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Diagnostic performance of cerebrospinal fluid free light chains in Lyme neuroborreliosis – a pilot study

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Abstract

Background: The aim of this study was to evaluate the diagnostic performance of cerebrospinal fluid (CSF) free light chains (FLCs) in the diagnosis of Lyme neuroborreliosis (LNB).

Methods: Serum and CSF levels of κ - and λ -FLC, albumin and total concentration of immunoglobulin M (IgM) were determined together with CSF chemokine CXCL13 in 23 patients with definite LNB, 35 inflammatory neurological disease control (INDC) and 18 non-inflammatory control (NIC) patients. Indices and intrathecal fractions (IFs) of FLC and IgM were calculated.

Results: Significant differences in FLC indices and IFs were found between the LNB group and both control groups, $p \leq 0.007$. Sensitivity of intrathecal κ - and λ -FLC synthesis reached 78%–87% in LNB patients with a specificity of 94%–100% in NIC patients, whereas specificity in INDC patients was 69%. The corresponding frequencies of positive results for IF and index of IgM and CSF CXCL13 in these three diagnostic groups were 74%–96% in LNB patients, 0% in NIC patients and 3%–6% in INDC patients at the chosen cut-off levels.

Conclusions: The findings of this study show a moderate to high sensitivity of CSF κ - and λ -FLC in LNB patients with a high specificity in NIC patients. However, overlap in CSF κ - and λ -FLC levels between LNB and INDC patients calls

for caution in the interpretation and limits the diagnostic usefulness in the LNB diagnosis. CSF CXCL13 appears to be the most valuable additional biomarker of LNB aside from routine parameters such as CSF pleocytosis and anti-*Borrelia* antibody index.

Keywords: cerebrospinal fluid; diagnosis; free light chain; Lyme neuroborreliosis.

Introduction

Lyme borreliosis (LB) is the most common known tick-borne infection in both Europe and North America, and is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex [1, 2]. Lyme neuroborreliosis (LNB) is an important disseminated manifestation of LB caused by spirochetes that have reached the central nervous system (CNS), and has been reported as the second most common clinical manifestation of LB in Europe [3, 4]. Diagnosis of definite LNB according to the European Federation of Neurological Societies' (EFNS) guidelines requires neurological symptoms compatible with LNB, pleocytosis of the cerebrospinal fluid (CSF) and demonstration of *Borrelia*-specific intrathecal antibodies such as anti-*Borrelia* antibody index (AI), whereas cases of possible LNB are defined as fulfilling only two of three of these criteria [5]. Cases of possible LNB are not unusual in clinical practice in an LB endemic area. CSF pleocytosis may be absent in very early disease, and AI in paired CSF/serum samples may also be negative in the first few weeks. In addition, positive AI may persist for years even after successful treatment of LNB, thus hampering the laboratory diagnosis of *Borrelia* re-infections as well as other CNS disorders [1, 6–10]. Therefore, there is a need for additional reliable markers of ongoing LNB. In CSF, B cell markers in LNB have been in focus over the past few years. The chemokine CXCL13 has been of special interest, recently evaluated in a meta-analysis in the diagnosis of LNB [11]. In addition, already some 20 years ago, it was shown that the total concentration of immunoglobulin M (IgM) in CSF is elevated in early LNB [12]. This has also been confirmed in two more recent studies expressed as elevated total IgM index

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[10, 13]. Other markers for B cell activation are Ig free light chains (FLCs). These chains, detected as κ -FLC and λ -FLC, are produced and secreted in excess over heavy Ig chains by B cells. In inflammatory CNS diseases, detection of FLC in CSF may have a diagnostic value, especially in multiple sclerosis (MS) and clinically isolated syndrome patients [14–16]. There is also some evidence for a diagnostic value of CSF FLC in LNB [17, 18].

The aim of this study was to evaluate the performance of intrathecal κ -FLC and λ -FLC in the laboratory diagnosis of LNB in well-characterized adult LNB and clinically relevant control patients.

Materials and methods

Patients and samples

From the laboratory databases at the Departments of Clinical Chemistry and Clinical Microbiology, Kalmar County Hospital in Sweden, 23 adult (≥ 18 years) patients who were diagnosed with definite LNB according to EFNS guidelines [5] from the years 2013 to 2015 were identified and, where sufficient volume of serum (≥ 1 mL) and CSF (≥ 500 μ L) were available, included in the study. These 23 definite LNB patients are hereafter called LNB patients. Furthermore, in the same manner, the following control patients (≥ 18 years) were included; 53 patients sampled with both CSF and serum in 2013–2015 in whom LNB was one differential diagnosis, but not confirmed, and CSF white blood cell (WBC) count as well as AI had been performed and sufficient volume of serum (≥ 1 mL) and CSF (≥ 500 μ L) were available. These 53 control patients consisted of 35 CNS inflammatory neurological disease controls (INDCs) with MS with or without CSF pleocytosis in combination with other conditions with CSF pleocytosis (defined as a WBC count in CSF of $\geq 6 \times 10^6$ /L) but with negative AI and 18 CNS non-inflammatory neurological disease controls (NINDCs), i.e. no CSF pleocytosis and negative AI. The 35 inflammatory control patients can thus be considered as CNS INDCs, while the remaining 18 controls represent a combination of NINDCs and symptomatic controls (SCs) according to Teunissen et al. [19], these 18 controls are hereafter called CNS non-inflammatory controls (NICs). All samples had been stored after original analyses (2013–2015) at -20 °C until analyses in this study.

Clinical information for all patients were obtained from the electronic patient records and included sex, age, duration of symptoms before the lumbar puncture, type of complaint/symptom, clinical diagnosis and treatment. Similarly, pre-existing routine laboratory results including IgM and IgG AI and WBC and red blood cell (RBC) counts in the CSF were obtained from the laboratory databases. Information was gathered systematically and by the same persons (IT and MJ).

Laboratory analyses

The following analyses were performed on all CSF and serum samples by nephelometry using BN ProSpec: albumin (N antisera for human albumin assay), total concentration of IgM (N Latex IgM

assay), κ -FLC and λ -FLC (N Latex FLC kappa and N Latex FLC lambda assays) according to the manufacturer's instructions (Siemens, Erlangen, Germany). Results for CSF IgM and CSF λ -FLC below the lowest detection limit were assigned half the value of the lowest detection limit, respectively. The lowest detection limits for these analyses in CSF were: for IgM 0.149 mg/L and for λ -FLC 0.0994 mg/L. For albumin and κ -FLC in both serum and CSF as well as IgM and λ -FLC in serum, all results were detectable, thus above the respective lowest detection limits. Furthermore, CSF CXCL13 was measured using ELISA (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. In order to save material for multiple analyses, samples with CXCL13 results >500 pg/mL were not diluted to obtain a final concentration and were therefore assigned the value 500 pg/mL. Results for CSF CXCL13 below the lowest concentration point on the standard curve (7.8 pg/mL) were assigned half that value.

The following precision data were found, presented as inter-assay coefficient of variation (CV): $<2\%$ for albumin, $<4\%$ for IgM, $<3\%$ for κ -FLC and $<3\%$ for λ -FLC. The precision data were determined at the Department of Clinical Chemistry, Kalmar County Hospital by repeating two relevant levels of controls 6 times per day for 5 days. In total, 30 results were obtained for each analysis and level. CV was calculated as standard deviation divided by mean, times 100 expressed as percentage. Precision of CXCL13 ELISA according to the manufacturer was $<9.6\%$ CV.

Calculations

In order to obtain ratios, indices and intrathecal fractions (IFs), the following formulas were used:

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

$$Q_{\text{FLC}} = \frac{\text{CSF FLC (mg/L)}}{\text{Serum FLC (mg/L)}}$$

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

$$\kappa\text{-FLC index} = \frac{\text{CSF } \kappa\text{-FLC (mg/L) / Serum } \kappa\text{-FLC (mg/L)}}{\text{CSF albumin (mg/L) / Serum albumin (mg/L)}}$$

$$\lambda\text{-FLC index} = \frac{\text{CSF } \lambda\text{-FLC (mg/L) / Serum } \lambda\text{-FLC (mg/L)}}{\text{CSF albumin (mg/L) / Serum albumin (mg/L)}}$$

A non-linear function was also used for the calculation of IF of κ - and λ -FLC according to Hegen et al. as well as for IF of IgM according to Reiber in which an IF above 0 is considered a positive result [17, 20]:

$$Q_{\text{lim } \kappa\text{-FLC}} = 3.1276 \times \left(\frac{\text{CSF albumin (mg/L)}}{\text{Serum albumin (mg/L)}} \right)^{0.8001}$$

$$Q_{\text{lim } \lambda\text{-FLC}} = 2.1138 \times \left(\frac{\text{CSF albumin (mg/L)}}{\text{Serum albumin (mg/L)}} \right)^{0.8650}$$

$$\text{FLC}_{\text{Loc}} = (Q_{\text{FLC}} - Q_{\text{lim FLC}}) \times \text{FLC}_{\text{Serum}}$$

$$\begin{aligned}
 \text{FLC}_{\text{IF}} &= \frac{\text{FLC}_{\text{Loc}}}{\text{FLC}_{\text{CSF}}} \times 100 \\
 Q_{\text{lim IgM}} &= \left[0.67 \times \sqrt{\left(\frac{\text{CSF albumin (mg/L)}}{\text{Serum albumin (g/L)}} \right)^2 + 120} - 7.1 \right] \times 10^{-3} \\
 \text{IgM}_{\text{Loc}} &= (Q_{\text{IgM}} - Q_{\text{lim IgM}}) \times \text{Serum IgM (mg/L)} \\
 \text{IgM}_{\text{IF}} &= \frac{\text{IgM}_{\text{Loc}}}{\text{CSF IgM (mg/L)}} \times 100
 \end{aligned}$$

Statistical analyses

In order to compare the ability of different parameters to discriminate between diagnostic groups, cut-off values and areas under curve (AUC) with 95% confidence intervals (CI) were calculated using the groups LNB and NIC (Table 1) in receiver-operating characteristic (ROC) analyses using MedCalc, version 18.6. Statistical analyses were performed using χ^2 test for proportions, either Pearson χ^2 or maximum likelihood ratio χ^2 when appropriate. Comparisons across all three groups for non-parametrical data were performed using the Kruskal-Wallis ANOVA by ranks, followed by a pairwise Mann-Whitney U-test in case of significance (Statistica version 13). A p-value <0.05 was considered statistically significant.

Ethics

The study was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr 03-129 and Dnr M47-06).

Results

Details on sex, age, clinical features, symptom duration and frequency of antibiotic treatment effective for LNB as well as routine laboratory data including CSF RBC and WBC counts and IgM and IgG AI results are shown for the three clinical groups in Table 1. In addition, the storage time, for paired serum and CSF at -20°C until the analyses were performed for this study, are shown for the respective study groups in Table 1. When antibiotic treatment was given, serum and CSF were collected prior to the initiation of treatment in all but one patient: an LNB patient (AI positive for both IgM and IgG with CSF pleocytosis at $130 \times 10^6/\text{L}$) in whom samples were collected the day after antibiotic treatment was commenced. No significant differences across groups were detected regarding sex and age. However, frequencies of cranial nerve palsy,

Table 1: Patient clinical characteristics.

Parameter	Lyme neuroborreliosis (n=23)	Inflammatory neurological disease controls (n=35)	Non-inflammatory controls (n=18)	p-Value ^a
Sex, female/male (% female)	13/10 (57)	14/21 (40)	9/9 (50)	0.45
Age at lumbar puncture, years				
Mean (SD)	58.1 (11.6)	49.4 (20.1)	51.6 (16.5)	
Median (range)	60 (39–80)	48 (19–88)	58.5 (25–76)	0.099
Cranial nerve palsy ^{ab} (%)	12 (52)	8 (23)	2 (11)	0.009
Radiculitis ^b (%)	11 (48)	3 (9)	0 (0)	<0.001
Meningitis ^b (%)	2 (9)	6 (17)	0 (0)	0.064
Other symptom ^b (%)	7 (30)	19 (54)	16 (89)	<0.001
Median duration of symptom before LP, days (range)	21 (7–180)	8 (1–1000)	75 (2–3650)	0.093
Median CSF RBC count, $\times 10^6/\text{L}$ (range)	3 (0–768)	3 (0–70700)	0 (0–296)	0.041
Median CSF WBC count, $\times 10^6/\text{L}$ (range)	170 (24–525)	18 (0–336)	2 (0–5)	n/a
Median CSF mononuclear proportion, % (range)	99 (79–100)	100 (0–100)	n/a	n/a
CSF IgM anti- <i>Borrelia</i> antibodies (%)	17 (74)	0 (0)	0 (0)	n/a
Median anti- <i>Borrelia</i> IgM antibody index ^c (range)	0.93 (0–23)	0.04 (0–0.12)	0.03 (0–0.14)	n/a
CSF IgG anti- <i>Borrelia</i> antibodies (%)	18 (78)	0 (0)	0 (0)	n/a
Median anti- <i>Borrelia</i> IgG antibody index ^c (range)	7.4 (0.03–220)	0.03 (0.02–0.08)	0.02 (0–0.08)	n/a
Either CSF IgM or IgG anti- <i>Borrelia</i> antibodies (%)	23 (100)	0 (0)	0 (0)	n/a
Antibiotic treatment effective for LNB ^d (%)	23 (100)	9 (26)	2 (11)	<0.001
Median sample storage time at -20°C , days (range)	1426 (919–1797)	1740 (1037–1943)	1372 (1211–1940)	0.072

n, numbers; CNS, central nervous system; LP, lumbar puncture; CSF, cerebrospinal fluid; RBC, red blood cell; WBC, white blood cell; LNB, Lyme neuroborreliosis. ^ap-Values were calculated using Pearson χ^2 , maximum likelihood ratio χ^2 or Kruskal-Wallis ANOVA by ranks when appropriate. ^bTen patients presented with a combination of two clinical findings from the categories cranial nerve palsy, radiculitis, meningitis and other symptoms, the remaining 66 with one clinical finding only. ^cPerformed using the IDEIA Lyme Neuroborreliosis test (Oxoid, Hampshire, UK). A positive index result is defined as ≥ 0.3 according to the manufacturer. ^dTreatment given according to Swedish medical products agency guidelines 2009.

radiculitis and other symptoms as well as RBC count of the CSF and the number of patients given antibiotic treatment effective for LNB significantly differed between groups. In contrast, occurrence of meningitis and the median duration of symptom at the time of lumbar puncture was not shown to differ. No significant difference in the storage time of serum and CSF samples between groups was noted. The spectrum of clinical conditions and diagnoses collected from medical records for the INDC and NIC groups was broad and is shown in Table 2.

In order to determine the diagnostic performance of the evaluated parameters, two approaches were used. First, cut-off levels previously described for the respective

tests were used: For CSF CXCL13, a cut-off of 162 pg/mL was used according to a recent systematic review and meta-analysis [11]. For total IgM index, a cut-off of 0.234 was applied [10], while a positive IgM IF was defined as above 0 [20]. For κ - and λ -FLC indices as well as IF, cut-off levels according to Hegen et al. were used [17] (see Table 3). In addition to this strategy, and in order to compare the diagnostic performance of tests and calculations on an equal basis, ROC analyses were performed as previously described in the Statistical analyses section of this paper. ROC-based cut-off levels were then applied on the respective tests in the various patient groups. The corresponding AUC with 95% CI, ROC-based cut-off levels and numbers

Table 2: Clinical findings in controls.

Clinical condition/diagnosis	Inflammatory neurological disease controls (n = 35)	Clinical condition/diagnosis	Non-inflammatory controls (n = 18)
Multiple sclerosis	9	Headache	6
Cranial nerve palsy ^a	7	Cognitive disorder	3
Aseptic meningitis	5	Paresthesia	2
Stroke ^a	3	Cranial nerve palsy	2
Possible Lyme neuroborreliosis	2	Myalgia	2
Tick-borne encephalitis	1	Amyotrophic lateral sclerosis	1
Arteria iliaca stenosis	1	Carpal tunnel syndrome	1
Encephalopathy	1	Stroke	1
Herniated cervical disc	1		
Neurosarcoidosis	1		
Pneumonia	1		
Posterior reversible encephalopathy syndrome	1		
Radiculopathy	1		
Spontaneous intracranial hypotension	1		
Vertebral osteomyelitis	1		

CNS, central nervous system. ^aOne patient with two diagnoses.

Table 3: Positive results based on the chosen cut-off levels for each analysis.

Analysis	Lyme neuroborreliosis (n = 23)	Inflammatory neurological disease controls (n = 35)	Non-inflammatory controls (n = 18)
CSF CXCL13 ^a , n (%)	22 (95.7)	2 (5.7)	0 (0)
Total IgM index ^b , n (%)	18 (78.3)	1 (2.9)	0 (0)
κ -FLC index ^c , n (%)	18 (78.3)	11 (31.4)	0 (0)
λ -FLC index ^d , n (%)	20 (87.0)	11 (31.4)	1 (5.6)
IgM IF ^e , n (%)	17 (73.9)	2 (5.7)	0 (0)
κ -FLC IF ^e , n (%)	18 (78.3)	11 (31.4)	0 (0)
λ -FLC IF ^e , n (%)	20 (87.0)	11 (31.4)	1 (5.6)

n, numbers; CSF, cerebrospinal fluid; FLC, free light chain; IF, intrathecal fraction. ^aCut-off at 162 pg/mL. ^bCut-off at 0.234. ^cCut-off at 7.9.

^dCut-off at 4.2. ^eCut-off at 0.

(%) positive in the respective groups are shown in Table 4. The ROC AUC for CSF CXCL13 was 1, but as shown in Table 4, 95% CI for all tests overlap each other.

Scatterplots depicting results for CSF CXCL13, total IgM index, IgM IF, κ - and λ -FLC index as well as IF of κ - and λ -FLC for the three patients groups are shown in Figure 1. Significant differences in levels of results for all studied parameters were found between the LNB group and both control groups, $p \leq 0.007$. Results above the measuring range for CSF CXCL13, i.e. 500 pg/mL, were noted for 22 out of 23 patients in the LNB group and one out of 35 patients in the INDC group, the remaining patients showed results either within or below the measuring range. To compare FLC indices with the corresponding IF, results categorized as negative or positive for indices and IF applying the suggested levels for cut-off for κ - and λ -FLC indices and IF according to Hegen et al. were pairwise compared for κ - and λ -FLC, respectively, in all samples from these 76 patients [17]. All positive and negative κ - and λ -FLC results were 100% concordant comparing the index with IF results in all samples (data not shown in Table). For detailed results within the both control groups according to clinical conditions, see scatterplots in Figures 2 and 3.

All MS patients (nine out of nine) in the INDC group showed the highest and elevated levels of κ -FLC index as well as κ -FLC IF according to the proposed levels of cut-offs by Hegen et al. [17]. Regarding λ -FLC, six out of nine of the same INDC MS patients were positive for λ -FLC index and λ -FLC IF.

As shown in Table 2 and Figure 2, two patients in the INDC group were considered as cases of possible LNB due to the clinical presentation but lack of positive AI. One of these two patients was diagnosed and treated with penicillin V for erythema migrans 16 days before the onset of peripheral facial nerve palsy for which the patient sought

medical care after another 5 days, when lumbar puncture was performed. Laboratory investigations at lumbar puncture in this patient showed a total CSF WBC count of $48 \times 10^6/L$, all mononuclear cells. Furthermore, CSF-CXCL13 was 36 pg/mL, and total IgM-, κ - and λ -FLC index were all positive. Interestingly, λ -FLC IF was positive while κ -FLC IF and IgM IF were negative. In addition, this patient's *Borrelia* serology in peripheral blood was positive (C6 Lyme ELISA kit, Immunetics, Inc., Boston, MA, USA). The patient received oral treatment with doxycycline for 2 weeks and had recovered at follow-up 3 months later. The other patient was treated with erythromycin for pneumonia 15 days prior to the onset of peripheral facial nerve palsy for which she sought medical advice and was lumbar punctured after an additional 4 days. No previous erythema migrans was noted. The laboratory investigation revealed an all mononuclear pleocytosis of the CSF with $26 \times 10^6/L$ WBC, CSF-CXCL13 was 3.9 pg/mL and total IgM index, IgM IF as well as κ - and λ -FLC index and κ - and λ -FLC IF were all negative. Moreover, *Borrelia* serology in peripheral blood was negative (C6 Lyme ELISA kit, Immunetics, Inc., Boston, MA, USA). This patient was treated with oral doxycycline for 2 weeks and reported improvement already some 10 days later.

Two other patients with infectious diseases (pneumonia and vertebral osteomyelitis) in the inflammatory control group had CSF-CXCL13 results of at least 500 pg/mL (Figure 2): one patient presented with vertigo, fever and headache with a duration of at least 4 days. Further investigations including chest X-ray supported a diagnosis of pneumonia and the patient recovered after treatment with cefotaxime followed by penicillin V. The total CSF WBC count in this patient was $16 \times 10^6/L$, all mononuclear cells. Furthermore, this patient was negative for total IgM-, κ - and λ -FLC index as well as κ - and λ -FLC

Table 4: Comparisons of the diagnostic performance based on Receiver-operating-characteristic analyses.

Analysis	ROC AUC (95% CI) ^a	Cut-off (>)	Numbers positive, %	Numbers positive, %	Numbers positive, %
			Lyme neuroborreliosis (n = 23)	Inflammatory neurological disease controls (n = 35)	Non-inflammatory controls (n = 18)
CSF CXCL13, pg/mL	1 (0.914–1.000)	9.0	23 (100)	14 (40)	0 (0)
Total IgM index	0.993 (0.900–1.000)	0.049	22 (95.7)	21 (60.0)	0 (0)
κ -FLC index	0.971 (0.864–0.999)	2.74	20 (87.0)	16 (45.7)	1 (5.6)
λ -FLC index	0.957 (0.842–0.996)	3.79	20 (87.0)	13 (37.1)	1 (5.6)
IgM IF	0.973 (0.868–0.999)	–339	21 (91.3)	19 (54.3)	0 (0)
κ -FLC IF	0.981 (0.880–1.000)	–201	22 (95.7)	16 (45.7)	1 (5.6)
λ -FLC IF	0.978 (0.876–1.000)	–10.6	22 (95.7)	13 (37.1)	2 (11.1)

ROC, receiver-operating characteristic analysis; AUC, area under curve; CI, confidence interval; n, numbers; CSF, cerebrospinal fluid; FLC, free light chain; IF, intrathecal fraction. ^aROC analyses performed in Lyme neuroborreliosis patients and non-inflammatory controls.

Figure 1: Laboratory results in 23 Lyme neuroborreliosis patients, 35 inflammatory neurological disease control patients and 18 non-inflammatory control patients.

Horizontal bars represent medians. For all laboratory parameters, the initial Kruskal-Wallis test was performed with $p < 0.001$ for all parameters, followed by a pairwise Mann-Whitney U-test performed, which are shown in the figure. *One outlier result at -10,234 for IgM IF in the INDC group not shown in the figure. CSF, cerebrospinal fluid; LNB, Lyme neuroborreliosis; INDC, inflammatory neurological disease control; NIC, non-inflammatory control; FLC, free light chain; IF, intrathecal fraction.

Figure 2: Laboratory results in 35 inflammatory neurological disease control patients according to clinical conditions.

*One patient with both stroke and peripheral facial palsy is depicted twice. **One outlier result at $-10,234$ for IgM IF in patient with vertebral osteomyelitis not shown in the figure. Horizontal bars represent medians. INDC, inflammatory neurological disease control; CSF, cerebrospinal fluid; PRES, posterior reversible encephalopathy syndrome; FLC, free light chain; IF, intrathecal fraction.

Figure 3: Laboratory results in 18 non-inflammatory control patients according to clinical conditions. Horizontal bars represent medians. NIC, non-inflammatory control; CSF, cerebrospinal fluid; FLC, free light chain; IF, intrathecal fraction.

and IgM IF. The other patient presented with headache, fever, fatigue and confusion since a week. Cervical magnetic resonance imaging showed vertebral osteomyelitis

and blood cultures were positive for *Staphylococcus aureus*. Lumbar puncture in this patient showed a total CSF WBC count of $36 \times 10^6/L$, of which $25 \times 10^6/L$ were

mononuclear cells and the remaining $11 \times 10^6/\text{L}$ were polynuclear cells. The total IgM-, κ - and λ -FLC index as well as κ - and λ -FLC and IgM IF were all negative in this patient. This patient was initially treated with cloxacillin followed by oral flucloxacillin for a total of 3 months at which time full recovery was reached.

One patient in the NIC group with headache showed elevated levels of λ -FLC index and IF, but not for κ -FLC index or IF (Figure 3).

Discussion

In this study, we conclude a similar and moderate to high sensitivity for κ - and λ -FLC (78%–87%) in the laboratory diagnosis of LNB with a high specificity in non-inflammatory CSF control patients (90%–94%). This is in line with the previous findings [17], in which the same definition of LNB was used. However, in some contrast to the findings by Hegen et al., in our study we show a considerable overlap in levels of κ - and λ -FLC indices as well as IF between LNB patients and inflammatory control patients including a variety of relevant clinical conditions. κ - and λ -FLC levels above the cut-off were found in 31% of the patients in the INDC group, consequently reducing specificity to 69%, thus limiting the diagnostic usefulness of κ - and λ -FLC in LNB diagnostics. To be able to determine the usefulness of CSF FLC in the laboratory diagnosis of LNB, we believe it is important to include clinically relevant patients representing various CNS conditions of which LNB is one important differential diagnosis especially in tick-endemic areas of Europe and North America. As there is no pathognomonic sign or combination of signs for LNB, diagnosis of the condition requires laboratory investigations including CSF WBC count in combination with AI. The clinical presentation of LNB depends on affected parts of the CNS and peripheral nervous system. Findings include cranial nerve palsy, radiculitis and meningitis and often in combination with general complaints such as muscle/joint pain, fatigue and fever [21, 22]. Consequently, the final diagnosis includes a broad spectrum of clinical conditions of which LNB is one differential diagnosis. In this study, laboratory testing for LNB, i.e. CSF cell count and determination of AI, was ordered and performed in all patients as LNB was one possible differential diagnosis. In this aspect, we believe the well-characterized patients in this study to be representative of a routine clinical setting. Thus, elevated results of κ - and λ -FLC may indicate LNB but indeed also other conditions, in particular clinically isolated syndrome and MS, as shown in this study and previous studies [15, 16, 18, 23, 24].

Previous reports of FLC do not indicate any additional value of determining both κ - and λ -FLC, thus focusing on κ -FLC [15, 16, 18, 23, 24]. However, in the publication by Hegen et al. results indicated at least a similar or even higher sensitivity at fixed specificity for λ -FLC compared with κ -FLC in LNB [17]. In our study, we could not show any superior performance of λ -FLC in this regard. Another aspect of CSF FLC is the reporting format. Both index and IF calculations have been performed and used, and for LNB no one seem to be superior [17].

The diagnostic performance of IgM index and IgM IF in this study showed a similar and moderately high sensitivity in LNB patients with a high specificity in both INDC and NIC groups. Different cut-off levels for a positive IgM index have previously been suggested, e.g. 0.045–0.234 [10, 13, 25], consequently affecting both the sensitivity and specificity in the diagnosis of LNB as well as other CNS conditions. The role of IgM index in the diagnosis of LNB in both children and adults remains to be settled as well as the optimal level of cut-off for this purpose with regard to methodological considerations.

Regarding CSF CXCL13, our results further support previous multiple publications summarized in a recent review and meta-analysis [11]. Levels of CSF CXCL13 were elevated in all but one of the LNB patients with preserved high specificity in both INDC and NIC groups.

This study is limited by its retrospective design, using samples stored at -20°C for up to 5 years. Although immunoglobulins are considered insensitive to long periods of storage at -20°C [26–29], the stability of FLC in CSF under these prolonged storage conditions is mainly unknown. Studies of FLC stability in plasma and serum indicate that FLCs are fairly stable at -20°C for 4 weeks [30, 31], but one study of FLC in urine showed a decrease in levels after 3 months at -20°C [32]. It is unknown if this decline also applies to serum and CSF; however, as there was no significant difference in storage time across groups and all samples were handled in the same way regardless of patient group, we believe the significant differences in levels of FLC between groups reflect actual differences although these may indeed be underestimated. In addition, the stability of CSF CXCL13 after storage for years at -20°C may also be questioned, but we believe that the FLC reasoning above also applies to our CSF CXCL13 findings, which are in line with a number of previous publications [11].

Furthermore, the number of investigated patients in our study is small and only includes adults. Therefore, our findings need to be confirmed, and in addition it would be interesting to investigate the diagnostic performance of CSF κ - and λ -FLC in children. Children with LNB often present with a short symptom duration, e.g. 37%–57%

with a symptom duration of less than 1 week, and in these patients a negative AI may occur in up to 49% [33, 34]. Thus, there is a need for additional diagnostic markers of pediatric LNB, and as MS is uncommon in children [35], FLC could be an interesting alternative.

Taken together, our findings show elevated κ - and λ -FLC in CSF of LNB patients with a high specificity in NIC patients, whereas levels in INDC patients, MS patients in particular, are elevated and overlapping. Hence, increased CSF levels of κ - and λ -FLC, as well as IgM expressed as index or IF, must be interpreted with caution and suggest CSF B cell activity that may be caused by either LNB, MS or other conditions. In addition, it is also known that CSF CXCL13 may be elevated in MS, although generally at lower levels than LNB [36–40]. This was confirmed in the present study; thus, CSF CXCL13 appears to be the most useful additional marker of LNB aside from CSF pleocytosis and AI.

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