Neurofilament Light in Cerebrospinal Fluid is Associated With Disease Staging in European Lyme Neuroborreliosis

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ABSTRACT

BACKGROUND: Drivers of differences in disease presentation and symptom duration in Lyme neuroborreliosis (LNB) are currently unknown.

OBJECTIVES: We hypothesized that neurofilament light (NfL) in cerebrospinal fluid (CSF) would predict disease location and sequelae in a historic LNB cohort.

DESIGN: Using a cross-sectional design and archived CSF samples from 185 patients diagnosed with LNB, we evaluated the content of NfL in the total cohort and in a subgroup of 84 patients with available clinical and paraclinical information.

METHODS: Individuals were categorized according to disease location: a. Central nervous system (CNS) with stroke (N=3), b. CNS without stroke (N=11), c. Peripheral nervous system (PNS) with cranial nerve palsy (CNP) (N=40) d. PNS without CNP (N=30). Patients with hospital follow-up more than 6 months after completed antibiotic therapy were categorized as having LNB associated sequelae (N=15).

RESULTS: At diagnosis concentration of NfL exceeded the upper reference level in 60% (105/185), especially among individuals above 30 years. Age-adjusted NfL was not found to be associated with symptom duration. Age-adjusted NfL was significantly higher among individuals with CNS involvement. Category a. (stroke) had significantly higher NfL concentrations in CSF compared to all other categories, category b. (CNS involvement without stroke) had significantly higher values compared to the categories of PNS involvement. We found no significant difference between the categories with PNS involvement (with or without CNP). Significantly higher NfL was found among patients with follow-up in hospital setting.

CONCLUSION: Comparison of NfL concentrations between the 4 groups of LNB disease manifestations based on clinical information revealed a hierarchy of neuron damage according to disease location and suggested evolving mechanisms with accelerated injury especially when disease is complicated by stroke. Higher values of NfL among patients with need of follow-up in hospital setting suggest NfL could be useful to identify rehabilitative needs.

KEYWORDS: central nervous system, encephalitis, infections, meningitis, pain, peripheral nervous system infections

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Introduction

Background

Lyme neuroborreliosis (LNB) is caused by infection with the spirochete *Borrelia burgdorferi* sensu lato complex (*B burgdorferi*) and is the most frequent tick-borne central nervous system (CNS) infection in Europe with incidence rates estimated to be 3.2-6.3 per 100,000/year.[1-7]

Typical symptoms of early European LNB include painful meningoradiculitis and facial palsy (called Bannwarth syndrome). Left untreated, the infection can evolve into the late stage. A condition involving chronic meningitis or chronic progressive encephalomyelitis sometimes complicated with lacunar infarction. Symptoms include headache, malaise, significant weight loss, progressive spastic-ataxic gait disturbance, sensorineural hearing loss and cognitive complaints.[8] Although antibiotic treatment causes symptom relief in most infected individuals and is associated with a good long-term prognosis,[9] the disease course is highly variable, rendering some with long-term sequelae or prolonged cognitive and emotional symptoms.[10] Currently, it is unclear what drives differences in disease presentation and symptom burden. Controversies exist as to whether persistent symptoms reflects active infection, post-infectious immune phenomena or other factors[11]

B.burgdorferi is thought to enter the CNS from the blood by crossing the blood-brain barrier, although dissemination through peripheral nerves may also occur.[12] *B burgdorferi* can be cultured from cerebrospinal fluid (CSF) but in low quantity.[13,14] The pathogenesis of LNB stems from the inflammatory response triggered by the spirochete.[15] In the rhesus macaque model and the few autopsies from humans with LNB, leptomeningitis with perivascular infiltration involving both the pia mater and arachnoid has been described[15] as well as neuritis, necrotizing myelitis with degeneration and inflammation in the spinal nerve roots and vasculitis located to the brainstem.[16]

Neurofilament light (NfL) is a biomarker of neuron damage.[17] Neurofilaments are intermediate filaments of the neuronal cytoskeleton and highly expressed in large-caliber myelinated axons.[17,18] Under physiological conditions, low amounts of NfL are released into the interstitial space and into the CSF, as well as into the blood.[19] NfL in CSF and serum has been shown to increase with age, presumably reflecting age-related neuronal and axonal injury and loss, while in patients with CNS pathology associated with axonal injury or degeneration, increased amounts are released from neurons into the interstitial space and into the CSF.[20] Pathologic amounts of NfL in CSF has been associated with neuron damage in multiple diseases including Alzheimer's disease, HIV-associated dementia, active multiple sclerosis, amyotrophic lateral sclerosis, frontotemporal dementia[21] and peripheral neuropathy.[22,23] NfL concentrations accurately reflects the amount of neuron damage, as shown in age-related brain changes[20] and infections such as herpes zoster and HIV-1.[24,25]

Objective

We hypothesized that the concentration of NfL in CSF would be associated with the degree of neuronal damage in patients diagnosed with LNB and be associated with delayed recovery.

Methods

Study design

The study was designed as a retrospective cohort study.

Ethical Statement

The study was granted permission by the Ethical Committee of the Capital Region of Denmark, (Journal number H-19027317) and the Regional Data Protection Center of the Capital Region, Denmark (P-2019-707). Because we were working with historic samples a dispensation from written informed consent from patients was granted by the Ethical Committee of the Capital Region of Denmark.

Setting

The study was initiated at the Department of Infectious Diseases at Rigshospitalet, University Hospital of Copenhagen, in December 2020. Patients were identified in collaboration with the Danish National Patient Registry, and CSF samples obtained in February 2021. Samples were sent to Gothenburg University, Laboratory of Neurochemistry for analyses in February 2021. Clinical information was recorded from electronic patient files. NfL analyses were completed in May 2021. Data analyses were conducted, and the manuscript prepared in summer and autumn 2021.

Participants

We identified all archived samples (stored at −20 °C) at the Danish Biobank Register[26] from individuals with a first-time positive *B burgdorferi*-specific intrathecal antibody test in the period 2001-2011 and with the ICD-10 diagnosis code Borreliosis: A69.2.

Variables

Verification of the Lyme neuroborreliosis diagnosis. To confirm the diagnosis of LNB, patient files were systematically reviewed by an infectious disease specialist with experience in LNB diagnostics.

A definite LNB diagnosis was based on: (i) The presence of typical neurological symptoms, (ii) CSF leucocytes ≥10 × 10^6 cells/L, and (iii) A positive intrathecal *B burgdorferi* antibody production index. Probable LNB was defined as 2 of the 3 above criteria.[27]

In the period 2001-2015 the IDEIA flagella antigen-based enzyme-linked immunosorbent assay LNB test (Oxoid

Hampshire, United Kingdom) was used for detection of intrathecal synthesis of *B burgdorferi* s.l.-specific IgG and IgM antibodies.[28] An antibody production index >.3 was considered as positive according to the manufacturer's instructions.

Symptom duration

Onset of LNB was defined as the onset of neurological symptoms and the duration of neurological symptoms was defined as the number of days from onset of neurological symptoms to first hospital contact.

Symptoms and signs of Lyme neuroborreliosis

Symptoms and signs were obtained from patient files. The following symptoms were registered: radiating pain, fever, headache, chronic headache (duration for more than 6 months), peripheral cranial nerve palsies (nerve III, VI or VII), uni- or bilateral cranial nerve palsy, limb palsies (upper, lower extremities, hemi- or paraparesis), consciousness, sensory disturbances, gait-disturbances, stroke (MRI verified), cognitive complaints, seizures, vomiting, encephalopathy or other (free text).

Categorizing neurological involvement of Lyme neuroborreliosis

Neurological complications were then categorized based on involvement of CNS or peripheral nervous system (PNS) into 4 categories:

a. CNS involvement with stroke b. CNS involvement without stroke c. PNS involvement with cranial nerve palsy (CNP) and d. PNS involvement without CNP.

CNS involvement was defined as disease duration for more than 6 months with symptoms of either chronic progressive encephalomyelitis (defined as fluctuating consciousness with or without cognitive deficits, spastic-ataxic gait, seizures), chronic meningitis (symptoms of chronic headache, fever, vomiting, mental cloudiness) with or without lacunar infarction (MRI-verified).

PNS involvement was defined as peripheral cranial nerve palsy (peripheral bi- or unilateral cranial nerve palsies of cranial nerve III, VI or VII), radiculitis (radiating pain in back, head, truncus, or extremities characteristic for LNB).

The grading was hierarchical in the sense that a patient identified with several neurological manifestations were categorized under the dominating clinical picture, eg a patient with chronic encephalomyelitis, stroke and bilateral facial palsies would be categorized under CNS involvement with stroke. A patient with unilateral facial palsy and radiating pains would be categorized under PNS involvement with peripheral cranial nerve palsy.

Sequelae to Lyme neuroborreliosis was defined as having follow-up under the diagnosis Borreliosis: A69.2 in hospital setting, 6 months after the end of antibiotic treatment.

Paraclinical information

Information on CSF leukocytosis and total protein count were retrieved from patient files at the initial spinal tap performed when the patient was diagnosed with LNB

Data sources/measurements

All clinical and paraclinical information was retrieved from patient files.

NfL measurements were performed at University of Gothenburg, at the institute of Neuroscience and Physiology, Laboratory of Neurochemistry using the NF-Light kit from Quanterix (Billerica, MA). The measurements were performed as follows. Calibrators were run in duplicates and obvious outlier calibrator replicates were masked before curve fitting. Samples were diluted 100-fold and run in singlicates. Results were compensated for the dilution. Two quality control (QC) levels were run in duplicates in the beginning and the end of each run. For QC with concentration 10 pg/mL, repeatability was 2.9% and intermediate precision was 5.2%. For QC with concentration 107.6 pg/mL, repeatability was 3.6% and intermediate precision was 3.9%. Dynamic range = 1.9-1800 pg/mL lower limit of quantification (LLOQ) = 1.9 pg/mL. To compare NfL values with established reference values, results were normalized to the assay described by Gaetani et al.[29]

Bias

We were not able to verify the LNB diagnosis in 86 cases where patient files were not available. Patient reported symptom duration rely on memory and may thus be inaccurate, especially for longer symptom durations. We did not have access to patient follow-up outside hospital setting, therefore the proportion of patients with sequelae may be underestimated in this study.

Study size

With an anticipation of differences of means of .5 Log NfL, alpha value of .05 and power of 80%, a sample size of minimum of 12 individuals in each group (of neurological deficiency) was estimated. With 4 categories of neurological deficits a total of 50 patients with LNB would have been sufficient we aimed at including about 200 to compensate for lack of access to patient files, lack of sufficient CSF material or NfL analysis failure.

Quantitative variables

Upper reference values for NfL were calculated based on Yilmaz et al. (201.2×1.031^{age}). When comparing CSF NfL values between groups, we performed an age adjustment of CSF NfL based on the formula (CSF NfL (pg/mL)=97.5×1.031^{age}). Adjusting to the median age of 52 years.

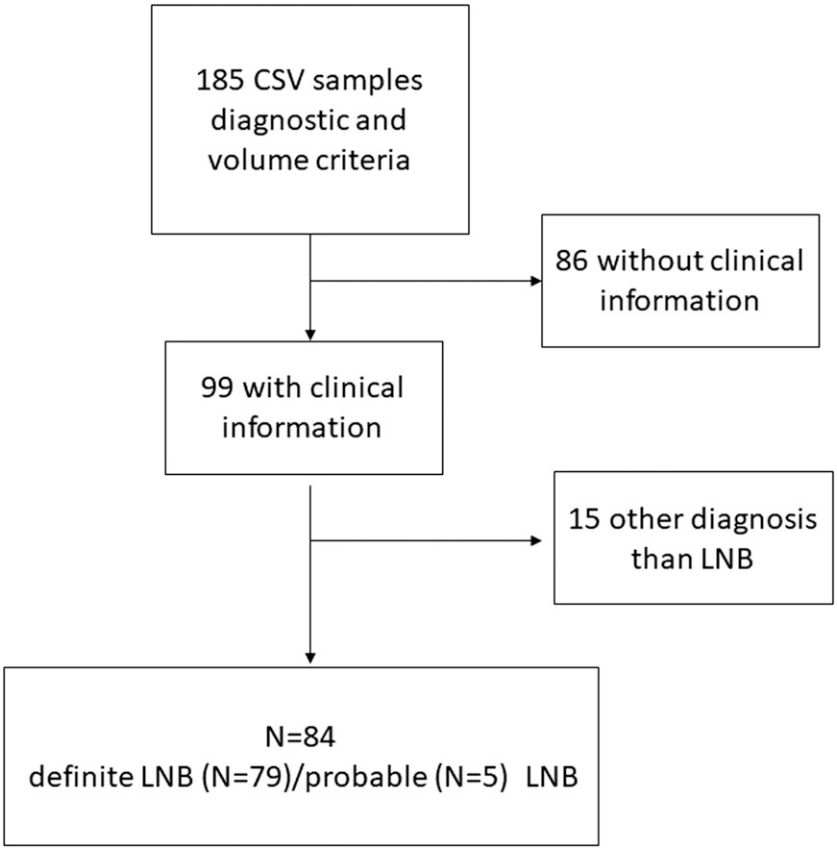

Figure 1. Flow chart of inclusion.

Statistical methods

Descriptive statistics are given in median values and interquartile ranges. For comparisons of groups two-tailed Mann Whitney test was used and a *P*-value below .05 was interpreted as significant.

For correlation analyses simple linear regression was applied, Goodness of Fit was estimated by R-squared test and *P*-values of below .05 was regarded significant for comparison of slope value deviation from zero.

Statistical test was performed using the GraphPad PRISM version 9.0.0 version (GraphPad Software, LLC).

Results

Participants

A total of 701 samples (serum and CSF) were identified at the at Danish Biobank Register. Of these 197 were CSF and 185 samples met our volume criterion of 200 µL (Figure 1).

Clinical data was available in 99 of the 185 patients. After careful revision of patient records, a total of 15/99 individuals were excluded because another diagnosis than LNB was found more likely (multiple sclerosis (n=4), hematologic cancer (n=2), epileptic seizure (n=1), viral meningitis (n=3), transitory ischemic attack (n=1), neurosarcoidosis (n=1) and history with LNB diagnosis, not meeting criteria for active infection (n=3)).

Of the remaining 84 patients, 5 had probable LNB and 79 had definite LNB based in accordance to guidelines.[27] Flow chart of patient inclusion and baseline clinical characteristics are illustrated in Figure 1 and Table 1.

Descriptive Data

Baseline characteristics and NFL values are listed in Table 1.

In total 105 of the total cohort of 185 individuals (57%) had NfL values above the upper reference at diagnosis. Of the 84 LNB patients with clinical information available, baseline characteristics (Table 1). A total of 50/84 (60%) LNB patients had NfL above upper reference level at diagnosis.

Outcome data

No association between age and symptom duration and age-adjusted Neurofilament Light. Unadjusted NfL values (pg/mL) as a function of age (years) with reference values is illustrated in punctuated line in Figure 2. As expected, a positive correlation between age and NfL was observed (P=.0099, R-squared .078, simple linear regression analyses). A larger fraction of patients having NfL above reference level was observed with increasing age. Median values of NfL (pg/mL) and interquartile ranges are listed in Table 1. Interestingly median value for patients younger than 30 was below the upper

Table 1. Baseline characteristics.

CHARACTERISTICS TOTAL COHORT (N=185)	NO. (%) OF PATIENTS OR MEDIAN (IQR)
Age, years	54 (38-66)
Male gender	93 (50)
NfL (pg/ml) measured	864.3 (459.3-1920)
NfL normalized to gaetani et al	1143 (640.5-2452)
Patients with clinical information (N=84)	
Age (years)	52 (34-63)
Male sex	47 (56)
NfL (pg/ml) measured	827.2 (475.6-1915)
NfL normalized to gaetani et al	1097 (660.8-2446)
Patients <30 (ref <380 pg/mL) (N=16)	215.6 (152.4-363.5)
Patients 30-<40 (ref<560 pg/mL) (N=10)	1033 (729.4-1963)
Patients 40-<60 (ref<890 pg/mL) (N=28)	1171 (748.2-3443)
Patients ≥60 (ref <1850 pg/mL) (N=30)	1639 (999.3-4292)
Symptom duration before diagnosis (days)	21 (9.5-45)
Symptoms	
Fever	15/84 (18)
Headache	5/84 (6)
Constipation	3/84 (4)
Cranial nerve paresis	43/84 (51)
Abducens (6th nerve)	2/84 (2)
Facial (7th nerve)	41/84 (49)
Unilateral	38/43 (88)
Radiating pain	42/84 (50)
CNS symptoms*	14/84 (6)
stroke	3/84 (3)
CSF information (N=72)	
Leukocytes (10⁶/L, ref<5)	121 (54-250)
Protein (g/L reference interval .15-.5)	1.03 (1.78-.63)

*defined as duration over 6 months with 1 or more of the following symptoms: fluctuating consciousness, cognitive deficits, spastic-ataxic gait, seizures or progressive weakness.

reference value, whereas all other age-groups had median values above the upper reference value. After adjusting for age related increase in NfL,[30] the association between age and NfL was no longer significant (P=.4220, R-squared .0079; data not shown).

We also investigated the relationship between symptom duration and the concentration of NfL in CSF. A simple linear regression analyses revealed no significant association between age-adjusted level of NfL in CSF and symptom duration and diagnosis in LNB (P=.13, R-squared = .03; data not shown).

Neurofilament Light in cerebrospinal fluid is associated with disease stage of Lyme neuroborreliosis. Patients with CNS involvement had significantly higher levels of age adjusted NfL in CSF compared to patients with PNS involvement only (Table 2, Figure 3).

Further analyses of the 4 subcategories a. CNS with stroke (N=3) b. CNS without stroke (N=11) c. PNS with CNP (N=40) and d. PNS without CNP (N=30) (Table 2, Figure 4) showed that patients with CNS involvement and stroke had significantly higher levels of age adjusted NfL in CSF compared to all other categories. Patients with CNS involvement but no stroke also had significantly higher levels of age adjusted NfL in CSF compared to patients with PNS involvement. We found no difference in age-adjusted NfL between patients with PNS involvement.

Age-adjusted NfL values as a function of age with disease location indicated in gray-scale colors are shown in Figure 5. Interestingly location to the PNS were almost exclusively observed among patients less than 30 years whereas disease involving CNS were observed with increasing age and associated with increasing NfL, especially among patients with stroke.

All analyses were re-run excluding the 5 patients with probable LNB, with no overall change in results.

Persistent symptoms 6 months after the end of antibiotic treatment is associated with higher NfL at diagnosis. Of the 84 patients, 15 had follow-up visits in a hospital setting 6 months after having completed antibiotic treatment due to sequelae after LNB (18%). Of the 15 patients who were followed-up in a hospital setting 10/15 had CNS involvement (2/10 stroke) and 5/15 had PNS involvement (3 radiculitis and 2 unilateral cranial nerve palsy).

Persistent symptoms 6 months after the end of antibiotic treatment were associated with higher levels of age adjusted NfL in CSF at admission (median 3.46 (IQR 3.05-3.73) vs median 3.06 (IQR 2.83-3.26), P=.02, Figure 5.

NfL correlates with total protein but not leukocytosis in CSF. Leukocyte count in CSF was available in 71 patients; in 13 we had information on CSF pleocytosis from patient files, but the exact leukocyte count could not be retrieved.

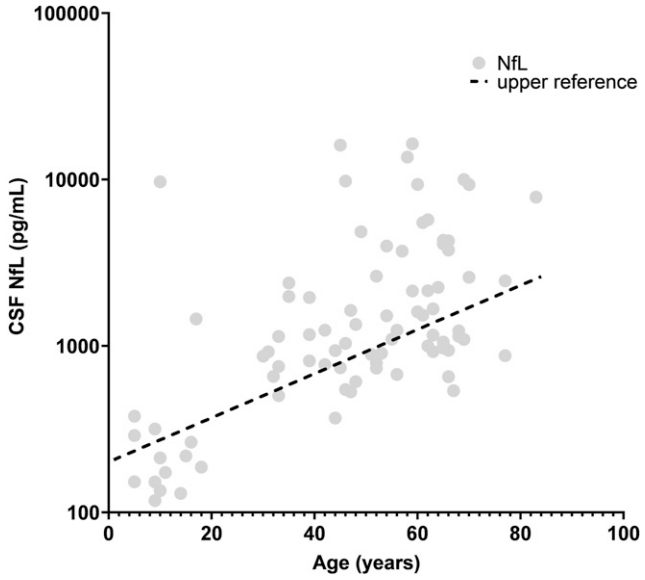

Figure 2. NfL in CSF as function of age in years with NfL in gray dots and upper reference limit in punctuated line.

No association was found between the total leukocyte count in CSF and age-adjusted levels of NfL in CSF by simple linear regression (P=.63, R square = .003; data not shown).

However, a simple linear regression analyses of age-adjusted NfL in CSF and total protein in CSF revealed a significant correlation (P<.0001, R-square = .27), see Figure 6. These results could indicate an association between the degree of blood-brain barrier disruption and neuroaxonal damage during LNB. Figure 7.

Discussion

In this retrospective study of patients with LNB we found NfL levels in CSF to be elevated at diagnosis in over half of patients and to be significantly associated with disease location and severity. Our study confirms late stage LNB is associated with parenchymatous CNS involvement with neuronal injury and accelerated neuronal loss when complicated with stroke.

Baseline information of this cohort of verified LNB with accessible clinical information was found to be as expected for Denmark in regards to age, sex and symptomatology.[4] Classical Bannwarth syndrome was the most prevalent disease presentation, seventeen percent had late stage disease, only 4 percent suffered stroke.

In Lyme disease, organ involvement most often arises sequentially; local skin manifestations are followed by involvement of the PNS in form of painful lymphocytic meningo-radiculitis. Chronic progressive encephalomyelitis, due to chronic lymphocytic meningitis arises as a late stage CNS manifestation.[8] The underlying pathologic processes driving late stage infection are unclear due to limited possibilities to access relevant tissue and difficulties quantifying infection with the spirochete – active infection, post-infectious immune phenomena or other factors are considered.[11]

Table 2. Compatison of age-adjusted NfL between patient catagories (wilcoxon test).

LOCALIZATION (NO.)	MEDIAN NFL (IQR)	*P*-VALUE		
CNS (14)	3.52 (IQR 3.15-4.08)		<.01	
PNS (70)	3.03 (IQR 2.82-3.24)			
Categories		vs a	vs b	vs c
a.CNS w. stroke (3)	4.30 (IQR 4.07-4.55)			
b.CNS no stoke (11)	3.42 (IQR 3.08-3.61)	.01		
c.PNS with CNP (40)	3.05 (IQR 2.82-3.20)	<.01	<.01	
d.PNS no CNP (30)	3.04 (IQR 2.82-3.45)	<.01	.03	.46
Hospital follow-up				
Follow-up (15)	3.46 (IQR 3.05-3.73)		.02	
No follow-up (69)	3.06 (IQR 2.83-3.26)			

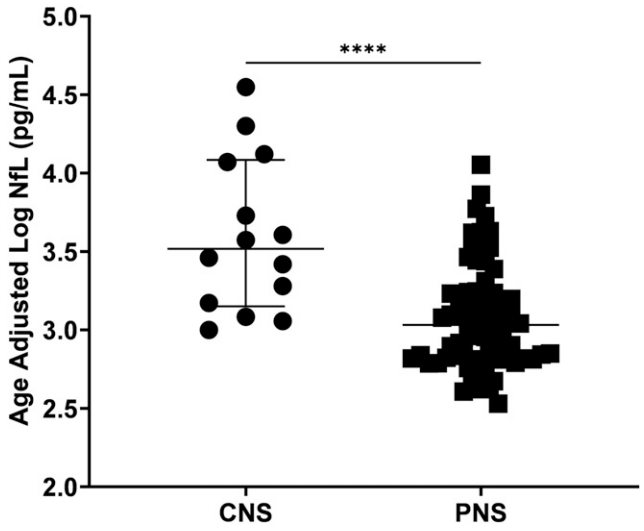

Figure 3. Scatter plot with age adjusted Log NfL (pg/mL) in patients with CNS (black dots) or PNS disease (black squares). lines indicate median with IQR, p-value indicated with asterisks. Asterisks representing P value classification: **** P value <0.0001, *** P value 0.0001 to 0.001, ** P value 0.001-0.01, * P value 0.01-0.05, ns P value >0.05.

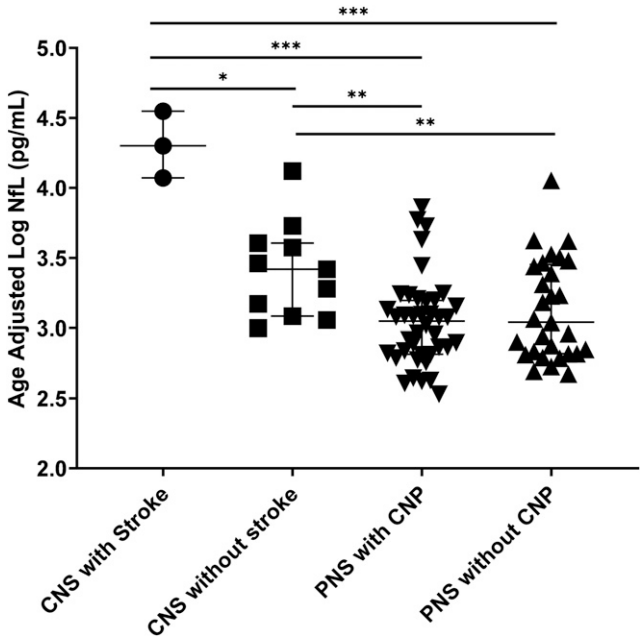

Figure 4. Scatter plot of age adjusted Log NfL (pg/mL) in four categories of disease location. a. CNS with stroke (black dots) b. CNS without stroke (black squares) c. PNS iwth CNP (inverted black triangles) d. PNS without CNP (black triangles). Lines indicating median with IQR. p-values indicated with asterisks. Asterisks representing P value classification: **** P value <0.0001, *** P value 0.0001 to 0.001, ** P value 0.001- 0.01, * P value 0.01-0.05, ns P value >0.05.

Interestingly, we found a clear association between quantities of NfL in CSF and disease location in the nervous system. NfL levels were generally high at time of diagnosis which has been reported in previous smaller studies.[31,32] Comparable concentrations of NfL have been observed in inflammatory demyelinating diseases such as active multiple sclerosis, advanced Alzheimer's disease[21,33] and herpes zoster meningo-encephalitis.[24] Highest quantities were observed in individuals with complicating stroke, approaching the levels reported in HIV associated dementia[25] and herpes simplex type-1 encephalitis.[34] Lowest quantities were observed in patients with PNS involvement. Here, we did not find significant differences between NfL concentrations among individuals with complicating peripheral cranial nerve palsy compared to those with radiculitis only. This finding probably reflects a relatively small contribution of peripheral cranial nerve injury to the relatively large nerve roots of the spinal canal, in early Lyme borreliosis.

Together, our results suggest that NfL as a biomarker accurately reflects the greater disease burden with resulting more neuron damage when infection locates to CNS. This finding is in good agreement with other studies showing NfL to be a sensitive marker of disease severity in herpes zoster and HIV[24,30] and disease activity in MS.[35] This current study cannot determine the underlying disease processes of late stage LNB but only validate the greater neuron loss associated with disease evolution. A longitudinal study will be helpful in evaluating the effect of antibiotic treatment and thus indirectly shed light on underlying pathology.

NfL above the upper reference level was more frequent in older age groups; only 20% individuals younger than 30 years of age had NfL above the upper reference limit at time of diagnosis, whereas 45-90% of patients older than 30 years had elevated NfL at diagnosis. However, age was also associated with more severe disease manifestations with higher frequency of CNS involvement. The cut-off for the reference value in this study was derived from Yilmaz et al.,[30] based on CSF NfL measures in 359 healthy individuals. The yearly increase in CSF NfL was observed to be 3.1%. Other studies have found a similar age-related increase.[36] However, recent studies of serum-NfL[20,35] reported a stable age-related increase op to 50-60 years but thereafter an accelerated increase. These estimates were based on serum values of NfL, which may be influenced by other factors such as BMI and glomerular filtration rates and may not be directly transferrable to our study.

Significantly higher levels of age-adjusted CSF NfL at diagnosis was observed in patients who later had follow-up in a hospital setting due to LNB-associated sequelae. Although, most of these individuals had a greater disease burden with CNS involvement, we speculate that NfL levels may provide a measure to identify individuals in need of rehabilitation. In this study we only had access to follow-up provided in hospital setting and thus a very rough estimate of sequelae. A more detailed prospective study with information on quality of life, pain and cognition is needed to clarify this aspect further.

In line with previous reports,[32] a significant association between the level of CSF total protein and age-adjusted levels of CSF NfL was observed. In LNB raised CSF total protein concentrations are likely caused by disruption of the blood-

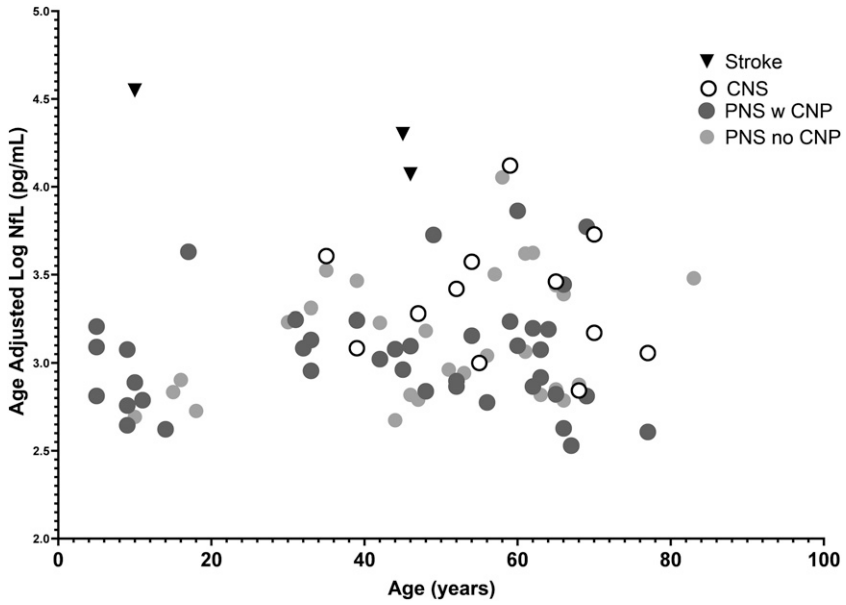

Figure 5. Age adjusted Log NfL (pg/mL) as a function of age with disease location indicated with symbols. CNS with stroke (inverted black triangles), CNS without stroke (white dots), PNS with CNP (dark gray dots) and CNS without CNP (light gray dots).

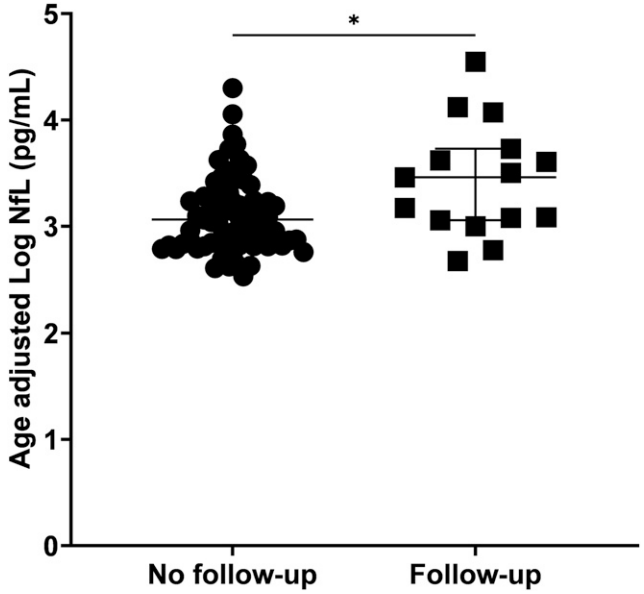

Figure 6. Scatter plot showing age adjusted Log NfL (pg/mL) in patients with no hospital follow-up for LNB (black dots) and with hospital follow-up for LNB (black squares), lines indicate median and IQR, p value indicated with asterisk. Asterisks representing P value classification: **** P value <0.0001, *** P value 0.0001 to 0.001, ** P value 0.001-0.01, * P value 0.01-0.05, ns P value >0.05.

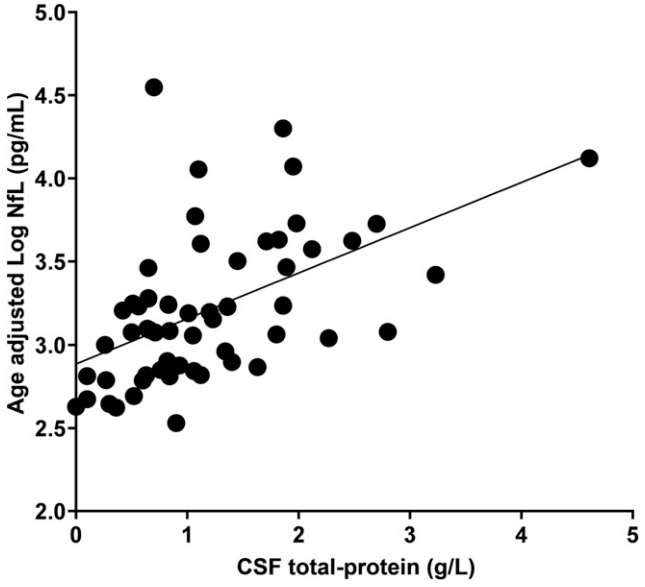

Figure 7. Age adjusted Log NfL (og/mL) plotted against total protein in CSF (g/L). Line simple linear regression R squared 0.27, p<0.0001.

brain, blood-CSF and blood-nerve barrier[37] as well as intrathecal immunoglobulin synthesis.[38,39] As albumin constitutes the largest fraction of total protein in CSF we speculate that the association is driven by albumin which is a surrogate maker for blood-brain, blood-CSF and blood-nerve barrier. However, we were not able to further investigate underlying drivers of this association, because serum and CSF albumin concentrations were not available.

Limitations

Although 185 CSF samples with a positive intrathecal synthesis of *B burgdorferi* specific antibodies were identified, we were only able to get clinical information on 99 of these and we verified the diagnosis in 84 individuals, which is a limitation of the study. Although good clinical data were available from these individuals at admission, we did not have detailed follow-up data, other than subsequent hospital contacts under the Borreliosis diagnosis, which is another limitation of the study.

Interpretation

The collective findings suggest a hierarchy of injury and its detection that not only have pathogenetic implications but also potential application in the clinical evaluation and staging of Lyme-related neuronal injury and possibly response to therapy.

Generalizability

B.burgdorferi sensu lato genospecies are restricted in geographical occurrence and organotropism. European LNB is associated with *B. Garinii* infection.[13] Currently, it is unknown if our finding holds true for North American LNB.

Author contribution

HM conceptualized and designed the work, collected and analyzed data and drafted the paper. AML conceptualized the study, helped draft and critically revised the article. HZ analyzed and interpreted the data and critically revised the article. LF, RG, MØ, ABA, UA, FS and KB critically revised the article

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