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Immunodiagnostics of Lyme neuroborreliosis

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GENERAL INTRODUCTION



INTRODUCTION

Lyme disease, also known as Lyme borreliosis (LB), is the most common tick-borne disease in temperate regions of the Northern Hemisphere [1]. LB is a multisystem disease, and the most frequent clinical symptom is an expanding skin rash also known as erythema migrans (EM). Other manifestations can involve the peripheral and/or central nervous system (Lyme neuroborreliosis [LNB]), joints (Lyme arthritis [LA]), skin (acrodermatitis chronica atrophicans [ACA]), and rarely the heart (Lyme carditis) and eyes (ocular Lyme) [1]. Except for a typical EM rash, the diagnosis of most other manifestations can be a challenge as symptoms are often non-pathognomonic [2]. This introduction provides background on the epidemiology, pathology and diagnostic challenges of LB. At the end of the chapter, the aims of this thesis are outlined.

THE HISTORY OF LYME BORRELIOSIS

The history of LB covers more than one century. The disease is named after the place Old Lyme (Connecticut, USA) where a clustered outbreak of presumed juvenile rheumatoid arthritis took place in the period between 1972 and 1975 [3]. Approximately one quarter of the cases reported an expanding skin rash currently known as EM in the weeks preceding the arthritis. In retrospect, this skin manifestation had already been described by Afzelius in 1910 [4] and by Lipschutz in 1913 [5] and was linked to the bite of a tick [6]. As the disease in Old Lyme did not match other known causes of arthritis it was considered to represent a new disease and was consequently called 'Lyme arthritis' [3]. In 1922, the first report of an association between EM and nervous system complaints appeared when Garin and Bujadoux reported a painful meningoradiculitis after the bite of a tick [7]. This was followed by a report from Hellerström in 1930 [8], who linked EM with meningitis. Nervous system complaints were also described by the German neurologist Bannwarth in 1941 and 1944 [9, 10]. He described a manifestation, now known as Bannwarth's syndrome, which was characterized by intense nerve pains that radiated to the extremities. Often patients also showed peripheral nervous system involvement (mainly facial nerve palsy) with an elevated number of lymphocytes in the cerebrospinal fluid (CSF).

A few years later, in 1948, Lennhoff [11] developed a staining technique that showed spirochetes in the lesions of patients with EM; however, these findings were not confirmed until 1981 when Burgdorfer et al. [12] discovered a new spiral-shaped bacterium (so-called spirochetes) in an *Ixodes dammini* (now: *Ixodes scapularis*) tick and called it *Borrelia burgdorferi*. He also suggested that this spirochete might be associated with Lyme disease as antibodies from Lyme disease patients bound to the bacterium. This association was confirmed in 1983 by Steere et al. [13], who showed that spirochetes that were isolated from ticks and Lyme disease patients had similar morphological and immunological features. They also showed increased levels of Lyme spirochete-specific immunoglobulin (Ig)M and IgG in Lyme disease patients that were absent in controls. Around the same time, Stiernstedt et al. [14] described 35 patients in Sweden who suffered from chronic meningitis, which was sometimes preceded by an EM or tick bite. Most of these patients had antibodies against the Lyme spirochete as well as against the *Ixodes ricinus* tick and, thus, an association between these spirochetes, a tick bite and chronic meningitis was demonstrated in Europe as well. Since then, numerous reports have been published on LB, which is now considered the most prevalent tick-borne infection with a wide geographic distribution in Northern America, Europe and parts of Asia [15].

THE VECTORS AND PATHOGENS OF LYME BORRELIOSIS

The genus *Borrelia* consists of two major phylogenetic groups: (i) the relapsing fever *Borrelia* group and (ii) the *B. burgdorferi* sensu lato (s.l.) complex group [16]. The genospecies which are part of the *B. burgdorferi* s.l. complex have been linked to LB and comprises at least 20 genospecies [16]. In North America, the most important genospecies causing LB is *B. burgdorferi* sensu stricto (s.s.) [16]. In Europe, the predominant genospecies causing LB are *Borrelia afzelii*, *Borrelia garinii*, *Borrelia bavariensis* and *B. burgdorferi* s.s. [16].

LB is a zoonosis and *B. burgdorferi* s.l. is transmitted by hard-bodied ticks of the *Ixodes* complex [17, 18]. The occurrence of LB in the world is dependent on the geographical distribution of its vector and reservoir hosts and is, therefore, mainly found in the Northern Hemisphere [19, 20]. In North America, the main tick species are *I. scapularis* in the Northeast and Upper Midwest and *Ixodes pacificus* in the West (Figure 1) [21]. In Europe, the main vector is *I. ricinus* and in Asia *Ixodes persulcatus*, although *I. persulcatus* has also been found in Eastern Europe [21].

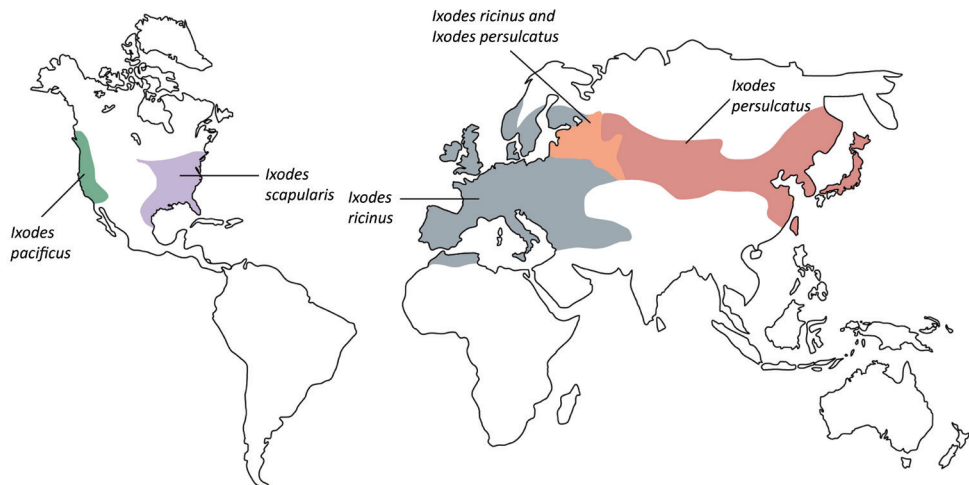


Fig. 1. Global distribution of the hard-bodied ticks of the *Ixodes* complex that can transmit *B. burgdorferi* s.l.. Figure reprinted from The Lancet, 379(9814), Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis, 461-73, ©2012 [21], with permission from Elsevier.

Ixodes ticks have a life cycle from 2 to 6 years that consists of four life stages: egg, larva, nymph and adult stage (Figure 2) [21]. Most tick activity is seen in spring and summer, which explains the seasonal incidence of LB as most cases occur between May and November [22, 23].

THE PREVALENCE OF *BORRELIA* GENOSPECIES IN TICKS IN THE NETHERLANDS

In the Netherlands, a study investigating questing *I. ricinus* ticks ($n = 5570$) from 22 different study sites showed a prevalence of *B. burgdorferi* s.l. of 11.8%, which could be subdivided in *B. afzelii* (6.7%), *B. garinii*/*B. bavariensis* (1.5%), *Borrelia valaisiana* (1.2%), *B. burgdorferi* s.s. (0.2%), and non-typeable *Borrelia* (2.2%) [24]. Another study investigated the prevalence of *B. burgdorferi* s.l. in 314 ticks that were obtained from 293 patients [25] in the Netherlands. The majority (94%) of these ticks were *I. ricinus* ticks, and almost one third (29.3%) contained *B. burgdorferi* s.l. DNA, which could be subdivided in *B. afzelii* (11.5%), *B. garinii* (3.5%), *B. burgdorferi* s.s. (2.2%), *B. valaisiana* (1.3%), and non-typeable *Borrelia* (11.5%) [25].

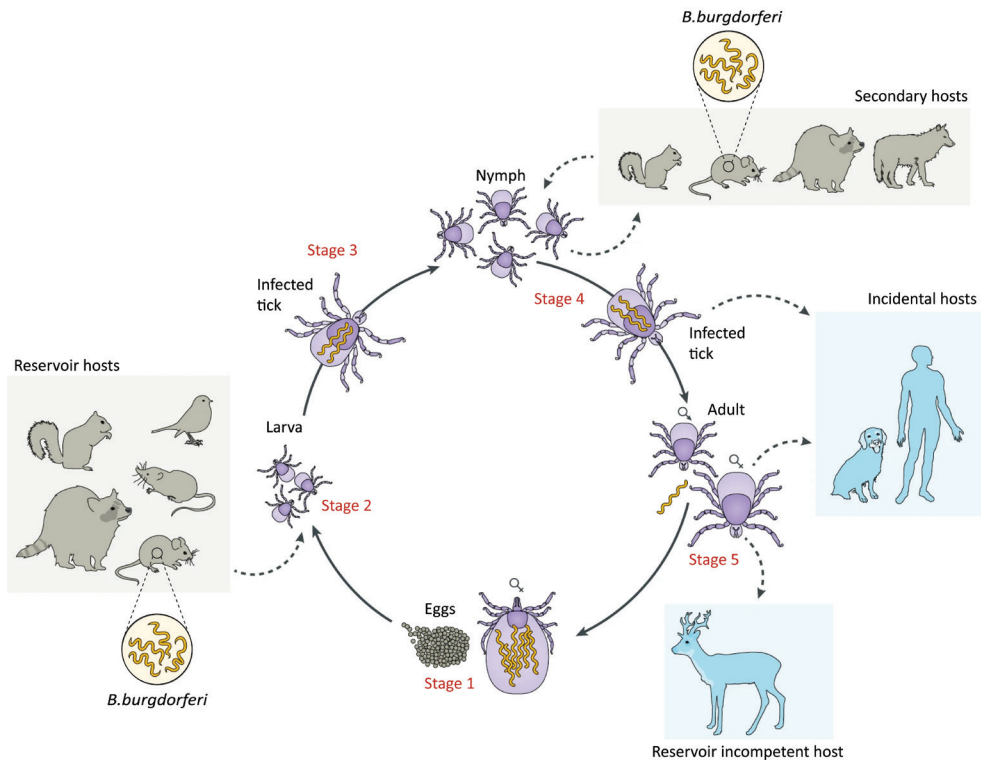


Fig. 2. The life cycle of the *Ixodes* tick and *B. burgdorferi* s.l.. The adult female tick lays up to 2000 eggs (stage 1), which will develop into six-legged larva (stage 2) [21, 26]. A blood meal is needed for development into the nymphal stage and this is probably the first moment the ticks may become infected with *B. burgdorferi* s.l. as transovarial transmission does not seem to occur [27, 28]. Larva typically feed on small mammals and birds and a blood meal normally takes 3-7 days. After the blood meal, the larva will drop off and molt into an eight-legged nymph (stage 3). Nymphs typically feed on medium to large-sized mammals and this is a second moment the tick may become infected with *B. burgdorferi* s.l.. After the blood meal, nymphs will drop off and molt into the adult stage (stage 4). Adult ticks typically seek a large animal host, such as deer, for mating and a last blood meal (stage 5). Since ticks cannot acquire *B. burgdorferi* s.l. from deer, they are called reservoir-incompetent hosts [26, 29]. Deer are, however, important for maintaining the tick population. For all feeding stages, humans can be an incidental host [26]. Figure adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Microbiology. Interactions between *Borrelia burgdorferi* and ticks. Kurokawa C, Lynn GE, Pedra JHF, Pal U, Narasimhan S, Fikrig E, ©2020 [26].

THE EPIDEMIOLOGY OF LYME BORRELIOSIS

LB has a bimodal age distribution with an incidence peak in childhood (range between 5 and 15 years) and one in adulthood (range between 50 to 75 years) [20, 30-35]. In North America, LB is a notifiable disease since 1991 and about 30,000 confirmed and probable LB cases are reported to the Centers of Disease Control (CDC) each year [35, 36]. Reported LB cases include dermatologic, rheumatologic, neurologic, and cardiac manifestations; the majority (70%-80%) are EM cases [35, 36]. As the number of reported LB cases is based on passive reporting, the reported incidence could be an underestimation, and recent CDC estimates suggest that the actual number of LB cases might be 10 times higher [36]. In Asia, reports on the incidence rates of LB are limited, although LB cases have been reported in Korea and Japan, where LB is a notifiable disease [37], and also in Russia [38], China [39] and Mongolia [40]. In most European countries, LB is not notifiable and to gain insight into the LB incidence rates, different strategies are used.

For the most part, incidence rates of LB in Europe are based on positive test results obtained from diagnostic laboratories [41]. Other ways to obtain LB incidence rates are based on voluntary reporting, surveys conducted among physicians, or by hospital diagnoses [41]. It is estimated that LB affects around 85,000 patients in Europe each year [20], although this is also most likely an underestimation. The incidence rate of LB in Western Europe was estimated at 22 per 100,000 population per year [42]. The highest incidences rates were found in Norway [32], Sweden [43], Switzerland [44], Austria [20], Slovenia [45] and the Netherlands [46], where the incidence rates ranged between 100 and 500 per 100,000 population per year. Incidence rates between 25 and 100 per 100,000 population per year were found in Finland [47], Germany [34], Czech Republic [48], France [49], Belgium [50] and Lithuania [51]. The lowest incidence rates, of less than 10 per 100,000 population per year, were found in the United Kingdom [52], Italy [53], Spain [54] and Iceland [55].

Reported incidence rates, however, should be interpreted with caution as these sometimes reflect only certain regions of a country or can be based on the mean incidence rate of the whole country. Use of non-standardized case definitions [42, 56] and unclear symptomology reporting [57] can also result in biased incidence rates. For EM, which is a clinical diagnosis, data might be lacking as not all LB cases will develop an EM or an EM remains unnoticed or unrecognized [42, 58, 59]. When serology is part of the case definition, this can also result in biased estimates: e.g., under-reporting due to false-negative test results can occur in the first weeks of infection when antibody levels are rising, but are still undetectable at the moment of blood sampling [42]. On the other hand, over-reporting can occur when positive serology results are interpreted as proof of active disease, while these can also result from a past infection [57]. Despite these pitfalls, in both North America [60] and Europe [56, 61], the incidence of LB seems to increase. Factors contributing to this increase include climate change, expansion of ticks and reservoir hosts, increased human tick exposure, more awareness as well as improved monitoring, detection, and reporting of both ticks and LB diagnoses [20, 62].

THE EPIDEMIOLOGY OF LYME BORRELIOSIS IN THE NETHERLANDS

In the period between 1994 and 2009, the incidence of tick bite consultations and EM diagnoses in the general practice in the Netherlands was investigated and both showed a 3-fold increase (Figure 3) [63-66]. This rise is likely due to, at least in part, an increase in disease awareness. In 2014, the number of tick bite consultations in the Netherlands slightly decreased and the number of EM cases seemed to stabilize [67], and in 2017, the number of tick bite consultations and EM diagnoses increased again [46].

The risk of transmission of *B. burgdorferi* s.l. among patients that visited the general practitioner (GP) for tick bites or EM in the Netherlands has been assessed in a nationwide study conducted in 2015 [68]. The findings indicate that the risk of developing EM after a tick bite was 2.6%, and synthesis of *Borrelia*-specific antibodies was shown in 3.2% of the tick bite cases. Analysis among patients who were bitten by a *B. burgdorferi* s.l.-positive tick showed an increased risk of developing EM as well as an increased risk of seroconversion (4.4% and 5.9%, respectively). Another Dutch study showed a similar risk of 2.6% for developing LB after a tick bite and showed that this was positively associated with tick attachment time, tick engorgement and the presence of *B. burgdorferi* s.l. DNA in ticks. The highest risk for developing LB was 14.4% and was linked to a substantially engorged *B. burgdorferi* s.l.-positive tick [69]. In Europe, transmission of *B. burgdorferi* s.l. by adult ticks is said to occur after at least 24 hours of tick infestation. However, in mouse models transmission was seen within 12 hours by nymphal ticks and this shorter infection time could be explained by *B. burgdorferi* s.l. already present in the salivary glands before a blood meal was initiated [70].

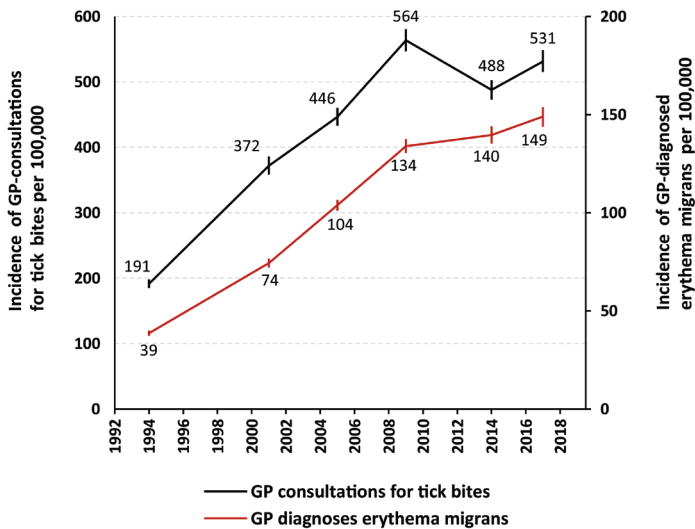


Fig. 3. The incidence of general practitioner (GP) reported tick bite consultations and erythema migrans (EM) diagnoses in the general practice between 1994 and 2017 in the Netherlands (17.1 million pop. in 2017). Figure adapted by permission from van den Wijngaard et al. [46].

THE CLINICAL MANIFESTATIONS OF LYME BORRELIOSIS

LB is a multisystem disease that can involve skin, nervous system, joints, heart, and eyes and is often classified as an early localized, early disseminated, or late disseminated disease (Figure 4A) [2]. Dissemination of *B. burgdorferi* s.l. can occur by flagellum-induced motility and chemotaxis [71, 72], and might, in part, be dependent on the genospecies causing LB. In North America, dissemination via blood seems more likely given the higher rates of spirochetes in the blood of patients from North America [73, 74]. In Europe, dissemination via peripheral nerves seems more likely, at least among patients with meningoradiculitis, which is most often caused by *B. garinii* [75, 76]. Patients with meningoradiculitis also have a higher frequency of EM located on the head, neck or torso compared to EM patients without neurological involvement and for the majority of these cases (79%), the location of the EM matched the location of the radicular pain [77].

The different Lyme manifestations (Figure 4B) might, in part, be explained by the different genospecies, which are linked to different tissue tropisms: e.g., *B. afzelii* is associated with skin manifestations, *B. burgdorferi* s.s. with joint manifestations, and *B. garinii* and *B. bavariensis* with nervous system manifestations [15, 16, 75, 78, 79]. The difference in clinical presentation might also be explained by the different genospecies as the EM of patients in North America, which is caused by *B. burgdorferi* s.s., expands more rapidly and is more often accompanied with other symptoms than EM in European patients, which is mainly caused by *B. afzelii* or *B. garinii* [73, 80]. Intra-species variation and host genetic factors have also been linked to differences in disease severity. For instance, in North America, *B. burgdorferi* s.s. outer surface protein (Osp)C type A in LA patients with a polymorphism in toll-like receptor 1 is associated with more inflammation and more severe LA [81, 82]. In Northeastern America, 65% of 291 strains isolated from EM lesions were attributable to four *B. burgdorferi* s.s. OspC types (A, B, I and K), and in the upper Midwest, *B. burgdorferi* s.s. OspC type H was mostly found (18.5% of 65 strains) [83]. In Europe, only certain *B. burgdorferi* s.l. sequence types (based on eight housekeeping genes) are found to cause LB in humans [79].

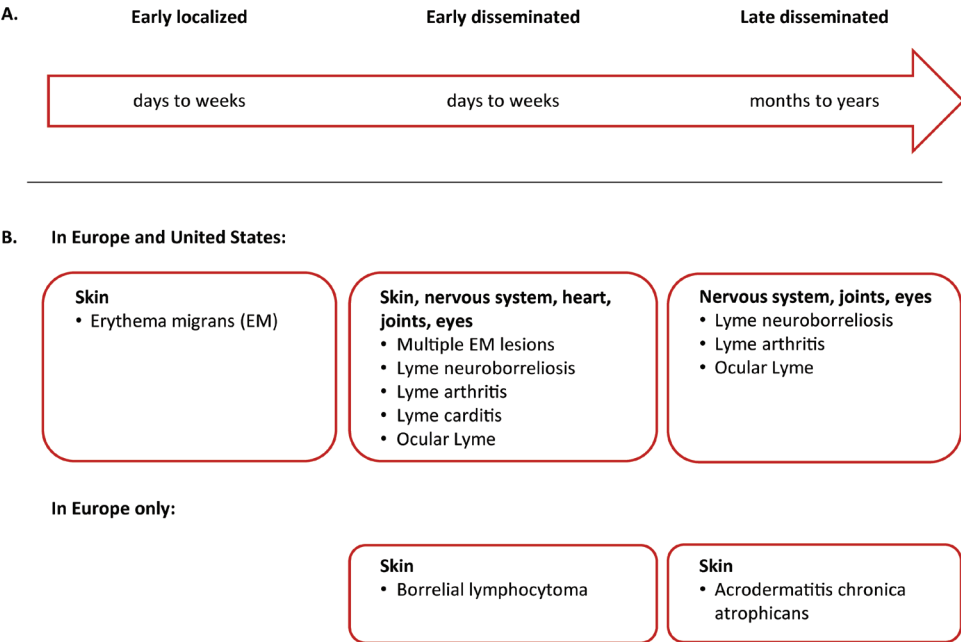


Fig. 4. The classification (panel A) and manifestations (panel B) of Lyme borreliosis seen in Europe and North America. Figure adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Disease Primers. Lyme borreliosis. Steere AC, Strle F, Wormser GP, Hu LT, Brandt JA, Hovius JW, Li X, Mead PS, ©2016 [80].

Asymptomatic infections occur as well and in Europe, these seem to be as common as symptomatic infections [25, 84]. In North America, however, asymptomatic infections are less common than symptomatic infections as was shown by Steere et al. [85], who reported that clinical symptoms were absent in less than 7% of the participants who showed seroconversion to *B. burgdorferi*.

ERYTHEMA MIGRANS

EM is the most prevalent LB manifestation and occurs in about 70% to 95% [2, 31, 34, 36, 50] of all LB cases. It is the only manifestation of early localized LB and occurs several days to weeks (typically 7 to 14 days) after the tick bite, when a skin rash appears that expands over time [16, 86]. This expanding skin rash is caused by the centrifugal migration through the skin of *B. burgdorferi* s.l. from the site of the tick bite [87]. A skin lesion that is equal to or more than 5 cm in diameter is often needed for a clinical diagnosis [21, 88]. Smaller lesions may be considered as EM when at least 2 days have past and the lesion is expanding. The most typical EM feature is a bull's-eye rash, but central clearing in EM lesions may not always occur (Figure 5) [21]. Other symptoms that can occur in approximately 50% of the patients are itching, burning or pain at the site of the EM, and, less often, fatigue, headache, malaise, arthralgias and myalgias [2]. In most areas of Europe, EM is caused by *B. afzelii* (70% to 90%), and less frequently by *B. garinii* (10% to 20%), except in Northeastern Europe where *B. garinii* predominates [2]. In North America, EM patients more often experience fever and lymphadenopathy than European EM patients [2, 89], and central clearing of EM is more often seen in Europe [16, 89]. If an EM remains unnoticed or unrecognized and left untreated, *B. burgdorferi* s.l. can spread to other body parts, and cause (early) disseminated LB.



Fig. 5. A classical bull's-eye rash (left panel) and a non-typical EM lesion (right panel). Photos from private collection.

MULTIPLE ERYTHEMA MIGRANS

The occurrence of multiple EM lesions at the time of presentation is found in 4% to 50% of the EM patients in Europe [86, 90] and in 20% to 40% of the EM patients in North America [91, 92]. In general, multiple EM lesions are smaller than single EM lesions and often lack the indurated center seen in single EM lesions [2]. The number of EM lesions in European patients often ranges between two to six lesions, which is lower than the number of EM lesions seen in patients from North America (>20 lesions) [93].

BORRELIAL LYMPHOCYTOMA

Borrelial lymphocytoma is mainly observed in Europe and occurs in less than 8% of LB cases [31, 44]. It is often caused by *B. afzelii* and, consistent with the geographical spread of the bacterium, not seen in North America [89]. Borrelial lymphocytoma appears as a painless small swelling of the skin that slowly enlarges to a diameter of up to several centimeters and is seen more often in children (1.5% to 7%) than in adults (0.5% to 2%) and is mostly located on the earlobe or areola mammae [2].

LYME CARDITIS

Heart involvement leading to Lyme carditis occurs in 0.3% to 4% of LB cases in Europe and in approximately 1% of LB cases in the U.S. [94], and mostly starts within 2 months after the onset of infection [2]. Lyme carditis most commonly involves conduction system disturbances of the atrioventricular (AV) node resulting in various degrees of AV block, but other beat disturbances, endomyocarditis and pericarditis have also been reported [1]. It is often preceded by an EM and can include symptoms of the nervous system or joints [1].

OCULAR LYME

Ocular manifestations have been reported during the early and later stages of disseminated LB. In the first few weeks, conjunctivitis has been reported in up to 10% of patients, and keratitis, uveitis and vitritis have also been reported [95]. Involvement of the eyes can also occur in conjunction with other Lyme manifestations, such as LA [96], and cause symptoms such as keratitis or uveitis. In patients with LNB, palpebral diastasis, blurred vision, strabismus and diplopia can occur [97].

LYME ARTHRITIS

LA is more commonly found among LB cases in North America (25%) [98] than in Europe (2% to 7%) [31, 34, 44]. Most patients have intermittent or persistent attacks of swelling and pain in one joint, most often the knee, although other large or small joints may also be affected, such as the ankle, hip, shoulder, elbow, or wrist [99, 100]. LA is seen as an early and late Lyme manifestation and in North America, and the onset of disease can range from 4 days to 2 years, with a mean of 6 months. In Europe, the onset of disease can range from 10 days to 16 months, with a mean of 3 months [2].

ACRODERMATITIS CHRONICA ATROPHICANS

ACA is a chronic skin manifestation, which occurs in 1% to 6% of European LB cases [31, 44, 101]. It is observed more often among women and rarely occurs among children [2]. ACA is mostly caused by *B. afzelii*, although infections caused by *B. garinii* and *B. burgdorferi* s.s. are also observed [2]. The affected skin mostly comprises the distal parts of the extremities and is characterized by a red or bluish-red discoloration with or without swelling that can become atrophic over time [2]. In contrast to EM and borrelial lymphocytoma, ACA is not self-limiting, and a substantial number of ACA patients develop a sensory peripheral neuropathy when left untreated [2].

THE CLINICAL MANIFESTATIONS OF LYME BORRELIOSIS IN THE NETHERLANDS

In 2010, the incidence rate of EM and disseminated LB was assessed by conducting a nationwide survey among GPs, company physicians, and medical specialists involved in LB diagnosis [68]. Based on the results of this study, the estimated number of EM cases was 22,000 and the estimated number of disseminated LB cases was 1,300. The majority of disseminated LB cases either had LNB or LA (34% and 39%, respectively). Other reported disseminated Lyme manifestations included ACA, borrelial lymphocytoma, Lyme carditis and ocular Lyme (16%, 8%, 1% and 1%, respectively). Based on the incidence rates of all LB cases in 2010, the relative proportion of patients with EM was 91%, with LA 2.1%, with LNB 1.8%, and with ACA 0.8% [67, 68]. A similar GP survey carried out in 2017 showed that the estimated number of EM cases had increased to 25,500, and that of disseminated LB to 1,500 cases nationwide (cf. Figure 3) [46].

THE CLINICAL MANIFESTATIONS OF LYME NEUROBORRELIOSIS

LNB is seen in <1% to 16% of LB cases in Europe [2, 31, 34, 44, 50, 102] and in approximately 12.5% of confirmed LB cases in North America [35]. Most LNB patients (90% to 95%) are classified as early LNB (symptom duration of less than 6 months) [103-105]. In Europe, less than 60% of the LNB patients noticed a tick bite and/or EM [76, 106-109]. For patients with early LNB, both the peripheral nervous system as well as the central nervous system can be affected. In Europe, the most common manifestation of LNB among adults is a lymphocytic meningoradiculitis (Bannwarth's syndrome), which presents as a severe sharp neuropathic pain of the skin that intensifies at night [76, 107, 109]. The radicular pain is often located at the site of the tick bite or EM [77], and is the only symptom present in almost half of the patients [107]. Approximately 40% of the patients have cranial nerve involvement comprising the facial nerve (nerve VII), that can result in either a unilateral or a bilateral (<30% of cases) paresis [76, 105, 107, 109]. Other cranial nerves that are less frequently involved include the abducens nerve (nerve VI), and the oculomotor nerve (nerve III), and rarely other cranial nerves [105, 107, 109, 110]. Limb, phrenic, abdominal wall, and bladder paresis have also been reported in a limited number of cases [105, 111-114]. Other frequently reported symptoms among adult LNB patients include meningitis, headache, sleep disturbance, fatigue, malaise, arthralgia, myalgia, paraesthesia, and concentration and/or memory disturbance [76]. Less common peripheral nervous system manifestations involve plexus neuritis and mononeuritis multiplex [115] or brachial plexopathy [116]. In less than 5% of the early LNB cases, central nervous system manifestations occur, such as encephalitis, myelitis and cerebral vasculitis [103].

If symptom duration is longer than 6 months, patients are classified as late LNB [117]. For late LNB cases in Europe, peripheral nervous system involvement include symptoms such as radiculopathy [105], mono- and polyneuropathy [103]. Polyneuropathy is caused by axonal degeneration involving the sensory nerves and has been observed in patients with ACA [103]. Central nervous system involvement among late LNB cases include symptoms such as myelitis, encephalitis [105], encephalomyelitis and cerebral vasculitis [103]. In some cases, a stroke or transient ischaemic attacks was reported [103].

The most common manifestations of LNB among children in Europe are facial nerve palsy and lymphocytic meningitis [118, 119]. Other frequently reported symptoms among European children include fatigue, headache, fever, loss of appetite, neck pain/stiffness, vertigo, radicular pain and EM and/or lymphocytoma (range between 20% to 75%) [108].

In North America, facial palsy is the most common manifestation of LNB (9%), followed by radiculoneuropathy (4%) and meningitis and/or encephalitis (3%) [120].

THE PATHOGENESIS OF LYME BORRELIOSIS

When a blood meal is taken from an infected reservoir host, *B. burgdorferi* s.l. will enter the *Ixodes* tick and migrate to the midgut and remain there until the next tick stage. When an infected tick takes another blood meal, *B. burgdorferi* s.l. multiplies and changes the expression of proteins located on the outer surface. In the midgut, *B. burgdorferi* s.l. expresses OspA, which binds to the tick receptor for OspA that is located on the epithelial cells in the midgut [71, 121]. Through temperature and pH changes during the blood meal, OspA will be downregulated and OspC will be upregulated [71, 122]. Consequently, *B. burgdorferi* s.l. will migrate to the salivary glands [21, 71], where OspC binds to the tick salivary gland protein (Salp)15. With the tick saliva, *B. burgdorferi* s.l. can enter the skin of the mammalian host [71]. Salp15 as well as other substances within the tick saliva have immunomodulatory properties to interfere with the hosts' innate and adaptive immune response and protect *B. burgdorferi* s.l. from complement- and antibody-mediated killing and reduce chemotaxis of immune cells [71, 123-125].

B. burgdorferi s.l. also has various mechanisms to evade the hosts' innate and adaptive immune response [71, 125, 126]. One of the mechanisms to evade the innate immune response is the expression of surface proteins that can interfere with the complement system, such as the fibronectin-binding lipoprotein BBK32 or the CD59-like complement inhibitory molecule [71, 125-130]. *B. burgdorferi* s.l. has also shown to be resistant to antimicrobial peptides (i.e., cathelicidin) [131, 132] and antimicrobial proteins (i.e., lactoferrin, proteinase 3, azurocidin) [133], that are part of the host immune response [125]. In addition, *B. burgdorferi* s.l. elicits the production of anti-inflammatory cytokines by mononuclear cells, such as IL-10, which suppresses phagocytosis and reduces the production of other pro-inflammatory mediators [71, 125, 126, 134]. Furthermore, *B. burgdorferi* s.l. has mechanisms to evade the immune system by hiding in phagocytic cells or in the extracellular matrix [71, 125, 126]. In the extracellular matrix, *B. burgdorferi* s.l. can attach to several proteins such as decorin, which is part of the connective tissue and enables the dissemination of *B. burgdorferi* s.l. and promotes its survival [71]. *B. burgdorferi* s.l. is also suggested by some to be pleomorphic, capable of forming biofilm-like structures [125, 135, 136], or exhibit conformational changes (i.e., round bodies, L-form bacteria, or microcolonies), which may play a role in escaping the immune system [125, 137].

To evade the adaptive immune response, *B. burgdorferi* s.l. can use antigenic variation resulting in the differential expression of surface proteins on the outer membrane [71, 125, 126], such as that

seen in the variable major protein-like sequence, expressed (VlsE) protein [138, 139]. Through recombination events in the Vls locus, the VlsE can change phenotypically. Consequently, this new VlsE protein cannot be recognized by the antibodies already formed. The adaptive immune response is also disabled through invasion of *B. burgdorferi* s.l. into the lymph nodes, as this may disrupt the formation of germinal centers, which are required for the formation of long-lived plasma cells and memory B cells [125, 140]. Furthermore, the strong and sustained IgM response suggests a failure of B cells to undergo a class switch from IgM to IgG [125, 140].

As *B. burgdorferi* s.l. does not have genes encoding toxins [141, 142], most of the clinical symptoms are expected to result from the tissue invasion of *B. burgdorferi* s.l. and the effects of the hosts' innate and adaptive immune response [2].

THE PATHOGENESIS OF LYME NEUROBORRELIOSIS

In 1984, *B. burgdorferi* s.l. was isolated from the CSF of a patient with meningoradiculitis for the first time [143]. Since then, *B. burgdorferi* s.l. has been detected in the CSF of LNB patients by culture and PCR [144]. The presence of *B. burgdorferi* s.l. in the CSF and subsequent activation of local immune cells results in lymphocytic pleocytosis (i.e., an increased number of lymphocytes in the CSF [normal cell count: <5 leucocytes per µl of CSF]), which comprises T cells, B cells, plasma cells and NK cells [145]. The exact location of *B. burgdorferi* s.l. in the central nervous system is unknown; however, using nonhuman primate models, *B. burgdorferi* s.l. has been localized in the leptomeninges, nerve roots, and dorsal root ganglia, but not in the parenchyma [146]. These findings were consistent with the pathological findings among LNB patients with Bannwarth's syndrome [114]. In the peripheral nervous system, *B. burgdorferi* s.l. was found in the endoneurium and in connective tissues of peripheral nerves and muscles using nonhuman primate models [146].

As mentioned previously, *B. burgdorferi* s.l. can enter the CSF via hematogenous dissemination [71], or via dissemination along the peripheral nerves [71, 76], as suggested by several studies which showed that radicular pain is often located in the region of the tick bite and/or EM [76, 147]. Ackermann et al. [148], however, reported no such association. The exact mechanisms that lead to the clinical symptoms found among LNB patients are not entirely clear. In mouse models, *B. burgdorferi* s.l. can bind to glial and neuronal cells, which could be affected by mechanisms such as (in)direct cytotoxicity, or autoimmune reactivity via molecular mimicry causing glial and neuronal cell death [72, 149, 150]. In nonhuman primate models, pro-inflammatory cytokines play a fundamental role in the pathogenesis of LNB [149, 151]. Studies on primary cells of the nervous system and tissue biopsies have shown that the neurotropism of *B. burgdorferi* s.l. and its binding to (but not invasion of) neuronal cells results in the production of inflammatory mediators, which can cause neurological damage [152-155].

THE LABORATORY DIAGNOSIS OF LYME BORRELIOSIS

The diagnosis of LB is mainly based on the presence of clinical findings or symptoms, and, for most Lyme manifestations, laboratory tests are used to support and confirm the clinical diagnosis. For EM, a clinical diagnosis is deemed sufficient to initiate treatment in case of typical lesions, otherwise, laboratory tests must be used to support the diagnosis [2, 156]. Laboratory tests for LB diagnosis can either be based on the direct detection of *B. burgdorferi* s.l., or on the indirect detection of its presence, by demonstrating a host immune response against *B. burgdorferi* s.l..

DIRECT DETECTION METHODS

Direct detection methods include microscopy, culture and PCR. Microscopy in blood or other infected tissues is difficult due to the often low numbers of spirochetes [157]. It is sometimes used for skin, cardiac and synovial tissue examination together with serology; however, for blood and CSF microscopy is not useful [157].

Culture is not routinely used for LB diagnostics due the long growth time, the need of complex media, and the low yield [156, 158, 159]. The yield is best for patients with early, untreated EM and the sensitivity of culture using skin biopsies from EM patients ranges between 40% and 90% [157, 159]. Skin biopsies are mostly taken from the edge of the EM lesion, but can also be taken from the center of the EM lesion as well as from the clinically normal perilesional site of the EM lesion [87, 159, 160]. The sensitivity of skin biopsies of borrelial lymphocytoma amounts to a maximum of 35%, and that of ACA to a maximum of 40% [156, 161, 162]. Blood cultures are mostly positive in the early phase of the infection consistent with the hematogenous spreading of the bacterium at that time, and results are best when large volumes of plasma are used (≥ 9 ml) [157]. In North America, the sensitivity of plasma culture ranges between 40% and 50%, while in Europe the sensitivity is below 10% [156, 157]. This could be related to the lower rate of multiple simultaneous EM manifestations in Europe [93]. The sensitivity of blood culture of disseminated LB cases and of CSF culture of LNB cases is low in both North America and Europe ($<10\%$ and $<15\%$, respectively) [156, 157], and culture of synovial fluid of LA patients has not been successful [157]. This suggests that the bacterium can only be readily detected in early Lyme manifestations, excluding ACA. For all these direct diagnostic approaches, specialized personnel is needed.

PCR can be used to diagnose LB, especially in the early phase of infection, prior to antibody synthesis and antibiotic treatment [159, 163]. Many studies have investigated the use of PCR detection of *B. burgdorferi* s.l. for LB diagnostics, and reported sensitivities varied extensively [159]. This might be explained by the PCR method, the target genes and primers used, and the clinical presentation [159]. Furthermore, much attention should be paid to the sample collection, sample transport and sample processing, as incorrect handling can negatively influence the sensitivity of the PCR [159]. The interpretation of PCR results can be complicated as a negative PCR result does not exclude LB [164], and due to the high sensitivity of PCR, false-positive PCR results may incidentally occur as well [164, 165]. In general, the sensitivity of PCR detection of *B. burgdorferi* s.l. in skin biopsies of EM and ACA patients is high, with a median of 69% (range: 36% to 88%) and 76% (range: 54% to 100%), respectively [159]. An equally high sensitivity was found for PCR detection of *B. burgdorferi* s.l. in synovial fluid of LA patients (median: 78% [range: 42% to 100%]) [159]. The sensitivity of PCR detection of *B. burgdorferi* s.l. in CSF of LNB patients is much lower (median: 38% [range: 12% to 100%]) and the best results are obtained for very early LNB cases [159, 166]. In the presence of lymphocytic pleocytosis, the sensitivity of PCR detection of *B. burgdorferi* s.l. in CSF is usually higher [163, 167]. PCR detection of *B. burgdorferi* s.l. in blood has the lowest sensitivity (median: 14% [range: 0% to 100%]), and is hampered by the low spirochetemia (estimated to approximately 0.1 spirochetes/ml of whole blood) [168] and the dissemination of *B. burgdorferi* s.l. in the tissues of joints, heart and the central nervous system [159, 163]. The specificity of PCR detection of *B. burgdorferi* s.l. was very high (approximately 100%) for all Lyme manifestations [159].

INDIRECT DETECTION METHODS

Indirect detection methods are based on the immune response of the host against *B. burgdorferi* s.l.. The most widely used tests in routine clinical practice to support the diagnosis of LB are based on the detection of *Borrelia*-specific antibodies in blood [169]. Most guidelines recommend the use a two-tier test strategy [86, 156]. This two-tier test strategy aims to improve the diagnostic performance of laboratory tests by combining a highly sensitive first test (i.e., a screening test)

with a highly specific second test (i.e., a confirmation test) to confirm equivocal and positive test results obtained in the first test [169-171]. The screening test often comprises an enzyme-linked immunosorbent assay (ELISA), and the confirmation test is based on either a western blot (North America) or an immunoblot (Europe) [169]. In North America, a modified two-tier test strategy has been approved by the United States Food and Drug Administration in which the western blot is substituted for a second ELISA [172, 173]. This modified two-tier test strategy has shown to be at least as sensitive as the conventional two-tier test strategy in diagnosing LB without a loss in specificity [174, 175]. Among patients with EM and early LNB, a single-tier test strategy using an ELISA based on the C6 peptide, which is derived from the immunodominant invariable region 6 of VlsE, has also proven to be effective [176]. The origin, type and number of antigens used in the various antibody assays may differ. These antigens can be based on whole-cell lysates, or on one or several purified native antigen(s), recombinant antigen(s), synthetic peptide(s) or a mixture of these [169]. As different *B. burgdorferi* s.l. genospecies are known to cause human disease in Europe, many European ELISAs use antigens derived from these different pathogenic genospecies [169].

The antibody response against *B. burgdorferi* s.l. is considered to be slow and delayed [177, 178]. The initial response is based on IgM against early antigens, such as p41, OspC, and BBK32 (Table 1), which are expressed by the bacterium in the initial stage to establish an infection [169]. Generally, IgM is detectable within 2 to 4 weeks after the start of infection and peaks after 6 to 8 weeks and then declines [169]. Persistence of IgM, however, also occurs [179-181], and may, at least in part, be explained by cross-reactive auto-antigens or antigens from other microorganisms or environmental factors [182]. The IgG response generally becomes detectable 2 to 6 weeks after the start of infection and peaks after 4 to 6 months and can remain elevated for years [183, 184]. The IgG response often starts with antibodies against VlsE, p41, OspC and BBK32, and is followed by antibodies against p18, p39 and p58 and later by p83/p100 [169].

Table 1. The expression of immunodominant *Borrelia* antigens during various stages of human infection. Table adapted from Talagrand-Reboul et al. [169]. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). Full terms at <https://creativecommons.org/licenses/by/4.0/>.

Early antigens	Early/late antigens	Late antigens
<ul style="list-style-type: none">• OspC (p21-p25, outer surface (lipo)protein C)• Flagellin (p41)• BBK32 (fibronectin-binding protein)	<ul style="list-style-type: none">• VlsE (p34-35, variable major protein-like sequence, expressed)• DbpA (p17-p18, decorin-binding protein A)• BmpA (p39, <i>Borrelia</i> membrane protein A)• OppA-2 (p58, oligopeptide-binding protein)• p14• p28• p43• p45	<ul style="list-style-type: none">• p83/100• OspA (p31, outer surface (lipo)protein A)• p30• p66• p93

Serology is not recommended for EM patients presenting with an expanding, annular, skin rash that is characterized by a bull's-eye rash, as this is pathognomonic. However, if the rash is atypical, the detection of *Borrelia*-specific antibodies can be helpful for diagnosis of LB. In Europe, the sensitivity of the two-tier test strategy among EM patients is moderate (~55%, 95% confidence interval [CI]: 32% to 77%) [169, 185, 186]. The sensitivity of the two-tier test strategy among LNB patients using serum is higher than that seen among EM patients (~87%, 95% CI: 60% to 98%), and among LA and ACA patients, the sensitivity of the two-tier test strategy is highest (~93%, 95% CI: 68% to 100%, and 100%, 95% CI: 77% to 100%, respectively) [169, 185, 186]. In North

America, the sensitivity of the two-tier test strategy is comparable to that in Europe and is ~46% (95% CI: 39% to 54%) for localized LB, ~90% (95% CI: 78% to 95%) for early disseminated LB, and ~99% (95% CI: 96% to 100%) for late disseminated LB [169, 187]. The specificity of the two-tier test strategy is very high (>98%) [169, 185, 187].

THE INTERPRETATION OF SEROLOGY RESULTS

The interpretation of serology results can be complicated. False-negative test results can be obtained in case the antigens present in the assay do not match the antigens expressed by the strain causing disease. This discrepancy can be explained by the intra- and interspecies heterogeneity of *B. burgdorferi* s.l. and/or the antigenic variation used by *B. burgdorferi* s.l. during the course of disease [71, 81, 82, 125, 126, 139, 188-190]. The composition of the expressed antigens in whole-cell lysates of cultured strains also depends on the environment in which these strains were cultured, as some antigens are only expressed in vivo or are lost during multiple culture passages [191, 192]. Consequently, a discrepancy can occur between the antigens applied in the antibody assay and those expressed during an active infection against which the antibodies are formed [159, 193]. As was shown in the previous paragraph, the sensitivity of antibody assays is positively correlated with disease stage. The sensitivity also seems positively correlated with the number of antigens applied in the assay [194, 195], although only to a certain extent [196]. Another complicating factor is the low sensitivity of antibody assays in the very early phase of the infection, which has a biological cause as the antibody response must be build up (Figure 6) [159]. Seroconversion occurs after 2 to 4 weeks and, in case of a negative test result at the initial disease phase, serology is advised to be repeated on a second blood sample taken 2 to 4 weeks later if symptoms persist [159]. As antibiotics can preclude or diminish the activation of the immune response, false-negative test results can be obtained, or a seroconversion from IgM to IgG might not occur if treatment starts before the moment of blood sampling [157, 197-200]. In mice, for instance, an abrogated immune response has been linked to the development of short-lived germinal centers incapable of forming memory B cells and long-lived plasma cells [201]. Absence of *Borrelia*-specific antibodies might also be caused by humoral immunodeficiency as was shown in some case reports [202, 203].

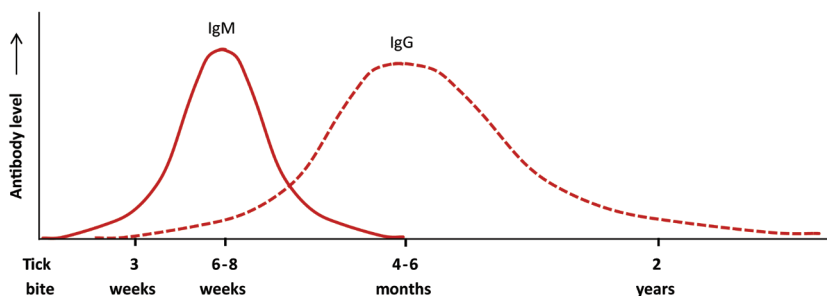


Fig. 6. Antibody response in Lyme borreliosis over time. Figure adapted from Studies in Health Technology and Informatics, Volume 116: Connecting Medical Informatics and Bio-Informatics, Hejlesen OK, Olesen KG, Dessau R, Beltoft I, Trangeled M., Decision Support for Diagnosis of Lyme Disease, 205 – 210, ©2016 [183], with permission from IOS Press. The publication is available at IOS Press through <https://ebooks.iospress.nl/volume/connecting-medical-informatics-andbio-informatics>.

False-positive test results might be explained by the use of epitopes that are shared by other related spiral micro-organisms, such as *Treponema pallidum*, the causative agent of syphilis [204-206] and various other *Treponema* species [207], or relapsing fever *Borrelia* [208, 209]. False-positive test results have also been reported among patients infected with Epstein-Barr virus [206, 210], cytomegalovirus [206], or *Helicobacter pylori* [206]. Other mechanisms known to cause false-positive (IgM) results are the presence of interfering substances such antinuclear

antibodies or rheumatoid factor [206], cross-reactive auto-antigens or environmental factors [182], or overreading of weak-positive immunoblot bands [211]. Another factor that complicates the interpretation of serology results is the persistence of antibodies after a cleared LB; thus, positive test results do not necessarily indicate active disease [179, 212]. In the literature, reported seroprevalences mostly range between 1% and 20% [213-219], but can be higher in certain regions and among certain risk groups [218, 220-222]. In the Netherlands, an IgG seroprevalence of 9% (regional range: 2% to 17%) was found among blood donors, and an IgG seropositivity of 15% (regional range: 10% to 29%) has been reported among risk groups such as owners of hunting dogs [223] of whom the majority (94%) were asymptomatic. Likely, such seropositivity represents a past infection, and has no (treatment) consequences.

Knowledge regarding the prevalence of LB and the performance characteristics of antibody assays is important in the use of serology for diagnostic purposes (Figure 7) [171]. In general, when serology is performed in a low endemic area, the probability of a positive test result (i.e., the positive predictive value [PPV]) being indicative of active LB is lower than in a high endemic area. In contrast, the probability of a negative test result (i.e., the negative predictive value [NPV]) excluding active LB is lower in a high endemic area than in a low endemic area.

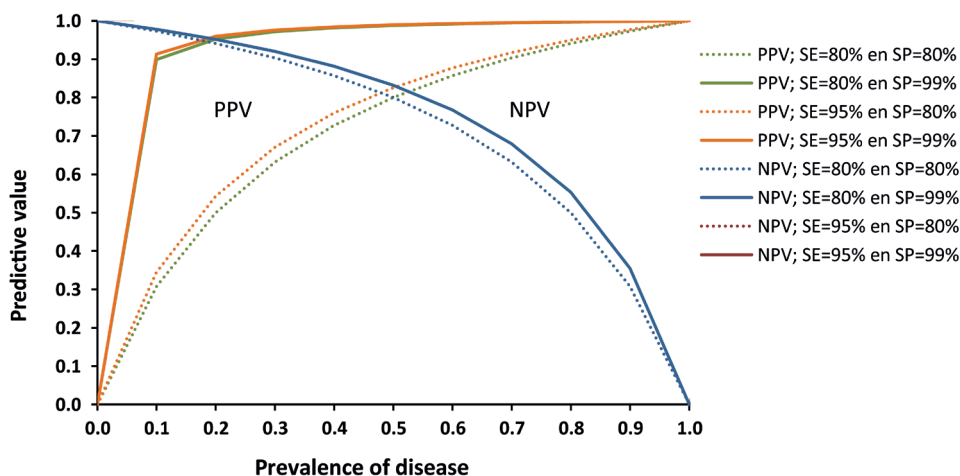


Fig. 7. The relationship between the pre-test probability and the positive- (PPV) and negative predictive value (NPV) of a test used to diagnose a disease [171]. Predictive values are shown using a test with a sensitivity (SE) of 80% (green and blue lines) and 95% (orange and red lines), and a specificity (SP) of 80% (dotted lines) and 95% (solid lines).

THE LABORATORY DIAGNOSIS OF LYME NEUROBORRELIOSIS

To diagnose LNB, clinicians seek to confirm clinical symptoms presumptive for LNB by the findings of (non-) specific immunological changes in the CSF. Non-specific changes in the CSF of LNB cases usually constitute a lymphomonocytic pleocytosis [224], although in a limited number of cases the absence of pleocytosis has been reported [105, 225]. Other non-specific changes in the CSF of LNB patients usually constitute the presence of oligoclonal IgG, the demonstration of intrathecal synthesis of total IgM and/or total IgG, elevated protein levels, and/or a dysfunctional blood-CSF barrier [226, 227]. A dysfunctional blood-CSF barrier is characterized by an increased CSF to serum ratio of albumin compared to the age-related CSF to serum ratio of albumin and reflects a reduced turnover rate of CSF [228]. An increased CSF to serum ratio of albumin can also implicate blood admixture of CSF and can be differentiated from a reduced CSF turnover rate via differential cell counts [228]. A dysfunctional blood-CSF barrier often intensifies during the course of disease

[227], and both the CSF leucocyte count and the blood-CSF barrier functionality will improve when symptoms resolve. Intrathecal synthesis of total IgM and/or total IgG, however, can remain present for a longer period of time [227, 229]. Low CSF glucose levels, which can be indicative for bacterial meningitis, are mostly seen in patients with chronic LNB [230]. Specific changes in the CSF of LNB cases comprise the presence of intrathecally produced *Borrelia*-specific antibodies. Normal CSF findings, however, do not exclude LNB and have been reported in very early cases of LNB and might be linked to an infection with *B. afzelii* [75, 144, 231]. Normal CSF findings are also observed in patients with ACA-associated polyneuropathy in Europe [232] or in patients with chronic neuropathy in North America [233].

The European Federation for Neurological Societies (EFNS) have constructed guidelines for the diagnosis and subsequent classification of LNB patients in definite or possible LNB [115]. A definite LNB patient must fulfill the following three criteria: (i) clinical symptoms suggestive of LNB (such as meningo-radicularitis, unilateral or bilateral facial palsy, other cranial neuropathies, meningitis, encephalitis, myelitis, or vasculitis) in the absence of another explanation for these symptoms, (ii) CSF pleocytosis, and (iii) intrathecal *Borrelia*-specific antibodies synthesis. A possible LNB patient must have clinical symptoms suggestive of LNB and either one of the other two criteria. In the absence of intrathecally produced *Borrelia*-specific antibodies, a possible LNB patient must have *Borrelia*-specific serum antibodies detectable 6 weeks post infection. If symptom duration is less than 6 weeks, then a positive PCR or culture may be supportive. Patients with late polyneuropathy (symptom duration of more than 6 months) can only be classified as a definite LNB patient if they have peripheral neuropathy, ACA and *Borrelia*-specific serum antibodies. In all other cases, patients are classified as non-LNB patient.

In North America, guidelines recommend that the diagnosis of LNB should be supported by the presence of *Borrelia*-specific serum antibodies (in case of peripheral or central nervous system involvement) and/or intrathecal synthesis of *Borrelia*-specific antibodies (in case of central nervous system involvement) [234]. In case the central nervous system is involved, pleocytosis can also support the diagnosis [234].

THE DETECTION OF INTRATHECALLY PRODUCED *BORRELIA*-SPECIFIC ANTIBODIES

In the absence of a gold standard test, the detection of intrathecally produced *Borrelia*-specific antibodies (IgM and IgG) is currently recommended for the diagnosis of LNB [115]. This is based on the measurement of the relative amounts of *Borrelia*-specific antibodies in CSF and serum and the subsequent calculation of a *Borrelia*-specific CSF/serum antibody index (AI) [115, 234]. For LNB diagnostics, one of two methods is often used. The first method is based on the calculation of a *Borrelia*-specific AI by determining the fraction of *Borrelia*-specific antibodies in the CSF and serum as part of the total amount of antibodies in the CSF and serum using the capture ELISA principle as described by Hansen and Lebech [235]. The second method is based on the calculation of a *Borrelia*-specific AI as described by Reiber and Peter [228]. Recently, the detection of *Borrelia*-specific antibodies in CSF only has also been evaluated [236-238].

Ideally, the presence of intrathecally produced *Borrelia*-specific antibodies is determined using methods that consider the functionality of the blood-CSF barrier and discriminate between blood-derived and brain-derived *Borrelia*-specific antibodies in the CSF [227]. Furthermore, methods are preferred that can correct for a poly-specific immune response [227]. Irrespective of the method used (i.e., a capture ELISA [235] or the method described by Reiber and Peter [228]), the calculation of the *Borrelia*-specific AI is complicated, and much attention must be paid to the individual measurements of the *Borrelia*-specific antibodies in the tested CSF/serum pairs, and consequently, to the CSF/serum pair to be used for determining the *Borrelia*-specific AI (Figure 8). When using the method described by Reiber and Peter [228], the relative amounts of total

antibodies in CSF and serum are also determined, and special attention is required with regard to the total antibody CSF/serum quotient to prevent false-negative AI results due to a poly-specific immune response [228].

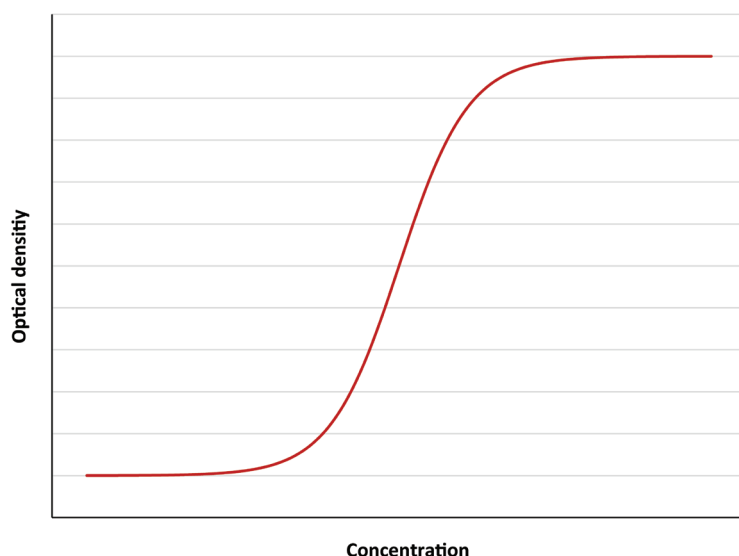


Fig. 8. The relationship between the optical density (OD) values (plotted on the y-axis) and the (log-transformed) concentrations (plotted on the x-axis) of serum and cerebrospinal fluid (CSF) is described by a sigmoid-shaped (S) curve [239]. Ideally, for an antibody index (AI) calculation, the concentrations of both serum and CSF are located in the middle (linear) part of the S-curve. When the OD values of CSF and serum are both located in the lower left corner (or both located in the upper right corner) of the S-curve, small differences between the OD values will result in larger differences between the corresponding concentrations, as is reflected by the almost horizontal lines in the S-curve in both corners [239]. Thus, when the OD values of CSF and serum are both located in the lower left part or upper right part of the curve on the horizontal line, and the serum-OD value is lower than the CSF-OD value, the difference in concentration will be larger, which will result in an increased CSF/serum quotient as well as an increased AI. This can lead to false-positive tests [239]. In contrast, when the serum-OD value is higher than the CSF-OD value, the difference in concentration will be smaller, which will result in a decreased CSF/serum quotient as well as a decreased AI. This can lead to false-negative test results [239]. Therefore, AI test results should be interpreted with care.

Overall, the detection of intrathecally produced *Borrelia*-specific antibodies requires a considerable amount of experience. Therefore, not all diagnostic laboratories are capable to perform such analysis. Furthermore, the interpretation of *Borrelia*-specific AI results is challenging for the same reasons as those encountered with interpreting the serology results performed on blood. Hence, a negative *Borrelia*-specific AI result does not exclude LNB, and a positive *Borrelia*-specific AI result is not an indication of active disease.

THE CELLULAR IMMUNE SYSTEM

Many studies have investigated the usefulness of measuring changes in the cellular immune response for the diagnosis of LB. Some of the diagnostic tools that were studied included the interferon-gamma (IFN- γ) based enzyme-linked immunospot (ELISpot) assay, the lymphocyte proliferation test (LTT), and the B-cell chemokine (C-X-C motif) ligand 13 (CXCL13) ELISA.

Changes in the cellular immune response can be detected by measuring the inflammatory response of the cellular immune system after exposure to pathogen-specific antigens. One of the inflammatory markers is IFN- γ , and for tuberculosis, a number of commercial IFN- γ release assays are available for use in diagnostics [240], among which the IFN- γ ELISpot assay [241]. In this assay,

peripheral blood mononuclear cells from patients are stimulated with antigens derived from *Mycobacterium tuberculosis*, which leads to the production of various inflammatory markers such as IFN- γ by T cells, which can be measured in vitro [242, 243]. For Q-fever, a research group in our hospital has shown the potential of using the cellular immune response in the diagnosis of this disease using an in-house IFN- γ ELISpot assay measuring the antigen-specific T-cell response induced by antigens derived from *Coxiella burnetii* [244], the causative agent of Q-fever. In both tuberculosis and Q-fever, T-cell assays can demonstrate whether a patient is infected; however, differentiation between active disease and a past infection is – as of yet – not possible. Whether this is any different for LB remains to be elucidated.

Before the start of our research into LB diagnostics in 2011, various studies had already shown that the cellular immune response against *B. burgdorferi* s.l. is characterized by a strong T helper (Th)1 response, in which IFN- γ is produced in the blood of patients with LNB and LA [245], in skin lesions of EM patients [246], in synovial fluid of LA patients [247], and in the CSF of LNB patients [248]. Similarly, by using an IFN- γ ELISpot assay, increased numbers of IFN- γ producing *Borrelia*-specific T cells were found in patients with early, late or chronic LB [249-251]. Increased numbers of IFN- γ producing *Borrelia*-specific T cells, however, were also found in seropositive, asymptomatic individuals [252, 253]. Furthermore, it has been put forward that the immune response in LNB patients is mainly localized in the CSF [249, 250, 254, 255], therefore suggesting that the investigation of CSF might be more suitable than that of blood. For LB, however, there are inconsistencies with regard to the results obtained with the IFN- γ ELISpot assay in the studies conducted thus far [256]. Studies often lack cutoff values [256], use different [251, 253, 255, 257], or unknown [258] *Borrelia* antigens and different study populations [256] leading to a large variability in results [253, 256, 257]. Most studies, however, did show that IFN- γ was associated with exposure to *Borrelia* [256].

Other studies reported the use of LTTs for the diagnosis of LB in which the proliferation of T cells is measured upon stimulation with *Borrelia*-specific antigens [259-263]. LTTs for diagnostic purposes are complex in their execution as these often require the use of radioactive substances, prolonged incubation times up to 5 days, and highly specialized personnel. Furthermore, LTTs are less sensitive and less specific than serology [259, 262, 263], although positive LTT results have been found among seronegative patients [259, 260, 263], which might be explained by the (antigen-composition of the) antibody assay used to detect the *Borrelia*-specific antibodies [259, 262]. Overall, studies on LTT showed a large variation in results and lacked clear performance characteristics and LTT is, therefore, not recommended in most LB guidelines [86, 256].

Despite the lack of published studies on clinically validated cellular assays using well-defined study populations, different laboratories offer commercial LTTs or IFN- γ based assays for the routine diagnosis of LB [264-270]. Despite the lack of a robust validation of these tests, patients may be offered prolonged courses of antibiotics assuming that a positive test result for LB is indicative for active disease [270]. This underlines the importance of a thorough clinical validation of these assays using a well-defined study population before implementation in routine diagnostics.

CXCL13 is considered a promising marker for the diagnosis of LNB as elevated levels of this chemokine have been found in the CSF of patients with early LNB, and CSF-CXCL13 levels rapidly decline after antibiotic treatment [271-274]. As illustrated in Figure 9, CXCL13 is produced by mononuclear cells upon entering of *B. burgdorferi* s.l. into the CSF and attracts B cells [71, 275]. Indeed, pleocytosis seen in LNB patients is characterized by a relatively large fraction of B cells [71, 145, 275, 276]. These B cells will mature into plasma cells and subsequently produce *Borrelia*-specific antibodies [71]. This hypothesis is supported by the detection of elevated CSF-CXCL13 levels prior to intrathecal *Borrelia*-specific antibody synthesis [271, 277], thereby showing its

potential for diagnosing early LNB cases. Furthermore, as CSF-CXCL13 levels rapidly decrease after antibiotic therapy, it is a potential marker for measuring disease activity [71, 271-274]. An international reference standard for the use of CXCL13 in CSF is lacking and a broad range of CSF-CXCL13 cutoff levels, ranging from less than 100 to more than 1229 pg/ml, have been published [271, 273, 274, 278, 279]. Thus, appropriate cutoff levels are warranted when CXCL13 is to be implemented for routine LNB diagnostics. However, since elevated CSF-CXCL13 levels are also found in other central nervous system diseases (infectious and non-infectious), results should be interpreted with care [273, 274, 280, 281].

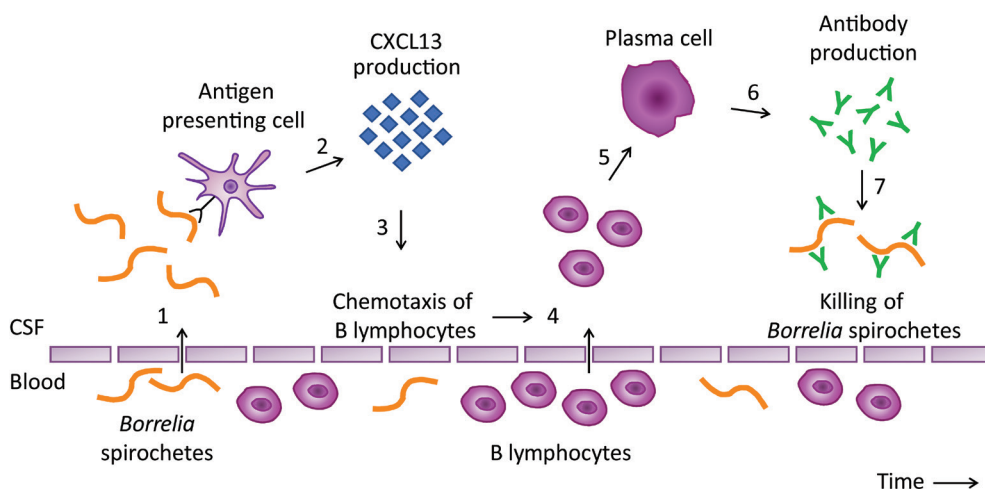


Fig. 9. The antibody response in the CSF in response to a *Borrelia* infection. *B. burgdorferi* s.l. enters the CSF and is recognized by antigen presenting cells (1). These cells subsequently produce CXCL13 (2), which attracts B cells (3). Consequently, B cells migrate into the CSF (4) and mature into plasma cells (5). Plasma cells will, then, produce *Borrelia*-specific antibodies (6) leading to the killing of *B. burgdorferi* s.l. (7). Figure adapted from Rupprecht et al. [71]. This article is published under license to BioMed Central Ltd.. This is an Open Access article distributed under the terms of the Creative Commons Attribution License. Full terms at <https://creativecommons.org/licenses/by/2.0>.

AIMS OF THIS THESIS

LB is the most commonly reported tick-borne infection and is mainly seen in the temperate regions of the Northern Hemisphere [1]. LB, caused by spirochetes belonging to the *B. burgdorferi* s.l. complex group, is a multisystem disease that can lead to local, early disseminated and late disseminated infection and can involve skin, nervous system, heart, joints and eyes [1]. Except for the typical EM lesions, the diagnosis of LB is based on clinical symptoms and should be confirmed by laboratory tests [86]. The diagnosis of LB, however, can be a challenge due to the large variety of clinical symptoms as these are mostly non-pathognomonic [2]. The variety of clinical symptoms might be caused by the intra- and interspecies heterogeneity of *B. burgdorferi* s.l. and/or host genetic factors [71, 81, 82, 125, 126, 139, 188-190]. The definite diagnosis of LB can also be a challenge due to the difficulty of interpreting laboratory test results, as a gold standard test is lacking. Negative test results do not exclude active disease and positive test results are no proof of active disease.

Due to these challenges for, and uncertainty of LB diagnosis, numerous civilians in North America [282-284] and Europe [285-289], who are affected by LB, have established support groups in a struggle for more recognition for patients with (possible) LB. These patient support groups provide a means to talk to and meet other patients affected by LB and to seek and provide

mutual support, and discuss issues regarding current diagnostics and research. The frustration regarding LB diagnostics among patients has even led to law suits against medical specialists [290]. In 2010, the Dutch Association for Lyme Disease Patients (NVLP) presented a petition to the Dutch Parliament, signed by over 70,000 people, to raise political attention and funding for research to improve diagnostics and treatment of LB, to increase knowledge about LB and its various manifestations, and to establish Lyme treatment centers to better serve patients with LB [291, 292]. Some of the concerns raised by the NVLP involve the use of antibody assays for LB diagnostics, including the lack of standardization of antibody tests, inter-laboratory test variation, and the inability of tests to detect all European *Borrelia* genospecies. Current diagnostic tests lack sensitivity and specificity leading to uncertainty for both medical specialists and patients whether an active infection with *Borrelia* can be excluded. Consequently, Lyme patients may feel unrecognized and seek help elsewhere (outside the main stream clinics) and rely on non-validated tests [264, 265] to ‘prove’ they have LB and often receive long-term antibiotic treatments [270]. Unfortunately, many of these tests lack proper validation and are not reimbursed by health insurance companies leading to unnecessary patient suffering and high costs [270, 291]. Consequently, the petition of the NVLP involved a number of requests. One of these requests included the development of more sensitive laboratory tests that better reflect the various *B. burgdorferi* s.l. genospecies and take into account the antigenic variation displayed by *B. burgdorferi* s.l.. Two other requests involved (i) the use of multiple tests for the diagnosis of LB, which should include tests based on direct detection methods, and (ii) the acceptance of tests offered abroad as these are currently not reimbursed by insurance companies. As a response on this petition, the Dutch government secured funding for the development of a Lyme expertise center to address these (diagnostic) challenges.

Medical specialists and patients alike are faced with the challenges surrounding LB diagnostics and better diagnostic tools are warranted, especially, since early and correct diagnosis of LB is essential for adequate treatment with antibiotics [21, 76, 293-296]. Therefore, the research in this thesis aims at addressing whether the diagnostic approach for LB (mainly LNB) can be improved and whether active disease can be distinguished from a previous - yet cleared - infection. To answer these questions, current and alternative diagnostic tests and/or algorithms are evaluated that include well-established diagnostic tests based on the humoral immune response (i.e., the detection of *Borrelia*-specific antibodies) as well as alternative diagnostic tests based on the cellular immune response. Consequently, this thesis is divided into two parts; one focusing on the humoral immune response against a *Borrelia* infection, and one focusing on the cellular immune response against a *Borrelia* infection. As clear case-definitions are defined for active LNB, well-defined patients with LNB are used as a proxy for patients with active LB.

OUTLINE OF THIS THESIS

PART I: THE CELLULAR IMMUNE RESPONSE

The research in this thesis, amongst others, involves the evaluation of the IFN- γ ELISpot assay for the routine diagnosis of LNB, as robust validations of such assays using well-defined study populations are lacking [264-270]. As our laboratory has a long-time experience in performing IFN- γ ELISpot assays [244, 297-304], an in-house *Borrelia* ELISpot assay was developed. To evaluate this assay for use in LNB diagnostics, a prospective case-control study (i.e., the T-cell response in Lyme (TRIL)-study) was set-up. The regional Medical Research Ethics Committees United approved the study (Nieuwegein, the Netherlands; MEC-U: NL36407.100.11), and all study participants gave their informed consent. Cases consist of well-defined active LNB patients (i.e., active LNB served as a proxy for active disease), and controls are divided into three groups

and consist of either patients treated for LNB in the past, healthy individuals treated for LB (mainly cutaneous) in the past, or untreated healthy individuals. In **chapter 2**, using the study population of the TRIL-study, the diagnostic potential of the in-house *Borrelia* ELISpot assay is evaluated. Therefore, the number of IFN- γ -producing T cells are measured after stimulating peripheral blood mononuclear cells with *B. burgdorferi* s.s. (strain B31) to investigate whether *Borrelia*-specific IFN- γ -producing T cells can be used as a marker for disease activity. Additional risk factors such as previous tick bites, clinical symptoms, and antibiotic treatment for LB are investigated to assess their contribution to the diagnostic performance of the in-house *Borrelia* ELISpot assay.

In **chapter 3**, the diagnostic performance of a commercial LymeSpot assay, that has not been validated previously, is compared to the diagnostic performance of the in-house *Borrelia* ELISpot assay studied in **chapter 2**. Again, active LNB patients are used as cases and the results are compared with those obtained from controls existing of treated LNB patients, and healthy individuals with or without a history of treated LB (mainly cutaneous).

In **chapter 4**, the diagnostic potential of two commercial CXCL13 assays on CSF is investigated. One of the assays under investigation has been studied extensively for testing CSF, even though the instruction manual for this assay does not mention the use of CSF for the detection of CXCL13. Consequently, this manual lacks a cutoff value for CSF-CXCL13. In the literature, a broad range of cutoff values are mentioned for this assay, which necessitates its validation before use in our hospital. To investigate the diagnostic potential of these two CXCL13 assays on CSF, a retrospective, cross-sectional study design was constructed using a well-characterized study population representative for the clinical setting in which these assays will be used.

PART II: THE HUMORAL IMMUNE SYSTEM

The detection of intrathecally produced *Borrelia*-specific antibodies should be assessed by the detection of these antibodies in CSF and serum within the same run and the subsequent calculation of a *Borrelia*-specific AI. To ensure optimal accuracy, the well-to-well variation (i.e., the intra-assay variation) of the test plate should be minimal. In **chapter 5**, the intra-assay variation of the commercial Enzygnost Lyme link VlsE/IgG ELISA that can be used for the diagnosis of LNB is investigated using a single dilution of the positive kit control (human serum with specific IgG to *B. burgdorferi*). The Enzygnost Lyme link VlsE/IgG ELISA measures IgG to a whole-cell lysate of *B. afzelii* PKo supplemented with recombinant VlsE. The possible impact of the intra-assay variation on LNB diagnostics is investigated through simulation using almost the same study population as the one used in **chapter 4** (i.e., the study population in **chapter 5** comprised 149 of the 156 patients used in **chapter 4**).

In **chapter 6**, the diagnostic performance of seven commercial antibody assays for LNB diagnostics is investigated using the same study population as the one used in **chapter 4**. Five antibody assays were based on the detection of *Borrelia*-specific antibodies (IgM and IgG) in CSF and serum and subsequent calculation of the *Borrelia*-specific (IgM and IgG) AI. Two antibody assays were based on the detection of these antibodies in CSF only as the calculation of a *Borrelia*-specific AI is rather complicated as was mentioned previously. Consequently, not all laboratories have the capacity and expertise to perform these analyses and, thus, a CSF-only assay to prove intrathecal *Borrelia*-specific antibody synthesis is to be preferred. Using multiparameter analysis, various routine CSF parameters (i.e., leucocyte count, total protein, blood-CSF barrier function, and intrathecal total-antibody synthesis) and a number of other parameters (i.e., *Borrelia*-specific serum antibodies, CSF-CXCL13, and a *Borrelia* species PCR on CSF) are investigated to assess their contribution to the diagnostic performance of each antibody assay.

In **chapter 7**, two standard two-tier test strategies for the detection of *Borrelia*-specific antibodies in serum are evaluated. The two strategies are based on an ELISA (either the C6 ELISA or the Serion ELISA), followed by confirmation of equivocal and positive ELISA results using the *recomLine* immunoblot. The C6 ELISA measures total immunoglobulin to the recombinant C6 peptide, and the Serion ELISA measures IgM and IgG to two whole-cell lysates of *B. burgdorferi* s.l. (i.e., *B. afzelii* Pko and *B. garinii*) with the addition of recombinant VlsE for the detection of IgG. A third test strategy is also evaluated and consists of a more unconventional approach based on the combination of both ELISAs as a screening test and immunoblot confirmation of all test results, except concordant negative test results. Again, active LNB patients are used as a proxy for active disease and results are compared to those obtained by testing the serum of treated LNB patients, and healthy individuals with or without a history of treated LB (mainly cutaneous).

REFERENCES

1. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, Kristoferitsch W, O'Connell S, Ornstein K, Strle F, Gray J. 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* 17:69-79. doi: 10.1111/j.1469-0691.2010.03175.x.
2. Stanek G, Strle F. 2018. Lyme borreliosis-from tick bite to diagnosis and treatment. *FEMS Microbiol Rev* 42:233-258. doi: 10.1093/femsre/fux047.
3. Steere AC, Malawista SE, Snyderman DR, Shope RE, Andiman WA, Ross MR, Steele FM. 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. *Arthritis Rheum* 20:7-17. doi: 10.1002/art.1780200102.
4. Afzelius A. 1910. Verhandlungen der dermatologischen Gesellschaft in Stockholm, December, 1909. *Arch Dermatol Syphil (Berlin)* 101:405-6. doi: 10.1007/978-3-662-34443-9_1.
5. Lipschütz B. 1913. Über eine seltene Erythemform (Erythema chronicum migrans). *Archiv für Dermatologie und Syphilis* 118:349-356. doi: 10.1007/BF02076105.
6. Afzelius A. 1921. Erythema chronicum migrans. *Acta Derm Venereol* 2:120-125.
7. Garin C, Bujadoux A. 1922. Paralysie par les tiques [tick paralysis]. *J Med Lyon* 71:765-767.
8. Hellerström S. 1930. Erythema chronicum migrans. *Acta Derm Venereol (Stockh)* 11:315-321.
9. Bannwarth A. 1941. Chronische lymphocytäre Meningitis, entzündliche Polyneuritis und „Rheumatismus“. *Archiv für Psychiatrie und Nervenkrankheiten* 113:284-376. doi: 10.1007/BF02095652.
10. Bannwarth A. 1944. Zur Klinik und Pathogenese der „chronischen lymphocytären Meningitis“. *Archiv für Psychiatrie und Nervenkrankheiten* 117:682-716. doi: 10.1007/BF01837711.
11. Lennhoff C. 1948. Spirochaetes in aetiologically obscure diseases. *Acta Derm Venereol* 28:295-324.
12. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. 1982. Lyme disease-a tick-borne spirochetosis? *Science* 216:1317-9. doi: 10.1126/science.7043737.
13. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE. 1983. The spirochetal etiology of Lyme disease. *N Engl J Med* 308:733-40. doi: 10.1056/NEJM198303313081301.
14. Stiernstedt G, Eriksson G, Enfors W, Jorbeck H, Svenungsson B, Skoldenberg B, Granstrom M. 1986. Erythema chronicum migrans in Sweden: clinical manifestations and antibodies to *Ixodes ricinus* spirochete measured by indirect immunofluorescence and enzyme-linked immunosorbent assay. *Scand J Infect Dis* 18:217-24. doi: 10.3109/00365548609032330.
15. Baranton G, Postic D, Saint G, I, Boerlin P, Piffaretti JC, Assous M, Grimont PA. 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* 42:378-383. doi: 10.1099/00207713-42-3-378.
16. Radolf JD, Strle K, Lemieux JE, Strle F. 2021. Lyme Disease in Humans. *Curr Issues Mol Biol* 42:333-384. doi: 10.21775/cimb.042.333.
17. Steere AC. 2001. Lyme disease. *N Engl J Med* 345:115-25. doi: 10.1056/NEJM200107123450207.
18. Lane RS, Piesman J, Burgdorfer W. 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annual review of entomology* 36:587-609. doi: 10.1146/annurev.en.36.010191.003103.
19. Margos G, Fingerle V, Reynolds S. 2019. *Borrelia bavariensis*: Vector Switch, Niche Invasion, and Geographical Spread of a Tick-Borne Bacterial Parasite. *Frontiers in Ecology and Evolution* 7. doi: 10.3389/fevo.2019.00401.

20. Lindgren E, Jaenson GTJ. 2006. Lyme Borreliosis in Europe: Influences of Climate and Climate Change, Epidemiology, Ecology and Adaptation Measures. Available at: https://www.researchgate.net/publication/252720714_Lyme_Borreliosis_in_Europe_Influences_of_Climate_and_Climate_Change_Epidemiology_Ecology_and_Adaptation_Measures. (Accessed March 2022).
21. Stanek G, Wormser GP, Gray J, Strle F. 2012. Lyme borreliosis. *Lancet* 379:461-73. doi: 10.1016/S0140-6736(11)60103-7.
22. Andreassen AM, Dehlendorff PB, Knudtzen FC, Bødker R, Kjær LJ, Skarphéðinsson S. 2020. Spatial and temporal patterns of Lyme Neuroborreliosis on Funen, Denmark from 1995–2014. *Scientific Reports* 10. doi: 10.1038/s41598-020-64638-5.
23. Septfons A, Goronflot T, Jaulhac B, Roussel V, De Martino S, Guerreiro S, Launay T, Fournier L, De Valk H, Fignon J, Blanchon T, Couturier E. 2019. Epidemiology of Lyme borreliosis through two surveillance systems: the national Sentinelles GP network and the national hospital discharge database, France, 2005 to 2016. *Euro Surveill* 24. doi: 10.2807/1560-7917.ES.2019.24.11.1800134.
24. Coipan EC, Jahfari S, Fonville M, Maassen CB, van der Giessen J, Takken W, Takumi K, Sprong H. 2013. Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. *Front Cell Infect Microbiol* 3:36. doi: 10.3389/fcimb.2013.00036.
25. Hofhuis A, Herremans T, Notermans DW, Sprong H, Fonville M, van der Giessen JW, van Pelt W. 2013. A prospective study among patients presenting at the general practitioner with a tick bite or erythema migrans in The Netherlands. *PLoS One* 8:e64361. doi: 10.1371/journal.pone.0064361.
26. Kurokawa C, Lynn GE, Pedra JHF, Pal U, Narasimhan S, Fikrig E. 2020. Interactions between *Borrelia burgdorferi* and ticks. *Nature Reviews Microbiology* 18:587-600. doi: 10.1038/s41579-020-0400-5.
27. Rollend L, Fish D, Childs JE. 2013. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: a summary of the literature and recent observations. *Ticks Tick Borne Dis* 4:46-51. doi: 10.1016/j.ttbdis.2012.06.008.
28. Richter D, Debski A, Hubalek Z, Matuschka FR. 2012. Absence of Lyme disease spirochetes in larval *Ixodes ricinus* ticks. *Vector Borne Zoonotic Dis* 12:21-7. doi: 10.1089/vbz.2011.0668.
29. Mysterud A, Stigum VM, Jaarsma RI, Sprong H. 2019. Genospecies of *Borrelia burgdorferi* sensu lato detected in 16 mammal species and questing ticks from northern Europe. *Scientific reports* 9:1-8. doi: 10.1038/s41598-019-41686-0.
30. Tulloch JSP, Semper AE, Brooks TJG, Russell K, Halsby KD, Christley RM, Radford AD, Vivancos R, Warner JC. 2019. The demographics and geographic distribution of laboratory-confirmed Lyme disease cases in England and Wales (2013-2016): an ecological study. *BMJ Open* 9:e028064. doi: 10.1136/bmjopen-2018-028064.
31. Berglund J, Eitrem R, Ornstein K, Lindberg A, Ringer A, Elmud H, Carlsson M, Runehegen A, Svanborg C, Norrby R. 1995. An epidemiologic study of Lyme disease in southern Sweden. *N Engl J Med* 333:1319-27. doi: 10.1056/NEJM199511163332004.
32. Eliassen KE, Berild D, Reiso H, Grude N, Christophersen KS, Finckenhagen C, Lindbaek M. 2017. Incidence and antibiotic treatment of erythema migrans in Norway 2005-2009. *Ticks Tick Borne Dis* 8:1-8. doi: 10.1016/j.ttbdis.2016.06.006.
33. CDC. Centers for Disease Control and Prevention. Lyme Disease Home. Data and surveillance. Recent surveillance data. Lyme disease-Reported cases by age group, United States, 2019. <https://www.cdc.gov/lyme/datasurveillance/charts-figures-recent.html>. Accessed 02-02-2022.
34. Enkelmann J, Bohmer M, Fingerle V, Siffczyk C, Werber D, Littmann M, Merbecks SS, Helmeke C, Schroeder S, Hell S, Schlotthauer U, Burckhardt F, Stark K, Schielke A, Wilking H. 2018. Incidence of notified Lyme borreliosis in Germany, 2013-2017. *Sci Rep* 8:14976.

- doi: 10.1038/s41598-018-33136-0.
35. Schwartz AM, Hinckley AF, Mead PS, Hook SA, Kugeler KJ. 2017. Surveillance for Lyme Disease - United States, 2008-2015. *MMWR Surveill Summ* 66:1-12. doi: 10.15585/mmwr.ss6622a1.
 36. CDC. Centers for Disease Control and Prevention. Lyme Disease Home. Data and Surveillance. <https://www.cdc.gov/lyme/datasurveillance/> [Accessed 26 October 2021].
 37. Lee WC, Lee MJ, Choi KH, Chung HS, Choe NH. 2019. A comparative study of the trends in epidemiological aspects of Lyme disease infections in Korea and Japan, 2011–2016. *Journal of Vector Borne Diseases* 56:268. doi: 10.4103/0972-9062.289396.
 38. Sabitova Y, Fomenko N, Tikunov A, Stronin O, Khasnatinov M, Abmed D, Danchinova G, Golovljova I, Tikunova N. 2018. Multilocus sequence analysis of *Borrelia burgdorferi* sensu lato isolates from Western Siberia, Russia and Northern Mongolia. *Infection, Genetics and Evolution* 62:160-169. doi: 10.1016/j.meegid.2018.04.015.
 39. Zhao GP, Wang YX, Fan ZW, Ji Y, Liu MJ, Zhang WH, Li XL, Zhou SX, Li H, Liang S, Liu W, Yang Y, Fang LQ. 2021. Mapping ticks and tick-borne pathogens in China. *Nature communications* 12:1-13. doi: 10.1038/s41467-021-21375-1.
 40. von Fricken ME, Rolomjav L, Illar M, Altantogtokh D, Hogan KM, Uyanga B, Ganbold D, Tsogbadrakh N. 2019. Geographic Range of Lyme Borreliosis in Mongolia. *Vector Borne Zoonotic Dis* 19:658-661. doi: 10.1089/vbz.2018.2421.
 41. Smith R, Takkinen J. 2006. Lyme borreliosis: Europe-wide coordinated surveillance and action needed? *Euro Surveill* 11. doi: 10.2807/esw.11.25.02977-en.
 42. Sykes RA, Makiello P. 2016. An estimate of Lyme borreliosis incidence in Western Europe. *J Public Health (Oxf)*. doi: 10.1093/pubmed/fdw017.
 43. 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* 25:426-32. doi: 10.1007/s10096-006-0167-2.
 44. Altpeter E, Zimmermann H, Oberreich J, Peter O, Dvorak C, Swiss Sentinel Surveillance N. 2013. Tick related diseases in Switzerland, 2008 to 2011. *Swiss Med Wkly* 143:w13725. doi: 10.4414/smw.2013.13725.
 45. Sočan M, Markič MB. 2020. [ZD25] Reported cases of Lyme borreliosis and tick-borne meningoencephalitis in Slovenia. National Institute of Public Health. 15.11.2020 [<http://kos.arso.gov.si/en/content/reported-cases-lyme-borreliosis-and-tick-borne-meningoencephalitis-slovenia-1>] (Accessed 2022 Jan 05).
 46. van den Wijngaard CC, Brown V, Harms MG, van Pelt W, Hofhuis A. Increase in the incidence and burden of Lyme borreliosis in the Netherlands between 2014 and 2017. https://www.rivm.nl/sites/default/files/2019-04/19.%20Hofhuis_Lyme%20borreliosis.pdf. (Accessed 12 January 2022).
 47. Sajanti E, Virtanen M, Helve O, Kuusi M, Lyytikäinen O, Hytonen J, Sane J. 2017. Lyme Borreliosis in Finland, 1995-2014. *Emerg Infect Dis* 23:1282-1288. doi: 10.3201/eid2308.161273.
 48. Orliková H, Kybicová K, Malý M, Kynčl J. 2021. Surveillance and epidemiology of Lyme borreliosis in the Czech Republic in 2018 and 2019. *Biologia* doi:doi: 10.1007/s11756-021-00868-w. doi: 10.1007/s11756-021-00868-w.
 49. Vandenesch A, Turbelin C, Couturier E, Arena C, Jaulhac B, Ferquel E, Choumet V, Saugeon C, Coffinieres E, Blanchon T, Vaillant V, Hanslik T. 2014. Incidence and hospitalisation rates of Lyme borreliosis, France, 2004 to 2012. *Euro Surveill* 19. doi: 10.2807/1560-7917.es2014.19.34.20883.
 50. Geebelen L, Van Cauteren D, Devleeschauwer B, Moreels S, Tersago K, Van Oyen H, Speybroeck N, Lernout T. 2019. Combining primary care surveillance and a meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Belgium, 2015-2017. *Ticks Tick Borne Dis* 10:598-605. doi: 10.1016/j.ttbdis.2018.12.007.

51. Petrulioniene A, Radzisauskiene D, Ambrozaitis A, Caplinskas S, Paulauskas A, Venalis A. 2020. Epidemiology of Lyme Disease in a Highly Endemic European Zone. *Medicina* (Kaunas) 56. doi: 10.3390/medicina56030115.
52. Tulloch JSP, Christley RM, Radford AD, Warner JC, Beadsworth MJB, Beeching NJ, Vivancos R. 2020. A descriptive epidemiological study of the incidence of newly diagnosed Lyme disease cases in a UK primary care cohort, 1998-2016. *BMC Infect Dis* 20:285. doi: 10.1186/s12879-020-05018-2.
53. Zanzani SA, Rimoldi SG, Manfredi M, Grande R, Gazzonis AL, Merli S, Olivieri E, Giacometti V, Antinori S, Cislighi G, Bestetti G, Nan K, Sala V, Gismondo MR, Atzori C, De Faveri E. 2019. Lyme borreliosis incidence in Lombardy, Italy (2000-2015): Spatiotemporal analysis and environmental risk factors. *Ticks Tick Borne Dis* 10:101257. <https://doi.org/10.1016/j.ttbdis.2019.07.001>.
54. Bonet Alaves E, Guerrero Espejo A, Cuenca Torres M, Gimeno Vilarrasa F. 2016. [Incidence of Lyme disease in Spain]. *Med Clin (Barc)* 147:88-9. doi: 10.1016/j.medcli.2016.01.021.
55. Vigfusson HB, Hardarson HS, Ludviksson BR, Gudlaugsson O. 2019. [Lyme disease in Iceland - Epidemiology from 2011 to 2015]. *Laeknabladid* 105:63-70. doi: 10.17992/lbl.2019.02.215.
56. Vandekerckhove O, De Buck E, Van Wijngaerden E. 2021. Lyme disease in Western Europe: an emerging problem? A systematic review. *Acta Clin Belg* 76:244-252. doi: 10.1080/17843286.2019.1694293.
57. Rizzoli A, Hauffe M, Carpi G, Vourc HG, Neteler M, Rosa R. 2011. Lyme borreliosis in Europe. *Euro Surveill* 16. doi: 10.2807/ese.16.27.19906-en.
58. Wormser GP. 2012. 329 - Lyme Disease, p 1930-1935. *In* Goldman L, Schafer AI (ed), *Goldman's Cecil Medicine* (Twenty Fourth Edition) doi:doi: 10.1016/B978-1-4377-1604-7.00329-8. W.B. Saunders, Philadelphia.
59. Feder HM, Jr., Whitaker DL. 1995. Misdiagnosis of erythema migrans. *Am J Med* 99:412-9. doi: 10.1016/s0002-9343(99)80190-9.
60. CDC. Centers for Disease Control and Prevention. Lyme Disease Home. Data and surveillance. Historical data. Lyme Disease Charts and Figures: Historical Data. Confirmed cases by month of disease onset, United States, 2008-2019. <https://www.cdc.gov/lyme/stats/graphs.html> [Accessed 26 October 2021].
61. WHO. World Health Organisation. Lyme Borreliosis in Europe. https://www.euro.who.int/__data/assets/pdf_file/0008/246167/Fact-sheet-Lyme-borreliosis-Eng.pdf. (Accessed 07 January 2022).
62. Stone BL, Tourand Y, Brissette CA. 2017. Brave New Worlds: The Expanding Universe of Lyme Disease. *Vector Borne Zoonotic Dis* 17:619-629. doi: 10.1089/vbz.2017.2127.
63. de Mik EL, van Pelt W, Docters-van Leeuwen BD, van der Veen A, Schellekens JF, Borgdorff MW. 1997. The geographical distribution of tick bites and erythema migrans in general practice in The Netherlands. *Int J Epidemiol* 26:451-7. doi: 10.1093/ije/26.2.451.
64. Hofhuis A, Harms M, van den Wijngaard C, Sprong H, van PW. 2015. Continuing increase of tick bites and Lyme disease between 1994 and 2009. *Ticks Tick Borne Dis* 6:69-74. doi: 10.1016/j.ttbdis.2014.09.006.
65. den Boon S, Schellekens JF, Schouls LM, Suijkerbuijk AW, Docters van LB, Van PW. 2004. Doubling of the number of cases of tick bites and lyme borreliosis seen by general practitioners in the Netherlands. [In Dutch]. *Ned Tijdschr Geneesk* 148:665-670.
66. Hofhuis A, Van der Giessen JWB, Borgsteede FHM, Welinga PR, Notermans DW, Van Pelt W. 2006. Lyme borreliosis in the Netherlands: strong increase in GP consultations and hospital admissions in past 10 years. *Euro Surveill* 11:2978. doi: 10.2807/esw.11.25.02978-en.
67. Hofhuis A, Bennema S, Harms M, van Vliet AJ, Takken W, van den Wijngaard CC, Van PW. 2016. Decrease in tick bite consultations and stabilization of early Lyme borreliosis in the

- Netherlands in 2014 after 15 years of continuous increase. BMC Public Health 16:425. doi: 10.1186/s12889-016-3105-y.
68. 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. doi: 10.1186/s13071-015-0777-6.
 69. Hofhuis A, van de Kastele J, Sprong H, van den Wijngaard CC, Harms MG, Fonville M, Docters van Leeuwen A, Simoes M, van Pelt W. 2017. Predicting the risk of Lyme borreliosis after a tick bite, using a structural equation model. PLoS One 12:e0181807. doi: 10.1371/journal.pone.0181807.
 70. Sertour N, Cotte V, Garnier M, Malandrin L, Ferquel E, Choumet V. 2018. Infection Kinetics and Tropism of *Borrelia burgdorferi* sensu lato in Mouse After Natural (via Ticks) or Artificial (Needle) Infection Depends on the Bacterial Strain. Front Microbiol 9:1722. doi: 10.3389/fmicb.2018.01722.
 71. Rupprecht TA, Koedel U, Fingerle V, Pfister HW. 2008. The pathogenesis of Lyme neuroborreliosis: from infection to inflammation. Mol Med 14:205-12. doi: 10.2119/2007-00091.Rupprecht.
 72. Coburn J, Garcia B, Hu LT, Jewett MW, Kraiczky P, Norris SJ, Skare J. 2021. Lyme Disease Pathogenesis. Curr Issues Mol Biol 42:473-518. doi: 10.21775/cimb.042.473.
 73. Maraspin V, Bogovic P, Ogrinc K, Rojko T, Ruzic-Sabljić E, Kastrin A, Strle K, Wormser GP, Strle F. 2021. Are Differences in Presentation of Early Lyme Borreliosis in Europe and North America a Consequence of a More Frequent Spirochetemia in American Patients? J Clin Med 10. doi: 10.3390/jcm10071448.
 74. Wormser GP. 2006. Hematogenous dissemination in early Lyme disease. Wien Klin Wochenschr 118:634-7. doi: 10.1007/s00508-006-0688-9.
 75. Strle F, Ruzic-Sabljić E, Cimperman J, Lotric-Furlan S, Maraspin V. 2006. Comparison of findings for patients with *Borrelia garinii* and *Borrelia afzelii* isolated from cerebrospinal fluid. Clin Infect Dis 43:704-10. doi: 10.1086/506936.
 76. 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. Reviews of Infectious Diseases 63:346-353. doi: 10.1093/cid/ciw299.
 77. Ogrinc K, Kastrin A, Lotric-Furlan S, Bogovic P, Rojko T, Maraspin V, Ruzic-Sabljić E, Strle K, Strle F. 2021. Colocalization of radicular pain and erythema migrans in patients with Bannwarth's syndrome suggests a direct spread of *Borrelia* into the central nervous system. Clin Infect Dis doi:doi: 10.1093/cid/ciab867. doi: 10.1093/cid/ciab867.
 78. Balmelli T, Piffaretti JC. 1995. Association between different clinical manifestations of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Research in Microbiology 146:329-340. doi: 10.1016/0923-2508(96)81056-4.
 79. Coipan EC, Jahfari S, Fonville M, Oei GA, Spanjaard L, Takumi K, Hovius JW, Sprong H. 2016. Imbalanced presence of *Borrelia burgdorferi* s.l. multilocus sequence types in clinical manifestations of Lyme borreliosis. Infect Genet Evol 42:66-76. doi: 10.1016/j.meegid.2016.04.019.
 80. 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. doi: 10.1038/nrdp.2016.90.
 81. Strle K, Jones KL, Drouin EE, Li X, Steere AC. 2011. *Borrelia burgdorferi* RST1 (OspC type A) genotype is associated with greater inflammation and more severe Lyme disease. Am J Pathol 178:2726-39. doi: 10.1016/j.ajpath.2011.02.018.
 82. Strle K, Shin JJ, Glickstein LJ, Steere AC. 2012. Association of a Toll-like receptor 1 polymorphism with heightened Th1 inflammatory responses and antibiotic-refractory Lyme arthritis. Arthritis Rheum 64:1497-507. doi: 10.1002/art.34383.
 83. Hanincova K, Mukherjee PO, N.H. Margos, G. Wormser, G.P., Reed KD, Meece JKV, M.F.

- Schwartz, I. . 2013. Multilocus sequence typing of *Borrelia burgdorferi* suggests existence of lineages with differential pathogenic properties in humans. *PloS one* 8:e73066. doi: 10.1371/journal.pone.0073066.
84. Wilhelmsson P, Fryland L, Lindblom P, Sjöwall J, Ahlm C, Berglund J, Haglund M, Henningsson AJ, Nolskog P, Nordberg M. 2016. A prospective study on the incidence of *Borrelia burgdorferi* sensu lato infection after a tick bite in Sweden and on the Åland Islands, Finland (2008–2009). *Ticks and tick-borne diseases* 7:71-79. doi: 10.1016/j.ttbdis.2015.08.009.
 85. Steere AC, Sikand VK, Schoen RT, Nowakowski J. 2003. Asymptomatic infection with *Borrelia burgdorferi*. *Clinical infectious diseases* 37:528-532. doi: 10.1086/376914.
 86. 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. doi: 10.1016/j.medmal.2018.11.011.
 87. Berger BW, Johnson RC, Kodner C, Coleman L. 1992. Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. *Journal of clinical microbiology* 30:359-361. doi: 10.1128/jcm.30.2.359-361.1992.
 88. CDC. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System (NNDS). Surveillance Case Definitions. Lyme disease. Lyme Disease (*Borrelia burgdorferi*) 2022 Case Definition. <https://ndc.services.cdc.gov/case-definitions/lyme-disease-2022/>. Accessed 18 Januari 2022.
 89. Marques AR, Strle F, Wormser GP. 2021. Comparison of Lyme Disease in the United States and Europe. *Emerg Infect Dis* 27:2017-2024. doi: 10.3201/eid2708.204763.
 90. Eriksson P, Schroder MT, Niiranen K, Nevanlinna A, Panelius J, Ranki A. 2013. The many faces of solitary and multiple erythema migrans. *Acta Derm Venereol* 93:693-700. doi: 10.2340/00015555-1549.
 91. 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* 71:3118-3124. doi: 10.1093/cid/ciz1215.
 92. Gerber MA, Shapiro ED, Burke GS, Parcels VJ, Bell GL. 1996. Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. *N Engl J Med* 335:1270-4. doi: 10.1056/NEJM199610243351703.
 93. Benedix F, Weide B, Broekaert S, Metzler G, Frick JS, Burgdorf WH, Rocken M, Schaller M. 2007. Early disseminated borreliosis with multiple erythema migrans and elevated liver enzymes: case report and literature review. *Acta Derm Venereol* 87:418. doi: 10.2340/00015555-0267.
 94. Forrester JD, Meiman J, Mullins J, Nelson R, Ertel SH, Cartter M, Brown CM, Lijewski V, Schiffman E, Neitzel D, Daly ER, Mathewson AA, Howe W, Lowe LA, Kratz NR, Semple S, Backenson PB, White JL, Kurpiel PM, Rockwell R, Waller K, Johnson DH, Steward C, Batten B, Blau D, DeLeon-Carnes M, Drew C, Muehlenbachs A, Ritter J, Sanders J, Zaki SR, Molins C, Schrieffer M, Perea A, Kugeler K, Nelson C, Hinckley A, Mead P, Centers for Disease C, Prevention. 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* 63:982-3.
 95. Lesser RL. 1995. Ocular manifestations of Lyme disease. *Am J Med* 98:60S-62S. doi: 10.1016/s0002-9343(99)80045-x.
 96. Huppertz HI, Munchmeier D, Lieb W. 1999. Ocular manifestations in children and adolescents with Lyme arthritis. *Br J Ophthalmol* 83:1149-52. doi: 10.1136/bjo.83.10.1149.
 97. Skiljic D, Gustavsson M, Dotevall L, Norrsell K, Gronlund MA. 2019. Ophthalmological

- findings in neuroborreliosis - a prospective study performed in Western Sweden. *Acta Ophthalmol* 97:44-52. doi: 10.1111/aos.13884.
98. CDC. Centers for Disease Control and Prevention. Lyme Disease Home. Lyme arthritis. <https://www.cdc.gov/lyme/treatment/LymeArthritis.html> [Accessed 26 October 2021].
 99. Arvikar SL, Steere AC. 2015. Diagnosis and treatment of Lyme arthritis. *Infect Dis Clin North Am* 29:269-80. doi: 10.1016/j.idc.2015.02.004.
 100. Huppertz HI, Karch H, Suschke HJ, Doring E, Ganser G, Thon A, Bentas W. 1995. Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. *Arthritis Rheum* 38:361-8. doi: 10.1002/art.1780380310.
 101. 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.
 102. Dahl V, Wisell KT, Giske CG, Tegnell A, Wallensten A. 2019. Lyme neuroborreliosis epidemiology in Sweden 2010 to 2014: clinical microbiology laboratories are a better data source than the hospital discharge diagnosis register. *Euro Surveill* 24. doi: 10.2807/1560-7917.ES.2019.24.20.1800453.
 103. Oschmann P, Dorndorf W, Hornig C, Schafer C, Wellensiek HJ, Pflughaupt KW. 1998. Stages and syndromes of neuroborreliosis. *J Neurol* 245:262-72. doi: 10.1007/s004150050216.
 104. 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. doi: 10.1093/brain/115.2.399.
 105. Schwenkenbecher P, Pul R, Wurster U, Conzen J, Pars K, Hartmann H, Suhs KW, Sedlacek L, Stangel M, Trebst C, Skripuletz T. 2017. Common and uncommon neurological manifestations of neuroborreliosis leading to hospitalization. *BMC Infect Dis* 17:90. doi: 10.1186/s12879-016-2112-z.
 106. Ljostad U, Skarpaas T, Mygland A. 2007. Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis. *Eur J Neurol* 14:873-6. doi: 10.1111/j.1468-1331.2007.01799.x.
 107. Nordberg CL, Bodilsen J, Knudtzen FC, Storgaard M, Brandt C, Wiese L, Hansen BR, Andersen AB, Nielsen H, Lebech AM, group Ds. 2020. Lyme neuroborreliosis in adults: A nationwide prospective cohort study. *Ticks Tick Borne Dis* 11:101411. doi: 10.1016/j.ttbdis.2020.101411.
 108. Skogman BH, Lager M, Henningsson AJ, Tjernberg I. 2017. The *recomBead Borrelia* antibody index, CXCL13 and total IgM index for laboratory diagnosis of Lyme neuroborreliosis in children. *Eur J Clin Microbiol Infect Dis* 36:2221-2229. doi: 10.1007/s10096-017-3049-x.
 109. Knudtzen FC, Andersen NS, Jensen TG, Skarphedinsson 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. doi: 10.1093/cid/cix568.
 110. Traisk F, Lindquist L. 2012. Optic nerve involvement in Lyme disease. *Curr Opin Ophthalmol* 23:485-90. doi: 10.1097/ICU.0b013e328358b1eb.
 111. Mormont EE, W., De Ronde T, Hanson P, Deltombe T, Laloux P. 2001. Abdominal wall weakness and lumboabdominal pain revealing neuroborreliosis: a report of three cases. *Clinical rheumatology* 20:447-450. doi: 10.1007/s100670170015.
 112. Djukic M, Larsen J, Lingor P, Nau R. 2013. Unilateral phrenic nerve lesion in Lyme neuroborreliosis. *BMC Pulm Med* 13:4. doi: 10.1186/1471-2466-13-4.
 113. Nibourg S, Beudel M, Trip J. 2021. Isolated proximal weakness of the legs due to neuroborreliosis. *BMJ Case Rep* 14. doi: 10.1136/bcr-2021-244431.
 114. Kaminsky AL, Maisonobe T, Lenglet T, Psimaras D, Debs R, Viala K. 2020. Confirmed cases of Neuroborreliosis with involvement of peripheral nervous system: Description of a

- cohort. *Medicine (Baltimore)* 99:e21986. doi: 10.1097/MD.00000000000021986.
115. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I. 2010. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol* 17:8-4. doi: 10.1111/j.1468-1331.2009.02862.x.
 116. Gómez-Eguílaz M, Gómez-Cerquera J, Calvo-Pérez L, Oteo JA. 2014. Neuroborreliosis: A single-hospital series of 7 cases. *Neurologia (Barcelona, Spain)* 31:137-139. doi: 10.1016/j.nrl.2014.03.008.
 117. Mygland A, Skarpaas T, Ljostad U. 2006. Chronic polyneuropathy and Lyme disease. *Eur J Neurol* 13:1213-5. doi: 10.1111/j.1468-1331.2006.01395.x.
 118. Tveitnes D, Natas OB, Skadberg O, Oymar K. 2012. Lyme meningitis, the major cause of childhood meningitis in an endemic area: a population based study. *Arch Dis Child* 97:215-20. doi: 10.1136/archdischild-2011-300526.
 119. Tveitnes D, Oymar K, Natas O. 2007. Acute facial nerve palsy in children: how often is it Lyme borreliosis? *Scand J Infect Dis* 39:425-31. doi: 10.1080/00365540601105764.
 120. CDC. Centers for Disease Control and Prevention. Lyme disease Home. Neurologic Lyme Disease. <https://www.cdc.gov/lyme/treatment/NeurologicLyme.html>. [Accessed 26 October 2021].
 121. Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, Desilva AM, Bao F, Yang X, Pypaert M, Pradhan D, Kantor FS, Telford S, Anderson JF, Fikrig E. 2004. TROSPA, an Ixodes scapularis receptor for *Borrelia burgdorferi*. *Cell* 119:457-68. doi: 10.1016/j.cell.2004.10.027.
 122. Ramamoorthy R, Scholl-Meeker D. 2001. *Borrelia burgdorferi* proteins whose expression is similarly affected by culture temperature and pH. *Infection and immunity* 69:2739-2742. doi: 10.1128/IAI.69.4.2739-2742.2001.
 123. Ramamoorthi N, Narasimhan S, Pal U, Bao F, Yang XF, Fish D, Anguita J, Norgard MV, Kantor FS, Anderson JF, Koski RA, Fikrig E. 2005. The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature* 436:573-7. doi: 10.1038/nature03812.
 124. Hovius JW, Schuijt TJ, de Groot KA, Roelofs JJ, Oei GA, Marquart JA, de Beer R, van 't Veer C, van der Poll T, Ramamoorthi N, Fikrig E, van Dam AP. 2008. Preferential protection of *Borrelia burgdorferi* sensu stricto by a Salp15 homologue in *Ixodes ricinus* saliva. *J Infect Dis* 198:1189-97. doi: 10.1086/591917.
 125. Anderson C, Brissette CA. 2021. The Brilliance of *Borrelia*: Mechanisms of Host Immune Evasion by Lyme Disease-Causing Spirochetes. *Pathogens* 10. doi: 10.3390/pathogens10030281.
 126. Embers ME, Ramamoorthy R, Philipp MT. 2004. Survival strategies of *Borrelia burgdorferi*, the etiologic agent of Lyme disease. *Microbes Infect* 6:312-8. doi: 10.1016/j.micinf.2003.11.014.
 127. Garcia BL, Zhi H, Wager B, Hook M, Skare JT. 2016. *Borrelia burgdorferi* BBK32 Inhibits the Classical Pathway by Blocking Activation of the C1 Complement Complex. *PLoS Pathog* 12:e1005404. doi: 10.1371/journal.ppat.1005404.
 128. Kraiczy P, Skerka C, Kirschfink M, Zipfel PF, Brade V. 2002. Immune evasion of *Borrelia burgdorferi*: insufficient killing of the pathogens by complement and antibody. *Int J Med Microbiol* 291 Suppl 33:141-6. doi: 10.1016/s1438-4221(02)80027-3.
 129. Alitalo A, Meri T, Lankinen H, Seppala I, Lahdenne P, Hefty PS, Akins D, Meri S. 2002. Complement inhibitor factor H binding to Lyme disease spirochetes is mediated by inducible expression of multiple plasmid-encoded outer surface protein E paralogs. *J Immunol* 169:3847-53. doi: 10.4049/jimmunol.169.7.3847.
 130. Pausa M, Pellis V, Cinco M, Giulianini PG, Presani G, Perticarari S, Murgia R, Tedesco F. 2003. Serum-resistant strains of *Borrelia burgdorferi* evade complement-mediated killing by expressing a CD59-like complement inhibitory molecule. *J Immunol* 170:3214-22. doi: 10.4049/jimmunol.170.6.3214.

131. Sambri V, Marangoni A, Giacani L, Gennaro R, Murgia R, Cevenini R, Cinco M. 2002. Comparative in vitro activity of five cathelicidin-derived synthetic peptides against *Leptospira*, *Borrelia* and *Treponema pallidum*. *Journal of Antimicrobial Chemotherapy* 50:895-902. doi: 10.1093/jac/dkf220.
132. Sarkar A, Tilly K, Stewart P, Bestor A, Battisti JM, Rosa PA. 2009. *Borrelia burgdorferi* resistance to a major skin antimicrobial peptide is independent of outer surface lipoprotein content. *Antimicrobial agents and chemotherapy* 53:4490-4494. doi: 10.1128/AAC.00558-09.
133. Lusitani D, Malawista SE, Montgomery RR. 2002. *Borrelia burgdorferi* are susceptible to killing by a variety of human polymorphonuclear leukocyte components. *The Journal of infectious diseases* 185:797-804. doi: 10.1086/339341.
134. Gautam A, Dixit S, Philipp MT, Singh SR, Morici LA, Kaushal D, Dennis VA. 2011. Interleukin-10 alters effector functions of multiple genes induced by *Borrelia burgdorferi* in macrophages to regulate Lyme disease inflammation. *Infect Immun* 79:4876-92. doi: 10.1128/IAI.05451-11.
135. Meriläinen L, Herranen A, Schwarzbach A, Gilbert L. 2015. Morphological and biochemical features of *Borrelia burgdorferi* pleomorphic forms. *Microbiology* 161:516. doi: 10.1099/mic.0.000027.
136. Sapi E, Balasubramanian K, Poruri A, Maghsoudlou JS, Socarras KM, Timmaraju AV, Filush KR, Gupta K, Shaikh S, Theophilus PAS, Luecke DF, MacDonald A, Zelger B. 2016. Evidence of in vivo existence of *Borrelia* biofilm in borrelial lymphocytomas. *European Journal of Microbiology and Immunology* 6:9-24. doi: 10.1556/1886.2015.00049.
137. Rudenko N, Golovchenko M, Kybicova K, Vancova M. 2019. Metamorphoses of Lyme disease spirochetes: Phenomenon of *Borrelia* persists. *Parasites & vectors* 12:1-10. doi: 10.1186/s13071-019-3495-7.
138. Liang FT, Yan J, Mbow ML, Sviat SL, Gilmore RD, Mamula M, Fikrig E. 2004. *Borrelia burgdorferi* changes its surface antigenic expression in response to host immune responses. *Infect Immun* 72:5759-67. doi: 10.1128/IAI.72.10.5759-5767.2004.
139. Zhang JR, Hardham JM, Barbour AG, Norris SJ. 1997. Antigenic variation in Lyme disease *Borreliae* by promiscuous recombination of VMP-like sequence cassettes. *Cell* 89:275-85. doi: 10.1016/s0092-8674(00)80206-8.
140. Hastey CJ, Elsner RA, Barthold SW, Baumgarth N. 2012. Delays and diversions mark the development of B cell responses to *Borrelia burgdorferi* infection. *The Journal of Immunology* 188:5612-5622. doi: 10.4049/jimmunol.1103735.
141. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390:580-6. doi: 10.1038/37551.
142. Takayama K, Rothenberg RJ, Barbour AG. 1987. Absence of lipopolysaccharide in the Lyme disease spirochete, *Borrelia burgdorferi*. *Infection and immunity* 55:2311-2313. doi: 10.1128/iai.55.9.2311-2313.1987.
143. 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* 231:141-4. doi: 10.1007/BF00313682.
144. Cerar T, Ogrinc K, Cimperman J, Lotric-Furlan S, Strle F, Ruzic-Sabljic E. 2008. Validation of cultivation and PCR methods for diagnosis of Lyme neuroborreliosis. *J Clin Microbiol* 46:3375-9. doi: 10.1128/JCM.00410-08.
145. 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 60:849-55. doi: 10.1001/archneur.60.6.849.
146. Cadavid D, O'Neill T, Schaefer H, Pachner AR. 2000. Localization of *Borrelia burgdorferi* in the nervous system and other organs in a nonhuman primate model of Lyme disease. Lab Invest 80:1043-1054. doi: 10.1038/labinvest.3780109.
 147. Pfister HW, Einhäupl KM, Wilske B, Preac-Mursic V. 1987. Bannwarth's syndrome and the enlarged neurological spectrum of arthropod-borne borreliosis. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology 263:343-347. doi: 0.1016/s0176-6724(87)80089-5.
 148. Ackermann R, Hörstrup P, Schmidt R. 1984. Tick-borne meningopolyneuritis (Garin-Bujadoux, Bannwarth). The Yale Journal of Biology and Medicine 57:485.
 149. Ramesh G, Bengel S, Pahar B, Philipp MT. 2012. A possible role for inflammation in mediating apoptosis of oligodendrocytes as induced by the Lyme disease spirochete *Borrelia burgdorferi*. Journal of Neuroinflammation 9:72. doi: 10.1186/1742-2094-9-72.
 150. Sigal LH. 1997. Immunologic mechanisms in Lyme neuroborreliosis: the potential role of autoimmunity and molecular mimicry. Semin Neurol 17:63-8. doi: 10.1055/s-2008-1040915.
 151. Ramesh G, Didier PJ, England JD, Santana-Gould L, Doyle-Meyers LA, Martin DS, Jacobs MB, Philipp MT. 2015. Inflammation in the pathogenesis of Lyme neuroborreliosis. Am J Pathol 185:1344-60. doi: 10.1016/j.ajpath.2015.01.024.
 152. Garcia-Monco JC, Fernandez-Villar B, Szczepanski A, Benach JL. 1991. Cytotoxicity of *Borrelia burgdorferi* for cultured rat glial cells. Journal of Infectious Diseases 163:1362-1366. doi: 10.1093/infdis/163.6.1362.
 153. Garcia-Monco JC, Fernandez-Villar B, Benach JL. 1989. Adherence of the Lyme disease spirochete to glial cells and cells of glial origin. Journal of Infectious Diseases 160:497-506. doi: 10.1093/infdis/160.3.497.
 154. Garcia-Monco JC, Villar BF, Alen JC, Benach JL. 1990. *Borrelia burgdorferi* in the central nervous system: experimental and clinical evidence for early invasion. Journal of Infectious Diseases 161:1187-1193. doi: 10.1093/infdis/161.6.1187.
 155. Garcia-Monco JC, Benach JL. 2019. Lyme neuroborreliosis: clinical outcomes, controversy, pathogenesis, and polymicrobial infections. Annals of neurology 85:21-31. doi: 10.1002/ana.25389.
 156. Kwaliteitsinstituut voor de Gezondheidszorg (CBO). 2013. Dutch guidelines Lyme disease 2013. Available at [In Dutch]: <https://www.rivm.nl/nieuws/cbo-richtlijn-lymeziekte-definitief>.
 157. Branda JA, Steere AC. 2021. Laboratory Diagnosis of Lyme Borreliosis. Clin Microbiol Rev 34. doi: 10.1128/CMR.00018-19.
 158. Marques AR. 2015. Laboratory diagnosis of Lyme disease: advances and challenges. Infect Dis Clin North Am 29:295-307. doi: 10.1016/j.idc.2015.02.005.
 159. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. 2005. Diagnosis of Lyme borreliosis. Clin Microbiol Rev 18:484-509. doi: 10.1128/CMR.18.3.484-509.2005.
 160. Jurca T, Ruzic-Sabljic E, Lotric-Furlan S, Maraspin V, Cimperman J, Picken RN, Strle F. 1998. Comparison of peripheral and central biopsy sites for the isolation of *Borrelia burgdorferi* sensu lato from erythema migrans skin lesions. Clin Infect Dis 27:636-8. doi: 10.1086/514715.
 161. Ogrinc K, Maraspin V, Lusa L, Cerar Kisek T, Ruzic-Sabljic E, Strle F. 2021. Acrodermatitis chronica atrophicans: clinical and microbiological characteristics of a cohort of 693 Slovenian patients. J Intern Med 290:335-348. doi: 10.1111/joim.13266.
 162. Maraspin V, Nahtigal Klevisar M, Ruzic-Sabljic E, Lusa L, Strle F. 2016. Borrelial Lymphocytoma in Adult Patients. Clin Infect Dis 63:914-21. doi: 10.1093/cid/ciw417.
 163. Dunaj J, Moniuszko A, Zajkowska J, Pancewicz S. 2013. The role of PCR in diagnostics of

- Lyme borreliosis. *Przegl Epidemiol* 67:35-9, 119-23.
164. Schmidt BL. 1997. PCR in laboratory diagnosis of human *Borrelia burgdorferi* infections. *Clinical microbiology reviews* 10:185-201. doi: 10.1128/CMR.10.1.185.
 165. Molloy PJ, Persing DH, Berardi VP. 2001. False-positive results of PCR testing for Lyme disease. *Clinical Infectious Diseases* 33:412-413. doi: 10.1086/321911.
 166. Gooskens J, Templeton KE, Claas EC, van Dam AP. 2006. Evaluation of an internally controlled real-time PCR targeting the *OspA* gene for detection of *Borrelia burgdorferi* sensu lato DNA in cerebrospinal fluid. *Clin Microbiol Infect* 12:894-900. doi: 10.1111/j.1469-0691.2006.01509.x.
 167. Ornstein K, Berglund J, Bergstrom S, Norrby R, Barbour AG. 2002. Three major Lyme *Borrelia* genospecies (*Borrelia burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*) identified by PCR in cerebrospinal fluid from patients with neuroborreliosis in Sweden. *Scandinavian journal of infectious diseases* 34:341-346. doi: 10.1080/00365540110080313.
 168. Wormser GP, Bittker S, Cooper D, Nowakowski J, Nadelman RB, Pavia C. 2001. Yield of large-volume blood cultures in patients with early Lyme disease. *J Infect Dis* 184:1070-2. doi: 10.1086/323424.
 169. Talagrand-Reboul E, Raffetin A, Zachary P, Jaulhac B, Eldin C. 2020. Immunoserological Diagnosis of Human Borreliosis: Current Knowledge and Perspectives. *Front Cell Infect Microbiol* 10:241. doi: 10.3389/fcimb.2020.00241.
 170. Centers for Disease Control and Prevention (CDC). 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 44:590-1.
 171. Dessau RB, van Dam AP, Fingerle V, Gray J, Hovius JW, Hunfeld KP, Jaulhac B, Kahl O, Kristoferitsch W, Lindgren PE, Markowicz M, Mavin S, Ornstein K, Rupprecht T, Stanek G, Strle F. 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. *Clinical Microbiology and Infection* 24:118-124. doi: 10.1016/j.cmi.2017.08.025.
 172. 2019. U.S. Food and Drug Administration (FDA) clears new indications for existing Lyme disease tests that may help streamline diagnoses [July 29, 2019]. <https://www.fda.gov/news-events/press-announcements/fda-clears-new-indications-existing-lyme-disease-tests-may-help-streamline-diagnoses>. Accessed 17 February 2022.
 173. Mead PS, Petersen J, Hinckley A. 2019. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 68:703. <http://dx.doi.org/10.15585/mmwr.mm6832a4>.
 174. Branda JA, Strle K, Nigrovic LE, Lantos PM, Lepore TJ, Damle NS, Ferraro MJ, Steere AC. 2017. Evaluation of Modified 2-Tiered Serodiagnostic Testing Algorithms for Early Lyme Disease. *Clin Infect Dis* 64:1074-1080. doi: 10.1093/cid/cix043.
 175. Davis IRC, McNeil SA, Allen W, MacKinnon-Cameron D, Lindsay LR, Bernat K, Dibernardo A, LeBlanc JJ, Hatchette TF. 2020. Performance of a modified two-tiered testing enzyme immunoassay algorithm for serologic diagnosis of Lyme disease in Nova Scotia. *Journal of clinical microbiology* 58:e01841-19. doi: 10.1128/JCM.01841-19.
 176. Wormser GP, Schriefer M, Aguero-Rosenfeld ME, Levin A, Steere AC, Nadelman RB, Nowakowski J, Marques A, Johnson BJ, Dumler JS. 2013. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. *Diagn Microbiol Infect Dis* 75:9-15. doi: 10.1016/j.diagmicrobio.2012.09.003.
 177. Kmety E. 2000. Dynamics of antibodies in *Borrelia burgdorferi* sensu lato infections. *Bratisl Lek Listy* 101:5Ě7.
 178. Branda JA, Linskey K, Kim YA, Steere AC, Ferraro MJ. 2011. Two-tiered antibody testing for Lyme disease with use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay. *Clin Infect Dis* 53:541-547. doi: 10.1093/cid/cir464.

179. Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. 2001. 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. doi: 10.1086/322669.
180. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. 1996. Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. *J Clin Microbiol* 34:1-9. doi: 10.1128/jcm.34.1.1-9.1996.
181. Hammers-Berggren S, Lebech AM, Karlsson M, Svenungsson B, Hansen K, Stiernstedt G. 1994. Serological follow-up after treatment of patients with erythema migrans and neuroborreliosis. *J Clin Microbiol* 32:1519-25. doi: 10.1128/jcm.32.6.1519-1525.1994.
182. Markowicz M, Reiter M, Gamper J, Stanek G, Stockinger H. 2021. Persistent Anti-*Borrelia* IgM Antibodies without Lyme Borreliosis in the Clinical and Immunological Context. *Microbiol Spectr* 9:e0102021. doi: 10.1128/Spectrum.01020-21.
183. Olesen, KG, Hejlesen, OK, Dessau, R, Beltoft, I, Trangeled, M. 2006. Diagnosing Lyme Disease: Tailoring patient specific Bayesian networks for temporal reasoning. In M. Studeny, & J. Vomlel (Eds.), *Proceedings of the Third European Workshop on Probabilistic Graphical Models (PGM '06* (pp. 231-238).
184. Biesiada G, Czepiel J, Lesniak MR, Garlicki A, Mach T. 2012. Lyme disease: review. *Arch Med Sci* 8:978-82. doi: 10.5114/aoms.2012.30948.
185. Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC. 2013. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. *Clin Infect Dis* 57:333-40. doi: 10.1093/cid/cit235.
186. Leeflang MMG, Ang CW, Berkhout J, Bijlmer HA, Van BW, Brandenburg AH, van Burgel ND, van Dam AP, Dessau RB, Fingerle V, Hovius JWR, Jaulhac B, Meijer B, Van PW, Schellekens JFP, Spijker R, Stelma FF, Stanek G, Verduyn-Lunel F, Zeller H, Sprong H. 2016. The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: a systematic review and meta-analysis. *BMC Infect Dis* 16. doi: 10.1186/s12879-016-1468-4.
187. 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. doi: 10.1371/journal.pone.0168613.
188. Kaiser R. 2000. False-negative serology in patients with neuroborreliosis and the value of employing of different borrelial strains in serological assays. *J Med Microbiol* 49:911-915. doi: 10.1099/0022-1317-49-10-911.
189. Wilske B, Hauser U, Lehnert G, Jauris-Heipke S. 1998. Genospecies and their influence on immunoblot results. *Wien Klin Wochenschr* 110:882-5.
190. Roessler D, Hauser U, Wilske B. 1997. Heterogeneity of BmpA (P39) among European isolates of *Borrelia burgdorferi* sensu lato and influence of interspecies variability on serodiagnosis. *Journal of clinical microbiology* 35:2752-2758. doi: 10.1128/jcm.35.11.2752-2758.1997.
191. Coleman AS, Pal U. 2009. BBK07, a dominant in vivo antigen of *Borrelia burgdorferi*, is a potential marker for serodiagnosis of Lyme disease. *Clinical and Vaccine Immunology* 16:1569-1575. doi: 10.1128/CVI.00301-09.
192. Ramamoorthy R, Philipp MT. 1998. Differential expression of *Borrelia burgdorferi* proteins during growth in vitro. *Infect Immun* 66:5119-24. doi: 10.1128/IAI.66.11.5119-5124.1998.
193. Weiner ZP, Crew RM, Brandt KS, Ullmann AJ, Schrieffer ME, Molins CR, Gilmore RD. 2015. Evaluation of Selected *Borrelia burgdorferi* lp54 Plasmid-Encoded Gene Products Expressed during Mammalian Infection as Antigens To Improve Serodiagnostic Testing for Early Lyme Disease. *Clin Vaccine Immunol* 22:1176-86. doi: 10.1128/CVI.00399-15.
194. Lahey LJ, Panas MW, Mao R, Delaney M, Flanagan JJ, Binder SR, Rebman AW, Montoya JG, Soloski MJ, Steere AC. 2015. Development of a multiantigen panel for improved detection

- of *Borrelia burgdorferi* infection in early Lyme disease. Journal of clinical microbiology 53:3834-3841. doi: 10.1128/JCM.02111-15.
195. Henningsson AJ, Christiansson M, Tjernberg I, Lofgren S, Matussek A. 2014. Laboratory diagnosis of Lyme neuroborreliosis: a comparison of three CSF anti-*Borrelia* antibody assays. Eur J Clin Microbiol Infect Dis 33:797-803. doi: 10.1007/s10096-013-2014-6.
 196. Dessau RB, Moller JK, Kolmos B, Henningsson AJ. 2015. Multiplex assay (Mikrogen recomBead) for detection of serum IgG and IgM antibodies to 13 recombinant antigens of *Borrelia burgdorferi* sensu lato in patients with neuroborreliosis: the more the better? J Med Microbiol 64:224-231. doi: 10.1099/jmm.0.000009.
 197. Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG. 1988. Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to *Borrelia burgdorferi*. N Engl J Med 319:1441-1446. doi: 10.1056/NEJM198812013192203.
 198. Baig S, Olsson T, Hansen K, Link H. 1991. Anti-*Borrelia burgdorferi* antibody response over the course of Lyme neuroborreliosis. Infect Immun 59:1050-6. doi: 10.1128/iai.59.3.1050-1056.1991.
 199. Ledue TB, Collins MF, Craig WY. 1996. New laboratory guidelines for serologic diagnosis of Lyme disease: evaluation of the two-test protocol. J Clin Microbiol 34:2343-50. doi: 10.1128/jcm.34.10.2343-2350.1996.
 200. Rebman AW, Crowder LA, Kirkpatrick A, Aucott JN. 2015. Characteristics of seroconversion and implications for diagnosis of post-treatment Lyme disease syndrome: acute and convalescent serology among a prospective cohort of early Lyme disease patients. Clinical rheumatology 34:585-589. doi: 10.1007/s10067-014-2706-z.
 201. Elsner RA, Hastey CJ, Olsen KJ, Baumgarth N. 2015. Suppression of Long-Lived Humoral Immunity Following *Borrelia burgdorferi* Infection. PLoS Pathog 11:e1004976. doi: 10.1371/journal.ppat.1004976.
 202. Wagemakers A, Visser MC, de Wever B, Hovius JW, van de Donk NWJC, Hendriks EJ, Peferoen L, Muller FF, Ang CW. 2018. Case report: persistently seronegative neuroborreliosis in an immunocompromised patient. BMC Infect Dis 18:362. doi: 10.1186/s12879-018-3273-8.
 203. Holl-Wieden A, Suerbaum S, Girschick HJ. 2007. Seronegative Lyme arthritis. Rheumatol Int 27:1091-3. doi: 10.1007/s00296-007-0333-6.
 204. Luft BJ, Dunn JJ, Dattwyler RJ, Gorgone G, Gorevic PD, Schubach WH. 1993. Cross-reactive antigenic domains of the flagellin protein of *Borrelia burgdorferi*. Res Microbiol 144:251-7. doi: 10.1016/0923-2508(93)90009-q.
 205. Kaiser R. 1995. Intrathecal immune response in neuroborreliosis: importance of cross-reactive antibodies. Zentralbl Bakteriell 282:303-14. doi: 10.1016/s0934-8840(11)80131-3.
 206. Hoeve-Bakker BJA, Jonker M, Brandenburg AH, den Reijer PM, Stelma FF, van Dam AP, van Gorkom T, Kerkhof K, Thijsen SFT, Kremer K. 2022. The Performance of Nine Commercial Serological Screening Assays for the Diagnosis of Lyme Borreliosis: a Multicenter Modified Two-Gate Design Study. Microbiology Spectrum 10:e00510-22. doi: 10.1128/spectrum.00510-22.
 207. Magnarelli LA, Miller JN, Anderson JF, Riviere GR. 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. Journal of clinical microbiology 28:1276-1279. doi: 10.1128/jcm.28.6.1276-1279.1990.
 208. Koetsveld J, Platonov AE, Kuleshov K, Wagemakers A, Hoornstra D, Ang W, Szekeres S, van Duijvendijk GLA, Fikrig E, Embers ME, Sprong H, Hovius JW. 2020. *Borrelia miyamotoi* infection leads to cross-reactive antibodies to the C6 peptide in mice and men. Clin Microbiol Infect 26:513 e1-513 e6. doi: 10.1016/j.cmi.2019.07.026.
 209. Luther B, Moskophidis M. 1990. Antigenic cross-reactivity between *Borrelia burgdorferi*, *Borrelia recurrentis*, *Treponema pallidum*, and *Treponema phagedenis*. Zentralbl Bakteriell 274:214-26. doi: 10.1016/s0934-8840(11)80104-0.

210. Molins CR, Sexton C, Young JW, Ashton LV, Pappert R, Beard CB, Schriefer ME. 2014. Collection and characterization of samples for establishment of a serum repository for Lyme disease diagnostic test development and evaluation. *J Clin Microbiol* 52:3755-62. doi: 10.1128/JCM.01409-14.
211. Seriburi V, Ndukwe N, Chang Z, Cox ME, Wormser GP. 2012. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clinical Microbiology and Infection* 18:1236-1240. doi: 10.1111/j.1469-0691.2011.03749.x.
212. Hammers-Berggren S, Hansen K, Lebech AM, Karlsson M. 1993. *Borrelia burgdorferi*-specific intrathecal antibody production in neuroborreliosis: a follow-up study. *Neurology* 43:169-75. doi: 10.1212/wnl.43.1_part_1.169.
213. Lernout T, Kabamba-Mukadi B, Saegeman V, Tré-Hardy M, de Laveleye M, Asikainen T, Dessau RB, Quoilin S, Litzroth A. 2019. The value of seroprevalence data as surveillance tool for Lyme borreliosis in the general population: the experience of Belgium. *BMC public health* 19:1-7. doi: 10.1186/s12889-019-6914-y.
214. Wilking H, Fingerle V, Klier C, Thamm M, Stark K. 2015. Antibodies against *Borrelia burgdorferi* sensu lato among Adults, Germany, 2008–2011. *Emerging infectious diseases* 21:107. doi: 10.3201/eid2101.140009.
215. Bušová A, Dorko E, Rimárová K, Diabelková J, Rovenská T, Feketeová E, Bereš M, Čellár R, Baranová Z, Kampe T. 2018. Seroprevalence of Lyme disease in Eastern Slovakia. *Cent Eur J Public Health* 26:S67-S71. doi: 10.21101/cejph.a5442.
216. Hjetland R, Nilsen RM, Grude N, Ulvestad E. 2014. Seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in healthy adults from Western Norway: risk factors and methodological aspects. *Apmis* 122:1114-1124. doi: 10.1111/apm.12267.
217. Carlsson SA, Granlund H, Nyman D, Wahlberg P. 1998. IgG Seroprevalence of Lyme borreliosis in the population of the land islands in Finland. *Scandinavian journal of infectious diseases* 30:501-503. doi: 10.1080/00365549850161520.
218. Dehnert MF, V., Klier C, Talaska T, Schlaud M, Krause G, Wilking HP, G. . 2012. Seropositivity of Lyme borreliosis and associated risk factors: a population-based study in children and adolescents in Germany (KiGGS). doi:doi: 10.1371/journal.pone.0041321. doi: 10.1371/journal.pone.0041321.
219. Cizman M, Avsic-Zupanc T, Petrovec M, Ruzic-Sabljic E, Pokorn M. 2000. Seroprevalence of ehrlichiosis, Lyme borreliosis and tick-borne encephalitis infections in children and young adults in Slovenia. *Wiener klinische Wochenschrift* 112:842-845.
220. Vestreheim DF, White RA, Aaberge IS, Aase A. 2016. Geographical differences in seroprevalence of *Borrelia burgdorferi* antibodies in Norway, 2011–2013. *Ticks and tick-borne diseases* 7:698-702. doi: 10.1016/j.ttbdis.2016.02.020.
221. Magnavita N, Capitanelli I, Ilesanmi O, Chirico F. 2022. Occupational Lyme Disease: A Systematic Review and Meta-Analysis. *Diagnostics* 12:296. doi: 10.3390/diagnostics12020296.
222. Barreiro-Hurlé L, Melón-García S, Seco-Bernal C, Muñoz-Turrillas C, Rodríguez-Pérez M. 2020. Seroprevalence of Lyme disease in southwest Asturias. *Enferm Infecc Microbiol Clin (Engl Ed)* 38:155-158. doi: 10.1016/j.eimc.2019.06.010.
223. Nohlmans MK, van den Bogaard AE, Blaauw AA, van Boven CP. 1991. [Prevalence of Lyme borreliosis in The Netherlands] [Article in Dutch]. *Ned Tijdschr Geneesk* 135:2288-92.
224. Hansen K, Crone C, Kristoferitsch W. 2013. Lyme neuroborreliosis. *Handb Clin Neurol* 115:559-75. doi: 10.1016/B978-0-444-52902-2.00032-1.
225. Rupprecht TA, Lechner C, Tumani H, Fingerle V. 2014. CXCL13: a biomarker for acute Lyme neuroborreliosis: investigation of the predictive value in the clinical routine. *Nervenarzt* 85:459-64. (In German). doi: 10.1007/s00115-014-4020-z.
226. Djukic M, Schmidt-Samoa C, Lange P, Spreer A, Neubieser K, Eiffert H, Nau R, Schmidt H. 2012. Cerebrospinal fluid findings in adults with acute Lyme neuroborreliosis. *J Neurol*

- 259:630-6. doi: 10.1007/s00415-011-6221-8.
227. Tumani H, Nolker G, Reiber H. 1995. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. *Neurology* 45:1663-70. doi: 10.1212/wnl.45.9.1663.
 228. Reiber H, Peter JB. 2001. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci* 184:101-22. doi: 10.1016/s0022-510x(00)00501-3.
 229. Kaiser R. 1994. Variable CSF findings in early and late Lyme neuroborreliosis: a follow-up study in 47 patients. *J Neurol* 242:26-36. doi: 10.1007/BF00920571.
 230. Stiernstedt GT, Skoldenberg BR, Vandvik B, Hederstedt B, Garde A, Kolmodin G, Jorback H, Svenungsson B. 1984. Chronic meningitis and Lyme disease in Sweden. *Yale J Biol Med* 57:491-7.
 231. Skogman BH, Wilhelmsson P, Atallah S, Petersson AC, Ornstein K, Lindgren PE. 2021. Lyme neuroborreliosis in Swedish children-PCR as a complementary diagnostic method for detection of *Borrelia burgdorferi* sensu lato in cerebrospinal fluid. *Eur J Clin Microbiol Infect Dis* 40:1003-1012. doi: 10.1007/s10096-020-04129-7.
 232. Kristoferitsch W, Sluga E, Graf M, Partsch H, Neumann R, Stanek G, Budka H. 1988. Neuropathy associated with acrodermatitis chronica atrophicans. Clinical and morphological features. *Ann N Y Acad Sci* 539:35-45. doi: 10.1111/j.1749-6632.1988.tb31836.x.
 233. Logigian EL, Steere AC. 1992. Clinical and electrophysiologic findings in chronic neuropathy of Lyme disease. *Neurology* 42:303-11. doi: 10.1212/wnl.42.2.303.
 234. Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Aguero-Rosenfeld ME, Auwaerter PG, Baldwin K, Bannuru RR, Belani KK, Bowie WR, Branda JA, Clifford DB, DiMario FJJ, Halperin JJ, Krause PJ, Lavergne V, Liang MH, Cody Meissner H, Nigrovic LE, Nocton JJ, Osani MC, Pruitt AA, Rips J, Rosenfeld LE, Savoy ML, Sood SK, Steere AC, Strle F, Sundel R, Tsao J, Vaysbrot EE, Wormser GP, Zemel LS. 2020. 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. *Clinical Infectious Diseases* 72:e1-e48. doi: 10.1093/cid/ciaa1215.
 235. 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. doi: 10.1002/ana.410300212.
 236. Skarpaas T, Ljostad U, Sobyte M, Mygland A. 2007. Sensitivity and specificity of a commercial C6 peptide enzyme immuno assay in diagnosis of acute Lyme neuroborreliosis. *Eur J Clin Microbiol Infect Dis* 26:675-7. doi: 10.1007/s10096-007-0336-y.
 237. van Burgel ND, Brandenburg A, Gerritsen HJ, Kroes AC, van Dam AP. 2011. High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients. *Clin Microbiol Infect* 17:1495-500. doi: 10.1111/j.1469-0691.2011.03459.x.
 238. Vermeersch P, Ressler S, Nackers E, Lagrou K. 2009. The C6 Lyme antibody test has low sensitivity for antibody detection in cerebrospinal fluid. *Diagn Microbiol Infect Dis* 64:347-9. doi: 10.1016/j.diagmicrobio.2009.03.013.
 239. van Gorkom T, van Arkel GHJ, Voet W, Thijsen SFT, Kremer K. 2021. Consequences of the Edge Effect in a Commercial Enzyme-Linked Immunosorbent Assay for the Diagnosis of Lyme Neuroborreliosis. *J Clin Microbiol* 59:e0328020. doi: 10.1128/JCM.03280-20.
 240. Lavani A, Pareek M. 2010. Interferon gamma release assays: principles and practice. *Enferm Infect Microbiol Clin* 28:245-252. doi: 10.1016/j.eimc.2009.05.012.
 241. Czerkinsky C, Andersson G, Ekre HP, Nilsson LÅ, Klareskog L, Ouchterlony Ö. 1988. Reverse ELISPOT assay for clonal analysis of cytokine production I. Enumeration of gamma-interferon-secreting cells. *Journal of immunological methods* 110:29-36. doi: 10.1016/0022-1759(88)90079-8.

242. Santin M, Munoz L, Rigau D. 2012. Interferon-gamma release assays for the diagnosis of tuberculosis and tuberculosis infection in HIV-infected adults: a systematic review and meta-analysis. *PLoS One* 7:e32482. doi: 10.1371/journal.pone.0032482.
243. Simsek H, Alpar S, Ucar N, Aksu F, Ceyhan I, Gozalan A, Cesur S, Ertek M. 2010. Comparison of tuberculin skin testing and T-SPOT.TB for diagnosis of latent and active tuberculosis. *Jpn J Infect Dis* 63:99-102. doi: 10.7883/yoken.63.99.
244. Limonard GJ, Thijsen SF, Bossink AW, Asscheman A, Bouwman JJ. 2012. Developing a new clinical tool for diagnosing chronic Q fever: the Coxiella ELISPOT. *FEMS Immunol Med Microbiol* 64:57-60. doi: 10.1111/j.1574-695X.2011.00890.x.
245. Oksi J, Savolainen J, Pene J, Bousquet J, Laippala P, Viljanen MK. 1996. Decreased interleukin-4 and increased gamma interferon production by peripheral blood mononuclear cells of patients with Lyme borreliosis. *Infect Immun* 64:3620-3. doi: 10.1128/iai.64.9.3620-3623.1996.
246. Salazar JC, Pope CD, Sellati TJ, Feder HM, Jr., Kiely TG, Dardick KR, Buckman RL, Moore MW, Caimano MJ, Pope JG, Krause PJ, Radolf JD, Lyme Disease N. 2003. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. *J Immunol* 171:2660-70. doi: 10.4049/jimmunol.171.5.2660.
247. Gross DM, Steere AC, Huber BT. 1998. T helper 1 response is dominant and localized to the synovial fluid in patients with Lyme arthritis. *J Immunol* 160:1022-8.
248. Ekerfelt C, Ernerudh J, Forsberg P, Bergstrom S. 1998. Augmented intrathecal secretion of interferon-gamma in response to *Borrelia garinii* in neuroborreliosis. *J Neuroimmunol* 89:177-81. doi: 10.1016/s0165-5728(98)00136-2.
249. Ekerfelt C, Ernerudh J, Bunikis J, Vrethem M, Aagesen J, Roberg M, Bergstrom S, Forsberg P. 1997. Compartmentalization of antigen specific cytokine responses to the central nervous system in CNS borreliosis: secretion of IFN-gamma predominates over IL-4 secretion in response to outer surface proteins of Lyme disease *Borrelia* spirochetes. *J Neuroimmunol* 79:155-62. doi: 10.1016/s0165-5728(97)00118-5.
250. Wang WZ, Fredrikson S, Sun JB, Link H. 1995. Lyme neuroborreliosis: evidence for persistent up-regulation of *Borrelia burgdorferi*-reactive cells secreting interferon-gamma. *Scand J Immunol* 42:694-700. doi: 10.1111/j.1365-3083.1995.tb03713.x.
251. Widhe M, Jarefors S, Ekerfelt C, Vrethem M, Bergstrom S, Forsberg P, Ernerudh J. 2004. *Borrelia*-specific interferon-gamma and interleukin-4 secretion in cerebrospinal fluid and blood during Lyme borreliosis in humans: association with clinical outcome. *J Infect Dis* 189:1881-1891. doi: 10.1086/382893.
252. Ekerfelt C, Masreliez C, Svenvik M, Ernerudh J, Roberg M, Forsberg P. 2001. Antibodies and T-cell reactivity to *Borrelia burgdorferi* in an asymptomatic population: a study of healthy blood donors in an inland town district in the south-east of Sweden. *Scand J Infect Dis* 33:806-8. doi: 10.1080/00365540110077376.
253. Ekerfelt C, Forsberg P, Svenvik M, Roberg M, Bergstrom S, Ernerudh J. 1999. Asymptomatic *Borrelia*-seropositive individuals display the same incidence of *Borrelia*-specific interferon-gamma (IFN-gamma)-secreting cells in blood as patients with clinical *Borrelia* infection. *Clin Exp Immunol* 115:498-502. doi: 10.1046/j.1365-2249.1999.00840.x.
254. Widhe M, Ekerfelt C, Jarefors S, Skogman BH, Peterson EM, Bergstrom S, Forsberg P, Ernerudh J. 2009. T-cell epitope mapping of the *Borrelia garinii* outer surface protein A in Lyme neuroborreliosis. *Scand J Immunol* 70:141-8. doi: 10.1111/j.1365-3083.2009.02285.x.
255. Forsberg P, Ernerudh J, Ekerfelt C, Roberg M, Vrethem M, Bergstrom S. 1995. The outer surface proteins of Lyme disease *Borrelia* spirochetes stimulate T cells to secrete interferon-gamma (IFN-gamma): diagnostic and pathogenic implications. *Clin Exp Immunol* 101:453-60. doi: 10.1111/j.1365-2249.1995.tb03134.x.

256. Raffetin A, Saunier A, Bouiller K, Caraux-Paz P, Eldin C, Gallien S, Jouenne R, Belkacem A, Salomon J, Patey O, Talagrand-Reboul E, Jaulhac B, Grillon A. 2020. Unconventional diagnostic tests for Lyme borreliosis: a systematic review. *Clin Microbiol Infect* 26:51-59. doi: 10.1016/j.cmi.2019.06.033.
257. Nordberg M, Forsberg P, Nyman D, Skogman BH, Nyberg C, Ernerudh J, Eliasson I, Ekerfelt C. 2012. Can ELISpot be applied to a clinical setting as a diagnostic utility for neuroborreliosis? *Cells* 1:153-67. doi: 10.3390/cells1020153.
258. Jin C, Roen DR, Lehmann PV, Kellermann GH. 2013. An enhanced ELISpot assay for sensitive detection of antigen-specific T cell responses to *Borrelia burgdorferi*. *Cells* 2:607-20. doi: 10.3390/cells2030607.
259. Valentine-Thon E, Ilsemann K, Sandkamp M. 2007. A novel lymphocyte transformation test (LTT-MELISA) for Lyme borreliosis. *Diagn Microbiol Infect Dis* 57:27-34. doi: 10.1016/j.diagmicrobio.2006.06.008.
260. Dressler F, Yoshinari NH, Steere AC. 1991. The T-cell proliferative assay in the diagnosis of Lyme disease. *Ann Intern Med* 115:533-539. doi: 10.7326/0003-4819-115-7-533.
261. Yoshinari NH, Reinhardt BN, Steere AC. 1991. T cell responses to polypeptide fractions of *Borrelia burgdorferi* in patients with Lyme arthritis. *Arthritis Rheum* 34:707-13. doi: 10.1002/art.1780340611.
262. Vaz A, Glickstein L, Field JA, McHugh G, Sikand VK, Damle N, Steere AC. 2001. Cellular and humoral immune responses to *Borrelia burgdorferi* antigens in patients with culture-positive early Lyme disease. *Infect Immun* 69:7437-44. doi: 10.1128/IAI.69.12.7437-7444.2001.
263. Horowitz HW, Pavia CS, Bittker S, Forseter G, Cooper D, Nadelman RB, Byrne D, Johnson RC, Wormser GP. 1994. Sustained cellular immune responses to *Borrelia burgdorferi*: lack of correlation with clinical presentation and serology. *Clin Diagn Lab Immunol* 1:373-8. doi: 10.1128/cdli.1.4.373-378.1994.
264. Arminlabs. *Borrelia* ELISpot (T-Cell-Spot/IGRA: Interferon-Gamma-Release Assay). Available at: <https://www.arminlabs.com/en/tests/elispot>. Accessed November 2018.
265. BCA-clinic. Is the LymeSpot revised more effective than the traditional ELISpot. 2018. Available at: <https://www.bca-clinic.de/en/is-the-lymespot-revised-more-effective-than-the-traditional-elispot/>. Accessed February 2019.
266. Nordic Laboratories. *Borrelia* Elispot LTT (previously named Lyme Elispot LTT). <http://www.nordiclabs.com/EDetail.aspx?id=2913>. Accessed 18 June 2018 and 28 February 2022.
267. Regenerus Labs. Partnering with BCA Labs - The Viral and Lyme Experts. The EliSpot (Interferon-Gamma-Test) and the "LymeSpot Revised": The new EliSpot. https://regeneruslabs.com/blogs/blog/partnering-with-bca-labs-the-viral-and-lyme-experts?_pos=1&_sid=afd85ce7f&_ss=r. Accessed 18 June 2018 and 28 February 2022.
268. Infectolab Americas. *Borrelia burgdorferi* ELISPOT. <https://www.infectolab-americas.com/borrelia-burgdorferi>. Accessed 28 February 2022.
269. IMD Labor Berlin. The lymphocyte transformation test LTT Lyme Disease. <https://www.imd-berlin.de/en/subject-information/diagnostics-information/lyme-disease-clinical-symptoms-and-diagnostics>. Accessed 18 June 2018 and 28 February 2022.
270. Alstrup K, Bech M. 2016. TV2_Documentar. [Snyd eller borrelia]. Available at: <https://www.dailymotion.com/video/x4wmdyj>. Accessed February 2019.
271. Ljostad U, Mygland A. 2008. CSF B-lymphocyte chemoattractant (CXCL13) in the early diagnosis of acute Lyme neuroborreliosis. *J Neurol* 255:782. doi: 10.1007/s00415-008-0974-8.
272. Rupprecht TA, Pfister HW, Angele B, Kastenbauer S, Wilske B, Koedel U. 2005. The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis. *Neurology* 65:448-50. doi: 10.1212/01.wnl.0000171349.06645.79.

273. Hytonen J, Kortela E, Waris M, Puustinen J, Salo J, Oksi J. 2014. CXCL13 and neopterin concentrations in cerebrospinal fluid of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammation. *J Neuroinflammation* 11:103. doi: 10.1186/1742-2094-11-103.
274. 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* 49:2027-30. doi: 10.1128/JCM.00084-11.
275. Kowarik MC, Cepok S, Sellner J, Grummel V, Weber MS, Korn T, Berthele A, Hemmer B. 2012. CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation. *J Neuroinflammation* 9:93. doi: 10.1186/1742-2094-9-93.
276. 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* 75:4351-6. doi: 10.1128/IAI.01642-06.
277. Wutte N, Berghold A, Löffler S, Zenz W, Daghofer E, Krainberger I, Kleinert G, Aberer E. 2011. CXCL13 chemokine in pediatric and adult neuroborreliosis. *Acta Neurol Scand* 124:321-8. doi: 10.1111/j.1600-0404.2010.01477.x.
278. Barstad B, Tveitnes D, Noraas S, Selvik Ask I, Saeed M, Bosse F, Vigemyr G, Huber I, Oymar K. 2017. Cerebrospinal fluid B-lymphocyte chemoattractant CXCL13 in the diagnosis of acute Lyme neuroborreliosis in children. *Pediatr Infect Dis J* 36:e286-e292. doi: 10.1097/INF.0000000000001669.
279. Ljostad U, Mygland A. 2010. Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. *Eur J Neurol* 17:118-123. doi: 10.1111/j.1468-1331.2009.02756.x.
280. Picha D, Moravcova L, Smiskova D. 2016. Prospective study on the chemokine CXCL13 in neuroborreliosis and other aseptic neuroinfections. *J Neurol Sci* 368:214-20. doi: 10.1016/j.jns.2016.05.059.
281. 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* 15:5968-73. doi: 10.1158/1078-0432.CCR-09-0108.
282. Lyme innovation. USA. <https://www.hhs.gov/cto/initiatives/innovation-and-partnerships/lyme-innovation/index.html>. Accessed 24 January 2022.
283. Lymedisease.org; powered by patients. USA. <https://www.lymedisease.org/>. Accessed 24 January 2022.
284. Global Lyme Alliance. USA. <https://www.globallymealliance.org/>. Accessed 24 January 2022.
285. Lyme Disease Action. UK. <https://www.lymediseaseaction.org.uk/>. Accessed 24 January 2022.
286. Association Vaincre Lyme. France. <https://www.vaincrelyme.com/>. Accessed 24 January 2022.
287. Stowarzyszenie Chorych na Boreliozę. Poland. <http://borelioza.org/onas.htm>. Accessed 24 January 2022.
288. ALCE Asociación Lyme Crónico España. Spain, <https://alcelyme.org/>. Accessed 24 January 2022.
289. Onlyme-aktion.org. Germany. <https://onlyme-aktion.org/>. Accessed 24 January 2022.
290. Haelle T. 2021. Judge dismisses Lyme disease lawsuit against IDSA, doctors, but the ordeal has left its scars. Available at: <https://www.medscape.com/viewarticle/961484>. (Accessed March 2022).
291. 2010. Nederlandse Vereniging voor Lyme patiënten. [Burgerinitiatief: de ziekte van Lyme]. <https://petities.nl/petitions/burgerinitiatief-de-ziekte-van-lyme?locale=en>. Accessed 24 January 2022.
292. den Oudendam WM, Broerse JEW. 2017. Lyme disease in the Dutch policy context:

- patient consultation in government research agenda setting. *Science and Public Policy* 44:303-316. doi: 10.1093/scipol/scw056.
293. Wormser GP, Nadelman RB, Dattwyler RJ, Dennis DT, Shapiro ED, Steere AC, Rush TJ, Rahn DW, Coyle PK, Persing DH, Fish D, Luft BJ. 2000. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. *Clin Infect Dis* 31 Suppl 1:1-14. doi: 10.1086/314053.
 294. 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* 117:273-80. doi: 10.7326/0003-4819-117-4-273.
 295. 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* 50:512-20. doi: 10.1086/649920.
 296. Torbahn G, Hofmann H, Rücker 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 dermatology* 154:1292-1303. doi: 10.1001/jamadermatol.2018.3186.
 297. Hofland RW, Thijsen SF, van Lindert AS, de Lange WC, van Gorkom T, van der Tweel I, Lammers JJ, Bossink AW. 2017. Positive predictive value of ELISpot in BAL and pleural fluid from patients with suspected pulmonary tuberculosis. *Infect Dis (Lond)* 49:347-355. doi: 10.1080/23744235.2016.1269190.
 298. Bossink AW, Dubbers M, Bouwman JJM, Thijsen SFT. 2011. Elispot On Cells From Bronchoalveolar Lavage, A Novel Approach To Diagnose " Bird Fanciers Lung". Conference: American Thoracic Society 2011 International Conference, May 13-18, 2011. Denver, CO, US. doi:doi: 10.1164/ajrccm-conference.2011.183.1_MeetingAbstracts.A4523.
 299. Limonard G, Bouwman JJM, Asscheman A, Thijsen SFT, Vlamincx BJM, Bossink A. 2011. Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection, vol 38, p 2470. *Eur Respiratory Soc*.
 300. Heemstra KA, Bossink AWJ, Spermon R, Bouwman JJM, van der Kieft R, Thijsen SFT. 2012. Added value of use of a purified protein derivative-based enzyme-linked immunosorbent spot assay for patients with *Mycobacterium bovis* BCG infection after intravesical BCG instillations. *Clinical and Vaccine Immunology* 19:974-977. <https://doi.org/10.1128/CVI.05597-11>.
 301. Arend SM, Thijsen SFT, Leyten EMS, Bouwman JJM, Franken WPJ, Koster BFPJ, Cobelens FGJ, van Houte AJ, Bossink AW. 2007. Comparison of two interferon- γ assays and tuberculin skin test for tracing tuberculosis contacts. *American journal of respiratory and critical care medicine* 175:618-627. doi: 10.1164/rccm.200608-1099OC.
 302. Hofland RW, Bossink AWJ, Bouwman JJ, Spermon R, van Houte AJ, Lammers JWJ, Thijsen SFT. 2018. Feasibility of Purified Protein Derivative based ELISpot for Urine and Bladder Fluid after Intravesical BCG Instillations. Part of Thesis ' Clinical Aspects of Immune Responses in Tuberculosis'. Available at: <https://dspace.library.uu.nl/handle/1874/364002.61-73>.
 303. Bouwman JJM, Thijsen SFT, Bossink AW. 2012. Improving the timeframe between blood collection and interferon gamma release assay using T-Cell Xtend. *J Infect* 64:197-203. doi: 10.1016/j.jinf.2011.10.017.
 304. Bathoorn E, Limburg A, Bouwman JJ, Bossink AW, Thijsen SF. 2011. Diagnostic potential of an enzyme-linked immunosorbent assay in tuberculous pericarditis. *Clin Vaccine Immunol* 18:874-7. doi: 10.1128/CVI.00031-11.

