Ivar Tjernberg*, Paula Gyllemark, Henrik Zetterberg, Kaj Blennow, Jan Ernerudh, Pia Forsberg, Johanna Sjöwall and Anna J. Henningsson

Cerebrospinal fluid markers of inflammation and brain injury in Lyme neuroborreliosis – a prospective follow-up study

https://doi.org/10.1515/cclm-2022-0097
Received February 4, 2022; accepted April 20, 2022;
published online May 2, 2022

Abstract

Objectives: The purpose of this study was to evaluate levels and kinetics of cerebrospinal fluid (CSF) markers of inflammation and brain injury in patients with Lyme neuroborreliosis (LNB).

Methods: Adult patients with clinically suspected LNB were enrolled, in a prospective clinical study in the South East of Sweden. Patients were classified according to the European Federation of Neurological Societies' guidelines. Definite cases of LNB were re-examined one month later including a repeat CSF investigation. Routine laboratory parameters were investigated along with CSF levels of neurodegenerative markers glial fibrillary acidic protein (GFAp), total tau (t-tau) and neurofilament light protein (NFL), as well as neuroinflammatory markers soluble triggering receptor expressed on myeloid cells 2 (sTREM2), YKL-40 and CXCL13. Non-LNB served as controls. An additional comparison group consisted of spinal anesthesia subjects (SAS) without known central nervous system conditions.

Results: CSF levels of sTREM2 and CXCL13 were elevated in definite LNB patients at diagnosis compared with non-LNB patients ($p<0.001$) and SAS ($p\leq0.01$). In addition, CSF levels of sTREM2, YKL-40 and CXCL13 rapidly declined in at follow-up after antibiotic treatment. In contrast, CSF levels of GFAp and t-tau did not differ across LNB groups, and did not change after treatment.

Conclusions: Although in a limited number of LNB patients, the results indicate a predominance of microglial and neuroinflammatory involvement rather than parenchymal CNS injury in CSF at diagnosis of LNB with a prompt decline after antibiotic treatment. The findings provide pathogenetic insights and may be of value in differential diagnosis of CSF findings.

Keywords: biomarkers; brain injury; cerebrospinal fluid; Lyme neuroborreliosis; pathogenesis.

***Corresponding author: Ivar Tjernberg**, MD, PhD, Department of Clinical Chemistry and Transfusion Medicine, Region Kalmar County, Kalmar, Sweden; and Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden, E-mail: ivar.tjernberg@liu.se. https://orcid.org/0000-0001-8657-2496

Paula Gyllemark, Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden; and Department of Infectious Diseases, Ryhov County Hospital, Region Jönköping County, Jönköping, Sweden

Henrik Zetterberg, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK; UK Dementia Research Institute at UCL, London, UK; and Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Kaj Blennow, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; and Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

Jan Ernerudh, Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden; and Department of Clinical Immunology and Transfusion Medicine, Linköping University, Linköping, Sweden

Pia Forsberg, Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden

Johanna Sjöwall, Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden; and Department of Infectious Diseases in Östergötland, Linköping University, Linköping, Sweden

Anna J. Henningsson, Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden; National Reference Laboratory for Borrelia and Other Tick-Borne Bacteria, Division of Clinical Microbiology, Laboratory Medicine, Region Jönköping County, Linköping University, Linköping, Sweden; and Department of Clinical Microbiology in Linköping, Linköping University, Linköping, Sweden

Introduction

Lyme borreliosis (LB) is considered the most common tick-borne disease in Europe and North America, an infection caused by spirochetes in the *Borrelia burgdorferi* sensu lato complex [1, 2]. If the spirochetes reach the nervous system, they may give rise to Lyme neuroborreliosis (LNB), which is the most common extracutaneous clinical manifestation of LB in Europe. Clinically, meningoradiculitis is a prominent feature of LNB, including radicular pain, meningitis, and palsies of cranial nerves, in particular the peripheral facial nerve [3, 4]. In Europe, diagnosis of LNB relies on the European Federation of the Neurological Societies' (EFNS) guidelines from 2010 stating the following three criteria for a definite LNB diagnosis: (i) neurological symptoms; (ii) cerebrospinal fluid (CSF) pleocytosis; (iii) presence of intrathecally produced anti-*Borrelia* antibodies. If only two of the three criteria are fulfilled the condition is considered possible LNB [5]. Importantly, a prompt diagnosis of LNB and subsequent antibiotic treatment has been shown to reduce the risk of development of persistent symptoms [3, 6]. Various theories for the cause of these long-term post-treatment symptoms have been proposed including autoimmune/inflammatory processes and central nervous system (CNS) tissue damage, conditions that may coexist. Thus, the diagnostics and pathogenesis of LNB is complex and the etiology of residual symptoms and complaints remains unknown [3]. A further understanding of the pathogenetic process in the CNS and possible biomarker candidates may emerge from mapping of neurodegenerative and neuroinflammatory proteins in CSF.

Already in 1999, Dotevall et al. reported that CSF levels of CNS proteins such as glial fibrillary acidic protein (GFAp) and neurofilament protein were related to clinical outcome in LNB and levels of these biomarkers declined after treatment [7]. Since then, additional CSF markers of brain injury have been established, related to the pathogenesis and course of Alzheimer disease, but also in neuroinflammatory disease like multiple sclerosis. These CSF markers include total tau (t-tau) reflecting neurodegeneration, neurofilament light protein (NFL), a biomarker for axonal damage, soluble triggering receptor expressed on myeloid cells 2 (sTREM2) reflecting microglial activation, and YKL-40 also known as chitinase-like-1 protein 3, reflecting neuroinflammation [8, 9]. Since these proteins reflect different aspects of neurodegeneration and neuroinflammation, and since information on their presence in LNB is limited, we examined the CSF levels of t-tau, NFL, sTREM2, YKL-40 and GFAp in a prospective follow-up study of patients investigated for LNB and compared with controls. In addition, routine CSF parameters were monitored, including CSF CXCL13, which is known to be markedly increased in LNB [10]. To assess the kinetics and influence of antibiotic treatment on neurodegenerative and neuroinflammatory markers, follow-up CSF samples were obtained in patients with definite LNB.

Materials and methods

Patients

Adult patients (≥18 years of age) presenting with clinically suspected LNB at the Departments of Infectious diseases in the County hospital Ryhov in Jönköping and the University hospital in Linköping in the South East of Sweden were included in the study from 2004 to 2011. The patients were recruited at the time of lumbar puncture as part of the routine diagnostic process. Type of symptoms, symptom duration, clinical findings and prescribed antibiotic treatment were recorded according to the study protocol. Patients were classified according to the EFNS guidelines [5]. Thus, the following criteria were used for classification: (i) neurological symptoms; (ii) CSF pleocytosis (total CSF white blood cell count $>5 \times 10^6$/L); (iii) positive *Borrelia*-specific CSF to serum (IgM and/or IgG) antibody index compatible with intrathecal *Borrelia*-specific antibody production. Based on this definition, the following groups of patients were defined in this study:

- Definite LNB – all three criteria fulfilled
- Possible LNB – neurological symptoms and CSF pleocytosis
- Non-LNB – neurological symptoms only

Patients with definite LNB underwent a follow-up investigation with lumbar puncture one month later. Follow-up investigations were also offered in selected cases in the other LNB groups for clinical and diagnostic purposes.

An additional comparison group, consisting of adult orthopedic patients scheduled for an elective lower limb surgery and requiring spinal anesthesia were recruited at the County Hospital Ryhov in Jönköping, i.e. spinal anesthesia subjects (SAS) as a previously proposed consensus definition [11]. Exclusion criteria for these patients were neurological disease, autoimmune disease, immunosuppressive treatment, diabetes and malignancy. The purpose of including this group was to determine levels of the selected laboratory analyses in a group of patients not under neurological investigation and to allow for additional comparison. CSF together with serum from SAS were obtained and stored at −80 °C.

Laboratory analyses

Routine CSF cell counts together with results of anti-*Borrelia* antibody CSF to serum index, (Lyme Borreliosis ELISA kit 2nd Generation, Dako Cytomation A/S, Glostrup, Denmark) performed as part of the initial investigation, were retrieved from the respective hospital laboratory

databases at Jönköping and Linköping hospitals in Sweden. Additional CSF and serum samples from included patients were stored at −80 °C until further analyses of CSF and serum albumin, total IgM and IgG together with CSF levels of t-tau, NFL, sTREM2, YKL-40, GFAp and CXCL13. These analyses were all performed at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden. CSF and serum albumin, IgG and IgM levels were measured on a cobas c 501 module instrument (Roche Diagnostics, Penzberg, Germany). CSF t-tau concentration was measured using a fully automated Lumipulse enzyme-linked immunosorbent assay (ELISA) (Fujirebio, Ghent, Belgium). CSF NFL and GFAP concentrations were measured using in-house ELISAs as described [12, 13]. CSF sTREM2 concentrations were measured using an in-house Meso Scale Discovery (MSD) immunoassay with electrochemiluminescence detection as described [14]. CSF YKL-40 concentrations were measured using a commercially available MSD assay according to the manufacturer's instructions (MSD, Rockville, MD). CSF CXCL13 concentrations were measured using a commercially available ELISA (Human CXCL13/BLC/BCA-1 Immunoassay), according to the manufacturer's instructions (R&D Systems Inc., Abingdon, United Kingdom). All measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation (CVs) were below 10%.

Calculations

In order to obtain ratios and indices, the following formulas were used:

$$\text{Albumin ratio} = \frac{\text{CSF albumin}\left(\frac{\text{mg}}{\text{L}}\right)}{\text{Serum albumin}\left(\frac{\text{g}}{\text{L}}\right)}$$

$$\text{Total IgM index} = \frac{\text{CSF IgM}\left(\frac{\text{mg}}{\text{L}}\right) \Big/ \text{Serum IgM}\left(\frac{\text{g}}{\text{L}}\right)}{\text{CSF albumin}\left(\frac{\text{mg}}{\text{L}}\right) \Big/ \text{Serum albumin}\left(\frac{\text{g}}{\text{L}}\right)}$$

$$\text{Total IgG index} = \frac{\text{CSF IgG}\left(\frac{\text{mg}}{\text{L}}\right) \Big/ \text{Serum IgG}\left(\frac{\text{g}}{\text{L}}\right)}{\text{CSF albumin}\left(\frac{\text{mg}}{\text{L}}\right) \Big/ \text{Serum albumin}\left(\frac{\text{g}}{\text{L}}\right)}$$

Statistical analyses

Statistical analyses were performed using maximum likelihood ratio Chi-square for multiple proportions (Statistica version 13.5.0.17). Comparisons across groups for non-parametrical data were performed using Kruskal–Wallis ANOVA by ranks, followed by pairwise Mann–Whitney's U-test when appropriate. Considering paired measurements for patients included in the follow-up analyses, Wilcoxon matched pairs signed rank test was used (GraphPad Prism version 8.4.2). A p-value <0.05 was considered statistically significant.

Results

Patient characteristics including sex, age, clinical features, symptom duration, routine laboratory results including CSF pleocytosis, anti-*Borrelia* antibody index, CSF to serum albumin ratio, total IgM and IgG CSF to serum index together with proportion of patients receiving antibiotic treatment effective for LNB are shown for the different patient groups in Table 1.

CSF and serum were collected prior to initiation of antibiotic treatment for all patients except for two patients in the non-LNB group. A statistically significant difference was noted for age across groups due to a higher age of the SAS group compared with all other patient groups ($p<0.001$). No significant difference in age was noted in pairwise comparisons between definite LNB, possible LNB and non-LNB groups (data not shown). Furthermore, expected differences were detected for CSF to serum albumin ratio, total IgM, and IgG index across groups ($p<0.001$), with the highest levels in the definite LNB group. Regarding clinical presentation, significant differences were found for frequencies of vertigo ($p=0.007$), radiculitis ($p=0.015$), and given antibiotic treatment ($p=0.005$) comparing the patient groups. In contrast, sex distribution, symptom duration at inclusion together with the remaining noted clinical symptoms and findings did not differ across groups (Table 1).

Scatterplots depicting results for CSF levels of t-tau, NFL, sTREM2, YKL-40, GFAp and CXCL13 at inclusion in the study are shown for the patient groups in Figure 1.

Differences comparing any or more of the patient groups with the SAS group were noted for all investigated CSF parameters except GFAp. Considering the definite LNB group, significantly higher levels were detected in CSF for sTREM2 and CXCL13 compared to SAS ($p\leq0.01$), non-LNB ($p<0.001$). In addition, higher levels of CXCL13 were also found in the definite LNB group compared with the possible LNB pleocytosis group ($p=0.008$), Figure 1. Moreover, the SAS group displayed higher levels of t-tau ($p<0.03$), NFL ($p=0.002$) and YKL-40 ($p<0.001$) compared with the non-LNB group.

One-month CSF and serum follow-up sampling were available for nine patients in the definite LNB group, all of whom had received oral doxycycline (200 mg/day for 14 days) for LNB given after the initial diagnostic lumbar puncture [15]. Clinically, improvement was noted in seven of these nine patients at the one-month follow-up visit (data not shown). In addition, the following patients were also sampled again after one month with lumbar puncture: Two patients in the possible LNB pleocytosis group and three patients in the non-LNB group. The two patients in the possible LNB pleocytosis group presented with short symptom duration at inclusion (1.0 and 1.4 weeks), and both developed an IgM positive anti-*Borrelia* antibody index at follow-up (data not shown). The laboratory CSF

Table 1: Patient clinical characteristics and routine laboratory findings.

Parameter	Definite LNB n=11	Possible LNB n=5	Non-LNB n=43	Spinal anesthesia subjects n=46	p-Value[a]
Positive LNB AI, n (%)	11 (100)	0 (0)	0 (0)	1 (2)	N/A
CSF WBC count, $\times 10^6$/L, median (range)	132 (6–421)	14 (6–1,030)	1 (0–4)	0 (0–6)	N/A
CSF-alb/S-alb median (range)	9.2 (6.5–48)	7.2 (4.3–25)	4.7 (2.2–12)	5.7 (2.9–13)	<0.001
Total IgM index, median (range)	1.01 (0.02–4.84)	0.14 (0.03–0.28)	0.04 (0.02–1.29)[b]	0.05 (0.03–0.10)	<0.001[b]
Total IgG index, median (range)	0.78 (0.46–1.11)	0.53 (0.43–0.61)	0.47 (0.35–0.81)	0.43 (0.34–0.50)	<0.001
Sex, female/male, (% female)	5/6 (45)	3/2 (60)	20/23 (47)	26/20 (57)	0.75
Median age years (range)	52 (25–69)	53 (37–60)	52 (17–81)	72 (50–84)	<0.001
Median duration symptoms before LP weeks (range)	4.0 (2.0–8.0)	5.0 (1.0–9.0)	8.0 (2.0–52)[c]	N/A	0.39
Fatigue, n (%)	6 (55)	2 (40)	19 (44)	N/A	0.80
Fever, n (%)	4 (36)	0 (0)	4 (9)	N/A	0.06
Nausea, n (%)	4 (36)	1 (20)	10 (23)	N/A	0.66
Vertigo, n (%)	0 (0)	1 (20)	17 (40)	N/A	0.007
Concentration difficulties, n (%)	0 (0)	1 (20)	8 (19)	N/A	0.13
Head -and/or neck pain, n (%)	9 (82)	2 (40)	22 (51)	N/A	0.12
Radiculitis, n (%)	7 (64)	2 (40)	8 (19)	N/A	0.015
Myalgia -and/or arthralgia, n (%)	6 (55)	2 (40)	17 (40)	N/A	0.67
Numbness, n (%)	5 (45)	1 (20)	15 (35)	N/A	0.59
Cranial nerve palsy, n (%)	5 (45)	2 (40)	9 (21)	N/A	0.23
Antibiotic treatment effective for LNB[d], n (%)	10 (91)	3 (60)	17 (40)[e]	N/A	0.005

LNB, Lyme neuroborreliosis; AI, anti-*Borrelia* antibody index; n, numbers; CSF, cerebrospinal fluid; WBC, white blood cell; S, serum; alb, albumin. [a]p-Values were calculated using maximum likelihood ratio Chi-square or Kruskal–Wallis ANOVA by ranks when appropriate. [b]One sample below measuring range for cerebrospinal fluid IgM not included from the non-LNB group. [c]Symptom duration missing for one patient in the non-LNB group. [d]Treatment given according to Swedish medical products agency guidelines 2009. [e]Two patients in the non-LNB group received doxycyclin ahead of lumbar puncture.

results for these two patients are high-lighted in red in Figure 1. None of the remaining re-examined patients developed laboratory confirmation of LNB. The paired CSF results are shown in Figure 2. A significant decrease in concentrations of sTREM2 (p=0.008), YKL-40 (p=0.008) and CXCL13 (p=0.004) were found at follow-up in the definite LNB group. Regarding the significant decrease in YKL-40, an additional statistical analysis was also performed excluding the patient with the highest values. The paired statistical significance remained (p=0.016, not shown in figure), thus confirming that this patient did not alone drive the statistical finding.

Discussion

In this prospective follow-up study of patients investigated for LNB we show elevated CSF levels of sTREM2 and CXCL13 in definite LNB that decline promptly after routine antibiotic treatment. To our knowledge, this is the first report showing that sTREM2 is elevated in CSF of LNB patients, and that the sTREM2 levels decrease rapidly after antibiotic treatment. Increased sTREM2 levels in CSF have previously been reported in multiple sclerosis and other inflammatory diseases affecting the CNS, such as viral meningitis, encephalitis, optic neuritis, Neuromyelitis Optica and acute disseminated encephalomyelitis indicating microglial activation in CNS in these conditions [16, 17]. Our findings are in line with previous studies reporting microglial involvement in LNB [18, 19]. CSF CXCL13 has been extensively investigated as a biomarker in the diagnostics of LNB over the past decade [10]. We were able to confirm elevated levels of CXCL13 at diagnosis also in our material, as well as to show declining concentrations after antibiotic treatment. In addition, a significant reduction in CSF level of YKL-40 was demonstrated in the definite LNB patients after antibiotic treatment. CSF YKL-40 is considered a neuroinflammatory biomarker that is upregulated in several inflammatory disorders and also in tumors. YKL-40, is produced by immune and nonimmune cells including neutrophils, macrophages, endothelial cells, fibroblasts and vascular smooth muscle cells and activated by cytokines [9]. Thus, our results further support a neuroinflammatory response in LNB involving a number of biomarkers that decline after antibiotic treatment and clinical improvement.

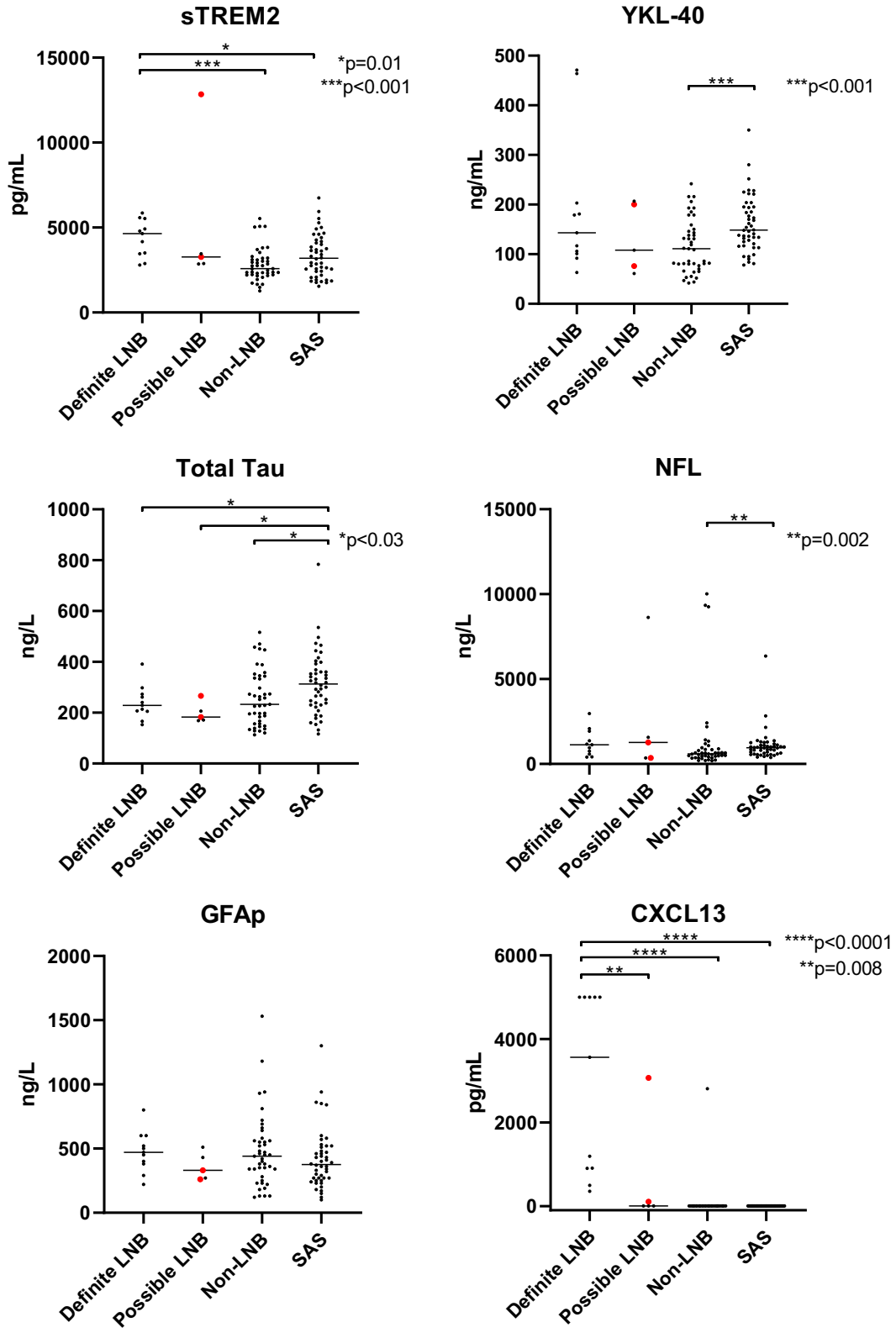

Figure 1: Cerebrospinal fluid results in 11 definite Lyme neuroborreliosis, 5 possible Lyme neuroborreliosis, 43 non-Lyme neuroborreliosis patients together with 46 spinal anesthesia subjects.
Horizontal bars represent medians. Two patients in the possible Lyme neuroborreliosis group developed positive IgM anti-*Borrelia* antibody index at follow-up – these two patients' results are high-lighted in red. Initial Kruskal–Wallis test followed by pairwise Mann–Whitney's U-test were performed and showed in Figure in case of significance. LNB, Lyme neuroborreliosis; SAS, spinal anesthesia subjects; NFL, neurofilament light protein; sTREM2, soluble triggering receptor expressed on myeloid cells 2; GFAp, glial fibrillary acidic protein.

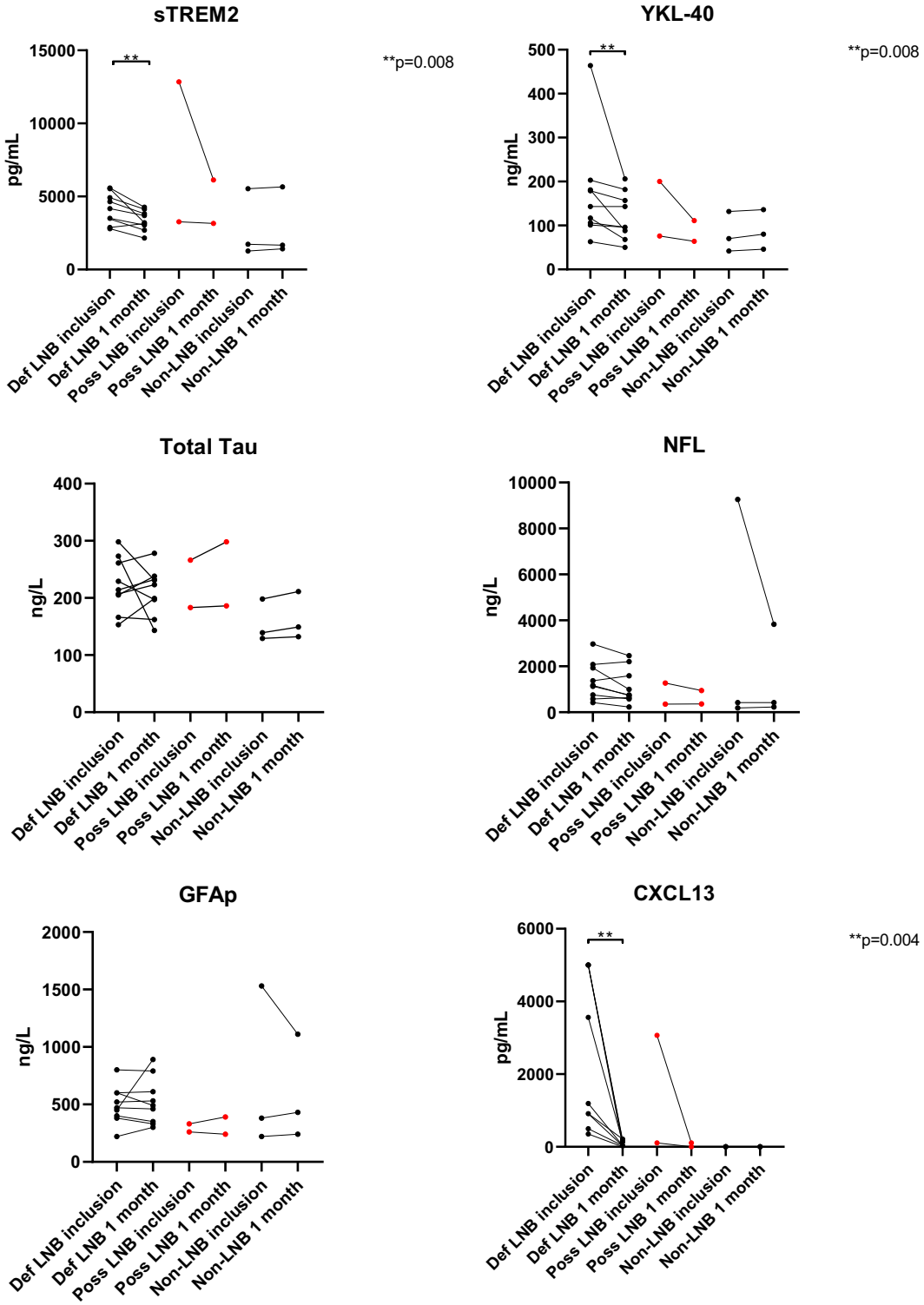

Figure 2: Paired cerebrospinal fluid at inclusion and after one month in 9 definite Lyme neuroborreliosis, 2 possible Lyme neuroborreliosis and 3 non-Lyme neuroborreliosis patients.

Only paired results for patients in the definite Lyme neuroborreliosis group were included in the statistical analysis, whereas results for the additional occasional patients of the other clinical groups are shown for information. Wilcoxon matched pairs signed rank test was used and showed in figure in case of significance. Two patients in the possible Lyme neuroborreliosis group developed positive IgM anti-*Borrelia* antibody index at follow-up – these two patients' results are high-lighted in red. Def LNB, definite Lyme neuroborreliosis; Poss LNB, possible Lyme neuroborreliosis; Non-LNB, non-Lyme neuroborrelisois; NFL, neurofilament light protein; sTREM2, soluble triggering receptor expressed on myeloid cells 2; GFAp, glial fibrillary acidic protein.

The baseline CSF levels of t-tau, NFL, YKL-40 and GFAp were not shown to differ significantly among the diagnostic LNB groups. However, significantly higher levels of NFL and YKL-40 were noted for SAS when compared to the non-LNB group. A reasonable explanation for these results is higher age of the participants in the SAS group. This is supported by the higher CSF levels of t-tau in SAS compared with all other groups and that all these CSF brain injury markers have been shown to increase with age [8]. Surprisingly, CSF levels of GFAp did not differ across the investigated groups, which is in contrast to previous findings [7, 20]. It could be speculated that the higher age of the subjects in the SAS group would complicate the detection of a possible difference in levels of GFAp comparing LNB with SAS. However, GFAp levels did not decline in the LNB group at the one-month follow-up further contradicting previous reports. Perhaps these discrepancies may be explained by differences in symptom duration at inclusion, duration between sampling, patient compositions and clinical manifestations [7, 20]. Although confirmatory studies are needed, our findings of non-altered levels in LNB of neurodegenerative markers GFAp, t-tau and NFL are of importance in differential-diagnostic evaluation of CSF since increased levels of these proteins would not support a diagnosis of LNB.

Strengths of this study include the prospective design with carefully investigated patients in combination with access to follow-up CSF after antibiotic treatment. The results cover different LNB diagnostic groups reflecting the patients under investigation for LNB in the routine clinic. In addition, a SAS group providing paired CSF and serum samples was included, further enabling important comparisons. On the other hand, limitations also need to be acknowledged. As the numbers of definite and possible LNB patients are limited, the results in this study call for follow-up studies to confirm our findings. Moreover, although the non-LNB group constitutes a clinically relevant comparison group, the non-inflammatory nervous system characteristics of this group, i.e. lack of CSF pleocytosis, must be considered when comparing the findings in relation to the LNB groups. Furthermore, although important for comparison, the SAS were significantly older compared to the LNB groups, thus complicating interpretation of the findings as some of the studied parameters show an age-dependent elevation. However, the inclusion of the SAS provides a broader perception of naturally occurring levels of the investigated CSF parameters in patients without known neurological conditions. Thus, taken the age difference into account, we still find the SAS group relevant and important. One additional factor to consider is that the samples had been stored at −80 °C for more than 10 years prior to performing the analyses, which could possibly affect the concentrations of the measured parameters. However, as all samples have been stored in the same manner, the detected differences across groups should still be valid.

In conclusion, the results of this study show elevated CSF levels of sTREM2 in patients with definite LNB, and the levels rapidly decrease after antibiotic treatment, indicating an initial microglial involvement in LNB. In addition, CSF levels of neuroinflammatory YKL-40 were shown to decrease in LNB after treatment, whereas concentrations of CSF GFAp neither differed across groups nor after antibiotic treatment in definite LNB cases. Also, the neurodegenerative markers t-tau and NFL were not increased in LNB, indicating a predominance of neuroinflammation rather than neurodegeneration. As previously shown, definite LNB cases displayed high CSF concentrations of CXCL13, which also rapidly decreased after treatment. Taken together, although in a limited number of LNB patients, our study shows prominent microglial and neuroinflammatory responses in CSF at diagnosis of LNB, with a prompt decline at follow-up sampling. These findings are of importance for understanding the pathogenesis of LNB.

Acknowledgments: We thank all involved patients and healthcare and laboratory staff for valuable assistance.
Research funding: This study was conducted with support from the Swedish Society of Medicine, the Medical Research Council of South East Sweden (FORSS) and Futurum – the Academy of Health and Welfare, Region Jönköping County. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union

Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA, (grant #1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). JE is supported by the Swedish Research Council (2018-02776), the Medical Inflammation and Infection Centre (MIIC), and ALF grants Region Östergötland (RÖ-936276).

Author contribution: IT had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: IT, HZ, KB, JE, AJH. Acquisition of data: IT, PG, JS, HZ, KB, JE, PF, AJH. Analysis and interpretation of data: IT, PG, JS. Laboratory analyses: HZ, KB. Drafting of the manuscript: IT, PG, JS. Critical revision of the manuscript for important intellectual content: IT, PG, JS, HZ, KB, JE, PF, AJH. Statistical analysis: IT. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: IT has served at advisory board for Pfizer Inc. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the work presented in this paper. JE has given lectures, unrelated to the present work, in symposia sponsored by Merck, Biogen och Abbvie. JS has given lectures and participated in advisory boards in collaboration with Merck and Biogen. PG and PF report no conflicts of interest. AJH has a collaborative research agreement with Abbott Laboratories, Chicago, IL and Reagena Ltd, Toivala, Finland.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Written consent was obtained from all participants before study inclusion. The study conforms with World Medical Association Declaration of Helsinki and was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr M106-04, 2011/65-32, 2015/192-32, 2018/388-32, 2019-02449).

References

1. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet 2012;379:461–73.
2. Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, et al. Lyme borreliosis. Nat Rev Dis Prim 2016;2:16090.
3. Koedel U, Fingerle V, Pfister HW. Lyme neuroborreliosis-epidemiology, diagnosis and management. Nat Rev Neurol 2015;11:446–56.
4. Stanek G, Strle F. Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiol Rev 2018;42:233–58.
5. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I, et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol 2010;17:8–16,e1–4.
6. 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.
7. Dotevall L, Hagberg L, Karlsson JE, Rosengren LE. Astroglial and neuronal proteins in cerebrospinal fluid as markers of CNS involvement in Lyme neuroborreliosis. Eur J Neurol 1999;6:169–78.
8. Mila-Aloma M, Salvado G, Gispert JD, Vilor-Tejedor N, Grau-Rivera O, Sala-Vila A, et al. Amyloid beta, tau, synaptic, neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's continuum. Alzheimers Dement 2020;16:1358–71.
9. McGrowder DA, Miller F, Vaz K, Nwokocha C, Wilson-Clarke C, Anderson-Cross M, et al. Cerebrospinal fluid biomarkers of Alzheimer's disease: current evidence and future perspectives. Brain Sci 2021;11:1–56.
10. Rupprecht TA, Manz KM, Fingerle V, Lechner C, Klein M, Pfirrmann M, et al. Diagnostic value of cerebrospinal fluid CXCL13 for acute Lyme neuroborreliosis. A systematic review and meta-analysis. Clin Microbiol Infect 2018;24:1234–40.
11. Teunissen C, Menge T, Altintas A, Alvarez-Cermeno JC, Bertolotto A, Berven FS, et al. Consensus definitions and application guidelines for control groups in cerebrospinal fluid biomarker studies in multiple sclerosis. Mult Scler 2013;19:1802–9.
12. Gaetani L, Hoglund K, Parnetti L, Pujol-Calderon F, Becker B, Eusebi P, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. Alzheimer's Res Ther 2018;10:8.
13. Rosengren LE, Ahlsen G, Belfrage M, Gillberg C, Haglid KG, Hamberger A. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. J Neurosci Methods 1992;44:113–9.
14. Jensen CS, Bahl JM, Ostergaard LB, Hogh P, Wermuth L, Heslegrave A, et al. Exercise as a potential modulator of inflammation in patients with Alzheimer's disease measured in cerebrospinal fluid and plasma. Exp Gerontol 2019;121:91–8.
15. Swedish Medical Products Agency. Läkemedelsbehandling av borreliainfektion - ny rekommendation. Läkemedelsverket, editor. Läkemedelsverket; 2009. p. 12–7.
16. Piccio L, Buonsanti C, Cella M, Tassi I, Schmidt RE, Fenoglio C, et al. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. Brain 2008;131:3081–91.

17. Zetterberg H. Fluid biomarkers for microglial activation and axonal injury in multiple sclerosis. Acta Neurol Scand 2017;136(201 Suppl): 15–7.
18. Duray PH. Histopathology of clinical phases of human Lyme disease. Rheum Dis Clin N Am 1989;15:691–710.
19. Fallon BA, Levin ES, Schweitzer PJ, Hardesty D. Inflammation and central nervous system Lyme disease. Neurobiol Dis 2010;37:534–41.
20. Dotevall L, Rosengren LE, Hagberg L. Increased cerebrospinal fluid levels of glial fibrillary acidic protein (GFAp) in Lyme neuroborreliosis. Infection 1996;24:125–9.