European neuroborreliosis

Long term follow-up

Randi Eikeland

Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

2012

Dissertation date: 150612

Scientific environment

Institute of Clinical Medicine University of Bergen, Bergen

Department of neurology, Sørlandet Hospital HF, Arendal

South Eastern Regional Health Authority, Norway

Acknowledgements

This dissertation was founded by the South Eastern Regional Health Authority and supported by the research department of Sørlandet Hospital. Without them it would not have been possible to give this work priority. The work was performed at the department of Neurology, Sørlandet Hospital, and I am grateful for the time and the flexibility provided by the department. I am also thankful for the good cooperation with the University of Bergen.

Thank you to the patients and their family and friends who stood up for research and gave of their goodwill and time. I dedicate this work to you, and I hope our effort will contribute to help patients with Lyme neuroborreliosis in the future.

I am filled with gratitude towards my three supervisors: Unn Ljøstad, Åse Mygland and Karen Herlofson. First of all I want to thank Unn for being available, always! Her accuracy and honesty is admirable, and I have never met anybody working as rapidly as her! Her insightful criticism and curiosity regarding our work inspirers. Åse`s creativity and experience give work scientific wings, but she manages to drag us back to clinical practice. I was proud the day you asked me to join your project. Karen, college and friend for years, made me believe that despite being a sworn clinician, I could also be a researcher. Her perfectionism is admirable, her English is splendid!

Without neuropsychologist and dear friend Gro C. Løhaugen I would have been lost in the jungle of NP. Thank you for collaboration, encouraging and wonderful meals. And thank you for sharing Jens with Clas and me.

Of all my helpers at the policlinic in Arendal and Kristiansand I thank especially Jorunn Berg and Bjørg Sollie. I thank my dear daughter Oda and our friend Victoria Pålsson for hours of boring paperwork! Thanks to Sølvi and Tone at the laboratory, statistician Are Hugo Pripp and friend and neuropsychologist Bjørg Øygarden for practical help.

Thanks to colleges at the department of neurology, among them Ragnar, Harald, Edith, Cornelia, Andreas, Antonia, Solgunn and Lars Kristian for never saying a hard word although you have to take over my work in the clinic. I love to work with you all! I also thank Per Monstad, Olaf Henriksen, Harald Gåskjenn and Ragnar Solhoff for showing me the thrill of neurology.

I thank my research colleges for inspiring talks and practical help, especially Svein Gunnar Gundersen, the leader of the research unit at Sørlandet Hospital, Anne, Marianne and Merethe. And thank you friend Jon Skranes for your optimism and help.

Thanks Arendal Dramatiske, Grane revyen and MixogComp for keeping my spirit up!

Dear family and friends, I thank you for supporting me and believing in me, especially my mother and father, Gunnar and Anne Gyro, my beautiful children Erlend and Oda, and, finally, my beloved husband and soul mate, Clas, for practical help, friendship and love.

.

Abstract

Lyme disease is the most common human tick borne disease in the northern hemisphere, and the southern coastline of Norway is a high endemic region regarding Lyme disease.
European Lyme neuroborreliosis (LNB) often presents with a sub acute painful lymphocytic meningoradiculitis (Bannwarth syndrome) with or without paresis in the abdominal wall, the limbs or muscles innervated by cranial nerves. Central nervous system involvement as encephalitis, myelitis and vasculitis is rare.
Most LNB patients experience marked improvement in neurological symptoms within weeks to a few months after antibiotic treatment, but years after treatment 10 – 50% report persisting or new symptoms including fatigue, concentration difficulties and musculoskeletal problems. Remaining complaints after adequately treated *Bb* infections are often named Post Lyme Disease Syndrome (PLDS). The prevalence and impact of PLDS is debated since similar symptoms are common in the general population, and there are few European controlled studies on the issue. Most studies on outcome after LNB are conducted in the US, and as Borrelia genotype and the clinical picture of Lyme disease in the US differ somewhat from what we find in Europe, the study results are not necessarily transferable to European patients.

Aims

Our aim was to assess the long-term impact of LNB on Health Related Quality of Life (HRQoL) in a controlled study of well-characterized adult European LNB patients.
We also wanted to compare the neuropsychological (NP) functioning by assessing executive/attention functions, processing speed and memory in a group of adult LNB patients 30 months after treatment to a matched control group. Finally, we wanted to identify clinical, demographical or laboratory factors associated with a reduced HRQoL and fatigue after treatment of LNB.

Patients and methods

A cohort of 50 patients was followed for 30 months after treatment for LNB. The patients were recruited from a treatment study conducted in southern Norway comparing per oral doxycycline to intravenous ceftriaxone. All patients were living in the geographical region of Agder Counties, and received treatment between May 2004 and December 2007.
The LNB patients brought a control person from the same geographical area, matched for age, gender and education level. Exclusion criterion for the controls was a history of acknowledged LNB.

At basis pre-treatment and at 4, 12 and 30 months we did a semi structured interview, a clinical score, and spinal and blood tap. At 30 months NP functioning was assessed, and all participants completed the following questionnaires: the Fatigue Severity Scale (FSS), the Montgomery-Åsberg-Depression Rating Scale (MADRS), and the Short Form-36 (SF-36: A HRQoL questionnaire including the sum scores Mental Component Summary (MCS) and Physical Component Summary (PCS)). They were asked about previous and current coexisting diseases, psychological distress and subjective complaints. A composite clinical score made for the treatment study, summarizing subjective complaints and objective findings, was used to assess clinical status. Non-complete recovery was defined as more than 1 point score on the composite clinical score.

The four NP tests in our test panel consisted of 23 subtasks, and we calculated a sumscore expressing the number of NP subtasks with scores ≤-1 SD from the mean in the control group (range 0-23). The sumscores were then categorized into three groups: Normal: 1-5 (≤ -1 SD from the mean sumscore in the control group), deficit: 6-8 (>-1-≤2 SD from the mean sumscore in the control group) and impairment: 9-23 (>-2 SD from the mean sumscore in the control group).
In the study regarding risk factors we did a univariate analysis comparing independent demographical, clinical and laboratory data to the PCS, MCS and FSS scores to look for associations. The variables which were associated with the outcome in the univariate analyses were analyzed further in a multiple regression model.

Before treatment 80 % of the patients had a complete or partial Bannwart syndrome, and 8 % had symptoms suggesting involvement of the central nervous system (myelitis, ataxia and confusion). Fifty percent were treated with oral doxycycline and 50 % with IV ceftriaxone. Sixty-eight percent were classified as definite LNB according to the criteria of the European Foundation of Neurological Society, and 32 % as possible LNB. Mean age at follow up was 55, and 58 % were male.

Results

Paper I: LNB treated patients had reduced HRQoL compared to controls as assessed with the SF-36 summary components PCS ($P<0.001$) and MCS ($p=0.010$) 30 months after treatment. The patients scored lower on all the eight subscales of the SF-36, except for bodily pain. The LNB patients who reported complete recovery (56%) had similar HRQoL scores as the controls.

Paper II: LNB treated patients scored lower on four NP subtasks assessing executive/attention functions, processing speed, visual and verbal memory, as compared to matched controls: Stroop test 4 (P=0.015), TMT 5 (P=0.004) Digit Symbol recall (P=0.038) and CVLT list B (P=0.003). The distribution of global NP function indicates that most of the LNB treated patients perform comparable to controls, while a small subgroup have a debilitating long-term course with cognitive impairment. Fatigue, depression, neurological deficits or HRQoL at 30 months after treatment were not associated with the global NP sum score. Eighteen out of 50 patients (36%) had objective findings in terms of neurological deficits and/or cognitive impairment.

Paper III: Delayed start of treatment and remaining complaints 4 and 12 months after treatment seem to predict a worse outcome with respect to HRQoL. Delayed start f treatment, a more severe disease pre-treatment and remaining complains at 4 and 12 months after treatment seem to predict more fatigue at 30 months. Age, gender, educational level, diagnostic accuracy, treatment option, signs of infection in the central nervous pre-treatment or coexisting somatic diseases or psychological distress were not associated with HRQoL outcome 30 months after treatment in our cohort, neither were any of the assessed CSF findings before treatment or during follow-up .

Conclusions

1. HRQoL was reduced in well-characterized European patients treated for LNB with a current recommended antibiotic regimen 30 months earlier, as compared to matched controls. The LNB treated patients were not more depressed and did not report more pain than the controls. Fatigue was the most disturbing persisting complaint, and was negatively associated with HRQoL. Mild neurological deficits were found in 28 % of the patients, and seemed to influence negative on the physical HRQoL and fatigue scores. The patients who reported subjective recovery had the same HRQoL as the controls.
2. Most of the patients who were treated for European LNB 30 months earlier had comparable NP functioning to matched controls, but a small subgroup had cognitive impairments regarding attention/ excecutive function, processing speed and memory that could affect their daily life. The LNB treated patients with complete recovery had similar NP functioning as the controls. We did not find any association between NP test results and HRQoL or fatigue.
3. It seems as a more serious LNB disease and a longer duration of symptoms before treatment can reduce HRQoL, and as symptom duration more than 6 weeks before treatment,

a more severe disease and non-complete recovery at four and 12 months predict a higher burden of fatigue 30 months after treatment. We did not find that any laboratory data predicted outcome after treated LNB, or that any CSF finding indicated an active *Bb* infection 30 months after treatment. Gender, age, comorbidity, signs of pre-treatment infection of the central nervous system or CSF findings before and during follow-up were not associated with HRQoL or fatigue at 30 months.

4. Thirty months after treatment of LNB 18 out of 50 patients (36%) had objective findings in terms of neurological deficits and/or cognitive impairment.

List of publications

Paper I: Eikeland R, Mygland Å, Herlofson K, Ljøstad U. European neuroborreliosis: quality of life 30 months after treatment. Acta Neurol Scand. 2011. 124: 349-354

Paper II: Eikeland R, Ljøstad U, Mygland Å, Herlofson K, Lohaugen GC. European neuroborreliosis: neuropsychological findings 30 months after treatment. Eur J Neurol. 2011. doi: 111/j.1468-1331.2011.03563.x. Epub 2011 oct. 15.

Paper III: Eikeland, Mygland Å, Herlofson K, Ljøstad U. Risk factors for a non-favorable outcome after treated European Neuroborreliosis. Submitted, November 2011

Contents

1. Background

1.1 Lyme disease.

1.1.1 The history and epidemiology of Lyme disease (LD)

The story of Lyme disease (LD), named from the region Lyme in Connecticut, is legendary. It shows us that important and unexpected solutions can often be founded upon the hard work of open-minded researchers who take the concerns of the public seriously. The descriptions of LD, the identifying of its vector; the hard shell tick *Ixodes*, and the discovery of the causative spirochete bacteria *Borrelia burgdorferi (Bb),* have occupied researchers all over the world for hundreds of years. The fact that ticks can cause diseases in humans has been known even from the antics and from the travel books of Dr. Livingston [1], and the most common clinical picture of disseminated disease in Europe, the Bannwart syndrome, was described by Bannwart in 1941 [2]. An important break-through came in the seventies when two observant mothers reported joint swelling and the typical rash Erythema Migrans in several children in their neighborhood, and field researchers connected the symptoms to the ticks in the bushes along the riverbanks in Lyme [3]. In 1981 the spirochete *Bb* was discovered [4], and since then the research field of "Borreliology" has become an expanding but also a controversial arena. There are still many questions regarding LD symptoms, treatment and prognosis to be answered.

The *Bb* bacterium generates various clinical diseases in humans and animals. In the northern hemisphere LD is the most common vector borne disease. Surveillances from different regions show us that the incidence of *Bb* infections varies considerably. In Norway the trend has been that the incidence of disseminated LD has grown slowly, but in the last years it seems to have stabilized with about 300 reported cases annually. In the high endemic regions of Aust- and Vest-Agder counties the incidence rates are 29.8 and 36.9 per 100 000 population respectively (numbers are from MSIS (MeldeSystemet for smittsomme (Infeksjons) Sykdommer) www.msis.no, Norwegian surveillance for infectious disease the Norwegian institute of Public Health). In the US the annual rate varies between 0.01 per 100 000 population in low endemic states, to 73.6 per 100 000 population in Connecticut in a 15 years period [5].

In general there has been a slightly growing prevalence of ticks, *Bb* and tick-borne diseases in the last decades. Farmland reverting to woodland, increasing number of deer and rodents, climate changes and bird immigration all contribute to this. Human behavior leading to the change of the landscape because of less agriculture in many countries is probably the main reasons for the increasing concentration of ticks and its parasites [6].

1.1.2 *Borrelia burgdorferi (Bb) sensu lato* and its vectors

The genus Borrelia consists of two major human pathogenic groups; *Borrelia recurrentis,* the agent of relapsing fever, and *Borrelia burgdorferi (Bb) sensu lato* group causing LD. New *Bb* species are discovered regularly, and there are regional differences in the distribution of ticks, hosts and *Bb* species. In Europe, at least five species of *Bb sensu lato* are pathogenic for humans, namely *Bb sensu stricto*, *Bb garinii, Bb afzelii, Bb spielmanii* and *Borrelia bavariensis* [7]. In the US LD is almost exclusively caused by the genotype *Bb sensu stricto*. In southern Norway, where this study was carried out, a study of prevalence and genotypes of *Bb* in ticks shows that we have mainly *Bb afzelii* (60%) and *Bb garinii* (23%), followed by *Bb sensu stricto* (11%) and *Bb valaisiana* (5%) [8].

A vector is an organism that carries a parasite or pathogen from host to host**,** and the gram negative *Bb* bacterium is totally dependent of its vectors and hosts and cannot survive outside them. In US the most common vectors are the ticks *Ixodes scapularis* and *Ixodes pacificus*. In Europe the tick *Ixodes ricinus* is the preferred vector, although other vectors can be found. The ticks are mainly active during summer and autumn, but if the temperature is above 4-5 degrees Celsius, and the surroundings are not too dry, you can find active ticks around the year along the southern coastline of Norway. The life cycle of the *Bb* typically lasts 2-3 years, and consists of three life stages, larva, nymph and adult. The primary *Bb* reservoir is mice and other small vertebras [5]. The ticks are inoculated with *Bb* when they feed blood, mainly from rodents and birds, and the ticks then transmit the *Bb* infection to other hosts like deer, or humans. The *Ixodes ricinus* needs two blood meals during one life cycle, and in addition the female tick needs a blood meal before she can produce eggs. Not all ticks are infected with *Bb*, and a recent study showed that the infection rate of the ticks in Agder counties was 22-31% [8]. Interestingly, some host animals, like deer, have developed strategies that protect them from infection with *Bb*, even if they are attractive hosts for ticks. Hunters in Norway tell stories about how they have found hundreds of ticks on one prey. The protection strategies are probably due to activation of the complement system in these animals [9]. The *Ixodes ricinus*

may also carry other much less frequent human pathogens such as *Tick borne encephalitis (TBE) virus*, *Anaplasma phagocytophilum* and *Babesia*.

1.1.3 Clinical stages of Lyme disease

Persons who were frequently out-of-doors in a high endemic region regarding LD in Sweden had a risk of 4 % per 10 hours spent outside of getting bitten by a tick [10], and a Germany study found that 2.6 % of tick-bitten individuals will develop LD [11]. The risk of getting infected with *Bb* after a tick-bite depends on several factors: how many ticks a person gets bitten by, for how long the tick feeds, if the tick is *Bb* infected, and what species of *Bb* are transferred [12].
In Norway the most common presentation of disseminated disease is Lyme Neuroborreliosis (LNB) (70%), followed by arthritis (20%) and the chronic skin affection Acrodermatitis Chronica Atrophicans (ACA) (numbers taken from MSIS). *Bb* heart affection is feared because of the potential serious conduction defects. The condition it is rare in Norway, but case reports do exist [13].

A stage classification based on the clinical presentations of LD, from localized disease with the typical skin infection Erythema Migrans (stage I), through the early disseminated stage with organ manifestations (stage II) to the late disseminated disease with symptoms persisting for more than 6 months (stage III) is commonly used. Even if this is the most typical progression of LD, it is important to know that LD may present primarily in any of these stages (see Table 01). Subclinical and asymptomatic courses are usual, and studies of blood donors in the high endemic region of southern Norway have shown that as much as 15-20 % of the population is positive for anti-*Bb* antibodies in serum despite no clinical history of LD [14].

Table 01. Stages of Lyme disease (LD). LD can present primarily in any of these stages, does not always progress from one stage to another, and can also have an asymptomatic course.

Localized disease LD	1-21 days after tick bite	Erythema migrans Lymfocytoma
Early disseminated LD	Starts 6-8 weeks after tick bite	Neuroborreliosis (LNB) Arthritis Carditis with conduction defects Other organ manifestations
Late disseminated LD	> 6 months after tick bite	Acrodermatitis Chronica Atroficans (ACA) Chronic arthritis Late neuroborreliosis
Post Lyme Disease Syndrome (PLDS)	Months to years after tick bite	Musculoskeletal pain Cognitive problems Fatigue

Localized disease, stage I: Erythema Migrans, the most characteristic skin manifestation (but not the only possible rash) is a slowly annular spreading rash around the site of the tick-bite 2-30 days after a person has been bitten. It can be accompanied by nonspecific “flu like” symptoms. At this stage the diagnosis is based on the history of tick-bite and clinical observations. Laboratory tests are seldom supportive of the diagnosis in stage I, and are not recommended. The Erythema Migrans normally resolves spontaneously after a few weeks and the chance of dissemination is considerably reduced if the patient is treated with antibiotics at this stage [15]. Another more rare manifestation is the Borrelia Lymphocytoma, a mass of lymphocytes in the skin, characteristically located on the ear or breast, and mainly found in children.

Early dissemination, stage II: In 10 % of the untreated infected patients, the infection spread to other organs of the body and musculoskeletal, neurologic and cardiovascular symptoms may occur a few weeks to six months after the tick-bite [5]. Only half of the persons developing LNB remember a tick-bite, and even fewer remember an Erythema Migrans (30%) [16].

Late dissemination stage III: If the infection last for more than 6 months, it is called late disseminated Lyme. The typical presentation of late dissemination in Europe is the chronic skin lesion ACA, sometimes with polyneuropathy [14]. Chronic arthritis, especially in the

larger joints (monoarthritis in the knee) and chronically progressive encephalomyelitis are rare manifestations [17].

Although most of the patients are diagnosed with LNB in the first weeks and months after the tick bite, up to 5 % get their first symptoms more than 6 months after the tick bite [18].

Post Lyme Disease Syndrome (PLDS): Wormser et al. have proposed a definition for PLDS. In short: Persisting subjective symptoms like cognitive complaints, fatigue, or widespread musculoskeletal pain starting within 6 months after completed treatment with a generally accepted antibiotic regime for a documented episode of Lyme disease fulfilling the definition given by the Centre of Disease Control and Prevention (CDCP) [19]. The symptoms should not be explained by any other condition, and they should be of such a severity that they reduce previous levels of everyday activities [20].

The term Chronic Lyme has been used to describe both symptoms and complaints in the late stage of the disease, and persisting complaints after treatment without any remaining signs of infection. In general, the neurological findings and symptoms in the early and late phase of the disease are successfully treated with antibiotics, and the infection is eradicated [21]. Researchers in the field borreliology have proposed diagnostic categories for persons who have symptoms attributed to “chronic Lyme disease” [22].

Table 02 The four predominant categories of diseases associated with chronic Lyme disease. Only patients with category 4 disease have Post–Lyme Disease Syndrome (PLDS)(Feder et al. NEngl J Med 2007) [22]

Category 1	Category 2	Category 3	Category 4
Symptoms of unknown cause, with no evidence of *Borrelia burgdorferi* infection	A well-defined illness unrelated to *B. burgdorferi* infection	Symptoms of unknown cause, with antibodies against *B. burgdorferi* but no history of objective clinical findings that are consistent with Lyme disease	Post–Lyme Disease Syndrome (PLSD)

Studies from Lyme referral centers in the US show that only one quarter to one third of the patients seeking guidance about suspected “chronic LD” has or have ever had LD [23]. A recent study from Germany highlights the importance of proper diagnostic workup in the group with suspected “chronic LD”. Out of 122 persons referred to a specialist center because

a “chronic LD” condition was suspected, 7% were diagnosed with acute LNB and 50% had other diseases that explained their complaints, among them 13 patients with findings suggestive of Multiple Sclerosis. Six out of the 122 referred persons were diagnosed with a Post Lyme Disease Syndrome (PLDS) [24].

To avoid misunderstandings, in our opinion, the term ”chronic Lyme” is ambiguous and should not be used.

1.1.4 Definition and clinical presentation of Lyme neuroborreliosis

In 2010 the European Federation of Neurological Societies (EFNS) reviewed the European guidelines on diagnosis and management of Lyme Neuroborreliosis, and suggested case definitions of definite and possible LNB [25].

Table 03 Suggested case definitions for Lyme Neuroborreliosis (LNB).
(Mygland et al. EFNS guidelines on the diagnosis and management of European Lyme Neuroborreliosis. Euro J Neurol 2010) [25]

Definite neuroborreliosis [a] All three criteria fulfilled	Possible neuroborreliosis [b] Two criteria fulfilled
1.Neurological symptoms suggestive of LNB without other obvious reasons 2.Cerebrospinal fluid pleocytosis 3.Intrathecal Bb antibody production	

a These criteria apply to all subclasses of LNB except for late LNB with polyneuropathy where the following should be fulfilled for definite diagnosis: (I) peripheral neuropathy (II) acrodermatitis chronica atrophicans (III) Bb-specific antibodies in serum.
b If criteria III is lacking; after a duration of 6 weeks, there have to be found Bb-specific IgG antibodies in the serum.

In most European countries, as reflected in the EFNS guidelines above, the detection of intrathecal production of anti-*Bb* antibodies is necessary to diagnose definite LNB. In the US this is not mandatory [26].

Another useful definition included in the EFNS guidelines is the distinction between early and late LNB [25].

Table 04 Classification of Lyme neuroborreliosis (LNB)
(EFNS guidelines of diagnostics and management of European Lyme neuroborreliosis Euro J Neurol 2010) [25]

Early LNB
Neurological symptoms for <6 months With manifestations confined to the peripheral nervous system (cranial nerves, spinal roots or peripheral nerves) (Bannwarth syndrome) With central nervous system manifestations
Late LNB
Neurological symptoms for more than 6 months With peripheral nervous system manifestations With central nervous system manifestations

Early LNB:

In Europe, the most common clinical presentation of an infection of LNB is the Bannwart syndrome, with painful radiculitis and lymphocytic meningitis [2]. The pain is often described as waning and waxing, radiating and severe, especially at night [27]. Paresis of muscles in the limbs, the abdominal wall, or in muscles innervated by cranial nerves, most commonly the facial nerve followed by abducens and oculomotor nerves, occur frequently in Europe [28]. Stiff neck and headache are sometimes present. LNB may also rarely present as peripheral mononeuritis multiplex or plexus neuritis. Involvement of the central nervous system (CNS) parenchyma is uncommon, but symptoms of myelitis and encephalitis as confusion, epileptic seizures, ataxia or tremor do occur [25].

Late LNB:

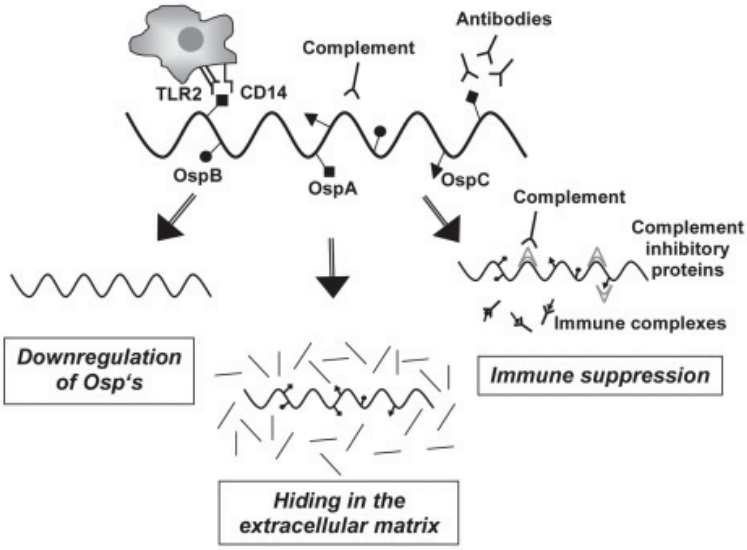
If untreated symptoms persist for more than 6 months, it is called late LNB. This can present as a progressive segmental myelitis, encephalitis or cerebral vasculitis, and cause symptoms like gait or bladder problems, spasticity or encephalopathy [29]. Mononeuropathy, radiculopathy and polyneuropathy may also occur. Distal symmetric polyneuropathy is rarely seen in Europe in *Bb* infections, but polyneuropathy may occur in connection with ACA [14].

1.1.5 Patophysiology of Lyme disease and Lyme neuroborreliosis

Bb lives in the mid-gut of the hard shell tick, attached to the tick-gut epithelial cells by the outer surface protein Osp A. When the tick bites into the host's skin the blood from the host flows into the tick gut and *Bb* is presented to the host's blood. During the first 24-48 hours of tick feeding, *Bb* multiplies and prepares itself for the meeting with the immune system of the host. Then *Bb* migrates to the salivary glands of the tick and is transmitted to the host through

the tick salvia. It normally takes time before *Bb* is transmitted to the host, and if the tick is removed within 24 hours, the risk of an infection with *Bb* is considerably reduced [30;31]. Rupprecht has made a figure describing which mechanisms Bb uses to evade the hosts immune system (see figure 01) [32].

Figure 01. Mechanisms for Borrelia burgdorferi to evade the hosts immune system. (Rupprecht et al. The Pathogenesis of Lyme Neuroborreliosis: From Infection to Inflammation. Mol med 2008, with permission) [32]



In humans *Bb* is attacked by the hostile immune system in several ways: *Bb* surface proteins are recognized by the CD14 and the toll like receptor 2 (TLR2) of the innate immune system. *Bb* is confronted with the hosts complement system. Antibodies against *Bb* outer surface proteins are produced by the activation of the acquired immune system. An important *Bb* defense mechanism against attacks from the hostile immune system is a change of the surface molecules on the bacteria`s outer membrane, so called antigenic variation. The most important switch is probably between the very immunogenic outer surface protein Osp A to Osp C. Osp C attaches to a tick salivary protein which prevent activation of the host`s complement system and builds immune complexes with the anti-*Bb* antibodies. *Bb* also hides in less accessible compartments to the immune system like the extracellular matrix [32].

If the infection is established in the skin the rash develops annularly around the site of the tick bite. At this time the antibody production has normally not started, and the diagnosis is

clinical. The infection might remain localized, or disseminate to other organs like the heart, eye, joints, the peripheral or central nervous system. If *Bb* disseminates into the nervous system by the blood stream or along other structures like the peripheral nerves is not known. It is possible that different species use different routes of dissemination, and that this phenomenon may be one of the reasons why the human clinical phenotype of LNB differs in different populations and geographical locations. The meningoradiculitis caused by the *Bb garinii* is often localized near the primary site of infection and might have spread along the nerve fibers to the dorsal roots. The multiple Erythema Migrans and the more diffuse meningitis seen in humans in the US where the *Bb sensu stricto* is the only known causative agent of LNB, point to a more hematogenous spread of *Bb* [32].

The cause of the injury to the nervous system is unknown [33]. *Bb* is difficult to find in human tissue, and to learn more about the pathogenesis there is a need of good animal models. The only successful induction of reliable clinically manifest LNB in an animal model is in the immunosupressed monkey rhesus Macaque [34]. Cadavid et al. found spirochetes in the leptomeningies, the nerve roots and dorsal root ganglia of the ape, but the CNS parenchyma was not affected. This seems to be in accordance with the clinical picture of meningoradiculitis.

Research has shown that the different *Bb* species have various affinities to different human tissue [35]. *Bb sensu stricto* is associated to arthritis, *Bb garinii* to LNB and *Bb afzelii* to dermatoborreliosis [36].

The ability of the *Bb* to survive in the host despite an intensive inflammation is a frequent cause of discussions, however, *Bb* is very susceptible to antibiotics like doxycycline, penicillin and ceftriaxone, and the main opinion is that when treated with adequate antibiotics, the *Bb* does not survive, and the infection is eradicated.

1.1.6 Laboratory diagnosis of Lyme neuroborreliosis

The gold standard of laboratory diagnostics in infectious diseases, namely direct detection of the bacteria by cultivation or PCR has low sensitivity in LNB. The sensitivity of cultivation is 10–30% in CSF in early LNB and less than 10% in blood of patients with an Erythema Migrans [37]. In Europe the PCR sensitivity is 23% in the CSF and 10% in the blood of LD patients (% is mean of several studies in Erythema migrans and early disseminated LD)[38] . Clinicians and researchers therefore have to depend on indirect laboratory methods. Most of

the time the diagnostic process is unproblematic if we evaluate clinical, anamnestic and laboratory findings together. Signs of inflammation are almost always present in the CSF in terms of elevated cell count to 10-1000 leucocytes / mm3, moderately elevated protein and CSF oligoclonal bands [39].
The anti-*Bb*-specific antibodies in serum and in CSF can be detected with an IgG- and IgM-differentiating enzyme-linked immunosorbent assay (ELISA). Diagnostic sensitivity of ELISA screening assays in early LNB is 70–90%, and 90-100% in late LNB [25]. Some laboratories confirm the positive test with an immune blot with a higher specificity.
There are several pitfalls when considering the anti-*Bb* antibody findings:
False positive IgM and IgG antibodies: The finding of anti-*Bb* IgM antibodies in serum without anti-*Bb* IgG antibodies must be interpreted with care as this can be result of cross-reactivity or a non-specific response to another disease [40]. Anti-*Bb* IgG antibodies can be present in serum and CSF years after symptomatic and asymptomatic *Bb* infections, also at high levels, and it is problematic to use anti-Bb antibodies as a marker for successful treatment because of this [41].

False negative IgM and IgG antibodies: It might take several weeks to produce detectable levels of anti-*Bb* antibodies, so it is possible to have an active LNB without anti-*Bb* antibodies. Later, within 6-8 weeks, anti-*Bb* antibodies are detected in almost 100 % of the LNB cases [42].

It is possible to have anti-*Bb* antibodies in CSF even if no antibodies are found in serum. This, combined with the fact that the clinical picture can be unclear, makes it necessary to do a lumbar puncture to look for inflammatory markers to diagnose a definite LNB [43].

Antibodies may leak from serum to CSF, and to prove that anti-*Bb* antibodies detected in CSF really are produced intrathecally, an antibody index is calculated which compares the antibody concentrations between the CSF and serum. A test like IDEIA Lyme Neuroborreliosis kit (DakoCytomation, Cambridgeshire, UK) corrects for the impairment of blood brain barrier. The antibody Index is calculated like this: (OD is optic density) OD CSF /OD serum x (OD CSF –OD serum [44].
The chemokine CXCL-13, a chemoattractant cytokine that attracts the B-lymphocytes to the site of the inflammation, has been extensively investigated during the last years, and seems to be useful in the early detection of LNB [45]. Detection of CSF CXCL-13 have a diagnostic

sensitivity of 100% in the early phase of LNB, as compared to a diagnostic sensitivity of 89% of a positive *Bb* antibody index in a study of Ljøstad et al [46].
The potential failure of making a positive diagnosis due to the insufficient tests available has led to a controversial marked for LNB testing, where many of the tests are used for diagnostic purposes before they have been properly evaluated. A critical evaluation of the available tests and recommendation of use are given in the EFNS guidelines. Currently the tests based on detection of antibodies are the most reliable and the ones recommended [25].

The use of imaging procedures as diagnostic tools, such as MRI, has not been very helpful until now [47]. If the clinical manifestation and the anamnesis points towards a diagnosis of LNB, a contrast MRI imaging technique that shows signs of nerve root and meningeal enhancement can support the diagnosis of LNB [48]. MRI of patients with acute LNB and encephalopathy might show white matter lesions [49]. The general lack of findings in structural imaging is in accordance with the fact that the *Bb* seldom affects the parenchyma of the CNS. Functional imaging techniques such as Single Photon Emission Computed Tomography **(**SPECT) have demonstrated abnormalities suggestive of reduced blood flow and metabolism [50;51]. More functional studies are necessary, not least because LNB patents with persisting complaints report functional problems like cognitive difficulties and fatigue.

1.1.7 Prevention and treatment of Lyme disease and Lyme neuroborreliosis

Avoiding known tick-infested areas may of course prevent LD, but this is a bit dramatic, and even though the chance of getting a tick bite is considerable in certain areas, the risk of serious disease is low. To prevent tick bites it is recommended to use insect repellant and wear long pants tucked into boots and long sleeves to protect the skin. Skin, clothing and pets should be examined for ticks, and ticks must be removed immediately with fingernails or with tweezers. A single dose of Doxycycline after the bite might prevent disease, but is not recommended because of the low chance of being infected combined with ecological and other considerations [52]. At the present time there are no available vaccines against infection of *Bb*.
The EFNS guidelines suggest treatment for14 days with per oral doxycycline (or penicillin) for LNB unless CNS involvement is suspected, in which case intravenous ceftriaxone for 14 days is recommended [25]. A non-inferiority study of the two treatment options in 112 unselected LNB patients with all stages and clinical manifestations has shown that the two treatment options are equally effective [39].

1.2 Prognosis after treated Lyme Neuroborreliosis

Most LNB patients who receive current recommended antibiotic treatment regimens have a decline in their CSF cell count and experience a marked relief of subjective symptoms and regression of objective neurological deficits within weeks to a few months after treatment, sometimes even after just a couple of days [39]. Despite this, up to 50% of the treated LNB patients complain about persisting symptoms. The most common post-LD complaints are arthralgia, fatigue, reduced quality of life and neurocognitive problems in addition to mild neurologic findings [52-55].

1.2.1 Health Related Quality of Life

The World Health Organization (WHO) defined quality of life as "an individual's perception of his or hers position in life in the context of the culture and value system in which he or she lives and in relation to his or hers goals, expectations, standards and concerns". It is a broad-ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationship, and relationship to salient features of the environment [56].

The term "quality of life" is incorporated into everyday vocabulary, but the concept of quality of life is not definitely defined. It has gradually come to mean subjectively assessed dimensions of life, mostly including physical and social functioning, emotional or mental state, tiredness, pain and sense of well being [57]. The concept of quality of life has different meanings within various fields. In a medical context we use the term "Health Related Quality of Life" (HRQoL), referring to how a person's wellbeing may be affected by a disease. A person diagnosed with a serious disease does not necessarily have a poor HRQoL, and the other way around, a person who have few objective signs of his or hers disease might have a poor HRQoL.

The need of standardized measurement of the patient's distress and functional impairment has increased. In the past, when most diseases were acute and life-threatening infectious diseases, the impact of medical care could be evaluated by mortality. Today the chronic diseases are more common, especially in the developed countries. The patient's subjective health perspective has become increasingly accepted as central to the monitoring and evaluation of medical care. The assessment of the relative improvement of a patient's condition is also

important when new medical care (like a new drug) is implemented. HRQoL measurements are regularly used to evaluate the benefit of new treatments and managements in cost-benefit analyses.

In clinical studies it is increasingly common to include a standardized questionnaire of HRQoL as one of the outcomes. This can contribute to uncover negative effects on a person's quality of life that more traditional measures as clinical, radiological and laboratory outcome measures might have missed [58].

HRQoL assessments can be used to compare groups and for longitudinal observations of populations. In populations with neurological diseases evaluation of a patient's quality of life is very useful. Neurological diseases are regularly chronic and incurable, and functional problems induced by the cerebral pathology that can affect daily life are common.

The most frequently reported complaints after LNB are pain, fatigue and cognitive problems. These symptoms are subjective and unspecific and in addition common in the general public. None of these complaints are easily evaluated, but they may all have an impact on HRQoL. Mental and physical problems including fatigue are known to influence Quality of Life[59].

1.2.2 Assessment of Health Related Quality of Life

There are several methods to measure quality of life, and the ideal method of assessing this subjective phenomenon does not exist. Some of the methods are disease specific or focus on special patient groups or areas of function, but most of them are generic, designed to be relevant to a wide range of health problems and to the general population. If a more global evaluation of HRQoL is warranted, a generic instrument that can be applied independently of diagnosis is recommendable. This can be used to compare a group of patients to healthy controls, to a control group with another disease or to the general population [60]. Generic instruments will be less specific to the actual disease, though.

To choose the right instrument for assessing HRQoL, reliability (reproducible and consistent results) and validity (the test measures what it intends to measure) are the most important factors. The instrument must be sensitive (detect real differences) and the scale must be responsive (able to detect changes). It is also important that validated translated versions in the language of the population of interest are available, and if possible, national normative data.

The use of self-administered questionnaires for assessment of HRQoL is less time consuming and cheaper than investigator-administered questionnaires.

Most HRQoL questionnaires are multi-item dimensions which assesses subjective experiences and functional status including at least physical, psychological and social factors. These dimensions can be used to evaluate different elements of quality of life, and to make a HRQoL profile in a person or a group. Because we often want to consider a global health outcome, sum components are often calculated based on the different dimensions. A typical example is the Physical Component Summary (PCS) and the Mental Component Summary (MCS) of the HRQoL questionnaire Short Form -36 (SF-36).

The assessed HRQoL change over time or difference between groups must be both statistically and clinically significant and reflect real changes that are of importance in a person's daily life. The results of previously conducted studies and experience from clinical praxis will help us to decide what differences are clinical meaningful to detect.

The impact of a person's reduced HRQoL on his or hers daily living is a matter of individual consideration, and many questionnaires also aim to evaluate this aspect by asking specifically how complaints like pain or fatigue influence the ability to do tasks, e.g. walking up steps. The subjective feeling of HRQoL can also change over time, and most questionnaires are restricted to a period of time. The SF-36 version 2 (that we used in our study), asks questions about the feelings and experiences the last four weeks. (For more detailed description of SF-36 see in method part of thesis).

1.2.3 Studies of Health Related Quality of Life after Lyme disease and Lyme neuroborreliosis

Several US studies on outcome after Lyme disease have included a HRQoL questionnaire in their evaluations, the most commonly used being the SF-36. The most important studies are listed in Table 05.

Table 05. Studies of Health Related Quality of Life (HRQoL) in Lyme Disease (LD) patients using Short Form -36

Studies	Material	Design	HRQoL results
[61] Shadick et al. 1994 Ipswich, Massachusetts, USA	38 LD patients 6.2 years after treatment 43 Healthy controls	Retrospective cohort study	Poorer global health status in treated LD patients
[62] Shadick et al. 1999 Nantucket Island, Massachusetts, USA	186 LD patients 6 years after treatment 167 controls	Retrospective cohort study	Lower scores in all 8 but energy dimension in treated LD patients, only bodily pain significant in final model. Physical dimensions were worse
[63] Kalish et al. 2001 Lyme, Connecticut, USA	Facial Palsy (31, treated 15) Erythema Migrans (25) Lyme Arthritis (28) Control Subjects (30)	Follow-up study of the Lyme population	Lower scores in body pain and physical limitations dimensions were found in some patients with untreated LD facial palsy
[64] Klempner et al. 2001 Boston, USA	78 seropositive LD patients with persistent symptoms 51 seronegative LD patients with persistent symptoms	Placebo controlled treatment trial	Impairment of HRQoL in all LD patients, no improvement with second antibiotic course
[65] Seltzer et al. 2000 Connecticut , USA	88 LD patients 88 controls	Cohort study, matched	No significant difference in HRQoL between controls and LD patients
[66] Krupp et al. 2003 Long Island, USA	28 patients with treated LD and fatigue 27 controls treated LD	Controlled treatment trial Ceftriaxone 28 days/ placebo	No significant improvement in HRQoL in LD patients after treatment
[67] Fallon et al. 2008 New York, USA	37 PLDS patients 20 PLDS controls	Double blind, placebo controlled treatment trial Ceftriaxone/placebo	Improvement of Physical Component Summary in ceftriaxone treated PLDS patient group, more improvement if severe disease
[68] Kowalski et al. 2011 USA	15 LD facial palsy patients 3 LD patients with localized disease 4.6 years after treatment	Retrospective double cohort study	Social function dimension scores were lower in LD facial palsy patient group.

In 2010 Kowalski et al. (USA) did a retrospective study and found that persons with longer duration of antibiotic treatment scored lower on the dimension social functioning of the SF-

35. They did not find differences in HRQoL between persons with definite or possible LD [69].

The US studies vary in design and inclusion criteria, making direct comparison of the studies difficult. Because of different *Bb*, the situation of the PLDS group regarding HRQoL might be different in Europe. We have not found European HRQoL studies of treated LNB patient which include a control group.

1.2.4 Fatigue

Definition

Fatigue can be described as a subjective overwhelming sense of tiredness, lack of energy or feeling of exhaustion [70]. Another definition is a subjective lack of physical and/ or mental energy that is perceived by the individual or caregiver, and pronounced enough to interfere with usual or desired activities [71]. Fatigue is distinct from limb weakness. It is not the same as sleepiness, and sleep does not give satisfactory relief of the symptoms. Fatigue is common in depression, and the symptoms of fatigue overlap with the symptoms of depression. The symptoms of low self-esteem, despair and feelings of hopelessness are more prominent in depressed than fatigued persons and this can be used to distinguish between these two conditions [72]. Fatigue and apathy are often confused with each other. Apathy is defined as a disorder of motivation with diminished goal directed behavior and cognition. In many neurological diseases, as Parkinson, both apathy and fatigue are frequent, often co-existing [73]. In studies regarding fatigue, both the assessment of depression and apathy should be considered.

Epidemiology

Fatigue is common in the general public [74] and in a Norwegian survey, 11.4% of the population reported chronic fatigue [75]. In a surveillance from 2005 using the Fatigue Severity Scale (FSS) it was found that 47 % of the Norwegian population was fatigued if they applied the recommended cut-off for significant fatigue of FSS ≥4 [76]! If FSS ≥5 was used as cut-off, however, the fatigue burden in Norwegians was 23 %, comparable to populations in other countries.

In clinical praxis physicians regularly meet patients complaining of fatigue. It is well known that many physical and mental conditions are complicated by fatigue, like hypothyroidism, anemia, cancer, neurological diseases, systemic diseases and psychiatric diseases [77;78]. Fatigue is also common as an adverse effect of medical treatment.

Fatigue in neurological diseases

In many neurological diseases fatigue is chronic and fluctuates in intensity, reduces quality of life and affects negatively social and occupational activities. Fatigue in Multiple Sclerosis (MS) patients is a classic example, and most of the fatigue related research is done on the MS population. Eighty to ninety per cent of patients with MS report fatigue [79]. The burden of fatigue is the most disturbing symptom for many MS patients, and may occur even if they have little or no physical problems related to the disease. Fatigue is one of the main reasons why many MS patients don't work [80].

Post-infectious fatigue

Post-infectious fatigue is also common. A prospective cohort study from 2006 conducted in a rural area in Australia found that a minority (12% of 253 patients at 6 months) of patients got a relatively uniform post- infective fatigue syndrome consisting of musculoskeletal pain, neurocognitive difficulties, mood disturbances and disabling fatigue after even the most common viral infections, and the condition might persist for 6 months and more. The most important predictive factor of this syndrome was a more severe illness. Demographic, psychological or microbiological factors did not predict fatigue in this cohort [81].

For many years now, patients have been diagnosed with Chronic Fatigue Syndrome (also called Myalgic Encephalomyelitis), a condition with unknown pathogenesis and etiology. No psychological or somatic explanation has been found. This syndrome, with an incidence of 0,2 to 2.6% [82], and with a number of changing definitions [83], has overlapping symptomatology with many other unexplained clinical conditions like fibromyalgia, the irritable bowel syndrome and multiple chemical sensitivity [84]. Studies of fatigue have shown that fatigue is an important factor in acute LD and in PLDS (see table 06). Subjects with anti-*Bb* antibodies report more fatigue than seronegative subjects [85]. There have been vivid discussions about LD and its role in the development of Chronic Fatigue Syndrome and "chronic LD" has been associated with the "medically unexplained symptoms" syndromes. A similarity between these conditions is that we do not know the pathophysiology causing the fatigue, and there is no cure. The chronic fatigued patients with ill defined conditions like Chronic Fatigue Syndrome feel even more insecure, confused and abandoned by the health care system [86]. A very interesting study found differences in the proteome profile in these Chronic Fatigue and PLDS [87].

Pathogenesis

The pathogenesis of fatigue is unknown, and many theories have been discussed during the last 20 years. The origin of fatigue is probably not connected to a specific anatomical region

in the brain and certainly not to one medical condition. The discussion of released inflammatory cytokines that starts tissue damage or change the pathways and balance of hormones and transmission substances are very interesting. In MS, often used as a model disease to illustrate different theories about fatigue, the role of the corticotrophin hormones are of special interest. Cortisones play an important role in the treatment of relapses in MS, and MS patients often describe an increased energy level during the cortisone cure. It is also proposed that changes in the metabolism in the frontal cortex or basal ganglia can cause fatigue [88].

Treatment

Secondary causes of fatigue like depression, poor sleep, pain, medications and deconditioning are probably the first issues to address [88]. Non-pharmacological approaches like exercise and cognitive behavioral therapy have shown to be effective treatment options in fatigued persons [89]. In MS pharmacological therapies like Amantadine, Pemoline and Modafinil have been tried out, but with questionable effect [88].

1.2.5 Assessment of fatigue

Fatigue is a subjective symptom, and difficult to measure. There are numerous assessment tools on the marked, but only two fatigue self-rating questionnaires are translated into Norwegian; the Fatigue Questionnaire (FQ) and the Fatigue Severity Scale (FSS). Both are validated in the Norwegian general population and are generic. Fatigue Severity Scale (FSS) is widely used, validated and reliable, and has main emphasize on physical fatigue and its impact on daily living. It is also used in several outcome studies after treated LD (see Table 06). A more detailed description of the FSS is to be found in the method section of this thesis.

1.2.6 Studies of fatigue in Lyme disease and Lyme neuroborreliosis

Table 06. Studies of fatigue in Lyme Disease (LD), Lyme neuroborreliosis (LNB) and Post Lyme Disease Syndrome (PLDS) using Fatigue Severity Scale (FSS)

Study	Material	Design	Results
[90] Krupp et al. 1991 New York, USA	15 LNB patients with memory disturbances 6.7 months after treatment 10 controls	Retrospective, matched controls	Fatigue correlated with memory deficiencies
[91] Bujak et al. 1996 USA	23 LNB 5.6 years after treatment 23 Recovered LD patients	Retrospective, matched controls	Fatigue in initial phase prognostic factor for poorer NP performance later
[92] Ravdin et al. 1996 USA	21 LD patients 21 Osteomyelitis 21 Healthy control	Prospective Controlled	Fatigue worse in LD patients, inverse correlated with memory deficiencies
[93] Gaudino et al. 1997 USA	38 LNB patients with fatigue 25 Chronic Fatigue Syndrome 56 Healthy controls	Retrospective Controlled	High incidence of fatigue in treated LNB and Chronic Fatigue Syndrome patients
[94] Pollina et al. 1999 USA	16 LD treated patients with persistent complains 15 Healthy controls	Retrospective Controlled	More fatigue in LD treated patients, but no impact on NP performance
[66] Krupp et al. 2003 USA	28 LD treated patients with fatigue (≥ 4 FSS score) 27 LD patients controls	Randomized Controlled treatment trial Ceftriaxone 28 days/placebo	FSS score reduced with more than 0.7 points in 69% of LD patients vs. 23 % in placebo group
[67] Fallon et al. 2008 New York, USA	23 PLDS patients Antibiotics 37 PLDS patients Placebo	Double blind, placebo controlled treatment trial	Improvement fatigue at 12 weeks in treated group, not at 24 weeks. Post-hock analysis found the same as Krupp (68% reduction 0,7 points on FSS scale vs. 25% placebo)

Based on these studies it is reasonable to conclude that fatigue is well known in LD and LNB treated patients, also years after treatment. The patophysiology is unknown, and repeated antibiotic cures have not shown convincing efficacy in reducing fatigue over time.

1.2.7 Cognitive problems and assessment of neuropsychological function

The science of neuropsychology aims to identify and quantify the cognitive consequences of brain injury. The definition of cognition varies throughout the literature. In general cognition is often assessed by Intelligence Quotient (IQ) tests presumed to reflect general cognitive ability. IQ refers to a derived score often included in Neuropsychological (NP) assessments. The NP tests assess NP functions like attention/executive functions, language, memory and visual motor skills. NP might help to detect more subtle problems and deficits in connection with diseases that affect the central nervous system. The knowledge of a person's cognitive abilities, deficits and impairments can help us to understand the complexity of the burden of disease, and to plan treatment strategies and interventions based on strengths and weaknesses based on the individual cognitive profile. Poorer test results in a group or on an individual level do not always implicate clinical consequences as this is highly dependent on the demands of the individual in everyday life and a person's ability to compensate.
The NP tests are often multifactorial, and the test results often rely on several functions, not only the single function it is primarily designed to measure. For example, one commonly used test, the Trail Making Test (TMT), where the participant has to draw lines to connect letters and numbers depend on motor skills, attention, executive functions and processing speed. The NP tests are sensitive but have a low specificity and assess cognitive functions with a great deal of overlapping. The different cognitive domains are also highly dependent on each other, an example is the executive functions that are highly dependent on attention, and it is therefore common to assess and analyze these aspects of cognition together [95].

The decision of what NP test to use can be difficult. There are many tests and test batteries available. Another challenge for Norwegian researchers is that few NP tests have been standardized nationally. In some tests as the Delis-Kaplan Executive Function System (D-KEFS) and the California Verbal Learning Test (CVLT) the original norms have been tried on a limited sample of Norwegians and found to be applicable [96;97].

Studies that apply NP assessments are not always easy to compare to each other because of the different tests used and the possibility of different interpretations of the test results. Many factors can influence the test results, like age, educational level, coexisting diseases, psychological conditions and test situations. The scores obtained from the individual tests have to be compared to a control group or normative data from the general population, mostly stratified by age.

The tests are usually administrated in a defined way in formal environments by a trained neuropsychologist. The Norwegian NP Commission recommends that a non-neuropsychologist who is going perform the NP tests has to go through training guided by an experienced neuropsychologist, and has to read the guidelines for NP testing given by the International Test Commission.

Tests assessing the functional areas of IQ, attention, executive functions , language, memory and visual motor skills are often included in a comprehensive NP assessment, but based on what information is considered most important in the given situation, focus may be restricted to only one or a few of these domains. Studies of neurocognitive functions often include an IQ test (most commonly a WAIS tests) or estimation of IQ and an evaluation of the premorbid functioning.

Attention/executive function

Attention/executive functions refer to higher order cognitive functions that are important to control and regulate goal directed behavior. Deficits in attention/executive functions may lead to problems in planning and initiation of activities, in interaction with other individuals and in the control of emotions and impulses. Attention/executive function problems are typical of brain injuries in the frontal lobes and/or its white matter pathways [95].
Executive functions are highly dependent on attention, and therefore these two aspects of cognition are often assessed and analyzed together.

The Stroop test and the TMT test are typical tests for assessing attention/executive functions, and the color-word interference, an adapted version of Stroop, and the TMT1-5 is translated into Norwegian as part of the D-KEFS test battery. Out of practical reasons the word-color interference test is called the Stroop-test in this thesis.

Processing speed

The term “processing speed” covers reaction time and perceptual-motor speed and deficits may be seen as cognitive slowing. Motor deficits in e.g. the hand reduce the fine motor speed, and can reduce processing speed even if there are no cognitive deficits. This must be kept in mind when using tests that requires drawing/ writing, like TMT and Digit symbol test. This problem can be avoided by using tests that assesses processing speed independently of the use of the hand (e.g. counting speed). Pollina did this to distinguish between reduced fine motor speed and cognitive slowing in LNB treated patients and found that the affected processing speed in these patients was independent of the hand function [94].
Typical tests for assessing processing speed are the TMT 5 and Digit symbol tests, both tests are translated into Norwegian, the Digit symbol test as part of the Wechsler Adult Intelligence Scale (WAIS) [98].

Memory functions and learning
Memory is the combination of processes where information is encoded, stored and retrieved, and consists of many components. Several models have been proposed to describe these processes, and although they differ, some major components seem to be consistent in current models.
Memory consists of long term memory and short term memory. Long term memory has an enormous capacity, and information can be actively brought up to the mind from previously gained information, sometimes for a lifetime. Recall or short term memory is like an echo of the immediate information gained, example a phone number or a message, allows recall for seconds only and is very limited without rehearsal capacity. Traditionally the explicit memory is assessed by NP tests, this part of the memory is available to the consciousness and can be actively brought to mind. It can be divided into memory for facts independent of context, like history, definitions and theories – semantic memory, and memory for events that are related to both time and space, the recollection of “what, where and when” of experiences we have had – so called episodic memory. The other part of long term memory, implicit memory, is not as available for testing, it is more procedural and no- conscious, like to remember how to ride a bike [99].

In NP research that includes individuals with LNB, the focus has mainly been on explicit memory, especially episodic memory. The tests of episodic memory often include free recall, cued recall and recognition of a list of e.g. words. Free recall is when a person has to remember a word list previously presented and recall as many words as he can. Cued recall is

tested when a hint, or a "cue word" is given that will remind the person what kind of words he has to remember, like "how many vegetables do you remember from the list presented". Recognition is tested when a person has to point out the words or pictures form a previously presented list among other words or pictures that were not presented in the first list (based on familiarity).

The Californian Verbal Learning Test (CVLT) is a test of episodic memory, widely used. It was the first clinical instrument that quantified multiple components of learning and memory and incorporated the principles from years of cognitive science. It tests verbal memory and verbal learning. It has also been used in many studies of outcome after LNB. The Digit symbol test is primarily a test of processing speed, but also includes assessment of incidental visual learning and visual memory.

Another much used memory test, the Wechsler Memory Scale (WMS), is also available in Norwegian. This test assesses both visual and verbal memory, but is much more time and effort demanding than CVLT-II.

The four neuropsychological tests we have used in our study (Californian Verbal Learning Test, Digit Symbol, Stroop and Trail Making Test) are presented in more detail in the method part of this thesis.

1.2.8 Studies of neuropsychological functioning after Lyme disease and Lyme neuroborreliosis

There are several studies on cognition after Lyme disease conducted during the last decades. A review from 2000 covers the controlled studies in which standardized NP tests has been used, starting with the Krupp et al. study from 1991, and ending with the two studies by Pollina et al. from 1999 [100]. Again, the lack of European controlled studies on long term outcome after LD is striking. The Benke et al. study from 1997 is the only European controlled study with standardized NP tests that we are aware of [101]. Several US studies have included an IQ test or an IQ estimation [41;61;90;92;102;103]. In addition to the studies on cognition in LD from 1991 to 2008 included in Table 07, three US treatment studies using cognitive function as primary or secondary endpoints are listed in Table 08. The table includes information about NP tests used, IQ assessments and premorbid IQ estimations.

Table 07. Studies including Neuropsychological (NP) testing in Lyme Neuroborreliosis (LNB) (except treatment studies, for these see Table 08)

Study	Material	Design	Assessments	Main results
[90] Krupp et al. 1991 New York, USA	15 LD patients 6.7 months after treatment with persisting complains 10 Healthy controls	Retrospective Matched controls	TMT WAIS-R (IQ estimate) Digit symbol COWA WMS-R SRT	LD treated patients scored worse on verbal fluency and memory tests, had more depression, with inverse effect on NP performance
[102] Kaplan et al. 1992 USA	20 LNB patients years after treatment with memory and mood problems 11 depressed controls 11fibromyalgia controls	Retrospective Controlled	CVLT WAIS-R(IQ-estimate) WMS	LNB treated patients were more depressed, but NP deficits cannot be explained solely by this. LNB patients had deficits in delayed recall, visual reproduction and associated learning. (Long-delay free recall, trial 5 on CVLT, no association to depression, or anxiety)
[61] Shadick et al. 1994 Ipswich, USA	38 LD patients 6.2 years after treatment 43 Healthy controls	Retrospective controlled cohort study	TMT Stroop Pegboard CVLT WMS IQ estimate (Shipley Abstraction)	LD treated patients scored worse on CVLT (Long delay recall) LD treated patients scored worse on tests assessing executive functioning and processing speed.Correlated with delayed treatment , more anti-*Bb* antibodies
[101] Benke et al. 1995 Austria	20 LNB patients 51 months after treatment (not all received treatment) 20 controls with other neurological problems without brain damage	Cohort study Matched controls	CVLT WAIS (Block design) COWA Reaction time Ravens progressive matrices	LNB treated patients scored lower on tests assessing learning, verbal memory, mental flexibility, verbal associative function and articulation. CVLT list B caused significant cued and free recall problems. Unimpaired: Intellect, psychomotor tempo, sustained attention
[91] Bujak et al. 1996 USA	23 LNB patients 5.6 years after treatment 23 patients who recovered from LD	Retrospective Matched controls	TMT CVLT WMS-R	LNB treated patients scored lower on tests assessing attention/ concentration/ and verbal and visual memory. More verbal than visual memory deficits
[92] Ravdin et al. 1996 USA	21 LD patients 15 months after treatment 21 controls with osteomyelitis 21 Healthy controls	Prospektive Controlled	CVLT IQ estimate (National Adult Reading test)	LD treated patients had more verbal memory and learning deficits. They had as much memory problems as the osteomyelitis controls. (Long delay free recall on CVLT, no association to depression or subjective complains)
[93] Gaudino et al. 1997 USA	38 LNB treated patients with fatigue 25 Chronic Fatigue Syndrome controls	Retrospective Controlled	WAIS-R (Digit Symbol) TMT COWA	LNB treated patients scored worse on the Digit Symbol, verbal learning, verbal fluency and fine motor speed

	56 Healthy controls		SRT WMS-R Benton Visual Retention test	
[94] Pollina et al. 1999 USA	16 LD treated patients 15 Healthy controls	Retrospective Controlled	Computer RT task Selective reminding test IQ estimate(Shipley Vocabulary test)	LD treated patients scored lower on tests assessing processing speed and memory .More depression and fatigue, but these factors did not have any impact on NP performance
[103] Pollina et al. 1999 USA	25 LD treated patients with persisting complains 23 Healthy controls	Retrospective Controlled	Computer RT PASAT TMT Digit symbol IQ estimate (ShipleyVocabulary test)	LD treated patients group had cognitive slowing independent of age, premorbid abilities, motor skill, and degree of depression
[104] Svetina et al. 1999 USA	44 LD patient at" second stage" 43 Healthy controls	Retrospectiv Controlled	COWA Boston naming test CVLT	LD treated patient claimed to have naming problems, and a subtle naming deficit was found secondary to memory retrieval deficit
[105] Kaplan et al. 1999 USA	14 patients with Lyme encephalopathy (objective infection) 18 PLDS patients 15 Healthy controls	Controlled matched	Gordon diagnostic system, SRT TMT Digit symbol	Both LD patient groups reported memory problems, Lyme encephalopathy group scored lower on memory test. PLDS group scored lower on attention and psychomotoric speed tests. Depression did not correlate to memory scores
[41] Kalish et al. 2001 Lyme, Connecticut, USA	84 LD patients 20-30 years after treatment (25 Erythema Migrans, 31 Facial palsy, 28 arthritis) 30 neighbors	Retrospective Controlled	CVLT COWA Digit symbol IQ estimation (Shipley Hartford Institute of Living Scale)	No significant differences on NP performances between the 4 groups
[106] Keilp et al. 2005 USA	81 LD patients with cognitive problems 7 years after treatment 39 Healthy controls	Retrospective Controlled	WAIS-III WMS-III	LD treated patients scored worse on memory and processing speed, but had normal global processing speed scores. No association to depression/ prolonged treatment/ delayed treatment found
[107] McAuliffe et al. 2008 USA	25 LD adolescents 24 months after treatment 25 Healthy controls	Retrospective Matched controls	WASI WRAML-II DKEFS	LD adolescents did score lower in tests assessing short term visual memory, short term and delayed verbal memory, recognition memory. They did have school problems

IQ; Intelligence Quotient, PASAT; Paced Auditory Serial Addition Test, TMT; Trail Making Test, WAIS; Wechsler Adult Intelligence, COWA; Controlled Oral Word Association SRT; Selective Reminding test, CVLT; California Verbal Learning test, WMS ;Wechsler Memory Scale, WASI;Wechsler Abbreviated Scale of

Intelligence, WRAML; Wide Range Test of Memory and Learning, D-KEFS; Delish-Kaplan Executive Functional System

The studies of cognition in LNB have different inclusion criteria and designs, and various tests have been used to assess cognitive function, so comparisons of the studies are not easy. The main impression is though, that some patients have cognitive functioning problems after LNB, even years after treatment. The studies have not revealed a specific cognitive deficit profile, but processing speed, memory and executive functions seem to be involved. There are until now no imaging studies that have shown parenchymal (CNS nerve tissue) changes that are specific for LNB [47] and repetitive and prolonged antibiotic treatment has not shown to improve cognitive function [64;66;67;108].

1.2.9 Pathophysiology of remaining complaints after Lyme neuroborreliosis

The pathophysiological explanation for remaining subjective and objective complaints after LNB is not known, but different theories have been discussed:

Slower response to therapy or incomplete resolution due to irreversible tissue damage:

Some patients have objective residual deficits like a partial facial palsy and/or subjective symptoms that may be due to slow resolution of the inflammatory process or irreversible tissue damage [41;52;109]. The study of Ljøstad et al. showed that patients with a higher CSF cell count and a higher CSF protein level had more complaints one year after treatment. It is possible that a more pronounced inflammatory process in these patients reflects a more aggressive infection that again causes more residual tissue damage [55].

Autoimmunity

Steere et al. have found that a few patients develop antibiotic-refractory Lyme arthritis after treatment that is most likely due to an autoimmune process triggered by the infection [110]. It has been discussed if an autoimmune process years after treated LNB can contribute to persisting complaints. This is not confirmed, but cross reactions of *Bb* specific antibodies with neuronal antigens are demonstrated [111] and a case report of autoimmune-mediated polyneuropathy in a LNB treated patient that responded well to immunoglobulin therapy is published by Rupprecht et al [112].

Endocrine dysfunction

Tuberculosis meningitis and various other infectious diseases can affect the endocrine system and cause dysfunction of the hypothalamus and/or the pituitary gland. A recent Swedish study evaluated hormone levels in previously treated LNB patients with and without persistent symptoms and compared the results to a healthy control group. They did not find any

corticotrophic insufficiency or other serious hormonal dysfunction associated with PLDS [113].

Ongoing infection

In the treatment and diagnostic guidelines from Wormser et al. arguments for and against an ongoing infection in PLDS patients are systematically evaluated [20]. The authors conclude that "There is no convincing biologic evidence for the existence of symptomatic chronic *Bb* infection among patients after they have received recommended treatment regimens for LD. Antibiotic therapy has not proven to be useful in PLDS, and is not recommended for patients with chronic (⩾6 months) subjective symptoms after recommended treatment regimens for Lyme disease".

Arguments against ongoing infection in these patients are that the *Bb* has not demonstrated antibiotic resistance, and there is no correlation between laboratory findings of inflammation and physical signs of disease in the treated LNB patients [20]. *Bb* can form cysts in vitro [114], but this has not been shown in vivo, and it is not considered to have any clinical significance [20]. Further it has not been proved that prolonged and repeated treatment courses have resulted in longer standing improvement of PLDS symptoms (see Table 08) [64;66;67;108].

Table 08. Treatment studies of Post Lyme Disease Syndrome (PLDS)

Study	Material	Design	Assessments	Result
[64] Klempner et al. 2001 [108] Kaplan et al. 2003 USA	78 seropositive LD patients with persistent symptoms 51 seronegative LD patients with persistent symptoms	Controlled treatment trial IV ceftriaxone 30 days, doxycycline 60 days /Placebo IV and oral	2001:Quality of life (SF-36) 2003:neurocognition (Symbol digit, calCAP, AVLT, BVRT,COWA)	No significant differences.
[66] Krupp et al. 2003 Long Island, USA	55 LD patients with persistent severe fatigue	Randomized, controlled treatment trial IV ceftriaxone/ placebo for 28 days	Fatigue (FSS) and cognitive function (WAIS, PASAT, TMT) at 6 months	No improvement in cognition. Temporary release fatigue. To severe AEs, treatment not recommended
[67] Fallon et al. 2008 New York, USA	37 treated LNB patients with cognitive impairment 20 matched controls	Controlled randomized treatment study 10 weeks IV ceftriaxone vs. placebo	Quality of Life (SF-36), fatigue (FSS) and neurocognition (TMT, Digit symbol, Stroop, COWA, Buschke Selective Reminding Test, Benton Visual Retention Test)	A improvement in cognition after 12 weeks was not present at 24 weeks Transient improvement of fatigue.

Short form-36;SF-36, CalCAP; California Computes Assessment Package, AVLT; Rey Auditory Verbal Learning Test, BVRT; Benton Visual Retention Test, COWA; Controlled Oral Word Association Test, WAIS; Wechsler Adult Intelligence Scale, PASAT; Paced Auditory Serial Addition Test, TMT; Trail Making Test, FSS; Fatigue Severity Scale, AE; Adverse Event

In 2011 a US study of 158 patients with NP symptoms for at least 3 months and positive testing for *Bb* previously received a extended antibiotic therapy (intravenous ceftriaxone), and reported improvement of cognition, arthralgias, myalgias and fatigue. This study did not include a control group, and did not demand intrathecal production of anti-*Bb* antibodies to prove the diagnosis of LNB. The outcome was assessed with a self-designed patient self-

administration symptom score with 28 separate questions about the severity of symptoms. They did not use standardized questionnaire for the assessment of fatigue, and they did not assess NP functioning. The authors recommended treatment duration for 25-52 weeks for LNB with cognitive symptoms, but we think this study conclusion must be considered with care because of all the methodological weaknesses mentioned [115].

Co-infections with other tick borne microorganisms like Babesia, TBE or Anaplasma

The possibility of a more severe disease if the patient has been infected with more than one tick borne pathogen is discussed, but until now studies have not confirmed this [64;67;116;117]. Symptomatic *Anaplasma* infections in humans appear to be rare in Norway; only two cases are documented, but a serological analysis of blood samples from *Bb* positive patients in Norway have shown that 10% are positive to both *Bb* and *Anaplasma* [118;119]. Between 1998 and December 2011 54 cases of tick borne encephalitis and no cases of human babesiosis are documented in Norway, but we have little knowledge about co-infections *(MSIS 20.12.2011*).

Psychiatric explanations

It is known that infection with *Bb* may cause psychiatric symptoms [120]. It is also discussed if psychological factors and psychiatric comorbidity might be an underlying explanation of PLDS. Solomon et al. found that psychiatric impairment before an episode of LNB could lead to more chronic problems after treated LNB [121]. Hajec et al. screened psychiatric patients for anti-*Bb* antibodies and found a higher proportion of seropositive individuals among psychiatric patients than in a control group, but found no association between seropositivity and specific psychiatric diagnostic categories [122]. Hassett et al. found that patients suspected to have “chronic LD” had more psychiatric comorbidity as compared to healthy controls, and controls with another chronic disorder. Forty-five per cent of the chronic LD patients had a depression, and 25% of them had anxiety, but they did not find psychiatric comorbidity in all “chronic LD” patients. Therefore they concluded that the symptoms associated with “chronic LD” in this patient group could not be described as a purely psychiatric condition [123].

In summary studies of long term outcome after LNB are mainly conducted in the US, and the studies are not easy to compare to each other mainly because of different inclusion criteria. The studies have found reduced HRQoL, fatigue and various cognitive deficits in LNB patients, but the pathophysiology of this non-favorable outcome is not known. Repeated and prolonged antibiotic treatment does not seem to help. In Europe there is a lack of controlled

studies on long term outcome after LNB. This is problematic as the results of the US studies are not necessarily transferable to European patients, because the *Bb* genotype and the clinical human LD phenotype in the US differ somewhat from what we find in Europe.

2. Aims of the study

The aim of this thesis was to investigate the long term outcome in European LNB treated patients regarding HRQoL, NP functioning and fatigue. We also wanted to identify possible risk factors for a non-favorable long term outcome in LNB treated patient.

Study hypotheses

Before study start we hypothesized that, compared to controls, patients treated for European LNB 30 months earlier had:

- Reduced HRQoL
- More cognitive deficits
- More fatigue

We also hypothesized that

- A non-favorable outcome after treated LNB could be predicted by factors like severity of the disease, time to treatment, comorbidity, gender, and sings of CNS infection involvement before treatment.

These hypotheses were tested in paper I-III as follows:

Aim of paper I: To assess HRQoL in well characterized adult European LNB patients 30 months after treatment in a case-control designed study.
Aim of paper II: To compare NP functioning in a group of well characterized adult European LNB patients 30 months after treatment to a matched control group.
Aim of paper III: To identify clinical, demographic or laboratory risk factors associated with a reduced HRQoL and fatigue 30 months after treatment in a cohort of well caracterized adult European LNB patients.

3. Material and methods

3.1 Study design

Paper I and II: Hospital based, age, gender and education level matched case-control follow-up study 30 months after treatment.

Paper III: Cohort study from time of diagnosis and treatment to follow-up at 4, 12 and 30 months.

3.2. Study population

Patients

From 2004 to 2008 we included 102 consecutive adult patients diagnosed with LNB in a randomized multicenter treatment trial conducted in the coastal areas of southern Norway. Out of these, 64 patients were recruited from two of the nine participating hospitals, covering the high endemic region of the Agder Counties. The 57 patients of the 64 patients who were included in the treatment trial between May 2004 and December 2007 were invited to the current follow-up study 30 (27-34) months after treatment. The inclusion criterion was definite or possible LNB according to criteria shown in Table 09.

Table 09. Diagnostic criteria used for inclusion of Lyme neuroborreliosis (LNB) patients in the treatment and follow-up study

Definite Lyme neuroborreliosis All three criteria fulfilled	**Possible Lyme neuroborreliosis** Criteria 1 and one or more of a- d
1. Neurological symptoms suggestive of LNB without other obvious reasons **2.** Lymphocytic pleocytosis (> 5 leucocytes/mm^3) **3.** Intrathecal anti-Borrelia antibody production	**1.** Neurological symptoms suggestive of LNB without other obvious reasons **a.** Lymphocytic pleocytosis (> 5 leucocytes/mm^3) **b.** Intrathecal anti-Borrelia antibody production **c.** Anti-Borrelia antibodies in serum **d.** Verified erythema migrans during the past four months

These diagnostic criteria comply with the diagnostic criteria from the EFNS guidelines (see Table 03 in background part of thesis) [25]. Thirty-four patients (68%) had definite and 16 (32%) patients had possible LNB according to these criteria.
Participants were invited by letter, and non-responders were phoned once. Fifty patients consented to participate and were included and analyzed.

Non-participants: Out of practical and logistically considerations we did not include all the participants from the treatment trial. Neither the patients from the treatment trial who were not recruited to the follow-up study, nor the seven patients who were invited but not included, differed from the 50 included patients regarding treatment or severity of the disease before and 4 months after treatment. The seven invited non-participants were in the mean 5 years older than the included patients.

The patient' pre-treatment characteristics are presented in Table 10.

Table 10. Lyme neuroborreliosis patient's characteristics before treatment

Mean age (years)	53 (SD=13) (range 18-77)
Gender (male)	29 (58%)
Coexsisting diseases	25 (50%)
Psychological diseases, past or present	10 (20%)
Tick bite past year	30 (60%)
Erythema migrans last year	11 (22%)
Neuroborreliosis diagnosis definite possible	 34 (68%) 16 (32%)
Pre-treatment Symptom duration (weeks)	9.2 (SD=19) (range 1-104)
Symptom duration> 6 weeks	10 (20%)
Symptom duration >6 months	4 (8%)
Mean CSF cell-count cells/mm3 (normal ≤5)	187 (SD=255) (range 0-1176)
Mean CSF protein level g/L (normal 1,5-5,0)	1.22 (SD=0.73) (range 0.3-2.8)
Mean clinical score (range 0-64)	8.3 (SD=4.3) (range 3-21)
Mean subjective score (range 0-12)	4.8 (SD=2.6) (range 1-11)
Mean objective findings	3.5 (SD=2.5) (range 0-11)
Bannwart syndrome	40 (80%)
Radiculitis	20 (40%)
Facial palsy unilateral bilateral	 7 (14%) 1 (2%)
Other cranial neuropathies	4 (8%)
Plexopathy	1 (2%)
Cerebral symptoms	4 (8%)
Ataxia	2 (4%)
Myelopathy	1 (2%)
Pareses in extremities	8 (16%)
Subjective complaints alone	5 (10%)
Treatment option doxycycline ceftriaxone	 26 (52%) 24 (48%)

Controls

Each LNB treated patient invited a control person at the time of follow-up, matched for age, education level and gender. If a patient failed to bring a control person, another patient was asked to bring two. The only exclusion criterion for the controls was previously

acknowledged LNB. Serological testing of the controls was not done as 15-20% of the inhabitants of the Agder Counties are known to have anti-*Bb* antibodies without any history of LNB [14].

Patient and control characteristics at follow up are shown in Table 11.

Table 11. Lyme neuroborreliosis treated patients and controls characteristics at follow-up 30 months after treatment

	LNB treated patiens (n=50)	**Controls (n=50)**
Age years (range)	55 (21-76)	56 (20-78)
Gender male	29 (58)	29 (58)
Married/partner yes	44 (88)	44 (88)
Employed	30 (60)	34 (68)
Secondary education 0-3 years	25 (50)	23 (46)
Secondary education 4-7 years	15 (30)	13(26)
Secondary education ≥7 years	10 (20)	14 (28)
Coexisting somatic diseases		
Total n (%)	25 (50)*	29 (58)*
Multiple sclerosis	1 (2)	0
Parkinson's disease	1 (2)	0
Diabetes mellitus	5 (10)	3 (6)
Atherosclerosis	4 (8)	2 (4)
Cancer	4 (8)	1 (2)
Disabling headache	0	1 (2)
Tinnitus	0	1 (2)
Head trauma	0	1 (2)
Hypertension	5 (10)	2 (4)
Asthma/COLD**	3 (6)	5 (10)
Fibromyalgia	2 (4)	2 (4)
Post-polio	1 (2)	1 (2)
Muscle pain/ischialgia	4 (8)	10 (20)
Previous/present psychiatric diseases	10 (20)	8 (20)

Data are number of patients (%), unless otherwise stated.*some participants had more than one disease**.** **
COLD = chronic obstructive lung disease

One patient had Multiple Sclerosis and one Parkinson's disease. In those patients the neurological findings attributed to LNB were new and accompanied by typical laboratory findings, and the criteria for definite LNB were fulfilled. The cancer diseases observed in both groups were treated and cured 10-20 years earlier except for in two patients, one who had a stable frontal astocytoma, and one patient who was diagnosed with an advanced cancer prostate between the treatment for LNB and follow-up.

3.3 Clinical and laboratory variables

Demographic, laboratory and clinical data was collected pre-treatment and at 4, 12 and 30 months after treatment (see figure 02).

Figure 02. Time axis "European neuroborreliosis. Long term follow up" study

Diagnosis and treatment	**X**			
Interview	X	X	X	X
Neurological examination	X	X	X	X
Spinal and blood tap	X	X (n=42)	X (n= 33)	X (n=29)
SF- 36				X
FSS,MADRS SAS, MMS				X
NP testing				X

SF-36: Short Form-36, FSS: Fatigue Severity Scale, MADRS : Montgomery and Åsberg Depression Rating Scale, SAS: Starkstein Apathy Scale, MMS: Mini Mental State, NP: Neuropsyhological

A composite clinical score was used to assess subjective complaints and clinical neurological findings, (range 0-64, 0= no complaints or findings) [39]. A clinical score at 30 months of ≤ 1 was regarded as complete recovery, and >1 as non-complete recovery.The clinical score data were normally distributed (see table 12).

Table 12 Composite clinical score in Lyme neuroborreliosis patients

Clinical score in LNB patients.	
Subjective complaints (maximum score = 12)	**Objective findings (maximum score = 52)**
Malaise Fatigue Pain Memory problems Concentration difficulties Paresthesia	Facial palsy * Reduced hearing* Vision loss l/r Reduced sensibility in face* Paresis of eye muscles* Other cranial neuropathies Tremor Ataxia Nuchal rigidity Confusion Other CNS findings Radiculopathy truncus * Radiculopathy arm* Radiculopathy leg* Paresis arm* Paresis leg*

Clinical outcome score is the sum of subjective and objective findings. Each item is scored 0 = no, 1 = mild, 2 = severe (maximum total score 64). * Left and right sides scored separately

All participants were asked the following at the 30 month follow up: “Do you have any other diseases?” ” Do you have, or have you previously had, psychological distress that made it necessary to use pharmacological therapy, consult a therapist or take sick leave?” ” Is fatigue an every-day problem for you?” “Did you recover completely from LNB?” We reviewed individual hospital records to confirm the patients past medical history.

To assess depression we used the Montgomery and Åsberg Depression Rating Scale (MADRS range 0-60. Scores 0-9 indicate no depression, scores 10 -19 indicate mild depression that does not need treatment, scores 20-34 indicate moderate depression that needs to be treated and scores > 35 indicate severe depression) [124]. This is a frequently used, reliable and validated questionnaire available in the Norwegian language.

To assess apathy we used the Starkstein Apathy Scale (SAS range 0-42 0= best, ≥ 14= significant apathy) which is translated into Norwegian [125].

To grade the cognitive functioning we used the Mini Mental State (MMS range 0-30, 30=no problems) [126] which is a reliable and validated test translated into Norwegian.

Laboratory variables

The patients underwent a lumbar puncture pre-treatment (n=50), at 4 months (n=42), at 12 months (n=33) and at 30 months (n=29) (see Figure 02). We analyzed cell-count, protein level, anti-*Bb* antibodies, oligoclonal bands, and CXCL-13 level in the CSF. Anti-*Bb* IgM and IgG antibodies in serum and anti-*Bb* IgG antibodies in CSF were first analyzed by an Enzyme-Linked Immunosorbent Assay (ELISA) test using *Bb* (PKo strain) as antigen, from November 2008 also including the VlsE antigen (Enzygnos Lyme link VIsE/IgG (Siemens, DADE Behring, Marburg, Germany).

CSF was examined for anti-*Bb* IgM antibodies and for intrathecal antibody production with the IDEIA *Bb* IgM and IDEIA Lyme Neuroborreliosis kit (DakoCytomation, Cambridgeshire, UK) using purified native flagella from the *B. Afzelii* DK1 strain as antigen. In these assays corrections for impairment of blood-brain barrier is unnecessary [44], and intrathecal production of antibodies is detected when, according to the manufacturers` recommendation, the antibody index of: OD optic density) CSF /OD serum x (OD CSF -OD serum) is > 0.3. For the CSF CXCL-13 detection we used an ELISA test (Quantikine, R&D Systems, Minneapolis, US). Based on the findings in a previously published study concentrations < 4 pg/ml were stated as not detectable (0 pg/ml) [46]. The patients were also tested for *Tick Borne Encephalitis (TBE)* virus antibodies. We did not test for *Anaplasma*, but none of the patients had a clinical picture or blood count suggestive of Anaplasma infection. All anti-*Bb* antibody negative patients were tested for *Herpes simplex, Varicella Zoster* and *entero-virus* by Polymerase Chain reaction (PCR) of CSF.

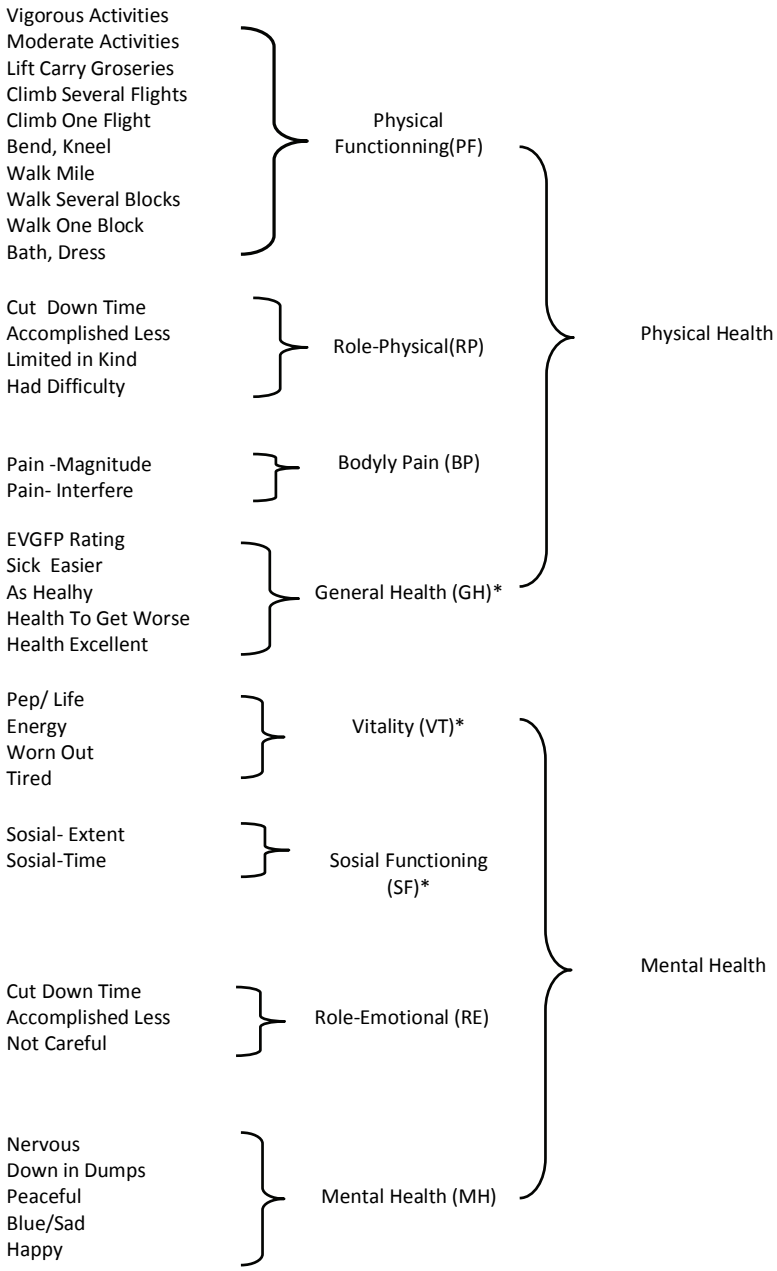
3.4 Outcome measures

HRQoL

At inclusion in the follow-up study all participants fulfilled the Norwegian translation of the HRQoL questionnaire SF-36 version 2, a short generic, 36 item questionnaire which measures eight multi-item dimensions covering functional status, well-being and over-all evaluation of health. The SF-36 is reliable, valid, and the most widely used questionnaire for assessing HRQoL in both neurological and other diseases. Version 2 relates its questions to the last four weeks. There are Norwegian normative data available [127]. Our primary outcomes were

mean scores in the two SF-36 summary measures; Physical Component Summary (PCS) and Mental Component Summary (MCS) scores. PCS is summarized from the four physical health dimensions; physical functioning (PF), role physical (RP), bodily pain (BP) and general health (GH), and MCS is summarized from the four mental health dimensions; vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH) (se Figure 03). The sumscores PCS and MCS are calculated from the 8 dimensions and related to normative data, in this study the Norwegian Normative data. The score range is 0 –100, with 100 as the best possible score [128].

Figure 03 Sort Form-36 Measurement model (adapted from Ware JE.SF-36 Health Survey Update 2000 Spine volume 25 3130-3139) [128]


Vigorous Activities
Moderate Activities
Lift Carry Groseries
Climb Several Flights
Climb One Flight
Bend, Kneel
Walk Mile
Walk Several Blocks
Walk One Block
Bath, Dress
Physical Functionning(PF)
Cut Down Time
Accomplished Less
Limited in Kind
Had Difficulty
Role-Physical(RP)
Pain -Magnitude
Pain- Interfere
Bodyly Pain (BP)
EVGFP Rating
Sick Easier
As Healhy
Health To Get Worse
Health Excellent
General Health (GH)*
Physical Health
Pep/ Life
Energy
Worn Out
Tired
Vitality (VT)*
Sosial- Extent
Sosial-Time
Sosial Functioning (SF)*
Cut Down Time
Accomplished Less
Not Careful
Role-Emotional (RE)
Nervous
Down in Dumps
Peaceful
Blue/Sad
Happy
Mental Health (MH)
Mental Health

It was not possible to calculate PCS in one control and one patient and MCS in one patient because of missing data on three of the SF-36 subscale dimensions. We decided not to impute data because we were able to use 48 full sumscores in patients and controls.

Secondary outcomes were the scores on the 8 dimensions of the SF-36. Score differences in the 8 dimension groups of 5-10 points can be regarded as modest and 10-20 as moderate changes [129].

Fatigue

The Fatigue Severity Scale (FSS) is a much used brief and simple self-assessment questionnaire which is translated into Norwegian, has satisfactory internal consistency, and is sensitive to clinically significant changes [76;130]. Each of the nine statements are rated on a scale from 1 (strong disagreement) to 7 (strong agreement) (see table 13). The individual FSS score is the mean of the scores on the 9 statements, ranging from 0-7 (0 =no problems) and assesses the effect of fatigue on activities of daily living. We defined a score $\geq$ 4 as significant fatigue and a score $\geq$5 as severe fatigue.

Table 13. Fatigue Severity Scale (FSS), Norwegian translation

FSS							
Siste uke har jeg følt at	**Skår**						
1. Mitt pågangsmot blir dårligere når jeg er utmattet	1	2	3	4	5	6	7
2. Jeg blir fort utmattet ved anstrengelser	1	2	3	4	5	6	7
3. Jeg har lett for å bli utmattet	1	2	3	4	5	6	7
4. Utmattelse nedsetter min fysiske funksjonsevne	1	2	3	4	5	6	7
5. Utmattelse skaper ofte problemer for meg	1	2	3	4	5	6	7
6. Utmattelse fører til at jeg har dårlig fysisk utholdenhet over lengre tid	1	2	3	4	5	6	7
7. Utmattelse virker negativt inn på mine gjøremål og forpliktelser	1	2	3	4	5	6	7
8. Utmattelse er ett av mine tre mest plagsomme symptomer	1	2	3	4	5	6	7
9. Utmattelse virker negativt inn på mitt arbeid, min familie og mitt øvrige sosiale liv	1	2	3	4	5	6	7

NP functioning

Cognitive assessment in patients and controls was carried out by one experienced neurologist (RE). All participants were informed that the NP tests assessed different aspects of cognition, like memory and attention, and they were encouraged to perform their best in every test. A trained neuropsychologist blinded to group adherence scored the NP tests. The NP tests were administered in a fixed order, but short breaks were permitted if needed. The NP testing lasted between 1.5 and 2.5 hours and was administrated between 09 and 14 all in the same day. The neurological examination and the lumbar punctures were done after the NP testing.
The NP test selection was based on previous relevant studies [61;106;108]. Tests that had been translated into Norwegian, had previously been used on a Norwegian population, and known to be reliable and validated were included (Stroop, TMT, Digit symbol and CVLT).

Attention/Executive functions

The trail making Test (TMT 1-4) assesses attention and flexibility in solving problems in visual-motor tasks. The first condition is visual scanning that requires the participant to cross out a number among an array of letters and numbers. In the second condition numbers must be connected in rising order. The TMT 3 requires the participant to connect letters in alphabetical order. The fourth condition is the primary attention/executive function task; the participant must switch between two sets of rules by connecting numbers and letters in correct order. Raw scores are the number of seconds taken to complete each task [99].

TMT 1-4 instructions:
TMT 1: Tick a specific number among an array of letters and numbers.
TMT 2: Connect numbers in rising order.
TMT 3: Connect letters in alphabetical order.
TMT 4: Connect numbers and letters in correct order.

The color-word interference, task 1-4, is an adapted version of the Stroop test that assesses the ability to inhibit a prepotent reaction (impulse control), and the ability to switch between rules. The first condition requires the participant to name colors, and in the second condition the task is to read color names. In the third condition the participant is required to inhibit the reading and focus on the ink color in which the word is written. In the last condition the participant is asked to name the ink color on all words except those that are placed within a square, those words must be read. The last two conditions are the primary attention/executive tests and require both the inhibition of reading and the ability to switch between rules. Raw scores are the time (seconds) used to complete the tasks [99].

Stroop1-4 instructions:
Stroop 1: Name colors.
Stroop 2: Read color names.
Stroop 3: Avoid reading the word and instead name the ink color in which the word is written.
Stroop 4: Name the ink color on all words, except those placed within a square, which should be read.

Processing speed

The Digit Symbol task is part of the Wechsler Adult Intelligence scale (WAIS-III), and measures processing speed. It requires the participant to copy symbols paired with numbers during a 120 seconds interval. Raw scores are the number of correctly copied symbols [99].

Digit symbol test instructions:
Copy symbols paired with numbers.

The fifth condition of the TMT (TMT 5) measures motor speed by asking the participant to draw a line as fast as possible. Raw score is the time (seconds) used to complete the task [99].

TMT 5 instructions:
Draw a line between dots as fast as possible.

Memory assessment

Visual memory and incidental learning was assessed by using the Digit symbol test free and cued recall and recognition. First, a row of symbols has to be combined with the numbers just copied in the first condition of this test. Recall condition was included by asking the participant to write down all of the symbols they could remember.

Digit symbol cued and free recall instructions:
Digit symbol cued recall test: Combine symbols and numbers recalled from the digit symbol test.
Digit symbol free recall test: Write down all symbols recalled from the digit symbol test.

The California Verbal Learning Test (CVLT) version II assesses verbal learning, short and long term memory, as well as recognition, through oral presentation of word lists that are to be learned during 5 repetitions. A second list is presented as a distracter once, and the participant has to recall the original list, in free and cued manner (short recall). After a 20 minute brake, the list must be recalled again, and at the end there is a recognition test (long recall). Raw score is the number of words recalled [131].
The learning effect in CVLT is represented by the repetition of the word list 5 times, and the test person is supposed to remember more words the last time as he did the first time.

CVLT instructions:
CVLT 1-5: Recall the list of words
CVLT list B: Recall a second list presented once
CVLT free and cued short recall: Recall the original list
CVLT free and cued long recall: Recall the original list after the break.

A problem to consider in every test situation is the level of individual effort. A person tested can be tempted to perform sub-optimally because of potential personal benefits, or to "please" the researcher. The optional trail of the CVLT-II, long delay forced-choice recognition, was included to examine the degree of individual effort:

CVLT-II, long delay forced-choice recognition instruction:
Pick the word from the original list among two different words presented immediately after the Long delay test.

The global NP sumscore

The four NP test in our test panel consisted of 23 subtasks, and we calculated a global NP sumscore expressing the number of NP subtasks with scores of -1 SD or more from the mean in the control group (range 0-23).

A challenge connected with the use of NP functioning assessments is that statistically lower results in one group compared to another does not necessarily have clinical consequences. To grade the severity of the results we categorized the global NP sumscores into three groups (see table 14).

Table 14 Neuropsychological tests sumscore, degree of severity

Normal: 1-5 (≤ -1 SD from the mean sumscore in the control group)
Deficit: 6-8 (>1-≤2 -SD from the mean sumscore in the control group)
Impairment: 9-23 (>2- SD from the mean sumscore in the control group).

Normal: Scores between mean and -1SD may also have an impact on a person's daily functioning, but to assess this, an individual evaluation is necessary.

Deficit: This implies that the person is not able to perform the task at hand at the level expected, and a deficit will often have consequences for daily life activities, for example at work/ school.

Impairment: The test person has major problems in performing the test/task at hand, or is not able to perform the task at all. NP impairment will cause functional problems in everyday life.

Missing data were dealt with by imputing the mean score from the respective patient or control group. If the scores were missing due to inability to perform the test at hand, we imputed the lowest obtained score in the respective group. Four patients missed in average 2.5 of the 23 subtasks, and four controls missed on average 1.5 of the 23 subtasks.

3.5 Statistics

Sample Size

Based on the results from previous studies we decided in advance of study start that a group difference of 7% in the SF-36 PCS and MCS scores between the two groups would be of clinical interest to detect [64]. With a two-sided test and a significance level of $P< 0.05$, 36 participants were required in each group to reject a null hypothesis of no difference with 80% power. To compensate for drop-outs and non-evaluable tests and questionnaires, we planned to enroll 50 persons.

Statistics used in paper I-III

Paper I: The groups were compared by using student t-test (continuous data) and Chi-square test (categorical data). P-values <0.05 were regarded as statistically significant. The results are reported as mean scores with SD or proportions.

Paper II: Because of the matched one-to-one cases study design we used paired t-tests for normally distributed continuous data and Wilcoxon signed-rank test for paired continuous data that were not normally distributed, and McNemar test for paired categorical data. P <0.05 were regarded as statistically significant. The results are reported as mean raw scores with SD or proportions.

To present a profile of the test results based on a dimensionless quantity, we transformed the test results of each of the NP-23 subtest raw scores into a Z-score. The Z score represents the distance between the patients' raw score and the mean in the control group (see Table 15).

Table 15. Definition of the Z score

Z score: ($Z_i=(X_i-Z_{con})/SD_{con}$ where Z_i is the individual Z score of the i^{th} patient, X_i is the individuals test raw score, Z_{con} is the mean test result of the control group and SD_{con} is the mean SD of the control group.)

The global NP sumscore (see above) was correlated to other findings by using Pearson's correlation coefficient for continuous data and Mann-Whitney test for categorical data.

Paper III: First we did univariate analyses for possible predictors using Pearson's correlation for continuous variables and student t-test for comparing means. Education level was split into 3 levels and analyzed with Analysis of Variance (ANOVA). To be able to do a simultaneous assessment of multiple potential risk factors, all variables with $P \leq 0.150$ were included stepwise in a multiple linear regression model, one model for each of the three outcomes, PCS, MCS and FSS. Because of missing data the CSF variables were analyzed separately. The results are presented with B-coefficient and 95% Confidence Interval (CI). For clinical implementation of the models, we also presented the coefficient of determination, R^2, which indicates how much of the variance in the outcome that is explained by the final model.

3.6 Ethics

The participants in the study should be protected from physical and psychological harm. Lumbar punctions might theoretically cause harm, and the controls were not asked to go through a spinal tap. At our hospital normal procedures require an LNB patient to go through a lumbar punition before treatment initiation, and commonly a second spinal is done to evaluate the treatment effect after some time. The EFNS guidelines include the detection of intrathecally produced anti-*Bb* antibodies to diagnose definite LNB [25]. We regard the risk of causing harm to the patients as minimal, but if the patients declined to do go through spinal taps at the follow-up, this was of cause respected, and several patients did decline.

The NP function assessments might have caused a mild psychological stress to some of the participants. Breaks were allowed when necessary, and the investigator informed the patients about the possible stressing effects of the NP tests. There was time to discuss the tests before and after assessment. If the controls scored poor on the tests they were advised about further diagnostics and treatment. The patients` general physicians were informed about the testing, and received information about test results.

When the patients and controls were asked to participate in the study, they were informed about the purpose of the study and the right to withdraw their participation at any time. Oral and written information were given.

All participants gave written informed consent. The study was approved by the Regional Committee for Medical Research Ethics in Southern Norway, and by the Norwegian Data Inspectorate.

This trial is a follow-up study on the treatment trial registered with ClinicalTrials.gov number NCT00138801

4. Summary of Results

Paper I: European Neuroborreliosis: A controlled Quality of Life study 30 months after treatment.

We found that HRQoL assessed with the SF-36 was reduced in 50 LNB treated patients compared to 50 matched controls. The group differences were 14% in mean PCS score and 9% in mean MCS score (PCS 44 (SD=9) vs. 51 (SD=6) P< 0.001, MCS 49 (SD=11) vs. 54 SD=6), P=0.001), both well above the pre-specified lower limit of 7% . The LNB treated patients scored worse than the controls on all the sub-dimensions on the SF-36 except bodily pain.

More LNB treated patients than controls reported fatigue to be an every-day problem (25 patients (50%) vs. 8 controls (16%), P= 0.001). Out of the 50% of patients with subjectively reported fatigue 15 had FSS scores ≥4. The 16 LNB patients who reported severe fatigue (n=16) had higher FSS scores (mean=5.6 (SD=1.1) versus the LNB patients reporting a little fatigued (n=9) (mean 2.7 SD=0.9). This difference was significant (P<0.000). We found more patients than controls with significant fatigue (21 vs. 5 scored ≥ 4 on the FSS, P<0.001) and severe fatigue (13 vs. 2 scored ≥ 5 on the FSS, P=0.001). The two controls with severe fatigue had other conditions commonly associated with fatigue, post-polio syndrome and chronic disabling cluster headaches, respectively.

There was no difference between the groups in the MMS scores.

The mean MADRS scores differed between LNB treated patients and controls (3.1 (SD=4.9) vs. 0.8 (SD=1.8), P=0.003), but the scores were below cut-off for depression in both groups and we did not consider the difference to be of clinical interest. One of the patients reached a MADRS score of 21 indicating a moderate depression in need of treatment. This participant suffered from relapsing depressions years before she got an LNB infection. We found no difference in mean MADRS scores between the patients reporting non-complete recovery and patients reporting complete recovery at 30 months.

The mean SAS score also differed between the groups (13 (SD=4) vs. 11(SD=4), P=0.016), but the scores were below cut-off for clinically significant apathy in both groups and we did not consider this difference to be of clinical interest either. We found no differences between the proportion of LNB treated patients and the proportion of controls that scored above the cut-off for significant apathy of 14 on the SAS (18 vs. 17, P=0.301). The primary and secondary endpoints are presented in Table 16.

Table 16. Primary and secondary endpoints in paper I, Quality of Life after treated Lyme neuroborreliosis

Questionnaires	**LNB treated patients (n=50)**	**Controls (n=50)**	**P-value**
SF-36 sum scores:			
PCS Physical component	44(9)	51(6)	<0.001
MCS Mental component	49(11)	54 (6)	0.010
SF – 36 subscores*			
Physical functioning, mean	80 (17)	90 (11)	0.001
Role physical	56 (43)	82 (31)	0.001
Role emotional	80 (34)	93 (17)	0.027
Bodily pain	66 (29)	75 (19)	0.116
General health	69 (21)	84 (14)	<0.001
Vitality	51 (22)	71 (14)	<0.001
Social functioning	84 (25)	95 (10)	0.005
Mental health	80 (15)	86 (11)	0.035
MADRS	3.1 (4.9)*	0.8 (1.8)*	0.003*
FSS	3.5 (17)	2.1 (10)	<0.001
MMS	28 (1.1)	29 (0.6)	0.106
SAS	13 (4)*	11 (4)*	0.016*
Reported subjective complaints	Number of patients (%)	Number of controls(%)	
Malaise	11 (22)	0	<0.001
Fatigue	25 (50)	8 (16)	0.001
Pain	16 (32)	21 (42)	0.408
Memory problems	23 (46)	5 (10)	<0.001
Concentration difficulties	17 (34)	4 (8)	0.003
Paresthesias	17 (32)	7 (14)	0.034

*The possible score range is 0-100. An increase in scores indicates an improvement in function.SF-36; Short Form-36, Health Related Quality of Life assessment questionnaire. SD; Standard Deviation, FSS ;Fatigue Severity Scale, MADRS; Montgomery and Åsberg Depression Rating Scale, SAS; Starkstein Apathy Scale; MMS ;Mini Mental State

Fourteen of our LNB treated patients had neurological findings. These findings are listed in Table 17.

Table 17. Number of Lyme neuroborreliosis patients with neurological findings 30 months after treatment. (N=50). Ten patients had more than one finding

Facial palsy	3
Reduced hearing	1
Reduced sensibility in the face	3
Anisocoria	1
Tremor	2
Ataxia	2
Nystagmus	2
Radiculopathy	6
Arm/leg paresis	4

Post-hoc and subgroup analyses

The 14 LNB treated patients 28% with neurological findings 30 months after treatment scored lower on PCS, but not on MCS as compared to those without persistent findings (PCS =38 (SD=8) vs. 47 (SD=9), P=0.003) MCS =48 (SD=12) vs. 53 (SD=10), P=0.2), and they had a higher FSS score (4.3 (SD=2.0) vs. 3.1 (SD=1.8), P=0.045).

The 22 LNB treated patients that reported persisting complaints (44%) had a lower mean PCS score (37 (SD=7) vs. 50 (SD=8), P=0.001), and FSS score (4.8 (SD=2) vs. 2.4 (SD=2) P< 0.001) but a comparable MCS score (47(SD=10) vs. 51 (SD=12),Pp=0.3) to the 28 (%) LNB treated patients reporting complete recovery.

The LNB treated patients who reported complete recovery had the same HRQoL as the controls in term of PCS, MCS and FSS scores.

There were no differences in mean PCS and MCS scores between patients with definite LNB (n= 34) and patients with possible LNB (n=16), (PCS 46 (SD=9) vs.43 (SD=12)P=0.5), MCS (50 (SD=12) vs. 52 (SD=11), P=0.4), or between patients treated with ceftriaxone and patients treated with doxycycline (PCS (45 (SD=11) vs. 44 (SD=9) P=0.6), MCS (48 (SD=13) vs. 52 (SD=10), P=0.2).

We found a correlation between FSS scores and PCS and MCS scores in the LNB treated patient group (PCS: Pearson's correlation coefficient -0.597 95% CI-0.753- -0.377, P<0.001, MCS: Pearson's correlation coefficient-0.468 95% CI -0.663- -0.213, P= 0.001), and in

controls (PCS: Pearson's correlation coefficient =-0.392 95% CI-0.608—0.122, P=0.005, MCS: Pearson's correlation coefficient-548, 95% CI-0.72—0.313 , P<0.001).

Paper II. European Neuroborreliosis: Neuropsychological findings 30 months post treatment.

We found that patients treated for LNB scored lower on tests assessing executive/attention functions (Stroop 4), processing speed (TMT5) visual (Digit Symbol recall) and verbal memory (CVLT list B) as compared to matched controls, and our hypothesis of cognitive deficits after treated LNB was confirmed. Mean NP test results are presented in Table 18.

Table 18. Neuropsychological test results in treated Lyme neuroborreliosis patients (n=50) and controls (n=50) 30 months after treatment. Numbers are raw scores (Standard Deviation)

Function	Measure	Patients	Controls	P-value
Executive	TMT 2 (seconds)	39.0 (18.2)	34.8 (14.1)	0.202
Functions	TMT 3 (seconds)	46.9 (38.6)	39.2 (21.5)	0.146
	TMT 4 (seconds)	101.4 (55.1)	101.9 (46.3)	0.332
	Stroop 1 (seconds)	31.6 (7.4)	30.6 (6.8)	0.558
	Stroop 2 (seconds)	23.0 (4.9)	22.0 (3.7)	0.312
	Stroop 3 (seconds)	64.8 (24.1)	59.2 (17.6)	0.102
	Stroop 4 (seconds)	77.6 (30.1)	67.0 (16.3)	**0.015**
Processing speed	Digit symbol (number of symbols.)	41.8 (12.8)	45.5 (11.5)	0.060
	TMT 5 (seconds)	28.4 (9.7)	19.2 (6.7)	**0.004**
Visual memory	Digit symbol, free recall (number of symbols)	6.6 (1.6)	7.2 (1.3)	**0.038**
	Digit symbol cued recall (number of symbols)	9.7 (4.7)	10.6 (4.6)	0.261
		Number of words recalled (SD)	Number of words recalled (SD)	
Verbal memory	CVLT trail 1	5.72 (1.9)	5.58 (2.0)	0.845
	CVLT trail 2	8.18 (2.4)	8.86 (2.7)	0.206
	CVLT trail 3	9.44 (2.4)	9.96 (2.8)	0.443
	CVLT trail 4	10.24 (2.1)	11.12 (2.6)	0.094
	CVLT trail 5	11.02 (2.4)	11.46 (2.6)	0.412
	CVLT trail 1-5	44.60 (9.1)	46.98 (11.5)	0.295
	CVLT list B	4.68 (1.9)	5.50 (2.0)	**0.014**
	CVLT Short delay	9.36(3.4)	10.18 (3.1)	0.255
	CVLT Short delay cued	11.58 (2.5)	11.33 (4,00)	0.780
	CVLT Long delay	10.34 (3.4)	11.14 (3.1)	0.426
	CVLT Long delay cued	11.50(3.4)	11.66 (3.4)	0.780

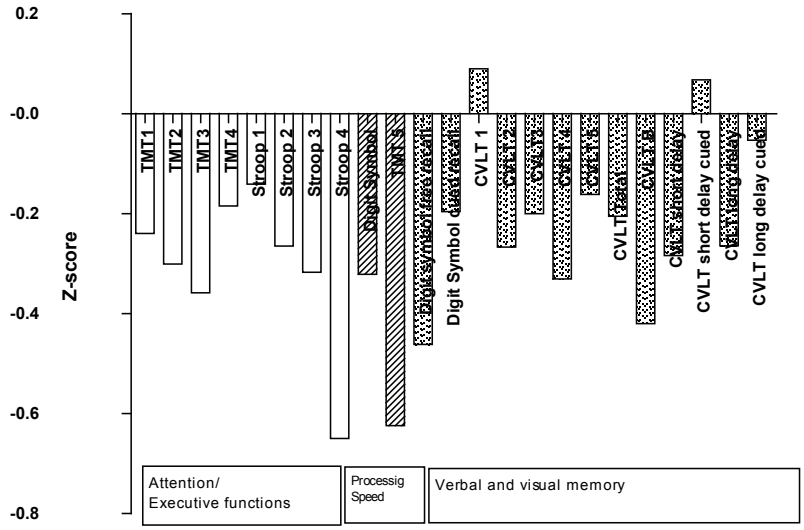
TMT; Trail Making Test, CVLT; California Verbal Learning Test

None failed the CVLT long delay forced-choice recognition test, indicating adequate test-effort in all participants.

The pattern of the NP deficits among our LNB treated patients was characterized by reduced impulse control, reduced processing speed and poorer ability of verbal learning. More patients than controls scored ≥-1 SD of the mean in the control group on processing speed (TMT 5) and visual memory (Digit Symbol recall), 14 vs. 7 (P=0.046) and 10 vs. 3 P=0.038), respectively.

The Z score profile demonstrates that the patients scored lower in all subtasks, except two (CVLT1 and CVLT short delay cued) and reflects a trend towards general lower test scores in the patients (see Figure 03).

Figure 03. Patients neuropychological test results displayed as z-scores, i.e. standard deviation above or below the mean in the control group. Y=0 is mean in control group.



TMT: Trail Making Test,CVLT: California Verbal learning Test

In order to investigate the clinical implications of the NP test scores further, we calculated the global NP sumscore expressing the number of NP subtasks with scores ≥-1 SD from the mean in the control group. There was no difference between the patient group and the control group regarding normal NP functioning (0-5 NP subtest with scores ≥-1 SD from mean sumscore in

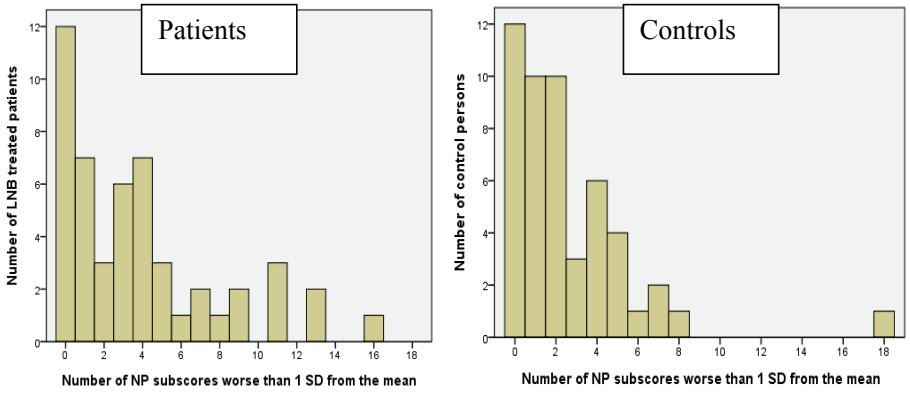
control group) or NP deficits (6-8 NP subtest with scores >-1SD- ≤-2 SD from the mean sumscore in the control group), but more patients than controls had an NP impairment (9-23 NP subtests with scores >-2 SD from the mean in the control group). The global sum score severities categories are presented in Table 19.

Table 19. Global NP sumscore categories

NP sumscore categories	Patients n=50	Controls n=50	P-value
Normal n (%)	38 (76)	45 (90)	0.067
Deficit n (%)	4 (8)	4 (8)	1.000
Impairment n (%)	8 (16)	1 (2)	**0.014**

The histogram of the global NP sumscores in the patient and the control group demonstrates that a small group of LNB patients had scores indicating cognitive impairment, and that most patients scored comparable to the controls (Figure 05). One control person had a global NP sumscore indicating NP impairment. In this person we suspected Alzheimer disease, and gave advises about further diagnostics and treatment.

Figure 04: Number of Neuropsychological (NP) subtask scores ≥ -1standard deviation (SD) from the mean in the control group in Lyme neuroborreliosis (LNB) treated patients and controls (range 0-23). LNB treated patients 3.9 (4.2) Controls 2.6 (3.1)



Post-hoc and subgroup analyses

Patients with subjective memory problems scored worse on Stroop 4 than those without subjective memory problems (mean 88.5 seconds (SD=35.4) vs. 68,3 seconds (SD=21.2), P= 0.023), and patients who reported non-complete recovery scored worse than patients who reported complete recovery on TMT 5 (mean 20.1 seconds (SD=6.4) vs. 27.5 (SD=10.3), P=0.014). We found no correlation between NP test results in LNB patients and HRQoL (PCS and MCS scores) fatigue (FSS score), depression (MADRS score and previously experienced psychological distress), reported malaise, reported concentration problems or persisting neurological findings 30 months after treatment.

Overall nine patients had NP impairment as assessed by the global NP sumscore and 14 patients had remaining objective neurological findings 30 months after treated LNB. Five patients had both NP impairment and remaining neurological findings, thus in total 18 (36%) of the patients had objective findings regarding NP impairment and/or neurological deficits after treated LNB.

Paper III: Risk factors for a non-favorable outcome after treated European Neuroborreliosis.

The results of the univariate analyses are presented in Table 20 and 21

Table 20. Associations between demographic and clinical variables and Health Related Quality of Life and fatigue 30 months after treated Lyme neuroborreliosis

*The difference is between the group with 4-6 years education and the group with more than 7 years of education after primary school. SD; Standard deviation, CI; Confidence interval, PCS; Physical Composite Summary and MCS; Mental Component Summary of the Health Related Quality of Life questionnaire SF-36 (Short Form-36), FSS; Fatigue Severity Scale

	PCS		**MCS**		**FSS**	
Variable (n)	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value
Gender Male (27)	45.2 (11.1)	0.738	50.9 (11.5)	0.501	3.5 (2.0)	0.950
Female (21)	44.2 (8.2)		48.6 (11.9)		3.5 (2.0)	
Education: years after primary school*						
0-3 (24)	44.1 (7.9)	**0.030**	48.7 (14.2)	0.646	3.7(2.0)	0.667
4-6 (16)	41.2 (12.1)		52.3 (9.6)		3.5 (2.4)	
>7 (10)	51.6 (7.4)		49.0 (7.7)		3.0 (1.3)	
Comorbidity pre-treatment						
Yes: (20)	39.9 (10.1)	**0.005**	48.5 (13.6)	0.576	4.4 (1.9)	**0.009**
No: (30)	47.9 (8.5)		50.8 (10.3)		2.9 (1.8)	
Treatment						
Ceftriaxone (26)	45.4 (10.9)	0,642	47.7 (12.6)	0.186	3.9 (1.9)	0.226
Doxycycline (24)	44.1 (9.0)		52.1 (10.3)		3.1 (2.1)	
Diagnostic accuracy						
Definite LNB (34)	45.5 (8.8)	0.471	49.0 (12.0)	0.426	3.4 (2.0)	0.627
Possible LNB (16)	43.3 (12.0)		51.8 (11.0)		3.7 (1.9)	
Pre-treatment CNS manifestations						
Yes: (4)	42.8 (4.8)	0.731	50.1 (18.3)	0.973	3.4 (2.5)	0.920
No: (46)	44.9 (10.2)		49.9 (11.4)		3.5 (2.0)	
Pre-treatment duration ≥ 6 weeks						
Yes: (10)	34.6 (8.5)	**<0.001**	48.1 (13.2)	0.590	5.4 (1.9)	**<0.001**
No: (38)	47.4 (8.5)		50.4 (11.3)		3.0 (1.7)	
Recovery 4 months						
Yes: (18)	49.9 (6.4)	**0.006**	55.6 (4.7)	**0.001**	2.1 (1.9)	**<0.001**
No: (32)	41.9(10.4)		46.8 (13.1)		4.2 (1.2)	
Recovery 12 months						
Yes: (29)	48.6 (9.1)	**0.002**	51.7 (8.3)	0.233	2.5 (1.6)	**<0.001**
No: (21)	39.8 (8.7)		47.6 (14.9)		4.9 (1.6)	
Continuous variables, correlation:	Pearson's correlation coefficient [95% CI]	P-value	Pearson's correlation coefficient [95% CI]	P-value	Pearson's correlation coefficient [95% CI]	P-value
Age, years , mean=56(SD=13)	-0.165 [-0.429-0.124]	0,262	0.029 [-0.257-0.31]	0.847	0.148 [-0.135-0.409]	0.305
Clinical score pre treatment mean, weeks mean 8,3(SD=4.3)	-0.251 [-0.499-0.035]	0.086	-0.243 [-0.493-0.044]	0.096	0.449 [0.196-0.646]	**0.001**

Table 21. Associations between laboratory findings and Health Related Quality of Life and fatigue after treated Lyme Neuroborreliosis

	PCS		**MCS**		**FSS**	
Categorical variables (n)	Mean (SD)	P-value	Mean (SD)	P-value	Mean (SD)	P-value
Intrathecal BAB production 30 months						
Yes:(17)	44.1(10.1)	0.875	51.9 (9.1)	0.195	3.4 (2.1)	0.788
No: (11)	43.3 (11.8)		45.3 (16.5)		3.6 (2.1)	
CSF OCBs pre-treatment						
Yes: (24)	45.9 (8.9)	0.498	52.4 (9.3)	0.127	3.3 (1.7)	0.725
No:(13)	43.9 (7.4)		46.4 (13.5)		3.5 (2.3)	
CSF OCBs30 months						
Yes: (12)	44.1(9.4)	0.888	44.7 (10.7)	0.101	4.0 (2.1)	0.906
No:(16)	43.5 (11.8)		52.8 (14.1)		3.9 (2.1)	
Continuous variables, correlation:	Pearson's correlation coefficient [95% CI]	P-value	Pearson's correlation coefficient [95% CI]	P-value	Pearson's correlation coefficient [95% CI]	P-value
CSF cell count pre-treatment (cells/mm^3) n=50 mean 187 (SD=255)	-0.079 [-0.355-0.209]	0.594	-0.088 [-0.363-0.201]	0.552	0.158 [-0.125-0.417]	0.273
CSF cell count 4 months (cells/mm^3) n=50 mean 7.8 (SD=11.4)	-0.031 [-0.339-0.283]	0.852	0.017 [-0.296-0.326]	0.981	0.046 [-0.261-0.345]	0.771
CSF cell count 12 months (cells/mm^3) n=29 mean 3.72 (SD=12.1)	0.011 [-0.363-0.382]	0.955	-0.333 [-0.628-0.045]	0.084	0.144 [-0.234-0.484]	0.458
CSF protein level pre-treatment (g/l) mean 1.22 (SD=0.73)	0.088 [-0.201-0.363]	0.551	0.114 [-0.175-0.385]	0.441	-0.020 [-0.296-0.259]	0.891
CSF protein level 4 months (g/l) (n=42) mean 0.61 (SD=0.24)	-0.017 [-0.326-0.296]	0.095	-0.086 [-0.387-0.231]	0.600	0.263 [-0.044-0.524]	0.093
CSF protein level 30 months (g/l) (n=29) mean 0.51 (SD=0.20)	0.064 [-0.316-0.426]	0.747	-0.237 [-0.56-0.149]	0.224	0.134 [-0.244-0.477]	0.488
CSF CXCL 13 level pre-treatment (n=32) mean 5227(SD=11915)	-0.194 [-0.513-0.172]	0.296	-0.002[-0.356-0.352]	0.990	0.232 [-0.126-0.537]	0.202

SD; Standard deviation, CI; Confidence interval, PCS; Physical Composite Summary and MCS; Mental Component Summary of the Health Related Quality of Life questionnaire SF-36 (Short Form-36), FSS ; Fatigue Severity Scale, BAB; Borrelia Antibodies, OCB; Oligoclonal Bands, CNS; Central Nervous System, CSF; Cerebrospinal Fluid.

Demographical and clinical variables

We found that pre-treatment symptom duration >6 weeks (B=-10.2, 95% CI -18.8- -6.7, P=0.002) and non-complete recovery at 12 months (clinical outcome score ≥ 1) (B=-5.6, 95% CI -10.8- -0.5, P=0.033) were associated with a worse PCS mean score. The final model explains 59% of the outcome variation (R^2=0.59). Non-complete recovery at 4 months (B=-8.9, 95% CI -15.5- -2.2, P=0.01) was associated with a worse MCS mean score. The final model explains 37% of the outcome variation (R^2 = 0.37). Pre-treatment symptom duration >6 weeks (B=1.3, 95% CI 0.15-2.4, P=0.028), a higher clinical score pre-treatment (B=0.1, 95% CI 0.002-0.2, P=0.019) and non-complete recovery at 12 months (B=1.7, 95% CI 0.7-2.6, P=0.001) were associated with a higher burden of fatigue. The final model explains 70% of the outcome variation (R^2=0.70). Our hypothesis that some of the demographical and clinical variables could predict outcome was met. Gender, comorbidity, treatment option, diagnostic accuracy, educational level and signs of infectious involvement pre-treatment of the CNS did not predict the outcome after treated LNB in our cohort.

CSF variables

Because of missing data at 4, 12 and 30 months, we analyzed the CSF findings separately. None of the CSF variables reached a significance level of P≤ 0.05 in the univariate analyses or in the regression model, so neither cell count, protein level, anti-*Bb* antibodies, presence of oligoclonal bands, nor CXCL-13 at baseline or during follow up predicted outcome regarding fatigue and HRQoL in this cohort.

We did not find evidences of active *Bb* infection at 30 months follow-up after treatment in any of the patients. One patient had significant pleocytosis at 30 months follow-up, but this patient had a re-infection with *Bb* with new emerging symptoms that resolved after treatment with intravenous ceftriaxone. Eight patients (28%) had slightly elevated CSF protein level, 17 (61%) had intrathecal anti-*Bb* antibody production, and 12 (43%) had presence of CSF oligoclonal bands 30 months after treatment. CSF CXCL-13 was measured in 32 (64%) of the patients pre-treatment and in 10 (20%) of the patients at 30 months follow-up. Out of these 32 (72 %) had an elevated CSF CXCL-13 levels before treatment, and none 30 months after treatment.

Post hoc analyses

Non-complete recovery at 30 months (composite clinical score >1) was correlated to the mean score on PCS (Pearson's correlation coefficient -0.474 95% CI- 0.668- -0.22 P= 0.001), MCS (Pearson's correlation coefficient -0.369 95% CI -0.591- -0.095, P=0.010) and FSS (Pearson's correlation coefficient 0.556 95% CI 0.329-0.722 P<0.001).

5. Discussion

5.1 Methodological aspects

Diagnostic accuracy of LNB cases

There is no gold standard for the diagnosis of LNB. Direct detection of *Bb* in blood and CSF is difficult, as the number of bacteria in these fluids is low, and culturing requires special conditions that can be hard to achieve. Clinicians and researchers therefore have to rely on a combination of clinical findings, patient history and laboratory results to diagnose LNB. The LNB patients in our cohort were selected according to strict diagnostic criteria meeting those from the European Foundation of Neurological Societies (EFNS) published in 2010 for definite and possible LNB [25]. To reflect everyday clinical praxis, and to avoid treatment delay, the patients were included in the treatment trial before the results of intrathecal anti-*Bb* antibody production were available. This is one of the reasons why one third of the patients were classified as having possible LNB, without anti-*Bb* antibody production. The patients with possible LNB (n=16) had other laboratory findings supporting the diagnosis, like CSF pleocytosis which normalized after treatment, high CSF CXCL-13 levels before treatment, and three patients developed intrathecal anti-*Bb* antibody production after some weeks. In addition, those with a possible LNB had typical clinical presentations; 14 (88% had a complete or partial Bannwart syndrome with radiculitis, palsy of cranial nerves, and/or pareses in the extremities. Two (12%) of the patients with possible LNB had only subjective symptoms pre-treatment (myalgias, asthenia, pain, malaise), they both had pleocytosis that normalized after treatment). For pre-treatment clinical and laboratory characteristics of the patients see Table 10 in the method part of this thesis. In summary we aimed for a high diagnostic accuracy of LNB cases to limit the possibility of including patients with other infectious diseases or disabilities with overlapping symptoms, and think we achieved this.

Control group

We followed a cohort of LNB patients prospectively, but the main outcomes in the present study (HRQoL, FSS, and NP functioning) were assessed only once, namely at the 30 month follow-up visit. A cross-sectional design like this gives information of the prevalence of our findings and associations between our observations, but does not allow any causative conclusions. Without comparable baseline scores of HRQoL, FSS or NP functioning, we don't know if our 30 month follow-up scores mainly reflect symptoms and signs that characterized the patients before they got LNB. To be able to relate our findings to LNB, we included a control group as similar as possible to our patient group, but without a history of

LNB. We recruited controls from the same geographical area as the patients, and because we wanted to assess variables that could be influenced by socioeconomically status and age, we matched the two groups for gender, age and educational level. The perfect control group is in practice almost impossible to obtain. HRQoL, fatigue and NP assessments might be influenced by several coexisting diseases or disorders found in the general population. The optimal control group should have the same frequency of coexisting diseases, and identical problems regarding pain, abilities and intelligence. We asked the patients to bring their own controls in order to improve the matching, especially regarding education. Of course the chance of getting more healthy controls is present (because it is easier to ask a healthy family member to come), and differences in outcome found between the two groups could be over-estimated.

On the other hand, we did not exclude controls with diseases or conditions that could influence the outcomes, like chronic diseases or a history of depression. The only exclusion criterion for the controls was a history of LNB. We did not test the control group for anti-*Bb* antibodies out of two reasons: Firstly, we know that the background seropositivity in our region is around 15-20% [14]. Secondly, we think that the reports from the patients about earlier LNB are reliable enough, as symptoms of LNB are normally easily recognized. Furthermore, the physicians in our region are familiar with the disease and ignored symptomatic LNB is probably a rare phenomenon.

The rate of diseases except LNB was comparable between patients and controls, so we think that the differences we found in outcome scores are not biased by more or less morbidity in the control group as compared to the patient group.

More controls than patients were retired from work, but mean age and educational level was similar in the two groups and we do not think that this affects our results either.

Overall, we believe that our control group is valid for our purpose, but we realize the limitations.

Main outcomes

The patient's point of view on how a disease affects their well-being and everyday functioning is essential. Our clinical experience was that many patients with LNB complained of symptoms like reduced Qality of Life, fatigue and neurocognitive impairments, in spite of correct treatment, and we therefore chose main outcomes in our study that measured these aspects.

We have used well established evaluation instruments described in the method chapter.

Even though the assessment of quality of life has gained more or less general acceptance as a measurable entity, it is optimistic to believe that all the dimensions of a patient's life can be

covered by a 36- item scheme like the SF-36. The HRQoL varies in different situations and at different times, and the individual's opinion of the importance regarding different aspects of HRQoL also varies. It is important to know if the issues we are measuring is important to the patient and have an impact on his or hers everyday life. Many questionnaires, including SF-36 consider these aspects and includes questions like what issue the person questioned finds the most important, and in which degree he or she thinks this affects his or her daily life. In this way we can assume that if a person scores poorly on the HRQoL questionnaire it does, in the patients opinion, affect his or her life.

The word "fatigue" is translated into "utmattelse" in Norwegian, a term that in my opinion seems to be understandable to most of the patients and distinguishable to "tired" (in Norwegian: "trett") and "worn-out" ("utslitt"). To assess fatigue we used the fatigue severity scale (FSS) which is a reliable, validated scale, and measures the impact of physical fatigue on daily life. Furthermore, FSS is a generic scale which gives us the opportunity to compare our results to the scores in the general population, and to FSS scores in studies of other diseases. FSS was developed to assess Multiple Sclerosis associated fatigue, and prior studies have shown acceptable internal consistency and stability over time, and also good sensitivity to changes caused by clinical improvement [132]. A more detailed description of the FSS is found in the method section of this thesis.

We based the selection of NP tests upon those used in relevant studies of cognition in LD, which had revealed significant differences between patients and control groups. We also wanted the test battery to cover a broad spectrum of neurocognitive domains, and if possible, use tests validated in Norway. Previous studies had reported deficits in memory, attention/executive functions, and in processing speed in LNB patients. The TMT test, the Digit symbol test and the Stroop tests attends to be good choices; as they were standardized, tried out in Norwegians, and they are commonly applied as part of NP assessments.

The CVLT assesses episodic verbal memory, and is commonly used, also in LD studies. Westervelt et al. concluded in their review that a memory test based on learning and recall of a word list seems to be more sensitive to detect memory deficits after LD than other techniques (like remembering a story) [100]. Several LD studies including the CVLT have found significant verbal memory problems in LD patients compared to controls, but the CVLT subtest performance profile vary.

The Digit symbol test primarily assesses processing speed, but it also tests visual memory and incidental learning. Several studies have found deficits after LD in verbal fluency, but tests assessing this function were not included in our study. Verbal fluency is a vulnerable function

connected with executive functions, and might have been important to assess. We have assessed executive functions with tests like Stroop 1-4 and TMT 1-4.
NP tests in general have a high sensitivity and are useful to detect even small NP deficits, but they have a low specificity. It can be challenging to decide which defined cognitive deficit is causing the problems, e.g. is it a memory problem or maybe an attention problem, or a combination of both?
A weakness of our study is that we did not assess IQ. However, several of the American studies included an IQ assessment or an estimation of IQ, and did not find IQ to be affected after LD [101]. To minimize this problem in the present study we matched the control group and the patients regarding age, educational level, and geographical region, known to correlate in some degree with intelligence [133]. The fact that the patients chose their controls in their own surroundings will also make the groups more comparable regarding IQ.

For the clinical status, during and after LNB, we used a composite clinical score based on a standardized interview and a clinical neurological examination. We regard this clinical score as a less sensitive and specific indicator of long-term outcome than HRQoL, FSS and NP test scores, and more susceptible to confounding. Consequently we chose not to use it as a main outcome measurement, rather as a measurement of clinical status throughout the study.

Potential biases

Selection of patients

We did not include all patient (n=112) from the treatment trial in this follow-up study. Out of economical and logistic considerations we had to include only a subgroup. Sixty-four of the 112 patients participating in the treatment trial were living in the geographical region of Agder counties, and we decided to include those of these 64 who were treated between May 2004 and December 2007, namely 57 patients. Fifty of these patients consented to participate and were included. To minimize the chance for selection bias, we compared our 50 included patients to the whole patient group from the treatment trial, and to the seven who were invited but declined to participate, and found no differences regarding illness severity before or after treatment, or regarding treatment options. Therefore, we think that our subgroup is representative for the whole cohort.
Mean pre-treatment symptom duration was 9.2 weeks in the whole cohort, and one interesting question is if a subgroup of patients with longer standing symptoms could have influenced our results. Ten patients had pre-treatment symptom duration more than 6 weeks, including four

patients with symptom duration more than 6 months. We do not think that our results are skewed by a these patients as the outcome regarding PCS, MCS, FSS and the NP tests did not differ between patients with and without pre-treatment symptom for more than 6 months.

Co-morbidity

Infections with other pathogens can also cause symptoms similar to those seen in LNB. Patients without anti-*Bb* antibodies were tested for *Herpes simplex, Varicella zoster* and *entero-virus* using CSF PCR with a negative result, and all patients were tested for anti-*TBE* antibodies, three patients were IgG positive and none were IgM positive for anti-*TBE* antibodies. Some researchers have discussed if a co-infection with *Anaplasma*, could give rise to a more serious disease. We did not test for *Anaplasma,* but symptomatic *Anaplasma* infections in humans are rare in Norway, and only two cases are documented. Serological analyses of blood samples of anti-*Bb* antibody positive patients in Norway, however, have shown that 10% are positive for both *Bb* and *Anaplasma* [118;134]. We still think that the chance of ignored cases of *Anaplasma* infections that might have altered our results is very little as such infections usually cause typical changes in the platelet and leukocyte count, and such changes were not present in our patients.

We asked all our patients and controls about somatic and psychological comorbidity. The mean age in the groups was 56 years, and of course some had other chronic diseases including psychological distress in their past history which could potentially alter the results of HRQoL, fatigue and NP assessments. We have, however, shown that our two groups are comparable regarding amount and severity of comorbidity, and we do not think that the differences found between LNB treated patients and controls in our study are confounded by coexisting diseases. Episodes of psychological distress during lifetime is quite common in the general population, and to be able to identify those who had a more serious psychiatric disease we asked about any psychiatric disease or psychological distress in the past or present which led to medical or therapeutic treatment or sick leave. Ten (20%) patients vs. 8 (16%) controls answered yes to this question, P=0.595), which is comparable to the prevalence in the general population in Norway [135].

Missing data

Missing data is a problem when dealing with epidemiological studies, and to be prepared for this we invited 14 patients more than the 36 required according to sample size calculations before study start. In contrast to the clinical and questionnaire obtained data which were almost complete, some laboratory data were missing. It was too demanding for some patients

to do all the planned spinal taps, and this resulted in a decreasing number of CSF samples available for analyses. In addition, by accident in our laboratory 19 CSF samples were destroyed before the final analyses of CSF CXCL-13 had been done. As a result of this, only 10 CSF samples were available for CSF CXCL-13 analyses. It is important to keep the missing CSF samples in mind when analyzing the laboratory data, and when considering the study results. In the risk factor study (paper III) we analyzed the laboratory data separately because of this.

Regarding the HRQoL SF-36 scores, two patients and one control missed a few items on the questionnaire, but in 48 patients we could calculate the sum scores MCS and PCS, so we did not impute missing data.

On the NP tests four patients had missing data on in average 2.5 of the 23 tests, and four controls missed in average 1.5 of the 23 subtasks, therefore we decided to impute the missing data to be able to include all patients and controls in the NP outcome analyses.

Under- and over-reporting of complaints

Self-rating questionnaires and NP assessments might give rise to information biases, as the tests can be administrated differently by different investigators. To minimize this problem we let one neurologist (the main investigator), trained as suggested by the NP foundation by an experienced neuropsychologist, do all the testing in patients and controls. The investigator was available for questions during the completion of the self-rating questionnaires to clarify misunderstandings, and tried to inform the participants as correctly, encouragingly and uniformly as possible. We also tried to keep the surroundings as comparable as possible from one participant to the other, including the time of the day, and interruptions and disturbing sounds from outside the room was avoided as far as possible.

At follow-up the patients` mean age was 55 years, and quite a lot of them were of an age where it is natural to start thinking about retirement from work. One can speculate if some of the five patients that reported that they retired from work because of problems after LNB (aged 61, 63, 63, 65 and 66 years at the time of treatment) would have become retirees anyway? It may be reasonable to think that the remaining complaints after LNB sort of "pushed them over the edge".

The fear of non-complete recovery and remaining complaints after a severe infection affecting the nervous system can bring people to over-report symptoms. Stories of dramatic consequences after LNB in the daily news do not make this less expectable

It can be difficult for a person to remember details of the disease history months to years later. Most of the demographical and many clinical variables from the early phases of the disease were available to us in the research database, and we also checked the patient's histories with the hospital records. In this way we think that the chance of recall biases was limited.

Internal validity

As our main outcome we used the validated and reliable version 2 of the SF-36 test. This is much used all over the world, and internal consistency and test-retest studies have been conducted. In Norway Loge and Kaasa reported reliability estimates in 1998, and Cronbachs alpha varied from 0.80 to 0.93 on the 8 subscales, which is very high [127]. The FSS showed satisfactory internal consistency (Cronbachs alpha 0.88) in a study of the Norwegian version [76].

The 2.version of the CVLT was translated into Norwegian in 2004, and in 2007 it was validated in 128 Norwegian patients who had previously been tested with CVLT version The conclusion was that CVLT 2 was found satisfactory and applicable in Norwegian patient populations. A validation in a larger population of healthy Norwegians is warranted, though [97]. The color-word interference test (modified Stroop test) has satisfactory reliability [99] and was translated to Norwegian in 2005 as part of the D-KEFS (Delis- Kaplan Executive Function System). Digit symbol is a part of the WAIS-III (Wechsler Adult Intelligence Scale-III) test battery. It has been translated into Norwegian- but uses American norms. The Digit symbol is reliable and valid and commonly used around world [136]. TMT is also a part of the D-KEFS, test reliability varies considerably depending on what kind of difficulties the test person has (e.g. motor problem in arm may interfere with test results) In the TMT version used in D-KEFS, as we used, TMT 5 is added to compensate for the fine motor component. Spreen and Straus have made a list of studies done on reliability of TMT without the compensating TMT 5, and most of them had a Cronbachs alpha over 0.60, many over 0.90 [99].

The calculating of a general NP score that combined all the 23 sub tasks can be criticized. The tests and subtasks assess different aspects of cognition and to put them together in one global outcome variable as if they were assessing one function may seem odd. However, previous studies have not revealed a typical NP deficit profile in treated LNB patients, and this method of counting the number of "failed" tests in a battery of NP tests (in the present study defined as minus one standard deviation from the mean of the control group) to form a global

cognitive function, is not uncommon, also in LD studies [90;101]. We therefore chose such an approach to get an impression of the severity of the neurocognitive overall symptom burden, and think this can be justified.

It can be problematic that the main investigator of the study did the testing. This can of course lead to false positive results in the direction of more substantial findings towards a "wanted" outcome. However, the neuropsychologist that scored the tests was blinded to group adherence. A great advantage of one person doing all the testing is the avoidance of inter-rater biases which can be substantial regarding NP testing.

Multiple tests

In our study we compared a lot of variables to several outcomes. The PCS and the MCS are sumscores of eight sub-dimensions of HRQoL, and these subscales are again summarized from 36 questions, each with several answer options. The NP assessments consist of four tests with 23 subtasks. It is important to realize that the chance of false positive and false negative differences is present. The probability of correlating variables that are dependent on each other and cover parts of the same qualities is obvious. We did not do a Bonferroni correction when analyzing the many scores of the SF-36 and the NP tests, and this should be brought into discussion when looking at the results. We think it is important to present our results without such a correction as this procedure would have been too conservative and inappropriate for our data, and many real differences could have been missed (type two error) [137]. The relatively big sample size, the inclusion of a control group, and the trend that the patient scored significantly lower as compared to controls in almost all the sub-dimension of the SF-36 (except pain) showed a difference in the two groups, strengthen our conclusions. Further the trend was that patients scored lower than controls in all NP sub-tasks except two. In the risk factor study (paper III) we chose to do a multiple regression. With this method we could analyze several presumably correlated variables in the same model. The significant associations between the outcome measures PCS, MCS and FSS and demographic, clinical and laboratory data found in the univariate analyses which were excluded in the multiple regression analyses were probably confounders. The outcome variation was better predicted by those variables which were included in the multiple regression models

External validity

We have tried to design our study, and choose our cohort as close up to everyday clinic as possible to be able to generalize the results. We have included patients with possible and definite LNB diagnosis, and patients with long and short pre-treatment duration of symptoms.

Moreover, we did not exclude patients with other somatic or psychiatric diseases that might affect cognitive function, fatigue burden or Quality of Life, and our group of patients is not restricted to those with persisting complaints after treatment of LNB. We have not included children; they normally have a better long term prognosis than adults.

5.2 Discussion of the results

We have compared our results to other previous conducted studies on treated LD, and we find that our study adds important information about long term outcome after treated LNB regarding HRQoL, neurocognition and fatigue. Few controlled studies are addressing these matters in Europe, which makes this study important.

HRQoL

We found that LNB treated patients had reduced HRQoL compared to controls as assessed with the SF-36 summary components PCS and MCS (PCS, 44 (SD=9) vs. 51 (SD=6) $P<0.001$ and MCS, 49 (SD=11) vs. 54 (SD=6), $P=0.010$) 30 months after treatment. The patients scored lower on all the eight sub-dimensions of the SF-36, except for bodily pain. LNB patients who reported complete recovery (56%) had similar HRQoL scores as the controls (paper I). Delayed start of treatment and remaining complaints at 4 and 12 months after treatment seem to predict a worse outcome with respect to HRQoL. Age, gender, educational level, diagnostic accuracy, treatment option, signs of infectious involvement of the central nervous system or coexisting somatic or psychological distress were not associated with HRQoL outcome 30 months after treatment in our cohort, neither were any of the assessed CSF findings before treatment or during follow-up (paper III).

Our findings of reduced HRQoL months and years after treated LNB are supported by some previous studies done in the US, but the patients' scoring profile differed some from our findings. Shadikc et al. did a small study of LD patients 6 years after treatment in 1994, and found a reduced global health status [61]. They did a larger study 6 years later, and found lower scores in all the eight SF-36 dimensions in LD treated patients as compared to healthy controls in the univariate analyses, but the only dimension surviving the multiple regression analysis was bodily pain. Interestingly, pain was the only dimension that did not differ between patients and controls in our study group. In addition to assessing pain as one of eight dimensions in SF-36, we also asked about pain in the semi-quantitative interview, and there

was no difference in the proportion of persons reporting pain among patients and among controls. The reason may be that *Bb afzelii* and *Bb garinii*, which are the dominating *Bb* species in our region, have less affinity to joints and muscles than *Bb sensu stricto*, which dominate in the US. The Bannwarth syndrome with radiculitis is indeed very painful, but the pain is often relieved in a couple of days after initiating antibiotic treatment, and vanishes after the treatment. Another possible explanation can be that cognitive deficits and fatigue overwhelmed pain.

Kalish et al. conducted a study in randomly selected patients from the original study from Lyme. They compared HRQoL, using SF-36, in healthy controls to three groups of patients; patients with LNB, patients with LD without affection of the nervous system (mainly EM), and patients with LD arthritis. They found that patients with involvement of the nervous system during the acute phase of the disease (facial palsy) that did not receive antibiotic treatment had more physical limitations (PCS) and bodily pain 20 years after disease than those who received antibiotic treatment. The antibiotic treated LNB patients had similar HRQoL scores as healthy controls. In contrast to this, we found physical limitation (low PCS scores) in our patients as compared to controls despite adequate antibiotic treatment [41].

In 2000 Seltzer et al. compared HRQoL in LD patients (not restricted to LNB) and healthy controls, and found no differences in SF-36 scores. Very few of the patients had signs of nervous system involvement (in more than 80 % EM was the main clinical manifestation), and lumbar puncture was not done. This study confirms that patients with EM have an excellent outcome after treatment, and the results cannot be directly compared to ours, as we only included patients with LNB [65].

SF-36 is also used as outcome measure in effect evaluation of prolonged antibiotic treatment in patients with remaining complaints after LNB. The treatment trial by Klempner et al. from 2001 reported an improvement of the HRQoL after 10 weeks with intravenous ceftriaxone, but the adverse events were substantial, and the treatment was not recommended [64].

We believe that none of the earlier studies of long term HRQoL in LD are directly comparable to our study, mainly because of the various inclusion criteria and study designs, and not least because they all are conducted in the US. After our study was published, a European research group applied the SF-36 questionnaire on German patients suspected to have “chronic Lyme “despite treatment [24]. They compared four groups of “chronic LD” patients to healthy controls, one seronegative group without any evidence of a *Bb* infection, one group of seropositive patients with other diseases that explained their complaints, one group of seropositive patients without other diseases that explained their complaints, and one

group with a proven history of LNB (PLDS patients). All three analyzed groups had reduced HRQoL and more depressive symptoms than the healthy controls, but as far as we can ascertain, the results of the HRQoL assessments in the six PLDS patients are not yet published.

Why some patients have reduced HRQoL after adequately treated LNB is much debated. It seems logical that the fear of serious problems after an infectious disease in the nervous system might reduce the chance of recovery and cause reduced HRQoL. When we planned the study, this was one of our main theories, and we decided to assess depression by the MADRS questionnaire and by interviewing the patients and controls about their psychological distress and psychiatric history. Others have reported association between psychiatric comorbidity and complaints after LNB [123]. Interestingly, our study results did not support the hypothesis that the patients' complaints are based on depressive symptoms. Despite a difference between the groups in mean MADRS scores, the mean scores were below cut-off for depression in both groups and only one patient had a MADRS score indicating depression in need of therapy (21), and one patient (16) and one control (10) had a slightly elevated MADRS score. The MADRS scores also did not differ between patients reporting recovery and patients reporting non-complete recovery at 30 months follow up, and the patients and controls had comparable histories regarding psychiatric and somatic comorbidity. Our findings are supported by a study by Hajek et al [122], but not by the study by Solomon et al. which found that psychiatric impairment before LNB resulted in more chronic symptoms afterwards [121].

Apathy is found in many neurological diseases, and is known to reduce HRQoL in Parkinson's disease [138]. We found a difference between mean scores in SAS between LNP patients and controls, but do not regard the measured difference to be of any clinical importance due to scores below cut-off for apathy in both groups. Further, we found comparable proportions of persons with high SAS (> 14) scores in the two groups.

Fourteen patients (28%) had objective neurological findings at 30 months follow-up. The neurological deficits were mild, but these 14 patients scored lower on PCS and on FSS, as compared to the rest, so the neurological deficits seem to have a functional impact. This is also reported in other Scandinavian studies, Berglund reported 12% and Hansen reported 5% of patients with disabling neurological deficits after treatment [28;53].

In conclusion our results demonstrate that European LNB patients have poorer HRQoL 30 months after treatment than matched controls. The underlying pathogenesis is unknown, but

pre-treatment symptom duration >6 weeks and non-complete clinical recovery during the first year after treatment seem to be risk factors for a non-favorable outcome regarding self-reported HRQoL.

Fatigue

The patients scored lower than controls on the FSS (mean FSS scores 3.5 vs. 2.1 P<0.001), and 50% of the patients and 16% of the controls (P=0.001) answered that fatigue was an everyday problem to them. The proportion of persons suffering from fatigue differed between patients and controls both when using four and five as cut-off (42 % vs. 10% scored ≥ 4 on the FSS, P<0.001 and 25% vs. 4% scored ≥ 5 on the FSS, P=0.001). Delayed start of treatment, a more severe disease before treatment and non-complete recovery at 4 and 12 months seem to predict more fatigue 30 months after treatment.

We did not find correlation between NP functioning and fatigue (P= 0.892) as was found in previous studies [90;92;94], but the FSS score in the LNB patients was correlated to PCS (P=<0.001) and MCS (P=0.001). In other conditions like Parkinson's disease fatigue is also shown to contribute to reduce HRQoL [59].

Although fatigue is a common problem both in the general population and in several acute and chronic diseases, we do not know the underlying patophysiology. This is of course frustrating, as it also restricts the possibilities of finding good treatment strategies. We found that a delayed treatment, more subjective symptoms and objective findings before treatment, and non-complete recovery at 12 months predicted more fatigue 30 months after treatment. The importance of starting the treatment early is supported by other reports [53;61;101;139]. Our findings are also in accordance with the theory that more severe disease gives rise to a less favorable outcome. Is this due to more serious tissue damage, psychological factors like fear of remaining problems, late inflammation, an active infection, disturbances of regulatory systems or other factors?

Fatigue is an important symptom in depression, and the overlapping symptoms might cause diagnostic challenges, but we did not find depression in our cohort as assessed with MADRS (see discussion of HRQoL).

Fatigue is in one study found to be more common in anti-*Bb* antibody seropositive persons as in anti-*Bb* antibody seronegative persons [85]. The incidence of chronic fatigue syndrome is increasing in Norway, as in many other countries. PLDS and Chronic Fatigue Syndrome are both syndromes with unknown patophysiology. Many patients and physicians ask themselves

about the possible role of an active or former *Bb* infection in patients with chronic fatigue syndrome. Regularly patients are referred for a second opinion about a "chronic Lyme" condition. Experiences from Lyme referral centers in the US show us that an active *Bb* infection based on medical history and CSF findings is rare in patients referred for a second opinion because of suspected "chronic Lyme" An interesting recent study in a European cohort looked at patients who were referred to a specialist centre for a second opinion of suspected "chronic LNB". Out of 112 referred patients nine had ongoing *Bb* infections, one of them LNB. They did a subgroup analysis of 95 of the remaining patients and found that 39% had another well defined illness which explained their symptoms (including 13 persons with Multiple Sclerosis), and only six patients had PLDS according to the proposed categories of Feder et al [24]. A recent study showed distinct CSF proteomes that might help us to differentiate Chronic Fatigue Syndrome from PLDS; this is interesting and might give us some diagnostic tools in the future [87].

One might ask how important it is to identify PLDS in patients with fatigue when we don't have a specific cure to offer them. I think that the patients will be more susceptible to other therapeutic approaches if they know as much as possible about the etiology of their disease. This is also supported by a study by Sigal et al., which suggests that the patients with a diagnosis of "unexplained syndromes" may be unable to benefit from therapy because of their anxiety [86].

Different treatment approaches for fatigue have been tried out, and most of the knowledge about this is probably due to research on fatigue in the Multiple Sclerosis population. First of all, secondary or additional causes of fatigue, like sleep problems, depression, metabolic disturbances, adverse effects of medications and so on must be eliminated. Several studies have looked into antibiotic treatment of fatigue after treated LD. Krupp et al. used FSS scores as main outcome to evaluate possible treatment effect of ceftriaxone intravenously for 28 days in previously treated LNB patients with persisting fatigue (all participants had a FSS of four and more before inclusion in the study) [66]. Fallon et al. also used FFS in a treatment trial with ceftriaxone for 10 weeks of PLDS patients with objective memory impairments. Both studies reported lower FSS scores after treatment. In the first study the treatment was not recommended because of side effects, and in the second study, the relief of fatigue found at 12 weeks, did not persist at 24 weeks [67]. Non-pharmacological treatments of fatigue include patient and caregiver education, psychological approaches and physical exercise. Cognitive behavioral therapy has shown to be effective in Chronic Fatigue Syndrome, and physical

exercise has shown that it may lighten the burden of fatigue in Multiple Sclerosis patients [89;140]. This should also be tested in PLDS patients.

In conclusion our results demonstrate that European LNB patients have more fatigue 30 months after treatment than matched controls. The underlying pathogenesis is unknown, but pre-treatment symptom duration >6 weeks and a more severe disease before treatment and non-complete clinical recovery during the first year after treatment seem to be risk factors for a non-favorable outcome regarding self- reported fatigue.

Neuropsychological (NP) functioning

We found that LNB treated patients scored lower on four NP subtasks assessing executive/attention functions, processing speed, visual and verbal memory, as compared to matched controls (Stroop test 4: 77.6 vs. 67.0, P=0.015, TMT 5: 23.4 vs. 19.2, P=0.004, Digit Symbol recall: 6.6 vs. 7.2, P=0.038 CVLT list B: 4.68 vs. 5.50, P=0.003). The distribution of global NP sumscores indicates that most of the LNB treated patients perform comparable to controls, while a small subgroup have a debilitating long-term course with cognitive impairment. Fatigue, depression, neurological deficits or HRQoL at 30 months after treatment were not associated with the global NP sum score. The subjective feeling of cognitive problems as memory problems or concentration problems does not always correlate with poor NP performances [141], but we found that subjective feeling of memory problems correlated with lower scores on Stroop 4. The subjective feeling of non-complete recovery correlated with poor scores on the TMT 5.

Westervelt et al. did an excellent review of controlled studies of NP outcome after treated LD conducted from 1996 to 2000, and found that the main deficits were related to memory, attention/executive function and processing speed [100]. Most studies which have found neurocognitive deficits in treated LD patients report memory problems [90-94;101;102;105;106], and most of the studies report problems with verbal memory and verbal learning. McAuliffe et al. found, in a study on adolescents, more visual memory and learning problems as we did in our cohort [107]. The LNB treated patients in our cohort had verbal memory recall problems when interfered with a distracting wordlist on the CVLT test, and visual memory and incidental learning problems on the Digit symbol test. They did not have any verbal learning deficits. Several studies have used the CVLT and have reported more verbal memory problems than we did: Kaplan et al. found deficits on verbal delayed recall and associated verbal learning years after treated LNB [102], Shadick et al. found verbal long delay recall in LD patients 6.2 years after treatment [61], Benke et al. found reduced verbal

learning, and reduced verbal memory (short time free and cued recall) in LNB patients (treated and not treated) years after the infection [101], Ravdin et al. found reduced verbal learning and long delay free recall deficits in LD treated patients a year after treatment [92]. We did not find any of these verbal memory deficits, but the patients in our LNB cohort had more recall problems when presented with a distraction word list as compared to controls. The CVLT test does not assess memory and learning functions alone, attention/ executive abilities influence on the verbal learning and memory [95]. The presentation of a second wordlist demands organization and attention skills even more, and one possible interpretation is that the observed memory problems in our cohort are strongly influenced by attention deficits. This might be supported by the findings in another test assessing attention/executive functions, the Stroop test. Our LNB treated patients scored lower than the controls on the most demanding test, Stroop 4, which requires inhibition of response and shifting between rules for solving the tasks. Attention/executive function deficits are also found in other studies [61;91;101;105].

Processing speed measured by Digit symbol and TMT-5 was lower in LNB patients than controls. The TMT-5 task is dependent on motor function in the arm, however, Pollina et al. have shown that a poor result in this task is independent of fine motor function and proprioception in LD patients, and can be regarded as a more disease specific cognitive deficit [94]. Reduced processing speed is also frequently reported in LD treated patients in accordance with our findings [61;93;103;105]. Four well designed treatment studies [64;66;67;108], have showed no improvement of neurocognitive functions after repeated and prolonged antibiotic therapy.

The main differences between our study and other studies on NP functioning in post-treatment LD patients are that we find less verbal memory problems and more visual memory problems. The findings of attention/ executive function deficits in our cohort seem to be in accordance with several previous conducted studies. We think that the main reasons that our study results differ from previously conducted studies are different inclusion criteria and the lack of a "disease specific NP profile". Several of the US studies have only included LNB treated patients with persisting complaints [67;90;93;102] while we have included patients with- and without persisting complaints. We would expect to find fewer differences in our cohort than in a cohort with pre-selected patients with known complaints. On the other hand, patients with affection of the nervous system might have more deficits than patients with LD without nervous system affection. We found that, a subgroup of LNB treated patients had NP

impairments, and that most of the patients performed as good as the control group on NP testing.

Imaging studies have not been conclusive regarding anatomical mapping of structural or functional changes of the PLDS [47]. Logigian et al. did a SPECT study in 13 patients with Lyme encephalopathy and found hypoperfusion of the subcortical basal ganglia and white matter [50]. Fallon et al. examined the regional blood flow and cerebral metabolic rate in 35 patients with persistent encephalopathy after LD and compared them to healthy controls, and found deficits in white and gray matter bilaterally, primarily in the temporal, parietal and limbic areas [142]. In another controlled study by Fallon et al. 11 LD treated patients with persisting subjective cognitive problems were tested with NP test while Xenon (133)-regional blood flow was registered. They found less blood flow changes in white matter especially in the posterior temporal and parietal lobes bilaterally. These flow reductions were associated with deficits in memory and visuospatial organization assessed by NP testing. They did not find any connection between these flow deficits and the small white matter lesions seen in some of the subjects [51].

Depression can affect NP function, and some of the previous studies included assessment of depression, as did we. Confusingly both negative, positive and non correlation was found! Two studies found better memory scores in depressed patients [90;93], others found no relationship [91;92;104;143], and some found depression in LNB patients, but not correlated to memory scores [105]. Barr found more reported subjectively memory problems in depressed patients with serological evidence of late-stage LD in a not controlled study, but all the 55 depressed LNB patients scored low on the depression rating scales, indicating that they were not severely depressed, and there was no correlation between the performances on CVLT and the subjective feeling of memory [141]. Elkins et al. tested 30 post-LNB patients but found no association between current depressive symptoms and NP test results. The study was limited by the lack of a control group [143].

In summary, these study results indicate that depression is not associated with cognitive problems in LNB treated patients. The LNB treated patients in our cohort were not depressed, and the cognitive problems found in our cohort cannot be explained by a coexisting depression. We think this is an important message to clinicians.

Only four of our patients had signs suggestive of infection of the CNS before treatment, and we found no correlation between CNS affection and global NP sum score functioning (3.6 vs. 7.3 P= 0.080). This must be interpreted with caution because of the low number of patients with CNS affection.

Fatigue can correlate with NP functioning [90;92;94] but it is uncertain if fatigue is causative of the NP test deficits or a confounder. Studies of fatigue and neurocognitive functioning in Multiple Sclerosis has shown that the NP deficits often appear at the end of the test situation when the patients have fulfilled many and often more demanding NP tasks [144]. This reflects poor vigilance. Our test battery consisted of a limited amount of tests to avoid test related fatigue, and we allowed breaks if needed. We found no correlation between NP test results and fatigue (self-reported fatigue and FSS score).

Five of our patients blamed persisting complaints after LNB for their retirement of work, but this was not associated to the NP scores.

In conclusion LNB treated patients scored lower on four NP subtasks assessing processing speed, visual and verbal memory and executive/attention functions, as compared to matched controls. The distribution of NP dysfunctions indicates that most LNB treated patients perform comparable to controls, while a small subgroup have a debilitating long-term course with cognitive problems.

6. Conclusions

1. Health Related Quality of Life was reduced in well-defined European patients treated for LNB with a current recommended antibiotic regimen 30 months earlier, as compared to matched controls. The LNB treated patients were not more depressed and did not report more pain than the controls. Fatigue was the most disturbing persisting complaint, and was negatively associated with Health Related Quality of Life. Mild neurological deficits were found in 28 % of the patients, and seemed to influence negatively on the physical Health Related Quality of life and fatigue scores. The patients who reported subjective recovery had the same Health Related Quality of Life as the controls.

2. Most of the patients who were treated for European LNB 30 months earlier had comparable neuropsychological (NP) functioning to matched controls, but a small subgroup had cognitive impairments regarding attention/excecutive function, processing speed and memory that could affect their daily life. The LNB treated patients with complete recovery had similar NP functioning as the controls. We did not find any association between NP test results and Health Related Quality of Life or Fatigue. NP testing should be applied when patients complain of memory problems, as these patients have poorer scores in some of the NP sub tasks. A test battery of Trail-making test, Stroop test and Symbol digit seems to reveal deficits, and a memory test including visual memory is recommended.

3. It seems that a more serious LNB disease and a longer duration of symptoms before treatment can reduce Health related Quality of Life, and that symptom duration more than 6 weeks before treatment, a more severe disease and non-recovery at four and 12 months predict a higher burden of fatigue 30 months after treatment. We did not find that any laboratory data predicted outcome after treated LNB, and there were no Cerebro Spinal Fluid (CSF) findings indicating an active *Bb* infection 30 months after treatment. Gender, age, comorbidity, signs of pre-treatment infection of the central nervous system or CSF findings before and during follow-up was not associated with Health Related Quality of Life or fatigue at 30 months.
4. Thirty months after treatment of LNB 18 out of 50 patients (36%) had objective findings in terms of neurological deficits and/or cognitive impairment.

Clinical implications

LNB should be treated as soon as possible to avoid a non-favorable outcome. Patients should be informed that the chances of a favorable outcome after treated LNB are good, but they also need to be informed about the possibility of cognitive impairments and fatigue months after treatment. If a PLDS is suspected, neurocognitive testing including assessment of memory and learning, attention/executive functions and processing speed might be helpful to document the condition and to help persons to find coping- strategies.
We probably need a national competence center on tick-borne diseases, situated in a high endemic region for Lyme disease and Tick-borne Encephalitis. The main responsibilities of such a competence center must be clinical research, diagnostic and treatment of persons with tick borne diseases, especially the patient in whom the diagnosis is uncertain, in patients with more severe disease and patients with a non-favorable outcome. We need practical guidelines for the diagnosis and treatment of tick borne diseases based on evidence based medicine, and at a national level.

7 Future perspectives

Further studies

1. Studies on the pathophysiology of LNB and long term complaints after treatment such as neurocognitive deficits and fatigue, including imaging studies like functional MRI are warranted. Especially the theories about a secondary autoimmune process or cytokine-driven immunological damage in the nerve system should be further explored. It is important to do studies in Europe as geographical differences on the genotype of the *Bb* species and phenotype of LD exist. Conclusions from US studies cannot be directly transferred to European conditions.
2. Studies that evaluate present and new diagnostic laboratory tests are needed to avoid over diagnostic and under diagnostic of LNB.
3. Our findings of possible risk factors for a non-favorable outcome after treated LNB need to be confirmed in a prospective study including a larger study population, especially regarding the laboratory findings.
4. The neuropsychological impairments found in our cohort may or may not represent a specific neuropsychological profile, and should be compared to neuropsychological profiles after other infectious or not-infectious diseases that affect the nervous system.
5. Further studies of treatment options of acute LNB and persistent complaints after treated LNB are recommended, both pharmacological and non-pharmacological approaches should be explored.
6. Strategies to reduce the fear of a non-favorable outcome after LNB in the public focusing on the favorable outcome in most LNB treated persons, and the fact that the diagnosis of LNB is commonly unproblematic.

8. References

Reference List

1. Livingston David. Missionary Travels and Reseaches in South Africa. eBook The prodject Gutenberg 2006.

2. Bannwarth A. Chronische lymphocytäre Meningitis, entzündlice Polyneuritis und Reumatismus. Arch Psychiatr Nervenkr 1941;113:284-376.

3. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, et al. The spirochetal etiology of Lyme disease. N Engl J Med 1983;308:733-40.

4. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? Science 1982;216:1317-9.

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

6. Randolph SE. To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? Vet Parasitol 2010;167:92-4.

7. Baranton G, De Martino SJ. Borrelia burgdorferi sensu lato diversity and its influence on pathogenicity in humans. Curr Probl Dermatol 2009;37:1-17.

8. Kjelland V, Stuen S, Skarpaas T, Slettan A. Prevalence and genotypes of Borrelia burgdorferi sensu lato infection in Ixodes ricinus ticks in southern Norway. Scand J Infect Dis 2010;42:579-85.

9. Bhide MR, Travnicek M, Levkutova M, Curlik J, Revajova V, Levkut M. Sensitivity of Borrelia genospecies to serum complement from different animals and human: a host-pathogen relationship. FEMS Immunol Med Microbiol 2005;43:165-72.

10. Stjernberg L, Berglund J. Risk of acquiring tick bites in south-eastern Sweden. Scand J Infect Dis 2002;34:840-4.

11. Maiwald M, Oehme R, March O, Petney TN, Kimmig P, Naser K, et al. Transmission risk of Borrelia burgdorferi sensu lato from Ixodes ricinus ticks to humans in southwest Germany. Epidemiol Infect 1998;121:103-8.

12. Rupprecht T, Fingerle V. Neuroborreliosis: pathogenesis, symptoms, diagnosis and treatment. Future neurology 2011;6:273-89.

13. Vegsundvag J, Nordeide J, Reikvam A, Jenum P. Late cardiac manifestation of infection with Borrelia burgdorferi (Lyme disease). BMJ 1993;307:173.

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

15. Wormser GP, Nadelman RB, Dattwyler RJ, Dennis DT, Shapiro ED, Steere AC, et al. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis 2000;31 Suppl 1:1-14.

16. Ljostad U, Mygland A, Skarpaas T. [Neuroborreliosis in Vest-Agder]. Tidsskr Nor Laegeforen 2003;123:610-3.

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

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

19. Case definitions for infectious conditions under public health surveillance. Centers for Disease Control and Prevention. MMWR Recomm Rep 1997;46:1-55.

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

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

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

23. Reid MC, Schoen RT, Evans J, Rosenberg JC, Horwitz RI. The consequences of overdiagnosis and overtreatment of Lyme disease: an observational study. Ann Intern Med 1998;128:354-62.

24. Djukic M, Schmidt-Samoa C, Nau R, von SN, Eiffert H, Schmidt H. The diagnostic spectrum in patients with suspected chronic Lyme neuroborreliosis--the experience from one year of a university hospital's Lyme neuroborreliosis outpatients clinic. Eur J Neurol 2011;18:547-55.

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

26. Steere AC, Berardi VP, Weeks KE, Logigian EL, Ackermann R. Evaluation of the intrathecal antibody response to Borrelia burgdorferi as a diagnostic test for Lyme neuroborreliosis. J Infect Dis 1990;161:1203-9.

27. Juvkam KH, Eikeland R. [A man with acutely starting pain in the right hip and thigh]. Tidsskr Nor Laegeforen 2011;131:959-61.

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

29. Pfister HW, Rupprecht TA. Clinical aspects of neuroborreliosis and post-Lyme disease syndrome in adult patients. Ijmm InterJ Med Microbiol 2006;296 Suppl 40:11-6.

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

31. Piesman J, Dolan MC. Protection against lyme disease spirochete transmission provided by prompt removal of nymphal Ixodes scapularis (Acari: Ixodidae). J Med Entomol 2002;39:509-12.

32. Rupprecht TA, Koedel U, Fingerle V, Pfister HW. The pathogenesis of lyme neuroborreliosis: from infection to inflammation. Mol Med 2008;14:205-12.

33. Cepok S, Zhou D, Vogel F, Rosche B, Grummel V, Sommer N, et al. The immune response at onset and during recovery from Borrelia burgdorferi meningoradiculitis. Arch Neurol 2003;60:849-55.

34. Cadavid D, O'Neill T, Schaefer H, Pachner AR. Localization of Borrelia burgdorferi in the nervous system and other organs in a nonhuman primate model of lyme disease. Lab Invest 2000;80:1043-54.

35. Strle F, Ruzic-Sabljic E, Logar M, Maraspin V, Lotric-Furlan S, Cimperman J, Ogrinc K, Stupica D, Nadelman RB, Nowakowski J, Wormser GP. Comparison of Erythema Migrans Caused by Borrelia burgdorferi and Borrelia garinii. Vector Borne Zoonotic Dis 2011.

36. Logar M, Ruzic-Sabljic E, Maraspin V, Lotric-Furlan S, Cimperman J, Jurca T, et al. Comparison of erythema migrans caused by Borrelia afzelii and Borrelia garinii. Infection 2004;32:15-9.

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

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

39. Ljøstad U, Skogvoll E, Eikeland R, Midgard R, Skarpaas T, Berglund J, et al. Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. Lancet Neurol 2008;7:690-5.

40. Ulvestad E, Kristoffersen EK. [False positive serological test in suspected borreliosis]. Tidsskr Nor Laegeforen 2002;122:88-90.

41. Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. Evaluation of study patients with Lyme disease, 10-20-year follow-up. J Infect Dis 2001;183:453-60.

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

43. Rupprecht TA, Pfister HW. What are the indications for lumbar puncture in patients with Lyme disease? Curr Probl Dermatol 2009;37:200-6.

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

45. Rupprecht TA, Pfister HW, Angele B, Kastenbauer S, Wilske B, Koedel U. The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis. Neurology 2005;65:448-50.

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

47. Aalto A, Sjowall J, Davidsson L, Forsberg P, Smedby O. Brain magnetic resonance imaging does not contribute to the diagnosis of chronic neuroborreliosis. Acta Radiol 2007;48:755-62.

48. Agarwal R, Sze G. Neuro-lyme disease: MR imaging findings. Radiology 2009;253:167-73.

49. Halperin JJ, Luft BJ, Anand AK, Roque CT, Alvarez O, Volkman DJ, et al. Lyme neuroborreliosis: central nervous system manifestations. Neurology 1989;39:753-9.

50. Logigian EL, Johnson KA, Kijewski MF, Kaplan RF, Becker JA, Jones KJ, et al. Reversible cerebral hypoperfusion in Lyme encephalopathy. Neurology 1997;49:1661-70.

51. Fallon BA, Lipkin RB, Corbera KM, Yu S, Nobler MS, Keilp JG, et al. Regional cerebral blood flow and metabolic rate in persistent Lyme encephalopathy. Arch Gen Psychiatry 2009;66:554-63.

52. Nowakowski J, Nadelman RB, Sell R, McKenna D, Cavaliere LF, Holmgren D, et al. Long-term follow-up of patients with culture-confirmed Lyme disease. Am J Med 2003;115:91-6.

53. Berglund J, Stjernberg L, Ornstein K, Tykesson-Joelsson K, Walter H. 5-y Follow-up study of patients with neuroborreliosis. Scand J Infect Dis 2002;34:421-5.

54. Vrethem M, Hellblom L, Widlund M, Ahl M, Danielsson O, Ernerudh J, Forsberg P. Chronic symptoms are common in patients with neuroborreliosis -- a questionnaire follow-up study. Acta Neurol Scand 2002;106:205-8.

55. Ljostad U, Mygland A. Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. Eur J Neurol 2009;17:118-23.

56. World Health Organization. Quality of life resulution. 1993. 2, 153-159.

57. Coulter A. Measuring Quality of life, in Kinmouth and Jones R (eds) Critical reading in general practice. Oxford: Oxford University press, 1993.

58. Spilker B. Quality of life assessments in clinical trials. New York: Raven Press, 1990.

59. Herlofson K, Larsen JP. The influence of fatigue on health-related quality of life in patients with Parkinson's disease. Acta Neurol Scand 2003;107:1-6.

60. Fayers P, Machin D. Quality of life : the assessment, analysis, and interpretention of patient-reported outcome. Chichester: John Wiley, 2007.

61. Shadick NA, Phillips CB, Logigian EL, Steere AC, Kaplan RF, Berardi VP, Duray PH, Larson MG, Wright EA, Ginsburg KS, Katz JN, Liang MH. The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. *Ann Intern Med* 1994;121:560-7.

62. Shadick NA, Phillips CB, Sangha O, Logigian EL, Kaplan RF, Wright EA, et al. Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Ann Intern Med 1999;131:919-26.

63. Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. Evaluation of study patients with Lyme disease, 10-20-year follow-up. J Infect Dis 2001;183:453-60.

64. Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 1912;345:85-92.

65. Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED. Long-term outcomes of persons with Lyme disease. JAMA 2000;283:609-16.

66. Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, et al. Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial.[see comment]. Neurology 2003;60:1923-30.

67. Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, Slavov I, Cheng J, Dobkin J, Nelson DR, Sackeim HA. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology 2008;70:992-1003.

68. Kowalski TJ, Berth WL, Mathiason MA, Agger WA. Oral antibiotic treatment and long-term outcomes of Lyme facial nerve palsy. Infection 2011;39:239-45.

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

70. Krupp LB, Pollina DA. Mechanisms and management of fatigue in progressive neurological disorders. Curr Opin Neurol 1996;9:456-60.

71. MS Councile for Clinical Practice Guidelines.Fatigue in Multiple Sclerosis. Washington DC: 1998.

72. Hawk C, Jason LA, Torres-Harding S. Differential diagnosis of chronic fatigue syndrome and major depressive disorder. Int J Behav Med 2006;13:244-51.

73. Starkstein SE, Leentjens AF. The nosological position of apathy in clinical practice. J Neurol Neurosurg Psychiatry 2008;79:1088-92.

74. Wessely S. Chronic fatigue: symptom and syndrome. Ann Intern Med 2001;134:838-43.

75. Loge JH, Ekeberg O, Kaasa S. Fatigue in the general Norwegian population: normative data and associations. J Psychosom Res 1998;45:53-65.

76. Lerdal A, Wahl A, Rustoen T, Hanestad BR, Moum T. Fatigue in the general population: a translation and test of the psychometric properties of the Norwegian version of the fatigue severity scale. Scand J Public Health 2005;33:123-30.

77. Greim B, Engel C, Apel A, Zettl UK. Fatigue in neuroimmunological diseases. J Neurol 2007;254 Suppl 2:II102-II106.

78. Liavaag AH, Dorum A, Fossa SD, Trope C, Dahl AA. Controlled study of fatigue, quality of life, and somatic and mental morbidity in epithelial ovarian cancer survivors: how lucky are the lucky ones? J Clin Oncol 2007;25:2049-56.

79. Krupp LB, Alvarez LA, LaRocca NG, Scheinberg LC. Fatigue in multiple sclerosis. Arch Neurol 1988;45:435-7.

80. Krupp L. Fatigue is intrinsic to multiple sclerosis (MS) and is the most commonly reported symptom of the disease. Mult Scler 2006;12:367-8.

81. Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 2006;333:575.

82. Reid S, Chalder T, Cleare A, Hotopf M, Wessely S. Chronic fatigue syndrome. Clin Evid (Online) 2011.

83. Christley Y, Duffy T, Martin CR. A review of the definitional criteria for chronic fatigue syndrome. J Eval Clin Pract may 2010. Doi10.111/j.1365-2753.2010.01512.x

84. Aaron LA, Buchwald D. A review of the evidence for overlap among unexplained clinical conditions. Ann Intern Med 2001;134:868-81.

85. Treib J, Grauer MT, Haass A, Langenbach J, Holzer G, Woessner R. Chronic fatigue syndrome in patients with Lyme borreliosis. Eur Neurol 2000;43:107-9.

86. Sigal LH, Hassett AL. Contributions of societal and geographical environments to "chronic Lyme disease": the psychopathogenesis and aporology of a new "medically unexplained symptoms" syndrome. Environ Health Perspect 2002;110 Suppl 4:607-11.

87. Schutzer SE, Angel TE, Liu T, Schepmoes AA, Clauss TR, Adkins JN, et al. Distinct cerebrospinal fluid proteomes differentiate post-treatment lyme disease from chronic fatigue syndrome. PLoS One 2011;6:e17287.

88. Braley TJ, Chervin RD. Fatigue in multiple sclerosis: mechanisms, evaluation, and treatment. Sleep 2010;33:1061-7.

89. White PD, Goldsmith KA, Johnson AL, Potts L, Walwyn R, DeCesare JC, et al. Comparison of adaptive pacing therapy, cognitive behaviour therapy, graded exercise therapy, and specialist medical care for chronic fatigue syndrome (PACE): a randomised trial. Lancet 2011;377:823-36.

90. Krupp LB, Masur D, Schwartz J, Coyle PK, Langenbach LJ, Fernquist SK, et al. Cognitive functioning in late Lyme borreliosis. Arch Neurol 1991;48:1125-9.

91. Bujak DI, Weinstein A, Dornbush RL. Clinical and neurocognitive features of the post Lyme syndrome. J Rheumatol 1996;23:1392-7.

92. Ravdin LD, Hilton E, Primeau M, Clements C, Barr WB. Memory functioning in Lyme borreliosis. J Clin Psychiatry 1996;57:282-6.

93. Gaudino EA, Coyle PK, Krupp LB. Post-Lyme syndrome and chronic fatigue syndrome. Neuropsychiatric similarities and differences. Arch Neurol 1997;54:1372-6.

94. Pollina DA, Sliwinski M, Squires NK, Krupp LB. Cognitive processing speed in Lyme disease. Neuropsychiatry Neuropsychol Behav Neurol 1999;12:72-8.

95. Anderson V, Jacobs, Anderson PJ. Executive functions and the frontal lobes. Studies on neuropsychology, Neurology and Cognition. Bieliauskas: 2008.

96. Delis D, Kaplan E, Kramer J. Dehlis-Kaplan Executive Function System. Norwegian manual. San Anthonio TX: The Psychological cooperation, 2005.

97. Bosnes O. California Verbal Learning Test-II (CVLT-II) utprøvd i et klinisk utvalg i Norge: Psykometriske egenskalper og krysskulturell validitet. Tidsskrift for Norsk Psykologforening 2007;44:887-92.

98. Engvik H, Hjerkinn O, Seim S. Handbook for WAIS Wechsler Adult Intelligence Scale. Oslo (N): The Norwegian psychological Association, 1992.

99. Spreen O, Strauss E. A compendium of neuropsychological tests: administration, norms, and commentary. New York: Oxford University Press, 1998:XVI, 736.

100. Westervelt HJ, McCaffrey RJ. Neuropsychological functioning in chronic Lyme disease. Neuropsychol Rev 2002;12:153-77.

101. Benke T, Gasse T, Hittmair-Delazer M, Schmutzhard E. Lyme encephalopathy: long-term neuropsychological deficits years after acute neuroborreliosis. Acta Neurol Scand 1995;91:353-7.

102. Kaplan RF, Meadows ME, Vincent LC, Logigian EL, Steere AC. Memory impairment and depression in patients with Lyme encephalopathy: comparison with fibromyalgia and nonpsychotically depressed patients. Neurology 1992;42:1263-7.

103. Pollina DA, Elkins LE, Squires NK, Scheffer SR, Krupp LB. Does process-specific slowing account for cognitive deficits in Lyme disease? Appl Neuropsychol 1999;6:27-32.

104. Svetina C, Barr WB, Rastogi R, Hilton E. The neuropsychological examination of naming in Lyme borreliosis. Appl Neuropsychol 1999;6:33-8.

105. Kaplan RF, Jones-Woodward L, Workman K, Steere AC, Logigian EL, Meadows ME. Neuropsychological deficits in Lyme disease patients with and without other evidence of central nervous system pathology. Appl Neuropsychol 1999;6:3-11.

106. Keilp JG, Corbera K, Slavov I, Taylor MJ, Sackeim HA, Fallon BA. WAIS-III and WMS-III performance in chronic Lyme disease. J Int Neuropsychol Soc 2006;12:119-29.

107. McAuliffe P, Brassard MR, Fallon B. Memory and executive functions in adolescents with posttreatment Lyme disease. Appl Neuropsychol 2008;15:208-19.

108. Kaplan RF, Trevino RP, Johnson GM, Levy L, Dornbush R, Hu LT, et al. Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology 2003;60:1916-22.

109. Clark JR, Carlson RD, Sasaki CT, Pachner AR, Steere AC. Facial paralysis in Lyme disease. Laryngoscope 1985;95:1341-5.

110. Steere AC, Glickstein L. Elucidation of Lyme arthritis. Nat Rev Immunol 2004;4:143-52.

111. Sigal LH. Immunologic mechanisms in Lyme neuroborreliosis: the potential role of autoimmunity and molecular mimicry. Semin Neurol 1997;17:63-8.

112. Rupprecht TA, Elstner M, Weil S, Pfister HW. Autoimmune-mediated polyneuropathy triggered by borrelial infection? Muscle Nerve 2008;37:781-5.

113. Tjernberg I, Carlsson M, Ernerudh J, Eliasson I, Forsberg P. Mapping of hormones and cortisol responses in patients after Lyme neuroborreliosis. BMC Infect Dis 2010;10:20.doi:10.86/1471-2334-10-10

114. Brorson O, Brorson SH. In vitro conversion of Borrelia burgdorferi to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium. Infection 1998;26:144-50.

115. Stricker RB, Delong AK, Green CL, Savely VR, Chamallas SN, Johnson L. Benefit of intravenous antibiotic therapy in patients referred for treatment of neurologic Lyme disease. Int J Gen Med 2011;4:639-46.

116. Wang TJ, Liang MH, Sangha O, Phillips CB, Lew RA, Wright EA, et al. Coexposure to Borrelia burgdorferi and Babesia microti does not worsen the long-term outcome of lyme disease. Clin Infect Dis 2000;31:1149-54.

117. Ramsey AH, Belongia EA, Gale CM, Davis JP. Outcomes of treated human granulocytic ehrlichiosis cases. Emerg Infect Dis 2002;8:398-401.

118. Kristiansen BE, Jenkins A, Tveten Y, Karsten B, Line O, Bjoersdorff A. [Human granulocytic ehrlichiosis in Norway]. Tidsskr Nor Laegeforen 2001;121:805-6.

119. Stuen S, Bergstrom K. [Human anaplasmosis--a hidden disease in Norway?]. Tidsskr Nor Laegeforen 2008;128:2579-81.

120. Fallon BA, Nields JA. Lyme disease: a neuropsychiatric illness. Am J Psychiatry 1994;151:1571-83.

121. Solomon SP, Hilton E, Weinschel BS, Pollack S, Grolnick E. Psychological factors in the prediction of Lyme disease course. Arthritis Care Res 1998;11:419-26.

122. Hajek T, Libiger J, Janovska D, Hajek P, Alda M, Hoschl C. Clinical and demographic characteristics of psychiatric patients seropositive for Borrelia burgdorferi. Eur Psychiatry 2006;21:118-22.

123. Hassett AL, Radvanski DC, Buyske S, Savage SV, Gara M, Escobar JI,et al. Role of psychiatric comorbidity in chronic Lyme disease. Arthritis Rheum 2008;59:1742-9.

124. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:382-9.

125. Starkstein SE, Mayberg HS, Preziosi TJ, Andrezejewski P, Leiguarda R, Robinson RG. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci 1992;4:134-9.

126. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.

127. Loge JH, Kaasa S. Short form 36 (SF-36) health survey: normative data from the general Norwegian population. Scand J Soc Med 1998;26:250-8.

128. Ware JE, Jr. SF-36 health survey update. Spine (Phila Pa 1976) 2000;25:3130-9.

129. Rohde G, Mengshoel AM, Wahl AK, Moum T, Haugeberg G. Is health-related quality of life associated with the risk of low-energy wrist fracture: a case-control study. BMC Musculoskelet Disord 2009;10:80.

130. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121-3.

131. Delis D, Kramer J, Kaplan E, Ober B. CVLT-II : California Verbal Learning Test-II. San Antonio, TX: The Psychological Corporation, 2000.

132. Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: a practical guide for clinicians and researchers. J Psychosom Res 2004;56:157-70.

133. Wilson RS, Rosenbaum G, Brown G, Rourke D, Whitman D, Grisell J. An index of premorbid intelligence. J Consult Clin Psychol 1978;46:1554-5.

134. Bakken JS, Krueth J, Tilden RL, Dumler JS, Kristiansen BE. Serological evidence of human granulocytic ehrlichiosis in Norway. Eur J Clin Microbiol Infect Dis 1996;15:829-32.

135. Psykiske lidelser i Norge: et folkehelseperspektiv (Mental health disorders in Norway: a public health perspective). Oslo: Nasjonalt folkehelseinstitutt, 2009:81.

136. Wechsler D. The Wechsler Adult Intelligence Scale. san Antonio TX: Psychological Corporation, 1997.

137. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.

138. Benito-Leon J, Cubo E, Coronell C. Impact of apathy on health-related quality of life in recently diagnosed Parkinson's disease: The ANIMO study. Mov Disord 2011.doi: 10.1002/mds.23872

139. Krabbe NV, Jensen TE, Nielsen HI. [Neuroborreliosis in North Jutland]. Ugeskr Laeger 2008;170:3420-4.

140. Andreasen A, Stenager E, Dalgas U. The effect of exercise therapy on fatigue in multiple sclerosis. Mult Scler 2011;9. 1041-54

141. Barr WB, Rastogi R, Ravdin L, Hilton E. Relations among indexes of memory disturbance and depression in patients with Lyme borreliosis. Appl Neuropsychol 1999;6:12-8.

142. Fallon BA, Keilp J, Prohovnik I, Heertum RV, Mann JJ. Regional cerebral blood flow and cognitive deficits in chronic lyme disease. J Neuropsychiatry Clin Neurosci 2003;15:326-32.

143. Elkins LE, Pollina DA, Scheffer SR, Krupp LB. Psychological states and neuropsychological performances in chronic Lyme disease. Appl Neuropsychol 1999;6:19-26.

144. Krupp LB, Elkins LE. Fatigue and declines in cognitive functioning in multiple sclerosis. Neurology 2000;55:934-9.