggestive of LB. In 1439 patients referred to a Dutch Lyme center, nonspecific symptoms were equally common in patients with positive and negative *B. burgdorferi* serology (Zomer et al. 2019). In contrast, in the diagnostic criteria of our retrospective study (III), we considered nonspecific subjective symptoms suggestive of acute *B. burgdorferi* dissemination, if these symptoms disappeared after antibiotic treatment, otherwise the symptom presentation of the patient was compatible with LB. The reason for this approach was based on previously described nonspecific symptoms in culture-confirmed EM patients (Nadelman et al. 1996). Nevertheless, according to guidelines, examination of *B. burgdorferi* serology in patients with only nonspecific subjective symptoms is highly discouraged (Dessau et al. 2018).

Diagnosis and treatment delays are common in LNB patients. Even if meningoradiculitis is one of the most common presentations of LNB, it is difficult to be differentiated from musculoskeletal disorders, at least if it occurs without other symptoms such as peripheral nerve palsy or EM. Treatment delay in patients with radicular pain has been significantly longer than in patients with other symptoms. Additionally, treatment delay in children has been shorter than in adults, mainly because children more often have an easily recognizable clinical presentation with cranial nerve palsy and meningitis (Knudtzen et al. 2017). In Denmark, the estimated median treatment delay of LNB was 21 days (IQR 10–42) in 2015–2017 (Nordberg et al. 2020). Treatment delay was longer if symptoms began between November and April compared to the other months. Surprisingly, treatment delay has not been shortened in Denmark during the last two decades (Knudtzen et al. 2017, Nordberg et al. 2020). Time from symptom onset to initiation of treatment in our patients with definite LNB was 4 weeks (II), which is slightly longer than in Denmark. Evaluated by another method, patients with definite and probable LB hade a median 4–5 contacts to healthcare because of LB symptoms one year before referral in the retrospective study (IV), which means that the disease is still difficult to diagnose.

Treatment delay is an important issue because it has been associated with worse outcomes (Nordberg et al. 2020); in particular, patients with radicular pain have been shown to have more residual symptoms if treatment delay is more than 30 days compared to patients with treatment delays less than 30 days (Knudtzen et al. 2017). Additionally, treatment delay greater than 30 days is associated with PTLDS (Hirsch et al. 2020).

Because of the nonspecific nature of many clinical manifestations of LB, laboratory proof of infection is crucial in many presentations apart from EM. Clinical laboratories use different serological assays targeting different antigens in the diagnosis of LB. Antibodies do not necessarily develop in the same way in different patients, and various serological tests detect different kinds of antibodies. These reasons lead to overall sensitivities and specificities of serum serology of less than 100%. In a previous study comparing 12 clinical laboratories in different Nordic countries, the average sensitivity was 88% with pretty homogenous results for IgG, but sensitivity for IgM was only 59% with more heterogenous results (Lager et al. 2019). A major issue in evaluating the performance (sensitivity/specificity) of an LB serology assay is the lack of gold-standard samples, i.e., serum/CSF samples originating from culture-confirmed patients. The limitations related to *B. burgdorferi* serology assays may sometimes cause false negative and false positive findings.

6.6 The role of cerebrospinal fluid analysis in the diagnostics of Lyme neuroborreliosis

CSF analysis is essential for the laboratory diagnosis of LNB, and it has been recommended in almost every guideline in Europe and America (Eldin et al. 2019). The most important parameters are intrathecal *B. burgdorferi* antibody production and leukocyte cell count, which are mandatory for the diagnosis of definite LNB (Mygland et al. 2010). Besides verifying LNB diagnosis, CSF analysis is beneficial in differential diagnostics. Frequently, other possible causes of meningitis, such as herpes simplex virus, enteroviruses, and other bacteria, need to be excluded. In addition, CSF findings may suggest other inflammatory CNS diseases like multiple sclerosis or autoimmune encephalitis. In our randomized LNB treatment trial (II), even if symptoms caused a suspicion of LNB, the CSF analysis with other laboratory findings revealed two cases of TBE, two cases of multiple sclerosis, and viral meningitis in addition to other conditions with normal CSF findings. These diseases cannot always be distinguished clinically, and laboratory proof is crucial.

The need of a lumbar puncture in patients with facial nerve palsy or typical Garin-Bujadoux-Bannwarth syndrome has been questioned by some physicians (Rupprecht and Pfister 2009). Doxycycline has proven to be equally effective as ceftriaxone in the treatment of LNB, meaning that CSF analysis is not necessary to

guide the correct choice of antibiotic treatment. Additionally, in many cases symptoms compatible with LB together with positive serum *B. burgdorferi*–specific IgG antibodies lead to LB treatment regardless of findings of CSF analysis. So, if there is no suspicion of other treatable conditions causing the symptoms, and the result does not have an influence on treatment, why expose the patient to an uncomfortable invasive procedure? If the patient does not improve expectedly, the lumbar puncture can be performed after a couple of weeks, when positive AI and probably also mild pleocytosis should still be present. On the other hand, organizing the lumbar puncture for a patient may take some time, which may cause more treatment delay.

In patients with Bell's palsy, the oral corticosteroid therapy should be initiated within 72 hours of symptom onset (Baugh et al. 2013). Approximately 70% of patients with Bell's palsy recover spontaneously in 6 months, and with corticosteroid treatment the rates of complete recovery are about 80–85% (Peitersen 1982, Yoo et al. 2020). In facial nerve palsy due to LNB, corticosteroids do not improve the outcome, rather they may even be harmful (Clark et al. 1985, Jowett et al. 2017, Kalish et al. 2001a). In our study (II), 89% and 95% of LNB patients with facial nerve palsy, with and without corticosteroid treatment, respectively, had complete recovery. In a previous study including only 15 patients with LNB-associated facial nerve palsy, 93% of patients treated with antibiotics had complete recovery, but it was not reported if patients received corticosteroids or not (Kowalski et al. 2011). In acute LNB, patients benefit from rapid initiation of antibiotic therapy. These viewpoints, deciding the necessity of corticosteroids and starting antibiotics promptly, support the role of lumbar puncture in patients with acute (< 72 hours) facial nerve palsy. CSF leukocyte count and protein-level results are available in a few hours after lumbar puncture, while serological assays usually take several days. In patients with EM and complaints suggesting nervous system involvement, the positive predictive value of pleocytosis for LNB has been 68%, while normal CSF leukocyte count has excluded LNB with a predictive value of 92% (Ogrinc et al. 2013). A concordant result was received in a retrospective Finnish study concerning facial nerve palsy of children less than 17 years old. The positive and negative predictive values of pleocytosis for LNB in children with acute facial nerve palsy were 69% and 90%, respectively (Poyhonen et al. 2019). The authors concluded that CSF examination is worthwhile in the diagnosis of LNB among children with facial nerve palsy. Though almost every author recommends lumbar puncture in the diagnostics of facial nerve palsy due to suspected LNB, differing opinions also exist. Garro et al. comments that they do not recommend a routine lumbar puncture for patients with suspected LB-associated facial nerve palsy, except if there is any concern of bacterial meningitis (Garro and Nigrovic 2018). They rely on two-tier

serology in diagnostics and information about tick exposure. If LB is suspected, they recommend doxycycline treatment while awaiting serological results.

CXCL13 is proven to be useful in the differential diagnostics of facial nerve palsy in children (Poyhonen et al. 2019). As an advantage, CXCL13 concentration starts to increase early at the beginning of the infection, even days to weeks before antibody production is detectable (Rupprecht et al. 2006). Elevated CXCL13 concentration together with pleocytosis indicates LNB more likely than pleocytosis without markedly increased CXCL13 concentration in early LNB before antibody production has started. The rapid CXCL13 lateral flow immunoassay (LFA) point-of-care test has been shown to be reliable and has since been introduced into clinical practice (Pietikainen et al. 2018). When the CXCL13 result is received immediately after the lumbar puncture, it is easier for the clinician to determine whether the patient will benefit from antibiotics or not.

Intrathecal antibody production is sometimes present before a patient develops detectable antibodies in serum. In acute LNB with a symptom duration of less than 6 weeks, 74–80% of patients have positive AI (Ljostad et al. 2007, Nordberg et al. 2020). In a previous patient cohort of 194 LNB patients, 15 patients were seronegative at the time of diagnosis, but 13 of them had positive CSF AI. Median duration of symptoms of these 15 patients was 21 days (range 1–120). Two patients were seronegative, although symptoms had lasted more than six weeks and CSF AI was positive. Another patient had a hematologic malignancy (Nordberg et al. 2020). In the LNB treatment trial (II), two patients with positive AI had very low or negative *B. burgdorferi* antibody levels in serum. They were over 70 years old but did not have any co-morbidities explaining the low serum antibody concentrations. Another patient had a very long-term disease of about 10 years, and it is unclear whether LNB was still active or spontaneously resolved because pleocytosis was lacking. Unfortunately, this patient was lost to follow-up, so the treatment response is unknown. Another patient with only IgM antibodies in CSF and acute presentation of LNB may have reported false symptom onset due to dementia.

The novel clinical practice guidelines by IDSA, AAN and ACR, published in November 2020, recommend an individualized decision about performing a CSF examination if LNB is suspected (Lantos et al. 2021). Serum antibody testing is recommended rather than PCR or culture of either CSF or serum. If CSF testing is performed, it is important to obtain a simultaneous serum sample for determining CSF AI. The rationale for the recommendation is that serum antibody testing is the most sensitive diagnostic test in early LNB, and a normal AI does not exclude the diagnosis.

A routine control lumbar puncture seems to be unnecessary. In all our patients in the LNB treatment trial (II) with control CSF examination, CSF leukocyte count, protein level and CXCL13 concentration decreased, indicating resolution of

infection. Relapse or chronic infection is extremely rare after adequate treatment (Ross Russell et al. 2018). However, relapse has to be considered if objective signs of a patient increase after improvement, or a patient continues to have symptoms months after treatment, in which case a new CSF examination is indicated (Rauer et al. 2020). Though *B. burgdorferi* AI reacts slowly and individually after treatment, CXCL13 concentration and pleocytosis decrease rapidly after eradication of *B. burgdorferi* from CSF, and if not, a new course of antibiotic treatment should be prescribed (Koedel et al. 2015, Rauer et al. 2020). Reinfections are not uncommon, being more frequent in patients with previous EM, but patients with neurologic or cardiac involvement may also become reinfected (Kowalski et al. 2010, Nadelman et al. 2012). In the case of suspected reinfection, standard diagnostic procedures should be repeated.

6.7 How to improve antibiotic usage in Lyme borreliosis

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis were published in 2010 (Mygland et al. 2010). According to the guidelines, acute LNB with PNS symptoms can be treated with a 14-day course of oral doxycycline. In Norway, health authorities have advised to use oral rather than intravenous administration whenever possible. Later, adherence to LNB treatment guidelines of Norwegian clinicians was evaluated (Lorentzen et al. 2017). The new recommendations did not have an effect on treatment duration or choice between oral or intravenous antibiotics. The conclusion was that adherence to evidence-based treatment guidelines of physicians is unsatisfactory. In Denmark, the median treatment duration of LNB was 14 days (IQR 14–21), and 39% of patients were treated with intravenous antibiotics for the entire course in 2015–2017 (Nordberg et al. 2020). Additionally, at the Department of Infectious Diseases in Helsinki University Hospital (IV), intravenous ceftriaxone was widely used in the treatment of LB despite the certainty of LB or whether the patient had previously received an appropriate LB treatment. Therefore, it seems that a practice of intravenous antibiotics in the treatment of LNB and even in other manifestations of LB firmly remains, despite evidence supporting the use of oral doxycycline. Further, 30 patients were prescribed oral doxycycline after ceftriaxone treatment, despite this procedure is not recommended in guidelines.

Oral antibiotics offer many benefits when compared to intravenous antibiotics. They are more economical and do not need any healthcare professional for administration. A patient is not bound to a daily time schedule for medicine administration. Some patients want to continue working, despite LB symptoms, while having antibiotic treatment, and it is easier without a cannula, at least if work

is physical. Complications related to intravenous cannula, for instance infections and thrombophlebitis, can be avoided by using oral regimens. Therefore, it is rational to use oral medication whenever possible.

There are several reasons for poor adherence to treatment guidelines, which can be divided into guideline-related factors, personal factors and external factors (Mol et al. 2004). Barriers related to guideline adherence have been inconvenient availability, lack of conciseness, and concerns about the up-to-dateness of the guidelines. The characteristics of physicians have also influenced the acceptance of the guidelines. In particular, more experienced senior physicians might consider guidelines as a threat to their professional autonomy, while residents usually adopt guidelines better. Long-term habits, beliefs and previous treatment culture may affect adherence to new guidelines (Mol et al. 2004). In the case of LB, the distrust of negative serological test results may be one reason for unwarranted antimicrobial treatments. In addition to these challenges, there is social pressure to use excessive antimicrobial treatments. Disinformation about diagnostics and recommended treatment of LB is shared through different patient advocacy organizations, activists, experienced experts and even some physicians (Lorentzen et al. 2017). For example, the International Lyme and Associated Diseases Society (ILADS) has provided alternative recommendations on the management of LB, which lack evidence-based knowledge and confuse patients (Kullberg et al. 2020). Unfortunately, such harmful and false arguments are also fed to patients also in Finland.

The purpose of AMS is to guide the optimal use of antimicrobials to improve patient outcomes, and simultaneously limit the risk of complications as well as antimicrobial resistance. Practical instructions on how to implement AMS and guidelines for hospitals are published, and many of them are also suitable in LB (Mendelson et al. 2020). Implementation strategies should concentrate on improving the credibility and dissemination of the guidelines, and focusing on the education of physicians treating the patients (Mol et al. 2004). The utilization of antibiotics should be monitored, and feedback helps physicians to correct their practice. In addition to physicians, the general public would benefit from education on the appropriate treatment of LB and the harms of unnecessary prolonged antimicrobials (Macauda et al. 2011).

6.8 Strengths and limitations

Our patient material consisted of ordinary LB patients. In the LNB treatment trial (II), the randomized patients had very similar signs and symptoms to patients reported to have LNB (Knudtzen et al. 2017, Ljøstad et al. 2008). Unfortunately, we did not have patients with ACA or parenchymal CNS involvement such as stroke or vasculitis. One patient had seizures, which caused the suspicion of epilepsy, but

might have also been due to encephalitis. Cerebrovascular events were lacking in our LNB patients, which is not surprising because such manifestations are very rare (Garkowski et al. 2017). Patients suffering from CNS manifestations are usually treated at the Department of Neurology, where patient recruitment was less active/successful. Additionally, patients with suspected severe CNS infection are generally treated with empirical antibiotics, which was an exclusion criterion in the study. The lack of CNS manifestations in our study reduces the ability to generalize the results of antibiotic treatment response to these uncommon forms of LNB. However, the effectiveness of doxycycline against the common presentation of LNB is now further validated.

Almost half of our patients in the LNB treatment trial (II) were possible LNB patients. Possible LNB patients form a significant part of LNB in clinical practise, which is the reason why they were important to be included in this trial. The primary outcome of the LNB treatment trial indicated that both definite and possible LNB patients improve equally well with doxycycline and ceftriaxone. Including possible LNB patients in the trial may have diluted our results. From the physician's perspective, it is crucial to have evidence showing a prescribed treatment can benefit different kinds of LNB patients. Additionally, the previous LNB study comparing doxycycline to ceftriaxone included approximately 30% possible LNB patients (Ljøstad et al. 2008).

Our method to evaluate the outcome of patients in the LNB treatment trial (II), VAS score, has both benefits and limitations. A patient assessed her/his own condition, with or without the assistance of a VAS ruler from 0 to 10. This simple method is an easy, quantitative, and fast tool for self-assessment. It can be used in many situations, like in the evaluation of pain and psychological malaise, and in the estimation of symptom variation in patients with allergic rhinitis (Collins et al. 1997, Demoly et al. 2013, Jollant et al. 2019). One of its limitations is a lack of precision, as it does not describe the quality of symptoms. In the previous study, a composite clinical score was used in the evaluation of LNB patients' conditions, which estimated the magnitude of 6 subjective complaints and 16 objective findings (Ljøstad et al. 2008). The composite clinical score used in that study presents a more accurate description of a patient's condition, but on the other hand, it is more time-consuming, and the outcome might be influenced by the treating physician.

Strengths of our retrospective cohort study (III and IV) are its population-based view of disseminated LB cases, thorough follow-up of included patients and complete information on antimicrobial purchases. Because all disseminated LB cases should be referred to special healthcare at the hospital District of Helsinki and Uusimaa, and the Department of Infectious Diseases is the only place serving diagnostics and treatment for LB, our patient cohort should be quite comprehensive. Some cases might have been treated in primary healthcare or left unrecognized,

however. Access to medical records of other healthcare providers was also comprehensive, with the exception of some minor healthcare providers who refused to participate. These reasons lead to the assumption that the quality of our follow-up data is probably good.

We designed our own classification for the certainty of LB in studies III and IV, which can reduce the generalizability of the results. In previous studies, authors have used slightly different classifications (Coumou et al. 2015, Haddad et al. 2019, Jacquet et al. 2019, Kobayashi T. et al. 2019). For example, Haddad et al. classified a patient as confirmed LB if four criteria were met: tick exposure or bite, clinical signs typical of LB, positive *B. burgdorferi*–specific IgM or IgG test, and recovery after antibiotic treatment. A patient was categorized as possible LB if three of these criteria were met. Our classification differs from others, especially concerning the effect of antibody findings on classification. We evaluated the *B. burgdorferi*–specific IgM and IgG antibodies separately and emphasized the importance of IgG antibodies. Additionally, the categorization of antibody levels was novel and experimental, although we have clinical experience in interpreting antibody levels and the method was introduced previously (Smit et al. 2015). Because classification was done retrospectively, all gathered information, including response to antibiotics and received differential diagnoses, were evaluated in the procedure. We assumed this would lead to the best accuracy in classification of LB. Because the area of Helsinki and Uusimaa is endemic for LB, we considered all our patients to be exposed to ticks.

Studies III and IV have the same limitations as many other retrospective studies. We collected data on previous and novel conditions based on patients' signs and symptoms documented in medical records, and on the diagnostic conclusions by the physicians. We were unable to ensure the reliability of diagnoses, and some symptoms may have been underreported. This may lead to inaccuracy or even mistakes in some diagnoses which are assumed to be behind suspected LB symptoms. Additionally, medical records were reviewed by only one researcher which is, of course, a subjective procedure.

7 Conclusions

The main conclusions of the study:

I. CXCL13 is a useful tool in diagnostics of acute LNB. CXCL13 concentration is markedly elevated in acute LNB and decreases rapidly after treatment. CXCL13 is also practical in the evaluation of treatment response.

II. The most common manifestations of LNB are painful meningoradiculitis, facial nerve palsy and Garin-Bujadoux-Bannwarth syndrome. Typical subjective symptoms are headache, nonspecific neck-shoulder pain and fatigue. Headache and fatigue are also common symptoms in patients with possible or unlikely LB.

III. Doxycycline is equally effective as ceftriaxone in the treatment of LNB with meningitis and PNS manifestations.

IV. Approximately half of the patients with presumed LB actually have definite, probable or possible LB. Among patients with unlikely LB, 2/3 had another condition explaining the symptoms. The most typical underlying conditions are musculoskeletal, neurological, psychological, and functional disorders.

V. Patients with initially suspected, but subsequently excluded, LB use more healthcare services than patients with possible, probable or definite LB both before and after infectious disease specialist consultation. Utilization of antibiotics is similar among the patient groups of different LB certainty.

Acknowledgements

This study was carried out at the Department of Clinical Medicine, Faculty of Medicine, University of Turku, and at the Departments of Infectious Diseases, Turku University Hospital and Helsinki University Hospital. Financial support was received from the Turku Doctoral program in Clinical Research (DPCR), Inflammation Center Research Fund of Helsinki University Hospital, TYKS-säätiö, the Finnish Medical Foundation, the Finnish Medical Society Duodecim, the Biomedicum Helsinki Foundation, the Orion Research Foundation, and the Finnish Society for the Study of Infectious Diseases.

I would like to express my sincere gratitude to my supervisors Professor Jarmo Oksi and Assistant Professor Jukka Hytönen, who have supported and guided me from the very beginning. Jarmo offered the idea of the LNB trial, and he has taught me the clinical principles of the diagnosing and treating of LB. Jukka has been priceless in questions regarding laboratory diagnostics, and he has given directions how to write good scientific text. You have both been available for my many questions and encouraged me when necessary.

I am very grateful to my senior support at Helsinki University Hospital, Professor Asko Järvinen and Docent Mari Kanerva. Asko accepted me to specialize in infectious diseases, offered me a topic on retrospective LB study as well as his expertise during the study, and enabled this research in many ways. Mari, who originally saw the need for our study, has been my rock in many issues from clinical problems to scientific methods. Without you both, this study would never have been completed.

I sincerely want to acknowledge the reviewers Docent Heikki Kauma and Docent Sari Hämäläinen, whose constructive comments and suggestions for corrections were of great value and clearly improved the final form of this thesis.

I would like to thank my co-authors for their contribution in our research. Satu Kurkela, thank you for lessons in clinical microbiology and other interesting discussions in the laboratory. Juha Puustinen, Anneli Lauhio, Ulla Hohenthal, Päivi Jalava-Karvinen, Tuomas Nieminen, Taru Finnilä, Tony Häggblom, Johanna Vilhonen, and Minna Marttila-Vaara, I am grateful for your crucial assistance in patient recruiting and follow-up. Saija Hurme, Mari Koivisto, Laura Airas, Annukka

Pietikäinen, Matti Waris, and Jemiina Salo are thanked for the help and advice in biostatistics, laboratory methods, and neurology. All my co-authors have had an important impact on the completion of the studies and the content of the publications.

I wish to also thank the other members of the *Borrelia* research group, especially Julia Cuellar, Eeva Feuth, Otto Glader, Tuula Rantasalo, and Meri Rouhiainen, who have made me feel welcome on my visits to Turku and helped me with practical matters and scientific questions.

I warmly thank my wonderful colleagues at the Department of Infectious Diseases at Helsinki University Hospital for clinical, scientific and social support. I feel privileged to work and have lunch breaks with you.

For twenty years, my fabulous friends Ursus-tyttäret, Jenni Anttila, Miia Aro, Eeva Elamo, Pia Ettala, Salla Fagerrud, Roosa Lankinen, Kristiina Pälve, Erika Storm and Milla Ylijoki have been there to share both the joys and sorrows of life. Concerning research, you offer important different perspectives. I am grateful to have you in my life.

I express my deepest gratitude to my dear parents, Riitta Laajasuo and Kalle Lamminen, who gave me a happy childhood and encouraged me to study medicine.

My family, husband Tomi and lovely, active children Kaapo and Selja, thank you for being here. I love you.

Espoo, May 2021

Elisa Kortela

References

Aarli JA, Skeie GO, Mygland A, Gilhus NE. 1998. Muscle striation antibodies in myasthenia gravis. Diagnostic and functional significance. Ann N Y Acad Sci 841:505-515.

Ackermann R, Gollmer E, Rehse-Kupper B. 1985. [Progressive Borrelia encephalomyelitis. Chronic manifestation of erythema chronicum migrans disease of the nervous system]. Dtsch Med Wochenschr 110:1039–1042.

Ackermann R, Horstrup P, Schmidt R. 1984. Tick-borne meningopolyneuritis (Garin-Bujadoux, Bannwarth). Yale J Biol Med 57:485–490.

Adrion ER, Aucott J, Lemke KW, Weiner JP. 2015. Health care costs, utilization and patterns of care following Lyme disease. PLoS One 10:e0116767.

Afzelius A. 1920. Erythema chronicum migrans. Acta Dermato-venereologica 1:120-125.

Agger WA, Callister SM, Jobe DA. 1992. In vitro susceptibilities of Borrelia burgdorferi to five oral cephalosporins and ceftriaxone. Antimicrob Agents Chemother 36:1788-1790.

Agre F, Schwartz R. 1993. The value of early treatment of deer tick bites for the prevention of Lyme disease. Am J Dis Child 147:945-947.

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

Ali A, Vitulano L, Lee R, Weiss TR, Colson ER. 2014. Experiences of patients identifying with chronic Lyme disease in the healthcare system: a qualitative study. BMC Fam Pract 15:79.

Amsden GW. 2005. Anti-inflammatory effects of macrolides--an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? J Antimicrob Chemother 55:10-21.

Ang CW, Brandenburg AH, van Burgel ND, Bijlmer HA, Herremans T, Stelma F, Lunel FV, van Dam AP, Dutch Working Group on Diagnosis of Lyme B. 2015. A Dutch nationwide evaluation of serological assays for detection of Borrelia antibodies in clinically well-defined patients. Diagn Microbiol Infect Dis 83:222-228.

Ang CW, Notermans DW, Hommes M, Simoons-Smit AM, Herremans T. 2011. 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 30:1027-1032.

Arancibia A, Guttmann J, Gonzalez G, Gonzalez C. 1980. Absorption and disposition kinetics of amoxicillin in normal human subjects. Antimicrob Agents Chemother 17:199-202.

Arnez M, Pleterski-Rigler D, Ahcan J, Ruzic-Sabljic E, Strle F. 2001. Demographic features, clinical characteristics and laboratory findings in children with multiple erythema migrans in Slovenia. Wien Klin Wochenschr 113:98–101.

Arnold RW, Schriever G. 1993. Lyme amaurosis in a child. J Pediatr Ophthalmol Strabismus 30:268–270.

Arvikar SL, Steere AC. 2015. Diagnosis and treatment of Lyme arthritis. Infect Dis Clin North Am 29:269–280.

Asbrink E, Hovmark A. 1988. Early and late cutaneous manifestations in Ixodes-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). Ann N Y Acad Sci 539:4–15.

Asbrink E, Hovmark A, Olsson I. 1986. Clinical manifestations of acrodermatitis chronica atrophicans in 50 Swedish patients. Zentralbl Bakteriol Mikrobiol Hyg A 263:253-261.

Ates L, Hanssen-Hubner C, Norris DE, Richter D, Kraiczy P, Hunfeld KP. 2010. Comparison of in vitro activities of tigecycline, doxycycline, and tetracycline against the spirochete Borrelia burgdorferi. Ticks Tick Borne Dis 1:30-34.

Aucott J, Morrison C, Munoz B, Rowe PC, Schwarzwalder A, West SK. 2009. Diagnostic challenges of early Lyme disease: lessons from a community case series. BMC Infect Dis 9:79.

Aucott JN, Soloski MJ, Rebman AW, Crowder LA, Lahey LJ, Wagner CA, Robinson WH, Bechtold KT. 2016. CCL19 as a Chemokine Risk Factor for Posttreatment Lyme Disease Syndrome: a Prospective Clinical Cohort Study. Clin Vaccine Immunol 23:757-766.

Babady NE, Sloan LM, Vetter EA, Patel R, Binnicker MJ. 2008. Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue. Diagn Microbiol Infect Dis 62:464-466.

Bacon RM, Kugeler KJ, Mead PS, (CDC) CfDCaP. 2008. Surveillance for Lyme disease--United States, 1992–2006. MMWR Surveill Summ 57:1-9.

Bakken JS, Bruun JN, Gaustad P, Tasker TC. 1986. Penetration of amoxicillin and potassium clavulanate into the cerebrospinal fluid of patients with inflamed meninges. Antimicrob Agents Chemother 30:481-484.

Baranton G, Assous M, Postic D. 1992. [Three bacterial species associated with Lyme borreliosis. CLinical and diagnostic implications]. Bull Acad Natl Med 176:1075-1085; discussion 1085–1076.

Bateman H, Sigal L. 2000. Update on Lyme Carditis. Curr Infect Dis Rep 2:299–301.

Batheja S, Nields JA, Landa A, Fallon BA. 2013. Post-treatment lyme syndrome and central sensitization. J Neuropsychiatry Clin Neurosci 25:176-186.

Baugh RF, et al. 2013. Clinical practice guideline: Bell's palsy. Otolaryngol Head Neck Surg 149:S1–27.

Bennet L, Halling A, Berglund J. 2006. Increased incidence of Lyme borreliosis in southern Sweden following mild winters and during warm, humid summers. Eur J Clin Microbiol Infect Dis 25:426-432.

Benninger F, Steiner I. 2017. CSF in acute and chronic infectious diseases. Handb Clin Neurol 146:187–206.

Berende A, ter Hofstede HJ, Vos FJ, van Middendorp H, Vogelaar ML, Tromp M, van den Hoogen FH, Donders AR, Evers AW, Kullberg BJ. 2016. Randomized Trial of Longer-Term Therapy for Symptoms Attributed to Lyme Disease. N Engl J Med 374:1209–1220.

Berger BW. 1989. Dermatologic manifestations of Lyme disease. Rev Infect Dis 11 Suppl 6:S1475–1481.

Bergloff J, Gasser R, Feigl B. 1994. Ophthalmic manifestations in Lyme borreliosis. A review. J Neuroophthalmol 14:15–20.

Berglund J, Eitrem R, Ornstein K, Lindberg A, Ringér A, Elmrud H, Carlsson M, Runehagen A, Svanborg C, Norrby R. 1995. An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med 333:1319–1327.

Beuchat I, Dunet V, Meylan P, Du Pasquier R. 2018. Late Lyme neuroborreliosis with chronic encephalomyelitis. Neurology 91:627-628.

Blanc F, Jaulhac B, Fleury M, de Seze J, de Martino SJ, Remy V, Blaison G, Hansmann Y, Christmann D, Tranchant C. 2007. Relevance of the antibody index to diagnose Lyme neuroborreliosis among seropositive patients. Neurology 69:953-958.

Blanton LS. 2019. The Rickettsioses: A Practical Update. Infect Dis Clin North Am 33:213–229.

Boerner J, Failing K, Wittenbrink MM. 1995. In vitro antimicrobial susceptibility testing of Borrelia burgdorferi: influence of test conditions on minimal inhibitory concentration (MIC) values. Zentralbl Bakteriol 283:49-60.

Borg R, Dotevall L, Hagberg L, Maraspin V, Lotric-Furlan S, Cimperman J, Strle F. 2005. Intravenous ceftriaxone compared with oral doxycycline for the treatment of Lyme neuroborreliosis. Scand J Infect Dis 37:449-454.

Borsic K, Blagus R, Cerar T, Strle F, Stupica D. 2018. Clinical Course, Serologic Response, and Long-Term Outcome in Elderly Patients with Early Lyme Borreliosis. J Clin Med 7(12):506.

Boulanger N, Boyer P, Talagrand-Reboul E, Hansmann Y. 2019. Ticks and tick-borne diseases. Med Mal Infect 49:87–97.

Brehmer-Andersson E, Hovmark A, Asbrink E. 1998. Acrodermatitis chronica atrophicans: histopathologic findings and clinical correlations in 111 cases. Acta Derm Venereol 78:207-213.

Bremell D, Dotevall L. 2014. Oral doxycycline for Lyme neuroborreliosis with symptoms of encephalitis, myelitis, vasculitis or intracranial hypertension. Eur J Neurol 21:1162-1167.

Bremell D, Mattsson N, Edsbagge M, Blennow K, Andreasson U, Wikkelsö C, Zetterberg H, Hagberg L. 2013. Cerebrospinal fluid CXCL13 in Lyme neuroborreliosis and asymptomatic HIV infection. BMC Neurol 13:2.

Briggs GG. 2002. Drug effects on the fetus and breast-fed infant. Clin Obstet Gynecol 45:6-21.

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. 1982. Lyme disease-a tick-borne spirochetosis? Science 216:1317-1319.

Burkhard PR, Burkhardt K, Haenggeli CA, Landis T. 1999. Plant-induced seizures: reappearance of an old problem. J Neurol 246:667-670.

Cairns V, Godwin J. 2005. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. Int J Epidemiol 34:1340-1345.

Cardenas-de la Garza JA, De la Cruz-Valadez E, Ocampo-Candiani J, Welsh O. 2019. Clinical spectrum of Lyme disease. Eur J Clin Microbiol Infect Dis 38:201-208.

Carvounis PE, Mehta AP, Geist CE. 2004. Orbital myositis associated with Borrelia burgdorferi (Lyme disease) infection. Ophthalmology 111:1023-1028.

Casado MJ. 1975. Doxycycline in respiratory tract infections. Report of a retrospective study in Spain during the winter 1972-1973. Chemotherapy 21 Suppl 1:76-90.

CDC. 2017. Lyme Disease (Borrelia Burgdorferi) 2017 Case Definition. (https://wwwn.cdc.gov/nndss/conditions/lyme-disease/case-definition/2017/)

CDC. 2019. Lyme Disease Maps: Most Recent Year. (https://www.cdc.gov/lyme/datasurveillance/maps-recent.html)

Cepok S, Zhou D, Vogel F, Rosche B, Grummel V, Sommer N, Hemmer B. 2003. The immune response at onset and during recovery from Borrelia burgdorferi meningoradiculitis. Arch Neurol 60:849-855.

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

Chandra A, Wormser GP, Klempner MS, Trevino RP, Crow MK, Latov N, Alaedini A. 2010. Anti-neural antibody reactivity in patients with a history of Lyme borreliosis and persistent symptoms. Brain Behav Immun 24:1018-1024.

Charles V, Duprez TP, Kabamba B, Ivanoiu A, Sindic CJ. 2007. Poliomyelitis-like syndrome with matching magnetic resonance features in a case of Lyme neuroborreliosis. J Neurol Neurosurg Psychiatry 78:1160-1161.

Chiodi F, Albert J, Olausson E, Norkrans G, Hagberg L, Sonnerborg A, Asjo B, Fenyo EM. 1988. Isolation frequency of human immunodeficiency virus from cerebrospinal fluid and blood of patients with varying severity of HIV infection. AIDS Res Hum Retroviruses 4:351-358.

Cimmino MA. 1998. Relative frequency of Lyme borreliosis and of its clinical manifestations in Europe. European Community Concerted Action on Risk Assessment in Lyme Borreliosis. Infection 26:298-300.

Clark JR, Carlson RD, Sasaki CT, Pachner AR, Steere AC. 1985. Facial paralysis in Lyme disease. Laryngoscope 95:1341–1345.

Collares-Pereira M, Couceiro S, Franca I, Kurtenbach K, Schafer SM, Vitorino L, Goncalves L, Baptista S, Vieira ML, Cunha C. 2004. First isolation of Borrelia lusitaniae from a human patient. J Clin Microbiol 42:1316-1318.

Collins SL, Moore RA, McQuay HJ. 1997. The visual analogue pain intensity scale: what is moderate pain in millimetres? Pain 72:95-97.

Costello CM, Steere AC, Pinkerton RE, Feder HM, Jr. 1989. A prospective study of tick bites in an endemic area for Lyme disease. J Infect Dis 159:136-139.

Cottle LE, Mekonnen E, Beadsworth MB, Miller AR, Beeching NJ. 2012. Lyme disease in a British referral clinic. QJM 105:537-543.

Coumou J, et al. 2015. Ticking the right boxes: classification of patients suspected of Lyme borreliosis at an academic referral center in the Netherlands. Clin Microbiol Infect 21:368 e311-320.

Coumou J, Hovius JW, van Dam AP. 2014. Borrelia burgdorferi sensu lato serology in the Netherlands: guidelines versus daily practice. Eur J Clin Microbiol Infect Dis 33:1803-1808.

Criswell D, Tobiason VL, Lodmell JS, Samuels DS. 2006. Mutations conferring aminoglycoside and spectinomycin resistance in Borrelia burgdorferi. Antimicrob Agents Chemother 50:445-452.

Cross R, Ling C, Day NP, McGready R, Paris DH. 2016. Revisiting doxycycline in pregnancy and early childhood--time to rebuild its reputation? Expert Opin Drug Saf 15:367-382.

Cunha BA, Baron J, Cunha CB. 2018. Similarities and differences between doxycycline and minocycline: clinical and antimicrobial stewardship considerations. Eur J Clin Microbiol Infect Dis 37:15-20.

Cunha BA, Sibley CM, Ristuccia AM. 1982. Doxycycline. Ther Drug Monit 4:115–135.

Czeizel AE, Rockenbauer M. 1997. Teratogenic study of doxycycline. Obstet Gynecol 89:524–528.

Dankner WM, Connor JD, Sawyer M, Straube R, Spector SA. 1988. Treatment of bacterial meningitis with once daily ceftriaxone therapy. J Antimicrob Chemother 21:637-645.

Dattwyler RJ, Halperin JJ, Volkman DJ, Luft BJ. 1988. Treatment of late Lyme borreliosis--randomised comparison of ceftriaxone and penicillin. Lancet 1:1191-1194.

Dattwyler RJ, Volkman DJ, Conaty SM, Platkin SP, Luft BJ. 1990. Amoxycillin plus probenecid versus doxycycline for treatment of erythema migrans borreliosis. Lancet 336:1404-1406.

de Almeida JR, et al. 2014. Management of Bell palsy: clinical practice guideline. CMAJ 186:917-922.

De Silva AM, Fikrig E. 1995. Growth and migration of Borrelia burgdorferi in Ixodes ticks during blood feeding. Am J Trop Med Hyg 53:397-404.

de Velde F, de Winter BC, Koch BC, van Gelder T, Mouton JW, consortium C-N. 2016. Non-linear absorption pharmacokinetics of amoxicillin: consequences for dosing regimens and clinical breakpoints. J Antimicrob Chemother 71:2909-2917.

Decazes JM, Bure A, Wolff M, Kitzis MD, Pangon B, Modai J. 1987. Bactericidal activity against Haemophilus influenzae of cerebrospinal fluid of patients given amoxicillin-clavulanic acid. Antimicrob Agents Chemother 31:2018-2019.

Demoly P, Bousquet PJ, Mesbah K, Bousquet J, Devillier P. 2013. Visual analogue scale in patients treated for allergic rhinitis: an observational prospective study in primary care: asthma and rhinitis. Clin Exp Allergy 43:881-888.

Dersch R, Freitag MH, Schmidt S, Sommer H, Rauer S, Meerpohl JJ. 2015a. Efficacy and safety of pharmacological treatments for acute Lyme neuroborreliosis - a systematic review. Eur J Neurol 22:1249-1259.

Dersch R, Hottenrott T, Senel M, Lehmensiek V, Tumani H, Rauer S, Stich O. 2015b. The chemokine CXCL13 is elevated in the cerebrospinal fluid of patients with neurosyphilis. Fluids Barriers CNS 12:12.

Dersch R, Sommer H, Rauer S, Meerpohl JJ. 2016. Prevalence and spectrum of residual symptoms in Lyme neuroborreliosis after pharmacological treatment: a systematic review. J Neurol 263:17-24.

Dessau RB, et al. 2018. 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 24:118-124.

Dever LL, Jorgensen JH, Barbour AG. 1992. In vitro antimicrobial susceptibility testing of Borrelia burgdorferi: a microdilution MIC method and time-kill studies. J Clin Microbiol 30:2692-2697.

Dever LL, Jorgensen JH, Barbour AG. 1993. Comparative in vitro activities of clarithromycin, azithromycin, and erythromycin against Borrelia burgdorferi. Antimicrob Agents Chemother 37:1704-1706.

Di Stefano A, et al. 2020. Cerebrospinal fluid biomarkers in patients with central nervous system infections: a retrospective study. CNS Spectr 25:402-408.

Dinerman H, Steere AC. 1992. Lyme disease associated with fibromyalgia. Ann Intern Med 117:281–285.

Dotevall L, Fuchs D, Reibnegger G, Wachter H, Hagberg L. 1990. Cerebrospinal fluid and serum neopterin levels in patients with Lyme neuroborreliosis. Infection 18:210–214.

Dotevall L, Hagberg L. 1989. Penetration of doxycycline into cerebrospinal fluid in patients treated for suspected Lyme neuroborreliosis. Antimicrob Agents Chemother 33:1078-1080.

Dotevall L, Hagberg L. 1999. Successful oral doxycycline treatment of Lyme disease-associated facial palsy and meningitis. Clin Infect Dis 28:569-574.

Drugline. 1992. Nr 08853 1992-09-24.

Eldin C, Raffetin A, Bouiller K, Hansmann Y, Roblot F, Raoult D, Parola P. 2019. Review of European and American guidelines for the diagnosis of Lyme borreliosis. Med Mal Infect 49:121-132.

Embers ME, Narasimhan S. 2013. Vaccination against Lyme disease: past, present, and future. Front Cell Infect Microbiol 3:6.

Estrada-Pena A. 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. Vet Parasitol 98:111-132.

Estrada-Pena A, de la Fuente J. 2014. The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Res 108:104-128.

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

Feder HM, Jr., et al. 2007. A critical appraisal of "chronic Lyme disease". N Engl J Med 357:1422-1430.

Feder HM, Jr., Whitaker DL. 1995. Misdiagnosis of erythema migrans. Am J Med 99:412-419.

Fekety FR. 1990. Safety of parenteral third-generation cephalosporins. Am J Med 88:38S-44S.

Feuth E, Virtanen M, Helve O, Hytonen J, Sane J. 2020. Lyme borreliosis in Finland: a register-based linkage study. BMC Infect Dis 20:819.

Figoni J, et al. 2019. Lyme borreliosis and other tick-borne diseases. Guidelines from the French Scientific Societies (I): prevention, epidemiology, diagnosis. Med Mal Infect 49:318-334.

Finn A, Straughn A, Meyer M, Chubb J. 1987. Effect of dose and food on the bioavailability of cefuroxime axetil. Biopharm Drug Dispos 8:519-526.

Fischer L, Korfel A, Pfeiffer S, Kiewe P, Volk HD, Cakiroglu H, Widmann T, Thiel E. 2009. CXCL13 and CXCL12 in central nervous system lymphoma patients. Clin Cancer Res 15:5968-5973.

Fish AE, Pride YB, Pinto DS. 2008. Lyme carditis. Infect Dis Clin North Am 22:275-288, vi.

Fitzgerald BL, et al. 2020. Metabolic Response in Patients with Post-Treatment Lyme Disease Symptoms/Syndrome. Clin Infect Dis. doi: 10.1093/cid/ciaa1455. Online ahead of print.

Forrester JD, Mead P. 2014. Third-degree heart block associated with lyme carditis: review of published cases. Clin Infect Dis 59:996-1000.

Forrester JD, et al. 2014. Notes from the field: update on Lyme carditis, groups at high risk, and frequency of associated sudden cardiac death--United States. MMWR Morb Mortal Wkly Rep 63:982-983.

Foulds G, Shepard RM, Johnson RB. 1990. The pharmacokinetics of azithromycin in human serum and tissues. J Antimicrob Chemother 25 Suppl A:73-82.

Francioli P, Ruch W, Stamboulian D. 1995. Treatment of streptococcal endocarditis with a single daily dose of ceftriaxone and netilmicin for 14 days: a prospective multicenter study. Clin Infect Dis 21:1406-1410.

Franco MP, Mulder M, Gilman RH, Smits HL. 2007. Human brucellosis. Lancet Infect Dis 7:775–786.
Galbraith KM, Ng AC, Eggers BJ, Kuchel CR, Eggers CH, Samuels DS. 2005. parC mutations in fluoroquinolone-resistant Borrelia burgdorferi. Antimicrob Agents Chemother 49:4354-4357.
Garkowski A, Zajkowska J, Zajkowska A, Kulakowska A, Zajkowska O, Kubas B, Jurgilewicz D, Hladunski M, Lebkowska U. 2017. Cerebrovascular Manifestations of Lyme Neuroborreliosis-A Systematic Review of Published Cases. Front Neurol 8:146.
Garro A, Nigrovic LE. 2018. Managing Peripheral Facial Palsy. Ann Emerg Med 71:618–624.
Gilson J, Khalighi K, Elmi F, Krishnamurthy M, Talebian A, Toor RS. 2017. Lyme disease presenting with facial palsy and myocarditis mimicking myocardial infarction. J Community Hosp Intern Med Perspect 7:363-365.
Girard YA, Fedorova N, Lane RS. 2011. Genetic diversity of Borrelia burgdorferi and detection of B. bissettii-like DNA in serum of north-coastal California residents. J Clin Microbiol 49:945-954.
Gooch WM, 3rd, Blair E, Puopolo A, Paster RZ, Schwartz RH, Miller HC, Smyre HL, Yetman R, Giguere GG, Collins JJ. 1996. Effectiveness of five days of therapy with cefuroxime axetil suspension for treatment of acute otitis media. Pediatr Infect Dis J 15:157-164.
Goodlet KJ, Fairman KA. 2018. Adverse Events Associated With Antibiotics and Intravenous Therapies for Post-Lyme Disease Syndrome in a Commercially Insured Sample. Clin Infect Dis 67:1568-1574.
Gray J, Zintl A, Hildebrandt A, Hunfeld KP, Weiss L. 2010. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. Ticks Tick Borne Dis 1:3-10.
Grillon A, et al. 2019. Characteristics and clinical outcomes after treatment of a national cohort of PCR-positive Lyme arthritis. Semin Arthritis Rheum 48:1105-1112.
Gronseth GS, Paduga R, American Academy of N. 2012. Evidence-based guideline update: steroids and antivirals for Bell palsy: report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 79:2209-2213.
Gudowska-Sawczuk M, Mroczko B. 2020. Chemokine Ligand 13 (CXCL13) in Neuroborreliosis and Neurosyphilis as Selected Spirochetal Neurological Diseases: A Review of Its Diagnostic Significance. Int J Mol Sci 21.
Haddad E, Chabane K, Jaureguiberry S, Monsel G, Pourcher V, Caumes E. 2019. Holistic Approach in Patients With Presumed Lyme Borreliosis Leads to Less Than 10% of Confirmation and More Than 80% of Antibiotic Failures. Clin Infect Dis 68:2060-2066.
Hadrup N, Lam HR. 2014. Oral toxicity of silver ions, silver nanoparticles and colloidal silver--a review. Regul Toxicol Pharmacol 68:1-7.
Hagberg L, Cinque P, Gisslen M, Brew BJ, Spudich S, Bestetti A, Price RW, Fuchs D. 2010. Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. AIDS Res Ther 7:15.
Hagberg L, Dotevall L, Norkrans G, Larsson M, Wachter H, Fuchs D. 1993. Cerebrospinal fluid neopterin concentrations in central nervous system infection. J Infect Dis 168:1285–1288.
Hakanen A, Jalava J, Kaartinen L. 2017:4. Mikrobilääkeresistenssin torjunnan kansallinen toimintaohjelma 2017–2021. Sosiaali- ja terveysministeriö.
Halperin JJ. 2003. Lyme disease and the peripheral nervous system. Muscle Nerve 28:133–143.
Halperin JJ. 2015. Nervous system Lyme disease. Infect Dis Clin North Am 29:241–253.
Hammers-Berggren S, Hansen K, Lebech AM, Karlsson M. 1993. Borrelia burgdorferi-specific intrathecal antibody production in neuroborreliosis: a follow-up study. Neurology 43:169-175.
Hansen K. 1994. Lyme neuroborreliosis: improvements of the laboratory diagnosis and a survey of epidemiological and clinical features in Denmark 1985-1990. Acta Neurol Scand Suppl 151:1-44.
Hansen K, Asbrink E. 1989. Serodiagnosis of erythema migrans and acrodermatitis chronica atrophicans by the Borrelia burgdorferi flagellum enzyme-linked immunosorbent assay. J Clin Microbiol 27:545-551.
Hansen K, Hindersson P, Pedersen NS. 1988. Measurement of antibodies to the Borrelia burgdorferi flagellum improves serodiagnosis in Lyme disease. J Clin Microbiol 26:338-346.

Hansen K, Lebech AM. 1991. Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal synthesis of Borrelia burgdorferi--specific immunoglobulin G, A, and M. Ann Neurol 30:197-205.

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

Harms M, Hofhuis A, Sprong H, Bennema S, Ferreira J, Fonville M, Docters van Leeuwen A, Assendelft W, Van Weert H, Van Pelt W, Van den Wijngaard C. 2021. A single dose of doxycycline after an ixodes ricinus tick bite to prevent Lyme borreliosis: An open-label randomized controlled trial. J Infect 82(1):98-104.

Harrer T, Geissdorfer W, Schoerner C, Lang E, Helm G. 2007. Seronegative Lyme neuroborreliosis in a patient on treatment for chronic lymphatic leukemia. Infection 35:110-113.

Hassler D, Zoller L, Haude M, Hufnagel HD, Heinrich F, Sonntag HG. 1990. Cefotaxime versus penicillin in the late stage of Lyme disease--prospective, randomized therapeutic study. Infection 18:16-20.

Hays R. 1998. RAND-36 health status inventory. The Psychological Corporation.

Heaton PC, Fenwick SR, Brewer DE. 2007. Association between tetracycline or doxycycline and hepatotoxicity: a population based case-control study. J Clin Pharm Ther 32:483-487.

Heim-Duthoy KL, Caperton EM, Pollock R, Matzke GR, Enthoven D, Peterson PK. 1990. Apparent biliary pseudolithiasis during ceftriaxone therapy. Antimicrob Agents Chemother 34:1146-1149.

Henningsson AJ, Malmvall BE, Ernerudh J, Matussek A, Forsberg P. 2010. Neuroborreliosis--an epidemiological, clinical and healthcare cost study from an endemic area in the south-east of Sweden. Clin Microbiol Infect 16:1245-1251.

Henry DC, Sydnor A, Jr., Settipane GA, Allen J, Burroughs S, Cobb MM, Holley HP, Jr. 1999. Comparison of cefuroxime axetil and amoxicillin/clavulanate in the treatment of acute bacterial sinusitis. Clin Ther 21:1158-1170.

Hinckley AF, Connally NP, Meek JI, Johnson BJ, Kemperman MM, Feldman KA, White JL, Mead PS. 2014. Lyme disease testing by large commercial laboratories in the United States. Clin Infect Dis 59:676-681.

Hirsch AG, Poulsen MN, Nordberg C, Moon KA, Rebman AW, Aucott JN, Heaney CD, Schwartz BS. 2020. Risk Factors and Outcomes of Treatment Delays in Lyme Disease: A Population-Based Retrospective Cohort Study. Front Med (Lausanne) 7:560018.

Hitt E. 2002. Poor sales trigger vaccine withdrawal. Nat Med 8:311–312.

Hofhuis A, Harms M, Bennema S, van den Wijngaard CC, van Pelt W. 2015. Physician reported incidence of early and late Lyme borreliosis. Parasit Vectors 8:161.

Hofmann H, Fingerle V, Hunfeld KP, Huppertz HI, Krause A, Rauer S, Ruf B, Consensus g. 2017. Cutaneous Lyme borreliosis: Guideline of the German Dermatology Society. Ger Med Sci 15:Doc14.

Hohman MH, Hadlock TA. 2014. Etiology, diagnosis, and management of facial palsy: 2000 patients at a facial nerve center. Laryngoscope 124:E283-293.

Holak H, Holak N, Huzarska M, Holak S. 2006. Tick inoculation in an eyelid region: report on five cases with one complication of the orbital myositis associated with Lyme borreliosis. Klin Oczna 108:220–224.

Holmgren AR, Matteson EL. 2006. Lyme myositis. Arthritis Rheum 54:2697–2700.

Holt SC. 1978. Anatomy and chemistry of spirochetes. Microbiol Rev 42:114–160.

Holzbauer SM, Kemperman MM, Lynfield R. 2010. Death due to community-associated Clostridium difficile in a woman receiving prolonged antibiotic therapy for suspected lyme disease. Clin Infect Dis 51:369-370.

Horne HW, Jr., Kundsin RB. 1980. The role of mycoplasma among 81 consecutive pregnancies: a prospective study. Int J Fertil 25:315-317.

Horowitz HW, Sanghera K, Goldberg N, Pechman D, Kamer R, Duray P, Weinstein A. 1994. Dermatomyositis associated with Lyme disease: case report and review of Lyme myositis. Clin Infect Dis 18:166-171.

Hovmark A, Asbrink E, Olsson I. 1986. The spirochetal etiology of lymphadenosis benigna cutis solitaria. Acta Derm Venereol 66:479-484.

Hu R, Lu C, Lu S, Hu Y, Ma H, Lai W, Zhu G, Feng P, Lu R, Li Y. 2016. Value of CXCL13 in diagnosing asymptomatic neurosyphilis in HIV-infected patients. Int J STD AIDS 27:141-146.

Hunfeld KP, Brade V. 2006. Antimicrobial susceptibility of Borrelia burgdorferi sensu lato: what we know, what we don't know, and what we need to know. Wien Klin Wochenschr 118:659-668.

Hunfeld KP, Kraiczy P. 2009. When is the best time to order a Western blot and how should it be interpreted? Curr Probl Dermatol 37:167-177.

Hunfeld KP, Kraiczy P, Kekoukh E, Schafer V, Brade V. 2002. Standardised in vitro susceptibility testing of Borrelia burgdorferi against well-known and newly developed antimicrobial agents--possible implications for new therapeutic approaches to Lyme disease. Int J Med Microbiol 291 Suppl 33:125-137.

Hunfeld KP, Kraiczy P, Wichelhaus TA, Schafer V, Brade V. 2000a. Colorimetric in vitro susceptibility testing of penicillins, cephalosporins, macrolides, streptogramins, tetracyclines, and aminoglycosides against Borrelia burgdorferi isolates. Int J Antimicrob Agents 15:11-17.

Hunfeld KP, Kraiczy P, Wichelhaus TA, Schafer V, Brade V. 2000b. New colorimetric microdilution method for in vitro susceptibility testing of Borrelia burgdorferi against antimicrobial substances. Eur J Clin Microbiol Infect Dis 19:27-32.

Hunfeld KP, Ruzic-Sabljic E, Norris DE, Kraiczy P, Strle F. 2005. In vitro susceptibility testing of Borrelia burgdorferi sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial chemotherapy. Antimicrob Agents Chemother 49:1294-1301.

Hunfeld KP, Weigand J, Wichelhaus TA, Kekoukh E, Kraiczy P, Brade V. 2001. In vitro activity of mezlocillin, meropenem, aztreonam, vancomycin, teicoplanin, ribostamycin and fusidic acid against Borrelia burgdorferi. Int J Antimicrob Agents 17:203-208.

Hunfeld KP, Wichelhaus TA, Rodel R, Acker G, Brade V, Kraiczy P. 2004. Comparison of in vitro activities of ketolides, macrolides, and an azalide against the spirochete Borrelia burgdorferi. Antimicrob Agents Chemother 48:344-347.

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

HUS. 2020. Mikrobilääkehoito-opas. (https://www.hus.fi/sites/default/files/2020-10/Mikrobil%C3%A4%C3%A4kehoito-opas.pdf)

Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, Jarlier V, Voss A, Pittet D. 2013. Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrob Resist Infect Control 2:31.

Hyatt JM, McKinnon PS, Zimmer GS, Schentag JJ. 1995. The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. Clin Pharmacokinet 28:143-160.

Ivacic L, Reed KD, Mitchell PD, Ghebranious N. 2007. A LightCycler TaqMan assay for detection of Borrelia burgdorferi sensu lato in clinical samples. Diagn Microbiol Infect Dis 57:137-143.

Jaaskelainen AE, Tikkakoski T, Uzcategui NY, Alekseev AN, Vaheri A, Vapalahti O. 2006. Siberian subtype tickborne encephalitis virus, Finland. Emerg Infect Dis 12:1568-1571.

Jacquet C, et al. 2019. Multidisciplinary management of patients presenting with Lyme disease suspicion. Med Mal Infect 49:112-120.

Jaenson TG, Varv K, Frojdman I, Jaaskelainen A, Rundgren K, Versteirt V, Estrada-Pena A, Medlock JM, Golovljova I. 2016. First evidence of established populations of the taiga tick Ixodes persulcatus (Acari: Ixodidae) in Sweden. Parasit Vectors 9:377.

Jarius S, Haas J, Paul F, Wildemann B. 2019. Myelinoclastic diffuse sclerosis (Schilder's disease) is immunologically distinct from multiple sclerosis: results from retrospective analysis of 92 lumbar punctures. J Neuroinflammation 16:51.

Jaruratanasirikul S, Hortiwakul R, Tantisarasart T, Phuenpathom N, Tussanasunthornwong S. 1996. Distribution of azithromycin into brain tissue, cerebrospinal fluid, and aqueous humor of the eye. Antimicrob Agents Chemother 40:825-826.

Jaulhac B, et al. 2019. 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 49:335-346.

Johnson RC, Kodner C, Russell M. 1987. In vitro and in vivo susceptibility of the Lyme disease spirochete, Borrelia burgdorferi, to four antimicrobial agents. Antimicrob Agents Chemother 31:164-167.

Johnson SE, Klein GC, Schmid GP, Feeley JC. 1984. Susceptibility of the Lyme disease spirochete to seven antimicrobial agents. Yale J Biol Med 57:549-553.

Jollant F, Voegeli G, Kordsmeier NC, Carbajal JM, Richard-Devantoy S, Turecki G, Caceda R. 2019. A visual analog scale to measure psychological and physical pain: A preliminary validation of the PPP-VAS in two independent samples of depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 90:55-61.

Jones KL, Muellegger RR, Means TK, Lee M, Glickstein LJ, Damle N, Sikand VK, Luster AD, Steere AC. 2008. Higher mRNA levels of chemokines and cytokines associated with macrophage activation in erythema migrans skin lesions in patients from the United States than in patients from Austria with Lyme borreliosis. Clin Infect Dis 46:85-92.

Jowett N, Gaudin RA, Banks CA, Hadlock TA. 2017. Steroid use in Lyme disease-associated facial palsy is associated with worse long-term outcomes. Laryngoscope 127:1451-1458.

Kaiser R. 1994. Variable CSF findings in early and late Lyme neuroborreliosis: a follow-up study in 47 patients. J Neurol 242:26-36.

Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. 2001a. Evaluation of study patients with Lyme disease, 10–20-year follow-up. J Infect Dis 183:453-460.

Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. 2001b. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10–20 years after active Lyme disease. Clin Infect Dis 33:780-785.

Kaplan RF, Trevino RP, Johnson GM, Levy L, Dornbush R, Hu LT, Evans J, Weinstein A, Schmid CH, Klempner MS. 2003. Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology 60:1916-1922.

Karkkonen K, Stiernstedt SH, Karlsson M. 2001. Follow-up of patients treated with oral doxycycline for Lyme neuroborreliosis. Scand J Infect Dis 33:259-262.

Karlsson M, Hammers-Berggren S, Lindquist L, Stiernstedt G, Svenungsson B. 1994. Comparison of intravenous penicillin G and oral doxycycline for treatment of Lyme neuroborreliosis. Neurology 44:1203-1207.

Karlsson M, Hammers S, Nilsson-Ehle I, Malmborg AS, Wretlind B. 1996. Concentrations of doxycycline and penicillin G in sera and cerebrospinal fluid of patients treated for neuroborreliosis. Antimicrob Agents Chemother 40:1104-1107.

Kindstrand E, Nilsson BY, Hovmark A, Pirskanen R, Asbrink E. 1997. Peripheral neuropathy in acrodermatitis chronica atrophicans - a late Borrelia manifestation. Acta Neurol Scand 95:338-345.

Klein NC, Cunha BA. 1995. Tetracyclines. Med Clin North Am 79:789–801.

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

Knudtzen FC, Andersen NS, Jensen TG, Skarphédinsson S. 2017. Characteristics and Clinical Outcome of Lyme Neuroborreliosis in a High Endemic Area, 1995-2014: A Retrospective Cohort Study in Denmark. Clin Infect Dis 65:1489-1495.

Kobayashi H. 1995. Biofilm disease: its clinical manifestation and therapeutic possibilities of macrolides. Am J Med 99:26S-30S.

Kobayashi T, Higgins Y, Samuels R, Moaven A, Sanyal A, Yenokyan G, Lantos PM, Melia MT, Auwaerter PG. 2019. Misdiagnosis of Lyme Disease With Unnecessary Antimicrobial Treatment Characterizes Patients Referred to an Academic Infectious Diseases Clinic. Open Forum Infect Dis 6.

Koedel U, Fingerle V, Pfister HW. 2015. Lyme neuroborreliosis-epidemiology, diagnosis and management. Nat Rev Neurol 11:446-456.

Koene R, Boulware DR, Kemperman M, Konety SH, Groth M, Jessurun J, Eckman PM. 2012. Acute heart failure from lyme carditis. Circ Heart Fail 5:e24-26.

Kohler J, Kasper J, Kern U, Thoden U, Rehse-Kupper B. 1986. Borrelia encephalomyelitis. Lancet 2:35.

Kohlhepp W, Oschmann P, Mertens HG. 1989. Treatment of Lyme borreliosis. Randomized comparison of doxycycline and penicillin G. J Neurol 236:464-469.

Konopka M, Kuch M, Braksator W, Walczak E, Jakucinski M, Lipowski D, Dluzniewski M. 2013. [Unclassified cardiomyopathy or Lyme carditis? A three year follow-up]. Kardiol Pol 71:283-285.

Kowalski TJ, Berth WL, Mathiason MA, Agger WA. 2011. Oral antibiotic treatment and long-term outcomes of Lyme facial nerve palsy. Infection 39:239-245.

Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. 2010. Antibiotic treatment duration and long-term outcomes of patients with early lyme disease from a lyme disease-hyperendemic area. Clin Infect Dis 50:512-520.

Kraiczy P, Weigand J, Wichelhaus TA, Heisig P, Backes H, Schafer V, Acker G, Brade V, Hunfeld KP. 2001. In vitro activities of fluoroquinolones against the spirochete Borrelia burgdorferi. 'Antimicrob Agents Chemother 45:2486-2494.

Kruger H, Reuss K, Pulz M, Rohrbach E, Pflughaupt KW, Martin R, Mertens HG. 1989. Meningoradiculitis and encephalomyelitis due to Borrelia burgdorferi: a follow-up study of 72 patients over 27 years. J Neurol 236:322-328.

Kullberg BJ, Vrijmoeth HD, van de Schoor F, Hovius JW. 2020. Lyme borreliosis: diagnosis and management. BMJ 369:m1041.

Laaksonen M, et al. 2018. Tick-borne pathogens in Finland: comparison of Ixodes ricinus and I. persulcatus in sympatric and parapatric areas. Parasit Vectors 11:556.

Laaksonen M, et al. 2017. Crowdsourcing-based nationwide tick collection reveals the distribution of Ixodes ricinus and I. persulcatus and associated pathogens in Finland. Emerg Microbes Infect 6:e31.

Lager M, et al. 2019. Serological diagnostics of Lyme borreliosis: comparison of assays in twelve clinical laboratories in Northern Europe. Eur J Clin Microbiol Infect Dis 38:1933-1945.

Lalor SJ, Segal BM. 2010. Lymphoid chemokines in the CNS. J Neuroimmunol 224:56–61.

Lamb HM, Ormrod D, Scott LJ, Figgitt DP. 2002. Ceftriaxone: an update of its use in the management of community-acquired and nosocomial infections. Drugs 62:1041–1089.

Lang CC, Moreland TA, Davey PG. 1990. Bioavailability of cefuroxime axetil: comparison of standard and abbreviated methods. J Antimicrob Chemother 25:645-650.

Lantos PM, et al. 2021. Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease. Clin Infect Dis 72:1–8.

Lenzlinger PM, Hans VH, Joller-Jemelka HI, Trentz O, Morganti-Kossmann MC, Kossmann T. 2001. Markers for cell-mediated immune response are elevated in cerebrospinal fluid and serum after severe traumatic brain injury in humans. J Neurotrauma 18:479-489.

Levin JM, Nelson JA, Segreti J, Harrison B, Benson CA, Strle F. 1993. In vitro susceptibility of Borrelia burgdorferi to 11 antimicrobial agents. Antimicrob Agents Chemother 37:1444-1446.

Li X, McHugh GA, Damle N, Sikand VK, Glickstein L, Steere AC. 2011. Burden and viability of Borrelia burgdorferi in skin and joints of patients with erythema migrans or lyme arthritis. Arthritis Rheum 63:2238-2247.

Lim DS, Murphy GM. 2003. High-level ultraviolet A photoprotection is needed to prevent doxycycline phototoxicity: lessons learned in East Timor. Br J Dermatol 149:213–214.

Lindgren E, Talleklint L, Polfeldt T. 2000. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick Ixodes ricinus. Environ Health Perspect 108:119-123.

Lindgren E, Jaenson TGT. 2006. Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. World Health Organization.

Liveris D, Schwartz I, Bittker S, Cooper D, Iyer R, Cox ME, Wormser GP. 2011. Improving the yield of blood cultures from patients with early Lyme disease. J Clin Microbiol 49:2166-2168.

Ljostad U, Skarpaas T, Mygland A. 2007. Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis. Eur J Neurol 14:873-876.

Ljøstad U, Skogvoll E, Eikeland R, Midgard R, Skarpaas T, Berg A, Mygland A. 2008. Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. Lancet Neurol 7:690–695.

Lode H, Borner K, Koeppe P, Schaberg T. 1996. Azithromycin--review of key chemical, pharmacokinetic and microbiological features. J Antimicrob Chemother 37 Suppl C:1-8.

Lode H, Magyar P, Muir JF, Loos U, Kleutgens K, International Gatifloxacin Study G. 2004. Once-daily oral gatifloxacin vs three-times-daily co-amoxiclav in the treatment of patients with community-acquired pneumonia. Clin Microbiol Infect 10:512-520.

Logigian EL, Kaplan RF, Steere AC. 1990. Chronic neurologic manifestations of Lyme disease. N Engl J Med 323:1438–1444.

Lohr B, Fingerle V, Norris DE, Hunfeld KP. 2018. Laboratory diagnosis of Lyme borreliosis: Current state of the art and future perspectives. Crit Rev Clin Lab Sci 55:219-245.

Lohr B, Muller I, Mai M, Norris DE, Schoffski O, Hunfeld KP. 2015. Epidemiology and cost of hospital care for Lyme borreliosis in Germany: lessons from a health care utilization database analysis. Ticks Tick Borne Dis 6:56-62.

Lomholt H, Lebech AM, Hansen K, Brandrup F, Halkier-Sorensen L. 2000. Long-term serological follow-up of patients treated for chronic cutaneous borreliosis or culture-positive erythema migrans. Acta Derm Venereol 80:362–366.

Lorentzen Å, et al. 2017. Lyme neuroborreliosis: do we treat according to guidelines? J Neurol 264:1506-1510.

Lovering AM, Pycock CJ, Harvey JE, Reeves DS. 1990. The pharmacokinetics and sputum penetration of ampicillin and amoxycillin following simultaneous i.v. administration. J Antimicrob Chemother 25:385-392.

Luft BJ, Dattwyler RJ, Johnson RC, Luger SW, Bosler EM, Rahn DW, Masters EJ, Grunwaldt E, Gadgil SD. 1996. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med 124:785-791.

Luger SW, Paparone P, Wormser GP, Nadelman RB, Grunwaldt E, Gomez G, Wisniewski M, Collins JJ. 1995. Comparison of cefuroxime axetil and doxycycline in treatment of patients with early Lyme disease associated with erythema migrans. Antimicrob Agents Chemother 39:661-667.

Luke DR, Foulds G. 1997. Disposition of oral azithromycin in humans. Clin Pharmacol Ther 61:641–648.

Macauda MM, Erickson P, Miller J, Mann P, Closter L, Krause PJ. 2011. Long-term Lyme disease antibiotic therapy beliefs among New England residents. Vector Borne Zoonotic Dis 11:857-862.

Macy E, Ngor EW. 2013. Safely diagnosing clinically significant penicillin allergy using only penicilloyl-poly-lysine, penicillin, and oral amoxicillin. J Allergy Clin Immunol Pract 1:258-263.

Maraspin V, Cimperman J, Lotric-Furlan S, Ruzic-Sabljic E, Jurca T, Picken RN, Strle F. 2002a. Solitary borrelial lymphocytoma in adult patients. Wien Klin Wochenschr 114:515-523.

Maraspin V, Cimperman J, Lotric-Furlan S, Ruzic-Sabljic E, Jurca T, Strle F. 2002b. Cerebrospinal fluid findings in adult patients with multiple erythema migrans. Wien Klin Wochenschr 114:505-509.

Marra CM, Tantalo LC, Sahi SK, Maxwell CL, Lukehart SA. 2010. CXCL13 as a cerebrospinal fluid marker for neurosyphilis in HIV-infected patients with syphilis. Sex Transm Dis 37:283–287.

Martin E, Koup JR, Paravicini U, Stoeckel K. 1984. Pharmacokinetics of ceftriaxone in neonates and infants with meningitis. J Pediatr 105:475-481.

Marzec NS, Nelson C, Waldron PR, Blackburn BG, Hosain S, Greenhow T, Green GM, Lomen-Hoerth C, Golden M, Mead PS. 2017. Serious Bacterial Infections Acquired During Treatment of Patients Given a Diagnosis of Chronic Lyme Disease - United States. MMWR Morb Mortal Wkly Rep 66:607-609.

Matei IA, Estrada-Pena A, Cutler SJ, Vayssier-Taussat M, Varela-Castro L, Potkonjak A, Zeller H, Mihalca AD. 2019. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. Parasit Vectors 12:599.

Mattingly TJ, 2nd, Shere-Wolfe K. 2020. Clinical and economic outcomes evaluated in Lyme disease: a systematic review. Parasit Vectors 13:341.

Matuschka FR, Fischer P, Heiler M, Richter D, Spielman A. 1992. Capacity of European animals as reservoir hosts for the Lyme disease spirochete. J Infect Dis 165:479-483.

May EF, Jabbari B. 1990. Stroke in neuroborreliosis. Stroke 21:1232-1235.

McAlister HF, Klementowicz PT, Andrews C, Fisher JD, Feld M, Furman S. 1989. Lyme carditis: an important cause of reversible heart block. Ann Intern Med 110:339-345.

Mead PS. 2015. Epidemiology of Lyme disease. Infect Dis Clin North Am 29:187-210.

Mendelson M, Morris AM, Thursky K, Pulcini C. 2020. How to start an antimicrobial stewardship programme in a hospital. Clin Microbiol Infect 26:447-453.

Meropol SB, Chan KA, Chen Z, Finkelstein JA, Hennessy S, Lautenbach E, Platt R, Schech SD, Shatin D, Metlay JP. 2008. Adverse events associated with prolonged antibiotic use. Pharmacoepidemiol Drug Saf 17:523-532.

Mikkila HO, Seppala IJ, Viljanen MK, Peltomaa MP, Karma A. 2000. The expanding clinical spectrum of ocular lyme borreliosis. Ophthalmology 107:581-587.

Mol PG, Rutten WJ, Gans RO, Degener JE, Haaijer-Ruskamp FM. 2004. Adherence barriers to antimicrobial treatment guidelines in teaching hospital, the Netherlands. Emerg Infect Dis 10:522-525.

Molloy PJ, Persing DH, Berardi VP. 2001. False-positive results of PCR testing for Lyme disease. Clin Infect Dis 33:412-413.

Moniuszko-Malinowska A, Czupryna P, Dunaj J, Pancewicz S, Garkowski A, Kondrusik M, Grygorczuk S, Zajkowska J. 2018. Acrodermatitis chronica atrophicans: various faces of the late form of Lyme borreliosis. Postepy Dermatol Alergol 35:490-494.

Moniuszko A, Czupryna P, Pancewicz S, Rutkowski K, Zajkowska O, Swierzbinska R, Grygorczuk S, Kondrusik M, Owlasiuk P, Zajkowska J. 2014. Evaluation of CXCL8, CXCL10, CXCL11, CXCL12 and CXCL13 in serum and cerebrospinal fluid of patients with neuroborreliosis. Immunol Lett 157:45-50.

Moore A, Nelson C, Molins C, Mead P, Schriefer M. 2016. Current Guidelines, Common Clinical Pitfalls, and Future Directions for Laboratory Diagnosis of Lyme Disease, United States. Emerg Infect Dis 22.

Moser B, Ebert L. 2003. Lymphocyte traffic control by chemokines: follicular B helper T cells. Immunol Lett 85:105-112.

Mouton JW, Vinks AA. 1996. Is continuous infusion of beta-lactam antibiotics worthwhile?--efficacy and pharmacokinetic considerations. J Antimicrob Chemother 38:5-15.

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

Mullegger RR, Glatz M. 2008. Skin manifestations of lyme borreliosis: diagnosis and management. Am J Clin Dermatol 9:355-368.

Muller DE, Itin PH, Buchner SA, Rufli T. 1994. Acrodermatitis chronica atrophicans involving the face. Evidence for Borrelia burgdorferi infection confirmed by DNA amplification. Dermatology 189:430-431.

Muller I, et al. 2012. Evaluating frequency, diagnostic quality, and cost of Lyme borreliosis testing in Germany: a retrospective model analysis. Clin Dev Immunol 2012:595427.

Murfin KE, Kleinbard R, Aydin M, Salazar SA, Fikrig E. 2019. Borrelia burgdorferi chemotaxis toward tick protein Salp12 contributes to acquisition. Ticks Tick Borne Dis 10:1124-1134.

Mursic VP, Wilske B, Schierz G, Holmburger M, Suss E. 1987. In vitro and in vivo susceptibility of Borrelia burgdorferi. Eur J Clin Microbiol 6:424-426.

Mygland A, Ljøstad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I, Societies EFoN. 2010. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol 17:8-16, e11-14.

Nadelman RB. 2015. Erythema migrans. Infect Dis Clin North Am 29:211-239.

Nadelman RB, Hanincova K, Mukherjee P, Liveris D, Nowakowski J, McKenna D, Brisson D, Cooper D, Bittker S, Madison G, Holmgren D, Schwartz I, Wormser GP. 2012. Differentiation of reinfection from relapse in recurrent Lyme disease. N Engl J Med 367:1883-1890.

Nadelman RB, Luger SW, Frank E, Wisniewski M, Collins JJ, Wormser GP. 1992. Comparison of cefuroxime axetil and doxycycline in the treatment of early Lyme disease. Ann Intern Med 117:273-280.

Nadelman RB, et al. 2001. Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an Ixodes scapularis tick bite. N Engl J Med 345:79-84.

Nadelman RB, Nowakowski J, Forseter G, Goldberg NS, Bittker S, Cooper D, Aguero-Rosenfeld M, Wormser GP. 1996. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. Am J Med 100:502-508.

Nadelman RB, Wormser GP. 1995. Erythema migrans and early Lyme disease. Am J Med 98:15S-23S; discussion 23S-24S.

Nakamura S. 2020. Spirochete Flagella and Motility. Biomolecules 10.

Nassal M, Skamel C, Vogel M, Kratz PA, Stehle T, Wallich R, Simon MM. 2008. Development of hepatitis B virus capsids into a whole-chain protein antigen display platform: new particulate Lyme disease vaccines. Int J Med Microbiol 298:135-142.

Nau R, Prange HW, Muth P, Mahr G, Menck S, Kolenda H, Sorgel F. 1993. Passage of cefotaxime and ceftriaxone into cerebrospinal fluid of patients with uninflamed meninges. Antimicrob Agents Chemother 37:1518-1524.

Nau R, Sorgel F, Eiffert H. 2010. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev 23:858-883.

Nelson C, Elmendorf S, Mead P. 2015a. Neoplasms misdiagnosed as "chronic lyme disease". JAMA Intern Med 175:132-133.

Nelson CA, Saha S, Kugeler KJ, Delorey MJ, Shankar MB, Hinckley AF, Mead PS. 2015b. Incidence of Clinician-Diagnosed Lyme Disease, United States, 2005-2010. Emerg Infect Dis 21:1625-1631.

Nemeth J, et al. 2016. Update of the Swiss guidelines on post-treatment Lyme disease syndrome. Swiss Med Wkly 146:w14353.

Nieto JC, Kim N, Lucarelli MJ. 2008. Dacryoadenitis and orbital myositis associated with lyme disease. Arch Ophthalmol 126:1165-1166.

Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. 1994. Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med 330:229–234.

Nordberg CL, et al. 2020. Lyme neuroborreliosis in adults: A nationwide prospective cohort study. Ticks Tick Borne Dis 11:101411.

Nowakowski J, McKenna D, Nadelman RB, Bittker S, Cooper D, Pavia C, Holmgren D, Visintainer P, Wormser GP. 2009. Blood cultures for patients with extracutaneous manifestations of Lyme disease in the United States. Clin Infect Dis 49:1733-1735.

O'Connell S, Granstrom M, Gray JS, Stanek G. 1998. Epidemiology of European Lyme borreliosis. Zentralbl Bakteriol 287:229-240.

Ogden NH, Maarouf A, Barker IK, Bigras-Poulin M, Lindsay LR, Morshed MG, O'Callaghan C J, Ramay F, Waltner-Toews D, Charron DF. 2006. Climate change and the potential for range expansion of the Lyme disease vector Ixodes scapularis in Canada. Int J Parasitol 36:63-70.

Ogrinc K, Logar M, Lotric-Furlan S, Cerar D, Ruzic-Sabljic E, Strle F. 2006. Doxycycline versus ceftriaxone for the treatment of patients with chronic Lyme borreliosis. Wien Klin Wochenschr 118:696-701.

Ogrinc K, Lotrič-Furlan S, Maraspin V, Lusa L, Cerar T, Ružič-Sabljič E, Strle F. 2013. Suspected early Lyme neuroborreliosis in patients with erythema migrans. Clin Infect Dis 57:501-509.

Ogrinc K, Lusa L, Lotrič-Furlan S, Bogovič P, Stupica D, Cerar T, Ružić-Sabljić E, Strle F. 2016. Course and Outcome of Early European Lyme Neuroborreliosis (Bannwarth Syndrome): Clinical and Laboratory Findings. Clin Infect Dis 63:346-353.

Ogrinc K, et al. 2017. Pathogenetic implications of the age at time of diagnosis and skin location for acrodermatitis chronica atrophicans. Ticks Tick Borne Dis 8:266-269.

Oksi J, Marjamäki M, Nikoskelainen J, Viljanen MK. 1999. Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis. Ann Med 31:225–232.

Oksi J, Mertsola J, Reunanen M, Marjamäki M, Viljanen MK. 1994. Subacute multiple-site osteomyelitis caused by Borrelia burgdorferi. Clin Infect Dis 19:891-896.

Oksi J, et al. 2007. Duration of antibiotic treatment in disseminated Lyme borreliosis: a double-blind, randomized, placebo-controlled, multicenter clinical study. Eur J Clin Microbiol Infect Dis 26:571-581.

Oksi J. and Koulu, L. 2017. Lymen borrelioosin ilmentymät iholla. Lääkärilehti 39:2153–2159.

Oliver JH, Jr., Owsley MR, Hutcheson HJ, James AM, Chen C, Irby WS, Dotson EM, McLain DK. 1993. Conspecificity of the ticks Ixodes scapularis and I. dammini (Acari: Ixodidae). J Med Entomol 30:54-63.

Oschmann P, Dorndorf W, Hornig C, Schäfer C, Wellensiek HJ, Pflughaupt KW. 1998. Stages and syndromes of neuroborreliosis. J Neurol 245:262-272.

Pages F, Dautel H, Duvallet G, Kahl O, de Gentile L, Boulanger N. 2014. Tick repellents for human use: prevention of tick bites and tick-borne diseases. Vector Borne Zoonotic Dis 14:85-93.

Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. 2014. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacol Ther 143:225-245.

Parola P, et al. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev 26:657-702.

Peitersen E. 1982. The natural history of Bell's palsy. Am J Otol 4:107-111.

Perry CM, Brogden RN. 1996. Cefuroxime axetil. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. Drugs 52:125-158.

Perry TR, Schentag JJ. 2001. Clinical use of ceftriaxone: a pharmacokinetic-pharmacodynamic perspective on the impact of minimum inhibitory concentration and serum protein binding. Clin Pharmacokinet 40:685-694.

Pessey JJ, Gehanno P, Thoroddsen E, Dagan R, Leibovitz E, Machac J, Pimentel JM, Marr C, Leblanc F. 1999. Short course therapy with cefuroxime axetil for acute otitis media: results of a randomized multicenter comparison with amoxicillin/clavulanate. Pediatr Infect Dis J 18:854-859.

Peter L, Jung J, Tilikete C, Ryvlin P, Mauguiere F. 2006. Opsoclonus-myoclonus as a manifestation of Lyme disease. J Neurol Neurosurg Psychiatry 77:1090-1091.

Pfister HW, Einhaupl K, Preac-Mursic V, Wilske B, Schierz G. 1984. The spirochetal etiology of lymphocytic meningoradiculitis of Bannwarth (Bannwarth's syndrome). J Neurol 231:141-144.

Pfister HW, Preac-Mursic V, Wilske B, Einhaupl KM. 1989. Cefotaxime vs penicillin G for acute neurologic manifestations in Lyme borreliosis. A prospective randomized study. Arch Neurol 46:1190-1194.

Pfister HW, Preac-Mursic V, Wilske B, Schielke E, Sorgel F, Einhaupl KM. 1991. Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis. J Infect Dis 163:311-318.

Pfister HW, Rupprecht TA. 2006. Clinical aspects of neuroborreliosis and post-Lyme disease syndrome in adult patients. Int J Med Microbiol 296 Suppl 40:11-16.

Pharmaca Fennica. 2008. Pharmaca Fennica: Pharmaceutical Information Centre. Rocephalin.

Phillips CJ, Gilchrist M, Cooke FJ, Franklin BD, Enoch DA, Murphy ME, Santos R, Brannigan ET, Holmes AH. 2019. Adherence to antibiotic guidelines and reported penicillin allergy: pooled cohort data on prescribing and allergy documentation from two English National Health Service (NHS) trusts. BMJ Open 9:e026624.

Picha D, Moravcova L, Smiskova D. 2016. Prospective study on the chemokine CXCL13 in neuroborreliosis and other aseptic neuroinfections. J Neurol Sci 368:214-220.

Picken RN, Cheng Y, Strle F, Picken MM. 1996. Patient isolates of Borrelia burgdorferi sensu lato with genotypic and phenotypic similarities of strain 25015. J Infect Dis 174:1112-1115.

Picken RN, Strle F, Ruzic-Sabljic E, Maraspin V, Lotric-Furlan S, Cimperman J, Cheng Y, Picken MM. 1997. Molecular subtyping of Borrelia burgdorferi sensu lato isolates from five patients with solitary lymphocytoma. J Invest Dermatol 108:92-97.

Piesman J, Gern L. 2004. Lyme borreliosis in Europe and North America. Parasitology 129 Suppl:S191-220.

Pietikainen A, Oksi J, Hytonen J. 2018. Point-of-care testing for CXCL13 in Lyme neuroborreliosis. Diagn Microbiol Infect Dis 91:226-228.

Pigrau C, Pahissa A, Gropper S, Sureda D, Martinez Vazquez JM. 1989. Ceftriaxone-associated biliary pseudolithiasis in adults. Lancet 2:165.

Poyhonen H, Lahdesmaki T, Hytonen J, Peltola V. 2019. Cerebrospinal Fluid Pleocytosis and Elevated C-X-C Motif Chemokine Ligand 13 Value Predict Lyme Borreliosis in Children With Facial Palsy. Pediatr Infect Dis J 38:1195-1198.

Preac-Mursic V, Weber K, Pfister HW, Wilske B, Gross B, Baumann A, Prokop J. 1989. Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis. Infection 17:355-359.

Price JR, Mitchell E, Tidy E, Hunot V. 2008. Cognitive behaviour therapy for chronic fatigue syndrome in adults. Cochrane Database Syst Rev:CD001027.

Puius YA, Kalish RA. 2008. Lyme arthritis: pathogenesis, clinical presentation, and management. Infect Dis Clin North Am 22:289-300, vi-vii.

Raffetin A, et al. 2020. Unconventional diagnostic tests for Lyme borreliosis: a systematic review. Clin Microbiol Infect 26:51-59.

Raja H, Starr MR, Bakri SJ. 2016. Ocular manifestations of tick-borne diseases. Surv Ophthalmol 61:726-744.

Randolph SE. 2011. Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm. Ticks Tick Borne Dis 2:179-182.

Randolph SE, Gern L, Nuttall PA. 1996. Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission. Parasitol Today 12:472-479.

Rauer S, Kastenbauer S, Hofmann H, Fingerle V, Huppertz HI, Hunfeld KP, Krause A, Ruf B, Dersch R, Consensus g. 2020. Guidelines for diagnosis and treatment in neurology - Lyme neuroborreliosis. Ger Med Sci 18:Doc03.

Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. 2012. Azithromycin and the risk of cardiovascular death. N Engl J Med 366:1881-1890.

Reed MD. 1996. Clinical pharmacokinetics of amoxicillin and clavulanate. Pediatr Infect Dis J 15:949-954.

Reid MC, Schoen RT, Evans J, Rosenberg JC, Horwitz RI. 1998. The consequences of overdiagnosis and overtreatment of Lyme disease: an observational study. Ann Intern Med 128:354–362.

Reimers CD, de Koning J, Neubert U, Preac-Mursic V, Koster JG, Muller-Felber W, Pongratz DE, Duray PH. 1993. Borrelia burgdorferi myositis: report of eight patients. J Neurol 240:278-283.

Richards DM, Heel RC, Brogden RN, Speight TM, Avery GS. 1984. Ceftriaxone. A review of its antibacterial activity, pharmacological properties and therapeutic use. Drugs 27:469-527.

Rijpkema SG, Tazelaar DJ, Molkenboer MJ, Noordhoek GT, Plantinga G, Schouls LM, Schellekens JF. 1997. Detection of Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia garinii and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol Infect 3:109-116.

Rizzoli A, Hauffe H, Carpi G, Vourc HG, Neteler M, Rosa R. 2011. Lyme borreliosis in Europe. Euro Surveill 16.

Robinson ML, Kobayashi T, Higgins Y, Calkins H, Melia MT. 2015. Lyme carditis. Infect Dis Clin North Am 29:255–268.

Roche. 2000. Rocephin (ceftriaxone sodium) for injection. Roche US Pharmaceuticals. Report no.

Rodel R, Freyer A, Bittner T, Schafer V, Hunfeld KP. 2007. In vitro activities of faropenem, ertapenem, imipenem and meropenem against Borrelia burgdorferi s.l. Int J Antimicrob Agents 30:83-86.

Ross Russell AL, Dryden MS, Pinto AA, Lovett JK. 2018. Lyme disease: diagnosis and management. Pract Neurol 18:455–464.

Rubenstein JL, et al. 2013. CXCL13 plus interleukin 10 is highly specific for the diagnosis of CNS lymphoma. Blood 121:4740-4748.

Rudenko N, Golovchenko M, Mokracek A, Piskunova N, Ruzek D, Mallatova N, Grubhoffer L. 2008. Detection of Borrelia bissettii in cardiac valve tissue of a patient with endocarditis and aortic valve stenosis in the Czech Republic. J Clin Microbiol 46:3540-3543.

Rupprecht TA, Kirschning CJ, Popp B, Kastenbauer S, Fingerle V, Pfister HW, Koedel U. 2007. Borrelia garinii induces CXCL13 production in human monocytes through Toll-like receptor 2. Infect Immun 75:4351–4356.

Rupprecht TA, Koedel U, Angele B, Fingerle V, Pfister HW. 2006. [Cytokine CXCL13--a possible early CSF marker for neuroborreliosis]. Nervenarzt 77:470-473.

Rupprecht TA, Manz KM, Fingerle V, Lechner C, Klein M, Pfirrmann M, Koedel U. 2018. Diagnostic value of cerebrospinal fluid CXCL13 for acute Lyme neuroborreliosis. A systematic review and meta-analysis. Clin Microbiol Infect 24:1234-1240.

Rupprecht TA, Pfister HW. 2009. What are the indications for lumbar puncture in patients with Lyme disease? Curr Probl Dermatol 37:200-206.

Rupprecht TA, Pfister HW, Angele B, Kastenbauer S, Wilske B, Koedel U. 2005. The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis. Neurology 65:448-450.

Rupprecht TA, Plate A, Adam M, Wick M, Kastenbauer S, Schmidt C, Klein M, Pfister HW, Koedel U. 2009. The chemokine CXCL13 is a key regulator of B cell recruitment to the cerebrospinal fluid in acute Lyme neuroborreliosis. J Neuroinflammation 6:42.

Ruskoaho H, Hakkola J, Huupponen R, Kantele A, Korpi ER, Moilanen E, Piepponen P, Savontaus E, Tenhunen O, Vähäkangas K. 2014. Lääketieteellinen farmakologia ja toksikologia. Duodecim.

Russo V, Puzio G, Siniscalchi N. 2006. Azithromycin-induced QT prolongation in elderly patient. Acta Biomed 77:30–32.

Ruzic-Sabljic E, Podreka T, Maraspin V, Strle F. 2005. Susceptibility of Borrelia afzelii strains to antimicrobial agents. Int J Antimicrob Agents 25:474-478.

Ryan MF, Thorn C. 2013. Lyme carditis in an immunocompromised patient. Case Rep Emerg Med 2013:380734.

Ryffel K, Peter O, Rutti B, Suard A, Dayer E. 1999. Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of Borrelia burgdorferi sensu stricto, B. garinii, B. afzelii, and B. Valaisiana in humans. J Clin Microbiol 37:4086-4092.

Sacco KA, Bates A, Brigham TJ, Imam JS, Burton MC. 2017. Clinical outcomes following inpatient penicillin allergy testing: A systematic review and meta-analysis. Allergy 72:1288–1296.

Sajanti E, Virtanen M, Helve O, Kuusi M, Lyytikäinen O, Hytönen J, Sane J. 2017. Lyme Borreliosis in Finland, 1995-2014. Emerg Infect Dis 23:1282-1288.

Sanchez E, Vannier E, Wormser GP, Hu LT. 2016. Diagnosis, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: A Review. JAMA 315:1767-1777.

Sanchez Navarro A. 2005. New formulations of amoxicillin/clavulanic acid: a pharmacokinetic and pharmacodynamic review. Clin Pharmacokinet 44:1097-1115.

Schmidt C, Plate A, Angele B, Pfister HW, Wick M, Koedel U, Rupprecht TA. 2011. A prospective study on the role of CXCL13 in Lyme neuroborreliosis. Neurology 76:1051-1058.

Schotthoefer AM, Frost HM. 2015. Ecology and Epidemiology of Lyme Borreliosis. Clin Lab Med 35:723–743.

Schwenkenbecher P, et al. 2017. Common and uncommon neurological manifestations of neuroborreliosis leading to hospitalization. BMC Infect Dis 17:90.

Scott LJ, Ormrod D, Goa KL. 2001. Cefuroxime axetil: an updated review of its use in the management of bacterial infections. Drugs 61:1455-1500.

Senel M, Rupprecht TA, Tumani H, Pfister HW, Ludolph AC, Brettschneider J. 2010. The chemokine CXCL13 in acute neuroborreliosis. J Neurol Neurosurg Psychiatry 81:929-933.

Sexton DJ, Tenenbaum MJ, Wilson WR, Steckelberg JM, Tice AD, Gilbert D, Dismukes W, Drew RH, Durack DT. 1998. Ceftriaxone once daily for four weeks compared with ceftriaxone plus gentamicin once daily for two weeks for treatment of endocarditis due to penicillin-susceptible streptococci. Endocarditis Treatment Consortium Group. Clin Infect Dis 27:1470-1474.

Shamim EA, Shamim SA, Liss G, Nylen E, Pincus JH, Yepes M. 2005. Constipation heralding neuroborreliosis: an atypical tale of 2 patients. Arch Neurol 62:671-673.

Shapiro ED, Gerber MA, Holabird NB, Berg AT, Feder HM, Jr., Bell GL, Rys PN, Persing DH. 1992. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. N Engl J Med 327:1769-1773.

Shepard RM, Falkner FC. 1990. Pharmacokinetics of azithromycin in rats and dogs. J Antimicrob Chemother 25 Suppl A:49-60.

Sicklinger M, Wienecke R, Neubert U. 2003. In vitro susceptibility testing of four antibiotics against Borrelia burgdorferi: a comparison of results for the three genospecies Borrelia afzelii, Borrelia garinii, and Borrelia burgdorferi sensu stricto. J Clin Microbiol 41:1791-1793.

Smit PW, Kurkela S, Kuusi M, Vapalahti O. 2015. Evaluation of two commercially available rapid diagnostic tests for Lyme borreliosis. Eur J Clin Microbiol Infect Dis 34:109-113.

Smith K, Leyden JJ. 2005. Safety of doxycycline and minocycline: a systematic review. Clin Ther 27:1329-1342.

Socolovschi C, Mediannikov O, Raoult D, Parola P. 2009. Update on tick-borne bacterial diseases in Europe. Parasite 16:259–273.

Sokolov AA, Lienhard R, Du Pasquier R, Erard V. 2015. Acute Lyme Neuroborreliosis With Transient Hemiparesis and Aphasia. Ann Emerg Med 66:60–64.

Solheim AM, Ljostad U, Mygland A. 2019. Six versus two weeks treatment with doxycycline in Lyme neuroborreliosis: the protocol of a multicentre, non-inferiority, double-blinded and randomised controlled trial. BMJ Open 9:e027083.

Sormunen JJ, Klemola T, Vesterinen EJ, Vuorinen I, Hytonen J, Hanninen J, Ruohomaki K, Saaksjarvi IE, Tonteri E, Penttinen R. 2016. Assessing the abundance, seasonal questing activity, and Borrelia and tick-borne encephalitis virus (TBEV) prevalence of Ixodes ricinus ticks in a Lyme borreliosis endemic area in Southwest Finland. Ticks Tick Borne Dis 7:208-215.

Stanek G, et al. 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 17:69-79.

Stanek G, Kahl O. 1999. Chemoprophylaxis for Lyme borreliosis? Zentralbl Bakteriol 289:655-665.

Stanek G, O'Connell S, Cimmino M, Aberer E, Kristoferitsch W, Granstrom M, Guy E, Gray J. 1996. European Union Concerted Action on Risk Assessment in Lyme Borreliosis: clinical case definitions for Lyme borreliosis. Wien Klin Wochenschr 108:741-747.

Stanek G, Strle F. 2003. Lyme borreliosis. Lancet 362:1639-1647.

Stanek G, Strle F. 2008. Lyme disease: European perspective. Infect Dis Clin North Am 22:327-339, vii.
Stanek G, Wormser GP, Gray J, Strle F. 2012. Lyme borreliosis. Lancet 379:461-473.
Stanek S, Strle F, Gray J, Wormser G. 2002. History and characteristics of Lyme borreliosis. Lyme borreliosis: biology, epidemiology and control.
Staniforth DH, Jackson D, Clarke HL, Horton R. 1983. Amoxycillin/clavulanic acid: the effect of probenecid. J Antimicrob Chemother 12:273-275.
Steere AC. 2001. Lyme disease. N Engl J Med 345:115-125.
Steere AC, Arvikar SL. 2015. Editorial commentary: what constitutes appropriate treatment of post-Lyme disease symptoms and other pain and fatigue syndromes? Clin Infect Dis 60:1783-1785.
Steere AC, Bartenhagen NH, Craft JE, Hutchinson GJ, Newman JH, Rahn DW, Sigal LH, Spieler PN, Stenn KS, Malawista SE. 1983. The early clinical manifestations of Lyme disease. Ann Intern Med 99:76–82.
Steere AC, Batsford WP, Weinberg M, Alexander J, Berger HJ, Wolfson S, Malawista SE. 1980. Lyme carditis: cardiac abnormalities of Lyme disease. Ann Intern Med 93:8-16.
Steere AC, Broderick TF, Malawista SE. 1978. Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am J Epidemiol 108:312-321.
Steere AC, Duray PH, Kauffmann DJ, Wormser GP. 1985. Unilateral blindness caused by infection with the Lyme disease spirochete, Borrelia burgdorferi. Ann Intern Med 103:382-384.
Steere AC, Levin RE, Molloy PJ, Kalish RA, Abraham JH, 3rd, Liu NY, Schmid CH. 1994. Treatment of Lyme arthritis. Arthritis Rheum 37:878-888.
Steere AC, Malawista SE, Hardin JA, Ruddy S, Askenase W, Andiman WA. 1977a. Erythema chronicum migrans and Lyme arthritis. The enlarging clinical spectrum. Ann Intern Med 86:685-698.
Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, Steele FM. 1977b. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum 20:7-17.
Steere AC, Schoen RT, Taylor E. 1987. The clinical evolution of Lyme arthritis. Ann Intern Med 107:725-731.
Steere AC, Sikand VK. 2003. The presenting manifestations of Lyme disease and the outcomes of treatment. N Engl J Med 348:2472-2474.
Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, Li X, Mead PS. 2016. Lyme borreliosis. Nat Rev Dis Primers 2:16090.
Steere AC, Taylor E, McHugh GL, Logigian EL. 1993. The overdiagnosis of Lyme disease. JAMA 269:1812-1816.
Sternbach G, Dibble CL. 1996. Willy Burgdorfer: Lyme disease. J Emerg Med 14:631-634.
Strle F, Maraspin V, Furlan-Lotric S, Cimperman J. 1996a. Epidemiological study of a cohort of adult patients with Erythema migrans registered in Slovenia in 1993. Eur J Epidemiol 12:503-507.
Strle F, et al. 1999. Comparison of culture-confirmed erythema migrans caused by Borrelia burgdorferi sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann Intern Med 130:32-36.
Strle F, Nelson JA, Ruzic-Sabljic E, Cimperman J, Maraspin V, Lotric-Furlan S, Cheng Y, Picken MM, Trenholme GM, Picken RN. 1996b. European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. Clin Infect Dis 23:61-65.
Strle F, Picken RN, Cheng Y, Cimperman J, Maraspin V, Lotric-Furlan S, Ruzic-Sabljic E, Picken MM. 1997. Clinical findings for patients with Lyme borreliosis caused by Borrelia burgdorferi sensu lato with genotypic and phenotypic similarities to strain 25015. Clin Infect Dis 25:273-280.
Strle F, Videcnik J, Zorman P, Cimperman J, Lotric-Furlan S, Maraspin V. 2002. Clinical and epidemiological findings for patients with erythema migrans. Comparison of cohorts from the years 1993 and 2000. Wien Klin Wochenschr 114:493-497.
Strle K, Strle F. 2020. Posttreatment Symptoms in Lyme Borreliosis. Clin Infect Dis.

Strle K, Stupica D, Drouin EE, Steere AC, Strle F. 2014. Elevated levels of IL-23 in a subset of patients with post-lyme disease symptoms following erythema migrans. Clin Infect Dis 58:372–380.

Strnad M, Honig V, Ruzek D, Grubhoffer L, Rego ROM. 2017. Europe-Wide Meta-Analysis of Borrelia burgdorferi Sensu Lato Prevalence in Questing Ixodes ricinus Ticks. Appl Environ Microbiol 83.

Stupica D, Veluscek M, Blagus R, Bogovic P, Rojko T, Cerar T, Strle F. 2018. Oral doxycycline versus intravenous ceftriaxone for treatment of multiple erythema migrans: an open-label alternate-treatment observational trial. J Antimicrob Chemother 73:1352-1358.

Sumner AJ. 1990. Aberrant reinnervation. Muscle Nerve 13:801-803.

Talleklint L, Jaenson TG. 1993. Maintenance by hares of European Borrelia burgdorferi in ecosystems without rodents. J Med Entomol 30:273-276.

Tateda K, Comte R, Pechere JC, Kohler T, Yamaguchi K, Van Delden C. 2001. Azithromycin inhibits quorum sensing in Pseudomonas aeruginosa. Antimicrob Agents Chemother 45:1930-1933.

Terekhova D, Sartakova ML, Wormser GP, Schwartz I, Cabello FC. 2002. Erythromycin resistance in Borrelia burgdorferi. Antimicrob Agents Chemother 46:3637-3640.

Thanassi WT, Schoen RT. 2000. The Lyme disease vaccine: conception, development, and implementation. Ann Intern Med 132:661–668.

THL. 2019. Tartuntatautien tilastotietokanta: Terveyden ja Hyvinvoinnin Laitos. https://sampo.thl.fi/pivot/prod/fi/ttr/shp/fact_shp?row=area-12260&column=time-12059&filter=reportgroup-12465

THL. 2020. Borrelioosin seuranta ja esiintyvyys Suomessa. (https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/taudit-ja-torjunta/taudit-ja-taudinaiheuttajat-a-o/borrelia/borrelioosin-seuranta-ja-esiintyvyys-suomessa)

Tjernberg I, Henningsson AJ, Eliasson I, Forsberg P, Ernerudh J. 2011. Diagnostic performance of cerebrospinal fluid chemokine CXCL13 and antibodies to the C6-peptide in Lyme neuroborreliosis. J Infect 62:149-158.

Torbahn G, Hofmann H, Rucker G, Bischoff K, Freitag MH, Dersch R, Fingerle V, Motschall E, Meerpohl JJ, Schmucker C. 2018. Efficacy and Safety of Antibiotic Therapy in Early Cutaneous Lyme Borreliosis: A Network Meta-analysis. JAMA Dermatol 154:1292–1303.

Tucker MH, Lomas CM, Ramchandar N, Waldram JD. 2017. Amoxicillin challenge without penicillin skin testing in evaluation of penicillin allergy in a cohort of Marine recruits. J Allergy Clin Immunol Pract 5:813-815.

Tuddenham S, Ghanem KG. 2018. Neurosyphilis: Knowledge Gaps and Controversies. Sex Transm Dis 45:147-151.

Turnidge JD. 1998. The pharmacodynamics of beta-lactams. Clin Infect Dis 27:10-22.

Ulu-Kilic A, Doganay M. 2014. An overview: tularemia and travel medicine. Travel Med Infect Dis 12:609-616.

Uzun S, Djamin RS, Kluytmans JA, Mulder PG, van't Veer NE, Ermens AA, Pelle AJ, Hoogsteden HC, Aerts JG, van der Eerden MM. 2014. Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2:361-368.

van Beek J, Sajanti E, Helve O, Ollgren J, Virtanen MJ, Rissanen H, Lyytikainen O, Hytonen J, Sane J. 2018. Population-based Borrelia burgdorferi sensu lato seroprevalence and associated risk factors in Finland. Ticks Tick Borne Dis 9:275-280.

van Burgel ND, Bakels F, Kroes AC, van Dam AP. 2011. Discriminating Lyme neuroborreliosis from other neuroinflammatory diseases by levels of CXCL13 in cerebrospinal fluid. J Clin Microbiol 49:2027-2030.

van Dam AP. 2011. Molecular diagnosis of Borrelia bacteria for the diagnosis of Lyme disease. Expert Opin Med Diagn 5:135-149.

van den Wijngaard CC, Hofhuis A, Wong A, Harms MG, de Wit GA, Lugner AK, Suijkerbuijk AWM, Mangen MJ, van Pelt W. 2017. The cost of Lyme borreliosis. Eur J Public Health 27:538-547.

van Dop WA, Kersten MJ, de Wever B, Hovius JW. 2013. Seronegative lyme neuroborreliosis in a patient using rituximab. BMJ Case Rep 2013.

Veinovic G, Cerar T, Strle F, Lotric-Furlan S, Maraspin V, Cimperman J, Ruzic-Sabljic E. 2013. In vitro susceptibility of European human Borrelia burgdorferi sensu stricto strains to antimicrobial agents. Int J Antimicrob Agents 41:288-291.

Veluscek M, Bajrovic FF, Strle F, Stupica D. 2018. Doxycycline-induced photosensitivity in patients treated for erythema migrans. BMC Infect Dis 18:365.

Verdon ME, Sigal LH. 1997. Recognition and management of Lyme disease. Am Fam Physician 56:427-436, 439-440.

Veve MP, Stuart M, Davis SL. 2019. Comparison of Neutropenia Associated with Ceftaroline or Ceftriaxone in Patients Receiving at Least 7 Days of Therapy for Severe Infections. Pharmacotherapy 39:809-815.

Viaccoz A, et al. 2015. CSF neopterin level as a diagnostic marker in primary central nervous system lymphoma. Neuro Oncol 17:1497-1503.

Viljanen MK, Oksi J, Salomaa P, Skurnik M, Peltonen R, Kalimo H. 1992. Cultivation of Borrelia burgdorferi from the blood and a subcutaneous lesion of a patient with relapsing febrile nodular nonsuppurative panniculitis. J Infect Dis 165:596-597.

Viljanen MK, Punnonen J. 1989. The effect of storage of antigen-coated polystyrene microwells on the detection of antibodies against Borrelia burgdorferi by enzyme immunoassay (EIA). J Immunol Methods 124:137-141.

Vlay SC, Dervan JP, Elias J, Kane PP, Dattwyler R. 1991. Ventricular tachycardia associated with Lyme carditis. Am Heart J 121:1558–1560.

Voordouw MJ. 2015. Co-feeding transmission in Lyme disease pathogens. Parasitology 142:290–302.

Waddell LA, Greig J, Mascarenhas M, Harding S, Lindsay R, Ogden N. 2016. The Accuracy of Diagnostic Tests for Lyme Disease in Humans, A Systematic Review and Meta-Analysis of North American Research. PLoS One 11:e0168613.

Wagner JN, Weis S, Kubasta C, Panholzer J, von Oertzen TJ. 2018. CXCL13 as a diagnostic marker of neuroborreliosis and other neuroinflammatory disorders in an unselected group of patients. J Neurol 265:74–81.

Wang G, van Dam AP, Schwartz I, Dankert J. 1999. Molecular typing of Borrelia burgdorferi sensu lato: taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev 12:633-653.

Warshafsky S, Nowakowski J, Nadelman RB, Kamer RS, Peterson SJ, Wormser GP. 1996. Efficacy of antibiotic prophylaxis for prevention of Lyme disease. J Gen Intern Med 11:329-333.

Webber BJ, Burganowski RP, Colton L, Escobar JD, Pathak SR, Gambino-Shirley KJ. 2019. Lyme disease overdiagnosis in a large healthcare system: a population-based, retrospective study. Clin Microbiol Infect 25:1233-1238.

Weber K, Neubert U. 1986. Clinical features of early erythema migrans disease and related disorders. Zentralbl Bakteriol Mikrobiol Hyg A 263:209-228.

Weisbord SD, Soule JB, Kimmel PL. 1997. Poison on line--acute renal failure caused by oil of wormwood purchased through the Internet. N Engl J Med 337:825-827.

Weismeier K, Adam D, Heilmann HD, Koeppe P. 1989. Penetration of amoxycillin/clavulanate into human bone. J Antimicrob Chemother 24 Suppl B:93-100.

Wetzig P. 1957. Aberrant regeneration of oculomotor and facial nerves. Rocky Mt Med J 54:347-348.

WHO. 2020. Antimicrobial resistance. (https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance)

Widner B, Leblhuber F, Fuchs D. 2002. Increased neopterin production and tryptophan degradation in advanced Parkinson's disease. J Neural Transm (Vienna) 109:181-189.

Widney DP, Breen EC, Boscardin WJ, Kitchen SG, Alcantar JM, Smith JB, Zack JA, Detels R, Martinez-Maza O. 2005. Serum levels of the homeostatic B cell chemokine, CXCL13, are elevated during HIV infection. J Interferon Cytokine Res 25:702-706.

Wilhelmsson P, Lindblom P, Fryland L, Ernerudh J, Forsberg P, Lindgren PE. 2013. Prevalence, diversity, and load of Borrelia species in ticks that have fed on humans in regions of Sweden and Aland Islands, Finland with different Lyme borreliosis incidences. PLoS One 8:e81433.

Wilske B, Fingerle V, Herzer P, Hofmann A, Lehnert G, Peters H, Pfister HW, Preac-Mursic V, Soutschek E, Weber K. 1993. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med Microbiol Immunol 182:255-270.

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

Wong C, et al. 2012. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. Lancet 380:660-667.

Wormser GP. 2006. Clinical practice. Early Lyme disease. N Engl J Med 354:2794–2801.

Wormser GP, et al. 2006. 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 43:1089-1134.

Wormser GP, Kaslow R, Tang J, Wade K, Liveris D, Schwartz I, Klempner M. 2005a. Association between human leukocyte antigen class II alleles and genotype of Borrelia burgdorferi in patients with early lyme disease. J Infect Dis 192:2020-2026.

Wormser GP, McKenna D, Carlin J, Nadelman RB, Cavaliere LF, Holmgren D, Byrne DW, Nowakowski J. 2005b. Brief communication: hematogenous dissemination in early Lyme disease. Ann Intern Med 142:751-755.

Wormser GP, McKenna D, Karmen CL, Shaffer KD, Silverman JH, Nowakowski J, Scavarda C, Shapiro ED, Visintainer P. 2020. Prospective Evaluation of the Frequency and Severity of Symptoms in Lyme Disease Patients With Erythema Migrans Compared With Matched Controls at Baseline, 6 Months, and 12 Months. Clin Infect Dis. 71(12):3118–3124.

Wormser GP, McKenna D, Scavarda C, Karmen C. 2018. Outcome of facial palsy from Lyme disease in prospectively followed patients who had received corticosteroids. Diagn Microbiol Infect Dis 91:336-338.

Wormser GP, et al. 2000. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis 31 Suppl 1:1-14.

Wormser GP, Ramanathan R, Nowakowski J, McKenna D, Holmgren D, Visintainer P, Dornbush R, Singh B, Nadelman RB. 2003. Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 138:697-704.

Xu L, Winn BJ, Odel JG. 2012. Lyme-associated orbital inflammation presenting as painless subacute unilateral ptosis. J Neuroophthalmol 32:246-248.

Yang R. 2018. Plague: Recognition, Treatment, and Prevention. J Clin Microbiol 56.

Yeung C, Baranchuk A. 2019. Diagnosis and Treatment of Lyme Carditis: JACC Review Topic of the Week. J Am Coll Cardiol 73:717-726.

Yim CW, Flynn NM, Fitzgerald FT. 1985. Penetration of oral doxycycline into the cerebrospinal fluid of patients with latent or neurosyphilis. Antimicrob Agents Chemother 28:347-348.

Yoo MC, Soh Y, Chon J, Lee JH, Jung J, Kim SS, You MW, Byun JY, Kim SH, Yeo SG. 2020. Evaluation of Factors Associated With Favorable Outcomes in Adults With Bell Palsy. JAMA Otolaryngol Head Neck Surg 146:256-263.

Zaidman GW. 1997. The ocular manifestations of Lyme disease. Int Ophthalmol Clin 37:13-28.

Zainal A, Hanafi A, Nadkarni N, Mubasher M, Lingutla D, Hoefen R. 2019. Lyme carditis presenting as atrial fibrillation. BMJ Case Rep 12.

Zeng YL, Lin YQ, Zhang NN, Zou CN, Zhang HL, Peng F, Liu ZJ, Zheng WH, Yan JH, Liu LL. 2016. CXCL13 chemokine as a promising biomarker to diagnose neurosyphilis in HIV-negative patients. Springerplus 5:743.

Zhou L, Dhopeshwarkar N, Blumenthal KG, Goss F, Topaz M, Slight SP, Bates DW. 2016. Drug allergies documented in electronic health records of a large healthcare system. Allergy 71:1305-1313.

Zomer TP, Barendregt JNM, van Kooten B, van Bemmel T, Landman GW, van Hees BC, Vermeeren YM. 2019. Non-specific symptoms in adult patients referred to a Lyme centre. Clin Microbiol Infect 25:67–70.

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama Oy, Turku, Finland 2021

ISBN 978-951-29-8503-6 (PRINT)
ISBN 978-951-29-8504-3 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)