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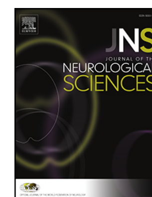

Prospective study on the chemokine CXCL13 in neuroborreliosis and other aseptic neuroinfections

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A B S T R A C T

The study evaluates the clinical significance of CXCL13 (leukocyte chemoattractant synthesized in CSF) in Lyme neuroborreliosis (LNB) and other aseptic CNS infections.

244 patients with symptoms of neuroinfection and/or LNB were divided into groups: A - patients with LNB-positive antibodies in serum and CSF (96) or CSF only (14); B - patients with aseptic non-borrelial neuroinfections (82); C - negative controls (52). Group A was divided into A1–A4 according to pleocytosis in CSF and AIIgG positivity.

The highest CSF CXCL13 concentrations (max. 81,287.60 pg/ml; median 1766.90 pg/ml) were in A1 (positive AI, pleocytosis) and A3 (negative AIIgG, pleocytosis; max. 7201,60 pg/ml, median 56.22 pg/ml). A2 (positive AI without pleocytosis) and A4 (negative AI without pleocytosis) had low CXCL13 levels - A2 max. 650.50 pg/ml (median < 7.80 pg/ml); A4 max. 118.56 pg/ml (median < 7.8 pg/ml). In B the median was 28.10 pg/ml (max. 595.87 pg/ml). In C the CXCL13 concentrations were the lowest (max. 83.83 pg/ml; median < 7.80 pg/ml). The lowest cut-off was 29 pg/ml (sensitivity 90.0%, specificity 72.2%), the highest one 400 pg/ml (sensitivity 59.6%, specificity 94.0%). The group differences of serum CXCL13 were insignificant.

The highest concentrations were at the beginning of the disease. In LNB CXCL13 correlates better with the CSF pleocytosis than AI positivity.

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1. Introduction

Diagnosis of Lyme neuroborreliosis (LNB) is complicated by the relatively long course of the disease, frequent unspecific clinical symptoms and the relatively slow response to antibiotic therapy comparing with some other bacterial CNS infections. Due to the low sensitivity of the direct laboratory methods, the basic mainstay of the diagnostic is serology. Frequent unspecific reactions, varying seroprevalence and many other problems bring additional variables. When the symptoms of cranial neuritis, painful radiculitis and lymphocyte meningitis supported by CSF-specific antibody synthesis (antibody index, AI_{IgG}) are manifested, the diagnosis is considered optimal [1]. However, AI diagnostic usability varies in the range 70–90% [2] and is limited to certain extent during the first 4–6 weeks of the disease [2–5]. Local antibody synthesis persists for weeks and months after infection [5,6]. Although the early Lyme borreliosis (LB) antibody response has an IgM pattern, the majority of LNB patients display only IgG or mixed antibody response. Sensitivity of CSF PCR varies in the range 20–40% and is substantially lower in blood [7]. As a result it is frequently complicated to recognize acute or active infection on the one hand, or to assess the effect of antibiotic therapy on the other [8]. Recently there have been attempts to solve both types of problems by examining the chemokine CXCL13 [9–16].

CXCL13 is a B-cell chemoattractant expressed in secondary lymphoid organs and it is synthesized in NB in considerable amounts into the CSF along with the less clinically relevant cyto- and chemokines (CCL3, CCL4, IL6, IL8, CXCL12, CXCL19…) [17,18]. CXCL13 promotes the secretion of cytokines facilitating humoral reactions and resulting in a higher number of B lymphocytes and plasmatic cells in CSF [19]. CXCL13 has been repeatedly shown in high concentrations in the acute phase of LNB in CSF [10], in contrast to blood levels which have not displayed this relationship [20] and are not indicative for active NB. Furthermore, this chemokine has been found elevated in CSF in many neurologic inflammations, mostly acute ones such as neurosyphilis, HIV encephalopathy, purulent meningitis and less frequently in malignancies, immunopathological CNS inflammations and

Abbreviations: AI, antibody index; APRIL, A proliferation inducing ligand; BAFF, B-cell activating factor; CNS, central nervous system; CC, chemokines motif C–C; CR, Czech Republic; CSF, cerebrospinal fluid; CXC, chemokines motif C–X–C; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; EV, enterovirus; HGA, human granulocytic anaplasmosis; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IL, interleukin; IT, intrathecal synthesis; LB, Lyme borreliosis; LBN, Lyme neuroborreliosis; PL, lymphocytic pleocytosis; PCR, polymerase chain reaction; ROC, receiver operating characteristic; TBE, tick-borne encephalitis; VZV, varicella-zoster virus; WB, western–blotting.

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other diseases [15,16,21–23]. Recently, for instance, Kowarik et al. [12] tested levels of CXCL12, CXCL19, BAFF and APRIL in neuroinfections and out of these only CXCL13 had the significantly highest correlation with the number of B cells, plasmablasts and intrathecal immunoglobulin synthesis (intrathecal synthesis, IT) in LNB and in the other neuroinfections. CXCL13 achieves the highest concentration just at the moment that IT synthesis is initiated. This phenomenon is known in both adults and children [11]. The reason why spirochete infection in particular often stimulates CXCL13 secretion has not yet been discovered, but the presence of a high proportion of lipoproteins in the spirochete cytoplasmic membrane has been mentioned [9,10,12].

In the present study, CXCL13 chemokine levels are tested in a group of neuroinfections, where patients with LNB make up a substantial part of the test group. This is an ongoing study targeted on diagnostic of neuroinfections; CXCL13 in CSF and serum was examined before the treatment initiation.

2. Materials and methods

2.1. Patient group

244 patients (230 adult, 14 children up to eighteen years) admitted with suspicion of neuroinfection have been included in this prospective study to date (Table 1). Patients with proven purulent neuroinfection and all patients with LB seropositivity without recent clinical symptoms of the disease were excluded. All included patients were examined and treated as inpatients and after discharging were followed-up (the first examination after 3 months). Basic cytological and biochemical examination of CSF was performed and additional tests were done for the presence of antiborrelial antibodies, including AI, PCR for LB DNA (data not shown), and the DNA of enteroviruses. DNA of HSV1,2 and VZV were tested in clinically relevant cases. A lumbar puncture was performed before treatment was initiated. Serum tick-borne encephalitis (TBE) and LB antibodies were tested in serum in all patients; a tick bite history was referred by 57 persons.

2.2. Study design

Patients were divided into groups as follows (Table 2). A patients considered for diagnosis LNB - positive antiborrelial antibodies in serum and CSF (96), and patients with specific antibodies in CSF only (14); B patients with the diagnosis of aseptic neuroinfection of non-borrelial origin (82); and C controls - LB seronegative patients, diagnosis of neuroinfection excluded (52). Group A was divided into subgroups in a second step: A1 CSF pleocytosis + local synthesis of antiborrelial antibodies (AI_{IgG} positive) (44 patients, definite NB[1]); A2 without CSF pleocytosis + AI_{IgG} positive (16, probable LNB*); A3 CSF pleocytosis + CSF antiborrelial antibodies positive (AI_{IgG} negative), (30, probable LNB*); A4 without CSF pleocytosis + CSF antiborrelial antibodies positive (AI_{IgG} negative), (20, possible LNB*). Basic clinical data (neurological involvement) of patients are shown in Table 3.

The clinical outcome of therapy was only assessed in group A (LNB) on an arbitrary scale of 1–5; the final outcome was compared with the initial clinical involvement: 1 normal status, 2 mild symptoms, 3 modest residues, 4 marked sequelae, able to work, 5 invalidity. In total, 85 patients out of 110 were examined, the final diagnosis in group A was not changed in any of the patients.

Patients were tested for CXCL13 in CSF and serum at the baseline before antibiotic treatment and were treated according to a standard treatment regime regardless of the result. Ceftriaxone was used in most cases in whom antibiotic treatment was indicated five patients were treated by doxycycline (allergy to betalactams, refusing IV therapy). Patients' data were stored electronically without personal identification. Patients were in detail informed about the purpose of the study and gave written informed consent before they had been enrolled into the study. In children, informed consent was given by parents. The study was approved by the Ethics Committee of the Hospital Na Bulovce, Prague, Czech Republic.

[1] Classification of LNB is not completely unified [24,25] categories “definite, probable, non-LNB” was based on [24]; “possible LNB” used only in this text.

Table 1
Stratification of patients into groups according to the diagnosis.

Group	Number	Adults/children
A (considered for neuroborreliosis)	110	96/14
B (CNS infection of other etiology)	82	82/0
C (control group)	52	52/0
Total	244	230/14

2.3. Laboratory tests

Samples of blood and CSF were centrifuged (1000*g*), aliquoted and frozen (−50 °C) within 2 h after taking. This procedure was chosen based on the experience that CXCL13 levels are not altered by freezing, whereas serum levels are decreased only slightly [11].

Measurement of CXCL13 levels was performed by the commercial ELISA test (Quantikine Human CXCL13/BLC/BCA-1 Immunoassay R&D Systems, Inc. Minneapolis MN, USA, local importer in ČR Biomedica ČR, s.r.o.). This test is not approved for routine clinical testing and is limited to research purposes. The CXCL13 analysis was conducted according to the manufacturer's recommendation, all tests were run in duplicate. The levels of CXCL13 were calculated from a standard curve of optical density versus concentration estimated for each set of samples. A concentration below 7.8 pg/ml was considered to be undetectable. Samples with concentrations over the standard curve of the assay (>500 pg/ml) were further diluted 10 to 100 times with the calibration diluent buffer of the kit and reanalysed [15].

Borrelia antibodies in serum and CSF were analysed by ELISA – VIDITEST anti-*Borrelia* recombinant IgG + Vlse/IgM (CSF) (VIDIA s.r.o. CR.); all positive results were confirmed by western-blotting (BlotLine Borrelia/ HGA IgM and Blot Line Borrelia/HGA IgG, TestLine, Clinical Diagnostic s.r.o. ČR). This kit is based on recombinants antigens of *Borrelia* sp. and *Anaplasma phagocytophyllum*. Only WB confirmed results were considered as positive. Albumin and IgG antibodies were detected by nephelometry (IMMAGE 800, Beckman Coulter Inc.). Antibody index (AI_{IgG}) was calculated by the VidiTab (Vidia s.r.o. CR) program; $AI_{IgG} > 1.5$ was considered positive.

CSF normal values: leukocyte total cell count <3/μl, erythrocytes <100/μl, total protein 0.2–0.45 g/l; this upper limit of total protein was chosen to simplify statistic calculations in different age groups; some low-end values are recognized as abnormally elevated due to older patients.

Statistical analysis: results were analysed by the statistical software Stata version 9.2 (Stata Corp LP, College Station, TX). The comparison of paired categorical data was calculated using a Kruskal-Wallis test, a

Table 2
Number of patients subgroup.

Group	Diagnosis	PL[a]	Ab in CSF	AI_{IgG}[b]	Ab in serum	Number
A1	LNB define	+	+	+	+	34
		+	+	−	−	10
A2	LNB probable	−	+	+	+	12
		−	+	−	−	4
A3	LNB probable	+	+	−	+	30
A4	LNB possible	−	+	−	+	20
B	CNS infection of other etiology	+	−	−	−	82
C	Control group	−	−	−	−	52

[a] PL lymphocytic pleocytosis >3 cells/mm^3 and/or CSF protein >0.45 g/l.
[b] AI_{IgG}- antibody index.

Table 3
Characteristics and basic clinical data of patients in subgroups.

Group	A				B	C
Subgroup	A1	A2	A3	A4		
Number of patients	44	16	30	20	82	52
Mean age/median (years)	42,55/46	53,06/60	39,8/35,5	48,1/44	36,1/35	44,9/41,5
Female/male ratio (%)	20/24 (45,5/54,5)	7/9 (43,8/56,2)	12/18 (40,0/60,0)	5/15 (25,0/75,0)	47/35 (57,3/42,7)	28/24 (53,8/46,2)
Diagnosis (%)						
Aseptic CNS infection	44 (100,0)	2 (12,5)	30 (100,0)	0 (0,0)	82 (100,0)	0
• Isolated meningitis	12 (27,3)	0	16 (53,3)	0	49 (59,8)	0
• Meningitis + facial palsy	15 (34,1)	0	8 (26,6)	0	2 (2,4)	0
• Meningitis + radicular involvement	14 (31,8)	0	1 (3,0)	0	0	0
• Meningoencephalitis, encephalitis, encephalomyelitis	3 (6,8)	2 (12,5)	5 (16,7)	0	31 (37,8)	0
Facial palsy only	0	2 (12,5)	0	3 (15,0)	0	6 (11,6)
Peripheral neuropathy only	0	9 (56,2)	0	7 (35,0)	0	12 (23,1)
Nonspecific symptoms only (arthralgia, myalgia, fatigue, headache, mental disorders)	0	3 (18,7)	0	9 (45,0)	0	34 (65,3)

Mann-Whitney test was used for multiple comparisons. Student's *t*-test was used to evaluate the unpaired data, Fisher's exact test for the effect of evaluating therapy. Results were rated in 95% confidence intervals, p-values <0.05 were considered statistically significant.

3. Results

Out of 244 patients included in the study, the diagnosis of LNB was considered in 110 (group A) (Table 1). Eighty-two patients were in the group with aseptic neuroinfections (group B) - 55 of known origin (26 TBE, 23 EV, 6 VZV) and 32 of unknown etiology. The rest – 52 patients without proven CNS infection – remained in group C.

CXCL13 levels differed considerably between the groups (Table 4, Fig. 1). The highest CXCL13 concentrations were found in LNB group A1 (pleocytosis, AI positive) and this parameter enabled this group to be differentiated from all other patients. Group A3 (pleocytosis, AI negative) displayed rather lower CXCL13 levels, but still high. Concentrations in group A2 (without pleocytosis, AI positive) and A4 (without pleocytosis, AI negative) were substantially lower.

Paired comparison proved that the CXCL13 concentrations in group A1 were significantly higher than in all groups ($p < 0.001$) except A3, where the significance level was $p < 0.04$. Similarly, the CXCL13 concentrations in A3 were significantly higher than in all other groups ($p < 0{,}001$ and $p = 0{,}005$) except for groups B (no significant difference) and A1. Although the maximal values in group A2 are slightly higher than in A4 these differences are not statistically significant (Table 4). Even the results from subgroup A2 did not differ from other neuroinfections or controls. Subgroup A4 levels were not significantly higher than controls.

In the group B (non-borrelial CNS infections) the median CXCL13 was 28.1 pg/ml. The median, p25 and p75 percentiles values were lower than in the A1 and A3 groups but the relation was only significant compared with group A1 ($p < 0.001$). Group C (controls) displayed the lowest concentrations of CXCL13 (Table 4). These concentrations mostly resemble the situation in A2 and A4 (non-significant difference).

In this report, using the ROC curve, the limits were searched that would enable us optimally to discriminate patients with LNB and other CNS infections. The cut-off values were determined according to the criterion maximization of the sum of sensitivity and specificity (Fig. 2, Table 5). Considering as abnormal groups A1 and A3 (LNB with pleocytosis) and normal all the other groups (A2, A4, B, C) the calculated discriminating cut-off level was 29 pg/ml, the levels above this limit can be considered elevated (Table 5, Fig. 2). Out of 74 patients signed as LNB (A1 + A3), 67 were above and 7 below this limit, while the rest of tested patients (A2, A4) displayed 47 values elevated and 123 below 29 pg/ml (244 patients in total). Calculated sensitivity was 90%, specificity 72.2%, the area under the ROC curve 0.881. As far as the 130 pg/ml limit is concerned (the part of the ROC curve where the characteristics do not change too much), the specificity rises to 88.2% with a drop of sensitivity at 67.1%. In the case of LNB patients with pleocytosis (A1, A2) the abnormal cut-off level of 400 pg/ml was calculated with specificity 94% and sensitivity 59.6% (ROC area 0.768). Naturally, when the abnormal group is enlarged, the values of sensitivity, specificity and ROC curve change (Table 5.). In the same table, the results of calculations of predictive values are shown. Positive predictive values varied between 57.3–95.6% in close relation to increasing specificity, and vice versa: the negative predictive values decreased in the interval 94.6–67.5%.

Table 4
CSF and blood CXCL13 chemokine concentrations in different patient groups (pg/ml).

Group of patients	Number of patients	p50	p25	p75	Minimal value	Maximal value
CSF						
A1	44	1766,90	645.50	11,440.00	7.8	81,287.60
A2	16	<7.80	<7.80	28.20	<7.80	650.50
A3	30	56.22	32.85	429.80	<7.80	7201.60
A4	20	<7.80	<7.80	7.87	<7.80	118.56
B	82	28.10	9.10	108.7	<7.80	595.87
C	52	<7.80	<7.80	3.90	<7.80	83.3
Blood						
A1	44	37.54	27.75	64.44	11.06	504.10
A2	16	34.40	24.38	66.12	14.10	143.40
A3	30	44.80	27.90	77.10	20.90	256.10
A4	20	32.94	23.00	42.48	9.40	53.40
B	82	39.05	29.91	54.14	12.30	310.85
C	52	29.25	23.41	48.51	14.59	228.62

p25, p50, p75 - percentiles.

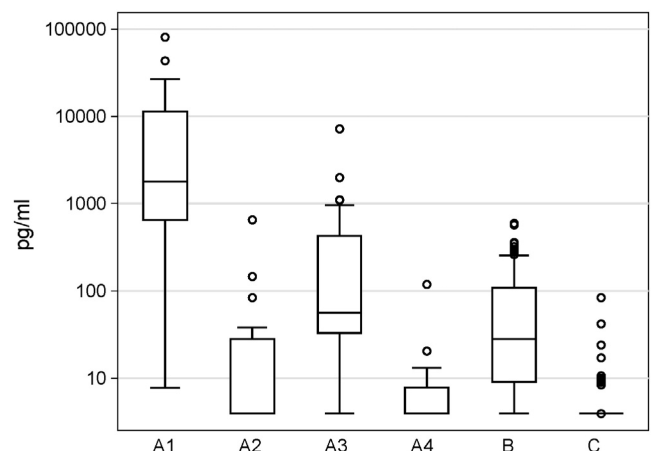

Fig. 1. CSF CXCL13 chemokine concentrations (pg/ml). A1, A2, A3, A4, B, C groups of patients.

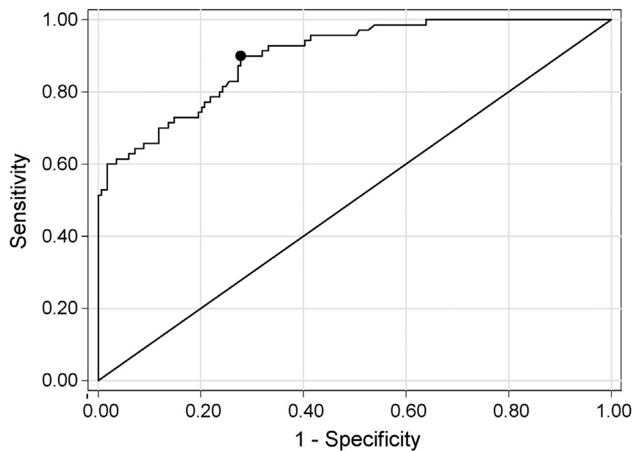

Fig. 2. Roc curve analysis using levels of CXCL13 to discriminate between group of A1 and A3 and other groups of patients (A2; A4; B; C).

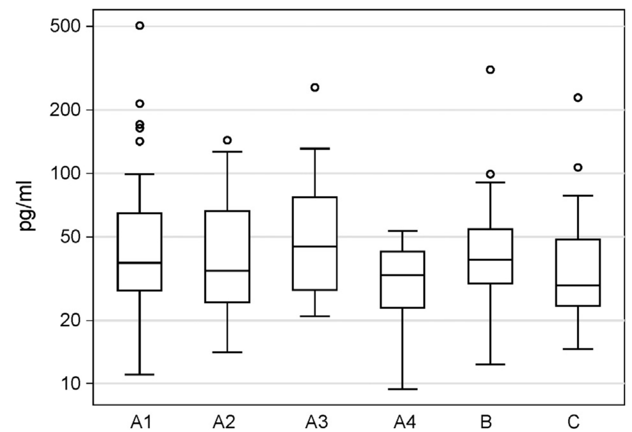

Fig. 3. Blood CXCL13 chemokine concentrations (pg/ml). A1, A2, A3, A4, B, C groups of patients.

Blood CXCL13 concentrations are very uniform in all groups of patients Fig. 3 (range 9.40–504.10; p50 = 36.33). There are noticeable differences between groups, but these are definitely not as important as in CSF. Even though according to the statistical comparison the groups cannot be considered homogenous ($p = 0.032$), none of the paired comparisons of doublets A1–A4/B/C was so high as to be statistically significant. Evaluating parallel blood and CSF CXCL13 concentrations we were able to find only three patients who had both elevated CXCL13 in blood and low concentration of CSF (<7.8 pg/ml). One such case was in A2 (126.50 pg/ml) and two in A3 (126.22 pg/ml; 256.1 pg/ml). Only one patient with a higher concentration of CXCL13 was found in control group C (228.6 pg/ml) and this result was just (and only) in blood. The cut-off value of 62 pg/ml was calculated from ROC for blood (A1 + A3 NB abnormal/rest of patients normal). Other calculations are not presented because they are of only minor importance.

Total protein concentration (Fig. 4) was the highest in LNB group A1 (range 0.35–7.09 g/l; mean 1.23 g/l; p75 = 1.57 g/l). The maximal value of 7.1 g/l was found in a patient with chronic borrelial encephalitis, normal CSF protein was only in four patients. The protein level in A1 was significantly higher than in all other groups except A3 (here with a non-significant difference of $p = 0.268$); other LNB groups (A2/A3/A4) did not differ significantly. When the protein concentration is compared with the normal range, elevated levels in A1, A3 and B can frequently be seen (94.3%, 85.2% and 79.4% respectively); the protein values in group A4 (60.0% of normal levels) were similar to the control group (67.3%; see the comment in methods).

The number of leukocytes and lymphocytes was assessed separately, but both of the parameters correlated so closely that only the total number of leukocytes was presented here (Fig. 5). However, in groups A1–A4 this figure only provides information about the spread of values because this pleocytosis was the classifying criterion. The range in the LNB groups (A) was 2–306 leukocytes/mm^3 (med. = 28.3 cell/mm^3). The difference between groups A1–A3-B on the one hand, and A2–A4-C on the other, are statistically significant ($p < 0.001$). The group of non-borrelial neuroinfections (B) had a leukocyte number comparable with LNB A1 and A3 (non-significant differences). Results in group C corresponded to normal CSF.

The duration of illness before treatment was less than 3 months in the majority of patients (Fig. 6), and more than 2/3 of patients (168 out of 244) had the symptoms for less than 1 month. According to this parameter, the NB patients (A1–A4) split into two units: patients with CSF pleocytosis (A1, A3) with an average history length of 2.1 months (A1) and 1.9 months, and cases without pleocytosis (A2, A4) where the average duration was 7.7 months (A2) and 3.8 months (A4). The history in group B was significantly shorter than in groups A2 ($p < 0.001$) A4 ($p < 0.001$) and C ($p < 0.001$). All other differences were non-significant.

The outcome for patients in this study was only evaluated in group A. All groups A1–A4 differed between themselves (i.e. were not homogenous; $p < 0.001$). A1 and A3 displayed a significantly better outcome than the remaining two – A2, A4 ($p < 0.001$ through $p = 0.027$). 72.2% of A1 and 85.7% of A3 patients had an outcome of "1" (ad integrum). In contrast, complete recovery was referred in only 14.3% and 28.6% of cases in A2 and A4, while 21.4% of patients remained without improvement after treatment (both A2, A4). In general, the outcome split the patients into two significantly different pairs, A1 + A3 and A2 + A4.

4. Discussion

It has already been published elsewhere showing that the CSF CXCL13 levels are elevated in an acute phase of aseptic and purulent CNS infections, especially in LNB [10,12,17,19,21], and that there is a good correlation with the proper function of this chemokine. The results of our study are in full accordance with this. CSF CXCL13 concentrations in aseptic neuroinfections (B) were significantly higher than in controls. The study reliably showed elevated CXCL13 in LNB even in higher concentrations, and moreover, there was remarkable diversification in borrelial patients. CXCL13 concentrations correlated with CSF pleocytosis. This is easy to explain by the arguments given above, but

Table 5
Calculation of the diagnostic sensitivity and specificity CSF CXCL13 measuring.

Abnormal[a]	Cut-off (pg/ml)	Prevalence (%)	Sensitivity (%)	95% CI[b]	Specificity (%)	95% CI[b]	ROC area	Positive Pr[c]	Negative Pr[c]
A1 + A3	29	29	90.0	80.5–95.9	72.2	64.8–78.8	0.811	57.3	94.6
A1 + A3	130	29	67.1	54.9–77.9	88.2	82.3–92.6	0.777	70.1	86.6
A1 + A2	400	24	59.6	45.8–72.4	94.0	89.4–96.9	0.768	75.6	88.1
A1 + A2 + A3	29	36	77.9	67.7–86.1	71.9	64.1–78.9	0.749	60.9	85.3
A1 + A2 + A3 + A4	400	44	40.6	31.1–50.5	98.5	94.7–99.8	0.695	95.6	67.5

[a] Group of patients.
[b] Confidence interval.
[c] Predictive value %.

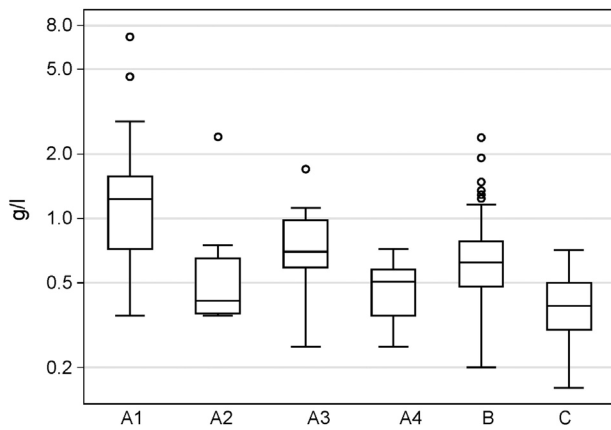

Fig. 4. Total CSF protein (g/l). A1, A2, A3, A4, B, C groups of patients.

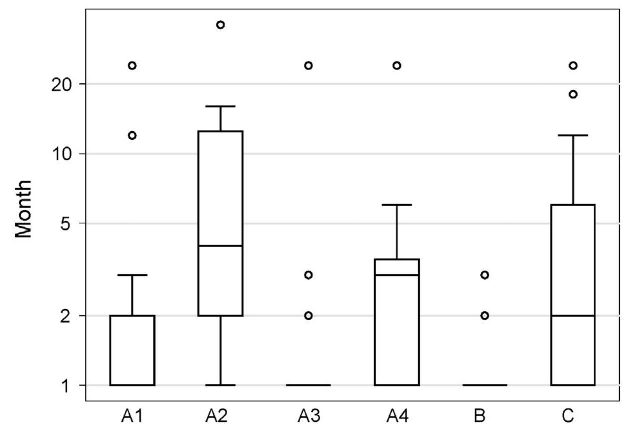

Fig. 6. Length of the disease before treatment (months). A1, A2, A3, A4, B, C groups of patients.

the patients differed in other parameters, which deserve further attention.

Patients with CSF pleocytosis and IT antibody synthesis (A1) represent ideally diagnosed patients – acute LNB ("definite" according to the EFNS classification). Only two patients were under the 29 pg/ml limit (and three under 130 pg/ml). In this case the great importance of CXCL13 examination can hardly be denied. Less obvious, but probably more interesting results from clinical point of view, are the remaining three LNB groups. Both of the groups with positive pleocytosis and negative AI and vice versa (A2, A3) fall into "possible" EFNS categories of LNB and this diagnosis is considered to be relatively well supported. But they differ substantially regarding the CXCL13 examination.

In A2, the chemokine concentrations did not differ either from the patients without pleocytosis and AI (A4), or from the controls. The lowest cut-off of 29 pg/ml was exceeded only in 25% patients of A2 (4/16), one patient in A4 (1/20) and three patients in controls (3/52). By contrast, the patients with pleocytosis and negative AI (A3) had the second highest levels of CXCL13, comparable only with acute neuroinfections (B) (16% of examined had a level under 29 pg/ml; 4/29). Although A3 represents the same LNB diagnostic "level" as A2 in A3, CXCL13 has higher diagnostic significance. It can be assumed that A2 can contain patients with a longer course of disease – in the subacute phase, perhaps spontaneously fading away. This hypothesis is also supported, besides the proper absence of pleocytosis, by the longer duration of illness. An additional argument for this is the lower clinical effect of antibiotic treatment than in the A1 and A3 groups, similar to group A4. This phenomenon could be caused by delayed treatment and/or by the exhaustion of the reparative possibilities of the organism during long, spontaneously running disease. The explanation described seems to be in good agreement with experiences published to date showing that CXCL13 declines quickly to normal values after the antibiotic treatment is initiated (along with normalisation of pleocytosis) [22,15,21,26]. Moreover, it is known that IT synthesis can persist for weeks or months later [2–5]. Results of CSF CXCL13 levels correlate well with previous study of Tjernberg et al. [27]. The groups A1, A2 that correspond fully with the similarly defined patients in this work displayed identical pattern – high percentage of elevated CXCL13 levels in "definite LNB" and low frequency of positives in patients with AI_{IgG} proven/without pleocytosis. Results of group A3, where the second highest positivity of CXCL13 was found, cannot be compared completely due to the rather different definition of these groups in the Tjernbergs' study.

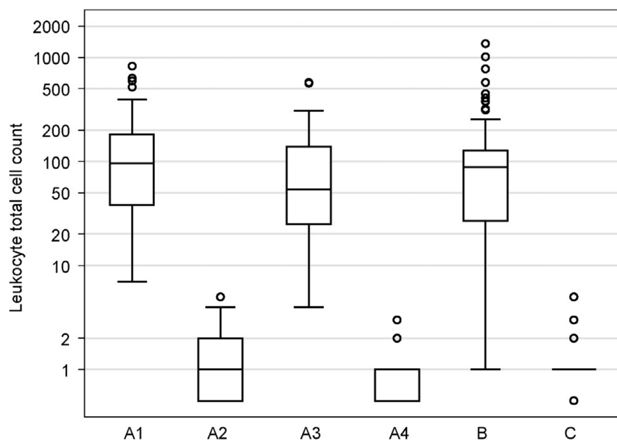

Fig. 5. Number of CSF leukocytes (cell/mm^3). A1, A2, A3, A4, B, C groups of patients.

The disease duration in patients with pleocytosis and negative AI (A3) does not differ from the cases in A1, but it is significantly shorter than in A2, A4. The high levels of CXCL13 can support the assumption that A3 patients have very early stage of LNB. The high levels of CXCL13 can support this assumption. The last LNB patient group without pleocytosis and AI positivity (A4) belongs, according to EFNS classification, to the non-LB category, the low CXCL13 levels in group A4 correlate well with the low probability of active LNB in these patients. Specific antibodies detected in CSF can originate from the blood, though a disrupted blood brain barrier and/or artificial bleeding was found in only 12 out of 20 A4 patients.

However, there is no generally accepted cut-off for diagnosing either LNB or other CNS infections based on CXCL13 levels in CSF, as reported; cut-offs range from less than 100 to more than 1229 pg/ml. Similarly, the cut-off level for the used diagnostic kit has not yet been defined by the manufacturer and despite the fact that all published results were obtained by the same kit, the recently published values differ substantially. Wutte et al. [11] considered the CSF levels above the >125 pg/ml limit as elevated and values above 500 pg/ml as highly elevated; the same limit was used by Ljøstad et al. [2]. A cut-off at 250 pg/ml was thus proposed by van Burgel et al. to distinguish between LNB and other diseases [16]. Results of Hytönen et al. show the cut-off of 415 pg/ml allowed to discriminate between borrelia (above the limit) and non-borrelia patients (below) [15]. Using the higher cut-off value of 1229 pg/ml was discussed by Schmidt et al. [28]. Blood cut-off levels are mentioned less frequently due to their minor clinical importance. Wutte et al. [20] showed the considerable variability of blood CXCL13: levels of 35–261 pg/ml were found among patients with dermatoses, while levels of 26–500 pg/ml were found in blood donors. The results of 29–400 pg/ml calculated in this report approximately correlate with those discussed above. Recent experiences obviously do not allow us to gain a more precise cut-off. Calculations of sensitivity and specificity, which are inversely related, vary as well. If only proven LNB cases were taken

for diagnostic sensitivity in this study, it would reach 90% (A1 and A3), but low specificity 72.2% and even more so if only A1 group would be considered as LNB according to [11,10,15,18,19]. Low sensitivity of 40.6% is caused by taking even probable cases of LNB together into the group of LNB.

Physiological CSF CXCL13 concentrations have not been defined sufficiently, it seems, for values less than 10 pg/ml. In our control group, only one measurement exceeding this value was observed (118.56 pg/ml). Published average pathological levels vary as well, as can be seen in the following examples: 500 pg/ml, 1183 pg/ml, 6480 pg /ml and 15,149 pg/ml (mean) [15,16,21]. These fluctuations are frequently justified on methodical grounds and so a better standardization is the condition (sine qua non) for future expansion of the test. Another reason for oscillations in values could be the differences in the structure of patient groups. Diversity in the form of clinical involvement, the method of LB diagnostics and the composition of control groups influences the final absolute CXCL13 concentration directly via cut-off values [29].

As far as the CXCL13 serum levels presented here are concerned, it is necessary to repeat previously published experience [20] that serum CXCL13 do not bring any essential additional clinical information either in LNB, or in aseptic neuroinfections to date. Although the CSF pleocytosis correlates very well with CXCL13 elevation, the absolute concentration of this chemokine is not related to the number of leukocytes (statistically verified here, $p = 1.000$). A similar result was published by Hytönen et al. [15]. The reason is not known.

It was shown that LNB patients with shorter disease duration, pleocytosis and also with elevated CXCL13 chemokine levels demonstrated better outcomes after therapy. In other words, the absence of these three markers in groups A2 and A4 indicates a statistically significant predisposition towards longer persisting disease symptoms. But the reasons for this are not necessarily the same. Whereas in the group with positive specific antibody synthesis the result could more probably be explained by the delayed antibiotic treatment, in the other group the cause could be in an incorrect diagnosis or unrecognised different illness. This cannot be resolved by the results presented here. The question raised by Senel [29] as to whether CXCL13 levels do correlate with clinical or laboratory disease activity and/or with its length remains without answer so far.

5. Conclusion

The results presented support the previously published experiences that the CSF CXCL13 chemokine levels closely correlate with pleocytosis (but not with absolute number of leukocytes) and total protein in patients with neuroborreliosis and other aseptic CNS infections. But in LNB there are a number of patients with intrathecally synthesized specific antibodies, in which the concentration of CXCL13 is low, and these patients have a longer disease course. The diagnostic role of CSF CXCL13 has not been definitely defined. Taking into account all three mentioned diagnostic markers of LNB which are easy accessible – CSF pleocytosis, total protein and AI – the diagnostic usage of CXCL13 seems to be most favorable in less clinically or laboratory defined cases, in unspecific symptoms or unequivocal laboratory results. However, the specificity of CXCL13 testing should be improved for this purpose because this chemokine has been proven in many other neuroinfections and neurological diseases. Of course, the CXCL13 levels are higher in NB than in the majority of the other pathological conditions and so it remains an important candidate diagnostic marker. The high diagnostic sensitivity and negative predictive value in A1 and A3 groups (for the cut-off 29 and 130 pg/ml) are reasonable supportive arguments. Better methodical standardization, especially concerning the cut-off discriminating values, would help in spreading this test more widely.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] K. Hansen, A.M. Lebech, Lyme neuroborerreliosis:a new sensitive diagnostic assay for intrathecal synthesis of *Borrelia burgdorferi* specific immunoglobulin G, A, and M, Ann. Neurol. 30 (1991) 197–205.
[2] U. Ljøstad, T. Skarpaas, A. Mygland, Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis, Eur. J. Neurol. 14 (2007) 873–876.
[3] R. Kaiser, S. Rauer, Analysis of the intrathecal immune response in neuroborreliosis to a sonicate antigen and three recombinant antigens of Borrelia burgdorferi sensu stricto, Eur. J. Clin. Microbiol. Infect. Dis. 17 (1998) 159–166.
[4] H. Tumani, G. Nölker, H. Reiber, Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis, Neurology 45 (1995) 1663–1670.
[5] B. Wilske, L. Bader, H.W. Pfister, V. Preac-Mursic, Diagnosis of Lyme neuroborreliosis. Detection of intrathecal antibody formation, Fortschr. Med. 109 (1991) 441–446.
[6] S. Hammers-Berggren, K. Hansen, A.M. Lebech, M. Karlsson, *Borrelia burgdorferi* - specific intrathecal antibody production in neuroborreliosis: a follow-up study, Neurology 43 (1993) 169–175.
[7] D. Pícha, L. Moravcová, D. Vaňousová, J. Hercogová, Z. Blechová, DNA persistence after treatment of Lyme borreliosis, Folia Microbiol. 59 (2) (2014) 115–125, http://dx.doi.org/10.1007/12223-013-0272-4.
[8] T.A. Rupprech, H.W. Pfister, B. Angele, S. Kastenbauer, B. Wilske, et al., The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis, Neurology 65 (2005) 448–450.
[9] B.M. Segal, E.L. Logigian, Sublime diagnosis of Lyme neuroborreliosis, Neurology 65 (2005) 351–352.
[10] U. Ljøstad, A. Mygland, CSF – lymphocyte chemoattractant (CXCL13) in the early diagnosis of acute Lyme neuroborreliosis, J. Neurol. 255 (2008) 732–773, http://dx.doi.org/10.1007/s00415-008-0785-y.
[11] N. Wutte, A. Berghold, S. Löffler, W. Zenz, E. Daghofer, et al., CXCL 13 chemokine in pediatric and adult neurroborreliosis, Neurologica 124 (2011) 321–328.
[12] M.C. Kowarik, S. Cepok, J. Sellner, V. Grummel, M.S. Weber, et al., CXL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation, J. Neuroinflammation 9 (2012) 93, http://dx.doi.org/10.1186/1742-2094-9-93.
[13] T. Cerar, K. Ogrinc, S. Lotrič-Furlan, J. Kobal, S. Levičnik-Stezinar, et al., Diagnostic value of cytokines and chemokines in Lyme neuroborreliosis, Clin. Vaccine Immunol. 20 (2013) 1578–1584.
[14] H. Sillapää, B.H. Skogman, H. Sarvas, I.J. Seppälä, P. Lahdenne, Cerebrospinal fluid chemokine CXCL13 in the diagnosis of neuroborreliosis in children, Scand. J. Infect. Dis. 45 (2013) 526–530, http://dx.doi.org/10.3109/00365548.2013.776700.
[15] J. Hytönen, E. Kortela, M. Waris, J. Puustinen, J. Salo, et al., CXCL13 and neopterin concentrations in cerebrospinal fluid of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammantion, J. Neuroinflammation 11 (2014) 103, http://dx.doi.org/10.1186/1742-2094-11-103.
[16] N.D. van Burgel, F. Bakels, A.C.M. Kroes, A.P. van Dam, Discriminating Lyme neuroborreliosis from other neuroinflammatory diseases by levels of CXCL13 in cerebrospinal fluid, J. Clin. Microbiol. 49 (2011) 2027–2030, http://dx.doi.org/10.1128/JCM.00084-11.
[17] G. Ramesh, A.G. MacLean, M.T. Philipp, Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain, Mediat. Inflamm. (2013), 480739 http://dx.doi.org/10.1155/2013/480739 Review.
[18] K. Narayan, D. Dail, L. Li, D. Cadavid, S. Amrute, et al., The nervous system ectopic germinal center: CXCL13 and IgG in Lyme beuroborreliosis, Ann. Neurol. 57 (2005) 813–823.
[19] T.A. Rupprecht, A. Plate, M. Adam, M. Wick, S. Kastenbauer, et al., The chemokine CXCL13 is a key regulator of B cell recruitment to the cerebrospinal fluid in acute Lyme neuroborreliosis, J. Neuroinflammation 6 (2009) 42, http://dx.doi.org/10.1186/1742-2094-6-42.
[20] N. Wutte, A. Berghold, I. Krainberg, E. Aberer, Serum CXCL13 chemokine is not a marker for active Lyme borreliosis, Acta Derm. Venereol. 91 (6) (2011) 724–725, http://dx.doi.org/10.2340/00015555-1144.
[21] R. Dersch, T. Hottenrott, M. Senel, V. Lehmensiek, H. Tumani, et al., The chemokine CXCL13 is elevated in the cerebrospinal fluid of patients with neurosyphilis, Fluids Barriers CNS 12 (2015) 12, http://dx.doi.org/10.1186/s12987-015-0008-8.
[22] D. Bremell, M. Mattsson, M. Edsbagge, K. Blennow, U. Andreasson, et al., Cerebrospinal fluid CXCL13 in Lyme neuroborreliosis and asymptomatic HIV infection, BMC Neurol. 13 (2013) 2, http://dx.doi.org/10.1186/1471-2377-13-2.
[23] P.M. Bossuyt, J.B. Reitsma, D.E. Bruns, C.A. Gatsonis, P.P. Glasziou, et al., The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. The standards for reporting of diagnostic accurancy group, Croat. Med. J. 44 (2003) 639–650.
[24] G. Stanek, V. Fingerle, K.P. Hunfeld, B. Jaulhac, R. Kaiser, et al., Lyme borreliosis: clinical case definitions for diagnosis and management in Europe, Clin. Microbiol. Infect. 17 (2011) 69–79, http://dx.doi.org/10.1111/j.1469-0691.2010.03175.x.

[25] A. Mygland, U. Ljøstad, V. Fingerle, T. Rupprecht, E. Schmutzhard, et al., EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis, Eur. J. Neurol. 17 (2010) 8–16, http://dx.doi.org/10.1111/j.1468-1331.2009.02862.x.
[26] C.M. Marra, L.C. Tantalo, S.K. Sahi, C.L. Maxwell, S.A. Lukehart, CXCL13 as a cerebrospinal fluid marker for neurosyphilis in HIV-infected patients with syphilis, Sex. Transm. Dis. 37 (2010) 283–287.
[27] I. Tjernberg, A.J. Henningsson, I. Eliasson, P. Forsberg, J. Ernerudh, Diagnostic performance of cerebrospinal fluid chemokine CXCL13 and antibodies to the C6-peptide in Lyme neuroborreliosis, J. Infect. 62 (2010) 149–158.
[28] C. Schmidt, A. Plate, B. Angele, H.W. Pfister, M. Wick, et al., A prospective study on the role of CXCL13 in Lyme neuroborreliosis, Neurology 79 (2011) 1051–1058.
[29] M. Senel, T.A. Rupprecht, H. Tumani, H.W. Pfister, A.C. Ludolph, et al., The chemokine CXCL13 in acute neuroborreliosis, J. Neurol. Neurosurg. Psychiatry 81 (2010) 929–933.