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Original Article

The predictive value of CXCL13 in suspected Lyme neuroborreliosis: a retrospective cross-sectional study.

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Declarations

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Conflicts of interest

All authors declare that they have no conflicts of interest. ICMJE forms for all authors are attached.

Ethics approval

The study was approved by the Danish Data Protection Agency (j.nr.19/4365) and as a quality development project at Odense University Hospital (j.nr.18/6688). The Danish National Committee on Health Research Ethics stated that no approval was required therefrom (j.nr.2019-2000-12).

Consent

As this was a retrospective study, no individual patient consent was required. This was stated at approval by the Danish Data Protection Agency (j.nr.19/4365) and Odense University Hospital (j.nr.18/6688).

Data availability

The dataset generated and analysed during the current study is not publicly available due to the Danish Data Protection Law in accordance with approval by the Danish Data Protection Agency (j.nr.19/4365). It is available from the corresponding author on reasonable request.

Authors´contributions

Conceptualization; Fredrikke C. Knudtzen, Anna Christine Nilsson, Joppe W. Hovius, Sigurdur Skarphedinsson. Data curation; Fredrikke C. Knudtzen, Anna Christine Nilsson. Formal Analysis; Fredrikke C. Knudtzen. Funding acquisition; Fredrikke C. Knudtzen, Sigurdur Skarphedinsson. Investigation; Fredrikke C. Knudtzen, Anna Christine Nilsson. Methodology; Fredrikke C. Knudtzen. Project administration; Fredrikke C. Knudtzen, Sigurdur Skarphedinsson. Resources; Anna Christine Nilsson. Supervision; Anna Christine Nilsson, Joppe W. Hovius, Sigurdur Skarphedinsson. Visualization; Fredrikke C. Knudtzen, Sigurdur Skarphedinsson. Writing – original draft; Fredrikke C. Knudtzen. Writing – review & editing; Fredrikke C. Knudtzen, Anna Christine Nilsson, Joppe W. Hovius, Sigurdur Skarphedinsson.

Abstract

Purpose

The role of CXCL13 as a marker of Lyme neuroborreliosis (LNB) is under investigation, and CXCL13 is not part of routine diagnostics in suspicion of LNB. Our aim was to find the optimal cut-off value of CXCL13 for LNB in a Danish population and to investigate the role of CXCL13 both in early LNB and as a discriminatory marker between LNB and other neuroinflammatory disorders.

Methods

A retrospective cross-sectional study including all patients with a cerebrospinal CXCL13 test performed at the Department of Clinical Immunology, Odense University Hospital, Denmark, between January 1st 2015 and December 31st 2018.

Results

We included 619 patients, of which 51 had definite LNB, 14 patients had possible LNB with neurological symptoms suggestive of LNB and pleocytosis but no intrathecal *Borrelia* antibodies, eight patients had prior LNB and 546 had no LNB. With an optimal CXCL13 cut-off of 49 ng/L we found a sensitivity of 100 % and specificity of 94 % (AUC 0.988, 95 % CI 0.980-0.996) when patients treated with antibiotics prior to lumbar puncture were excluded (n=130). All patients with possible LNB had a CXCL13 value above the cut-off value, 18/546 patients (3.3 %) without LNB had a CXCL13 value ≥ 50 ng/L.

Conclusion

While CXCL13 cannot be used as a stand-alone test, it can be used as a reliable additional marker in treatment-naive patients suspected of LNB. CXCL13 can be used to monitor treatment response in LNB patients.

Keywords

Lyme neuroborreliosis; CXCL13; predictive marker; cerebrospinal fluid

Introduction

Lyme neuroborreliosis (LNB) is a frequent and potentially severe manifestation of Lyme borreliosis. It occurs in 10 - 15 % of European patients infected with *Borrelia burgdorferi* sensu lato complex (*Bb*). The diagnosis of LNB is based on the combination of 1) relevant neurological symptoms, 2) cerebrospinal fluid (CSF) with lymphocytic pleocytosis and 3) evidence of intrathecal synthesis of *Bb* IgM and/or IgG antibodies measured through the *Bb* Antibody Index (*Bb* AI) [1-4]. The diagnosis is deemed definite LNB when all three criteria are present. Two out of three criteria reflects a possible diagnosis, strengthened by response to antibiotic treatment.

There is room for improvement of the current diagnostics of LNB. The *Bb* AI is considered the clinical reference standard, but only has a sensitivity of around 80 % in the early stages of the disease [5,6,4,1,7]. This can lead to patients being overlooked and not receiving timely treatment. On the other hand, *Bb* specific antibodies can remain in the CSF for months to years after treatment, and do not always represent an active infection [4,2]. Persisting CSF-*Bb* antibodies may result in patients with prior LNB receiving unnecessary treatment.

The most promising biomarker for improving LNB diagnostics is the B-lymphocyte attracting chemokine CXCL13. CSF-CXCL13 (from here on referred to as CXCL13) is elevated in the early stages of LNB before the production of *Bb* antibodies can be measured in the CSF [8-10]. CXCL13 levels also decline rapidly after initiation of relevant antibiotic treatment, hereby distinguishing between current and prior infection [8,11,9,12]. Previous studies have found CXCL13 to have a sensitivity of 88 - 100%, surpassing that of the *Bb* AI [8,13,14,9,10,15,16]. With a specificity of 89 – 99 % CXCL13 is superior to other inflammatory markers in the CSF when differentiating between LNB and other neuroinflammatory diseases [13,8,11,10,15,16]. Though generally seen with lower levels than in LNB, CXCL13 can be elevated in a range of other neuroinfections such as neurosyphilis and viral encephalitis, autoimmune neuroimmunological disorders and even CNS malignancies [8,11,14,9,16]. A recently published meta-analysis found an optimal CXCL13 cut-off of 162 ng/L across all published studies on LNB, and an optimal cut-off of 91 ng/L when only cross-sectional studies were included [8].

Even though these data are promising, CXCL13 is not yet part of the routine diagnostics of LNB and there is no consensus on an optimal cut-off value. As a result the European guidelines request more evidence on the role of CXCL13 in LNB [1]. We aimed 1) to quantify CXCL13 in patients with definite and possible LNB and to establish an optimal cut-off value for CXCL13 in Danish LNB

patients, 2) to investigate CXCL13 as a marker in early LNB and 3) to investigate CXCL13 as a differential diagnostic marker between LNB and other causes of neurological symptoms.
Our hypothesis was that the use of CXCL13 would improve the diagnostic yield in patients with suspected LNB and short symptom duration, help differentiate between ongoing and prior LNB and between LNB and other causes of neurological symptoms.

Materials and methods

Study population and design

For CXCL13 analyses, the Department of Clinical Immunology at Odense University Hospital serves the entire Region of Southern Denmark with a population of 1.223.348 inhabitants on January 1st 2019 [17]. This project was a retrospective cross-sectional study including all patients with a CXCL13 result registered in the laboratory information system at the Department of Clinical Immunology in the period of 01.01.2015 - 31.12.2018. Indication is not stated when CXCL13 tests are ordered, and laboratory technicians did not have access to clinical information or *Bb* AI results. The patients´ charts were retrieved through electronic hospital records. The study was approved by the Danish Data Protection Agency (j.nr.19/4365) and as a quality development project at Odense University Hospital (j.nr.18/6688). The Danish National Committee on Health Research Ethics stated that no approval was required therefrom (j.nr.2019-2000-12). The study sought to adhere to the STARD criteria for reporting diagnostic accuracy studies [18].

Registered data

Through review of patient charts we registered age, gender, date of symptom onset, neurological symptoms, date of CXCL13 sampling (lumbar puncture), sample arrival date at the laboratory, date and result of CXCL13 assay, antibiotic treatment prior to lumbar puncture, antibiotic treatment and duration and final diagnosis code after course of disease.
From blood tests C-reactive protein, white blood cell count, platelets, alanine aminotransferase, *Bb* antibodies (LIAISON Borrelia IgG/Borrelia IgM Quant from DiaSorin), HIV-1 status, *Mycobacterium tuberculosis* complex interferon-gamma release assay and serology for tick-borne co-infections (*Rickettsia* species (spp.), Tick Borne Encephalitis virus (TBEV), *Bartonella* spp., *Anaplasma phagocytophilum*, *Francisella tularensis*, *Babesia microti* and *Coxiella burnetii*) were registered when available.
From CSF the leukocyte count, protein, glucose, *Bb* IgM/IgG antibody index (IDEIA™ Lyme Neuroborreliosis Kit, Oxoid, Hampshire, United Kingdom), oligoclonal bands, bacterial growth,

polymerase chain reaction (PCR) for viruses (Herpes Simplex virus, Varicella Zoster virus, Enteroviruses, Cytomegalovirus, Epstein Barr virus, TBEV), pathology results, syphilis and autoimmune/paraneoplastic markers were registered when available.

CXCL13 assay

The CSF CXCL13 concentrations were determined using a CE-labelled CXCL13 ELISA assay (EUROIMMUN AG, Lübeck, Germany) in a routine setting in an accredited laboratory (DS/EN ISO 15189:2013 standard, accreditation nr.456), according to the manufacturer's instructions. The assay had been validated by the manufacturer and the Department of Clinical Immunology prior to being offered as a routine analysis. The manufacturers proposed reference range was normal range < 20 ng/L, borderline range 20-29 ng/L, increased 30-100 ng/L and strongly increased > 100 ng/L. These reference ranges were adopted locally. Concentrations < 10 ng/L were reported as such. For statistical analyses, samples with concentrations below the lowest level of detection were arbitrary set at 9 ng/L. Samples with concentrations above the highest level of detection were titrated to the endpoint if CSF was available (n=37). As incorrect handling of CSF samples might lead to a considerable reduction in CXCL13 concentration, samples were kept at +2 - +8°C for maximum 14 days after sampling before being analysed. Otherwise samples were frozen and stored at -20°C, and gently thawed at room temperature prior to analysing. When CSF samples were stored differently this was documented. Measurements were done in singles.

Definitions

Definite LNB was defined as patients fulfilling all three diagnostic criteria for LNB according to European guidelines [1].

Possible LNB was defined as patients with neurological symptoms suggestive of LNB, CSF pleocytosis, negative *Bb* AI, response to relevant antibiotic treatment and no other diagnosis found.

Relevant neurological symptoms were defined as symptoms known to be associated with LNB; radicular pain, paresis (cranial nerves and/or abdominal wall or limbs), symptoms of meningitis, myelitis and/or encephalitis [1,2].

Pleocytosis was defined as $\geq 5 \times 10^6$ white blood cells/L CSF.

Statistical analyses

Statistical analyses were carried out using STATA version 15. To test significance between groups, we used Pearson's chi-squared test and t-test for normally distributed data, Wilcoxon-Mann-Whitney for non-normally distributed data. Receiver operating characteristics (ROC) analysis and Youden's Index were used to establish CXCL13 cut-offs. A p-value < 0.05 was considered statistically significant.

Results

Patient inclusion and sub-groups

Patient inclusion is shown in **Fig 1**; the seven patients from outside the Region of Southern Denmark were excluded due to lack of access to patient data. We included 619 patients who were divided into four sub-groups of definite, possible, prior or no LNB based on CSF results and clinical data. Of the eight patients with prior LNB three had received antibiotic treatment for LNB 2-4 months prior to lumbar puncture and five had been diagnosed with LNB 4-10 years earlier.

Patient characteristics

The majority of patients were women (56.1 %), but in the prior LNB group seven of eight patients were men (p=0.012) (Table 1). The median age was 48 years (IQR 28), the patients with definite or possible LNB were significantly older (median 57 [IQR 24] years) than the patients without LNB (median 46 [IQR 27] years) (p=0.001). There were 30 children below 18 years of age.

CXCL13 cut-off

CXCL13 results are displayed in Table 1. In total, 520 samples were within the manufacturer's normal reference range, 13 in the borderline range, 23 were increased and 63 strongly increased. There were no significant differences in mean CXCL13 values between children (126.6 ng/L, 95%CI -49.1 – 302.2 ng/L) and adults (358.5 ng/L, 95%CI 182.0 - 535.1 ng/L, p=0.24). When calculating an optimal cut-off using the Youden's Index we found a sensitivity of 82 % and specificity of 94 % with a CXCL13 cut-off 49.5 ng/L (AUC 0.899, 95 % CI 0.842-0.956) if all patients were included. When patients with antibiotic treatment prior to lumbar puncture were excluded (n=130, including 14 patients with definite LNB) the optimal cut-off hardly changed (49 ng/L), but CXCL13 sensitivity and specificity were now 100 % and 94 % (AUC 0.988, 95 % CI 0.980-0.996) (**Fig 2**). This correlates to a positive predictive value (PPV) of 0.58 and a negative predictive value (NPV) of 1.00 if the patients in the definite LNB group were set as true positives, PPV 0.78 and unchanged NPV if the possible LNB patients were included.

Overall 74 patients (12.0 %) had a CXCL13 above cut-off, including all patients in the possible LNB group and 42 patients (82.4 %) in the definite LNB group. In all the nine patients in the definite LNB group with CXCL13 below cut-off, antibiotic treatment preceded lumbar puncture (**Fig 3**). The patients in the definite and possible LNB groups had significantly higher CXCL13 means than patients in the prior and non LNB groups (p<0.001). The optimal cut-off and sensitivity and specificity were not altered when paediatric patients were excluded.

CXCL13 in early LNB

All 14 patients with possible LNB had increased CXCL13 (range 63 – 2550 ng/L). Median delay from symptom debut to lumbar puncture was 10 days (IQR 20 [2-24] days), significantly shorter than in patients with definite LNB (median 22 [IQR31] days, p=0.019). All 14 patients reported a marked improvement of clinical symptoms after antibiotic treatment, the majority (64.3 %) within one month of treatment initiation.

CXCL13 as a differential diagnostic marker

In the no LNB group 18 patients (3.3 %) had a CXCL13 above our calculated cut-off at 49.5 ng/L. Their diagnoses and symptoms are displayed in Table 2.

Based on the final diagnoses given to each patient at the end of disease course, mean CXCL13 values for different diagnostic groups were calculated (Table 3). LNB patients had a significantly higher mean CXCL13 (3071.7 (SD 5927.0) ng/L) compared with the other diagnostic groups ($p<0.001$). Of the other diagnostic groups the patients with malignancies and autoimmune disorders had the highest mean CXCL13 concentrations with 316.4 ng/L (SD 887.2 ng/L) and 313.2 ng/L (SD 802.0 ng/L), respectively. In 144 patients, a lumbar puncture was done on suspicion of LNB which was ruled out, and another diagnosis was not found. All of these patients had a CXCL13 within normal range, yielding a specificity of 100 % in this group.

CXCL13 in repeated lumbar punctures

Only 36 patients had multiple measurements of CXCL13 registered; 33 had two measurements, three had three measurements (Table 4). The median time from first to second measurement was 38.5 days (IQR 175 [10.5 - 185.5] days). All patients in the definite and possible LNB group had declining CXCL13 over time. In the four cases where the second CXCL13 measurement was not normalized the lumbar puncture had been repeated 2 - 9 days after the first. The majority of no LNB patients had a normal CXCL13 value at both first and second measurement. The four patients with an increase in CXCL13 over time were diagnosed with multiple sclerosis, encephalomyelitis of unknown origin, rheumatoid meningitis and a cerebral infarction. Of 14 patients in the possible LNB group, only three had repeated lumbar punctures after 36, 165 and 180 days, respectively. Here, two were still *Bb* AI negative; the third patient was not re-tested for *Bb*.

CXCL13 handling

The mean delay from lumbar puncture to sample arrival at the laboratory was 1.82 days (SD 4.86,

range 0-60 days). To our knowledge, in four cases (0.6 %) the samples had not been handled correctly and had been in room temperature for > 24 hours. Two of these cases had LNB and strongly increased CXCL13 values of 4130 ng/L and 7900 ng/L. In one patient with encephalitis of unknown origin with pleocytosis of 163 $x10^6$/L, CXCL13 was 29 ng/L. The last patient had a peripheral paresis and sensitivity disturbance of unknown origin, pleocytosis (55 $x10^6$/L) and a CXCL13 < 20 ng/L. The mean time from sample arrival at the Department of Clinical Immunology to CXCL13 results (turnaround time) was 4.77 days (SD 3.58, range 0-22 days).

Discussion

In this large retrospective study investigating the use of CXCL13 in suspected LNB, our primary aim was to quantify CXCL13 in patients with definite and possible LNB in a real life clinical setting. When patients who had received antibiotic therapy prior to CXCL13 sampling were excluded we found an optimal cut-off value at 49 ng/L, providing a sensitivity of 100 %, specificity of 94 %, PPV of 0.58 and NPV of 1.00, respectively. All patients with possible and definite LNB had CXCL13 concentrations above this cut-off.

As previously mentioned a standardized CXCL13 cut-off value does not yet exist, and cut-offs vary substantially between studies [15,11,9,19-21,14,22,16]. Our optimal cut-off is lower than in most published studies including the 2018 meta-analysis where the optimal cut-off was 162 ng/L [8]. Choice of assay might influence CXCL13 results. Studies comparing two different assays on the same CSF-samples have found considerable differences in optimal cut-offs between tests [23,19]. Though local cut-offs based on each laboratory's results would be optimal, it is not feasible and will likely represent a barrier towards disseminating the use of CXCL13 globally.

Our second aim was to validate CXCL13 in early LNB, before intrathecal production of *Bb* antibodies had occurred. In the 14 patients with suspected early LNB, all had a short delay from symptom debut to lumbar puncture, and interestingly all had a CXCL13 above our optimal cut-off value. An alternative diagnosis was not found in any of these patients, and they were all treated as having LNB with clinical improvement after treatment. Unfortunately, only three had a repeated lumbar puncture. We could not confirm conversion to positive *Bb* antibodies in any of them, and *Bb* PCR was not available at the time of the study. Studies with systematically repeated lumbar punctures assessing CXCL13 and *Bb* AI in this patient group are scarce and of small sizes [9,24]. Larger studies are necessary to prove the added value of CXCL13 in early LNB. This area should be a priority in future research if we are to confirm these patients as having true LNB. It can, however, be challenging to

convince patients in remission to have repeated lumbar punctures done.
In our study, 130 patients had received antibiotic treatment before lumbar puncture, likely affecting their CXCL13 values [22,25,26]. In these patients the CXCL13 values were low, reconfirming the role of CXCL13 as an acute phase marker in untreated disease [5,27,26,11,9]. This also confirms that repeated CXCL13 measurements may be used as a marker for treatment response in LNB. The use of antibiotic treatment prior to testing should be kept in mind both when ordering CXCL13 and when interpreting the results. It is an important limitation to the use of the test.

Our third and final aim was to investigate CXCL13 as a differential diagnostic marker. Using our optimal cut-off 49.5 ng/L, 18 patients not deemed as having LNB had a CXCL13 above cut-off. The range of diseases in this group matches findings from previous studies of CXCL13 performance [14,11,19,20,22,9,28,29,26,30,31,16]. Due to the large study size and the majority of tests being ordered from highly specialized departments in a tertiary referral hospital, some of the diseases found are rare. Neurosyphilis, an important LNB differential diagnosis, can be seen with significantly elevated CXCL13 [29,11,28,31]. In neurosyphilis the CXCL13 value is related to symptom severity and possibly plays a role in the neurological damage seen due to B-cell mediated inflammation [32,33]. In primary CNS lymphoma, CXCL13 is expressed by malignant B-cells in the CNS, and is a prognostic marker of response to chemotherapy and survival [34,30]. All but one of the 18 patients had a *Bb* AI performed, and they were all negative. However, in two patients aged 14 and 63 years with less than four weeks of symptoms at lumbar puncture, another diagnosis was never found. Though unlikely and not deemed or treated as LNB, LNB cannot be ruled out as a differential diagnosis. Ruling out other relevant differential diagnosis is crucial in patients with suspicion of LNB and an elevated CXCL13, and relevant symptomology is still an important consideration. Of the 11 no LNB patients with strongly increased CXCL13 values, none had radicular pain, and only two had fascial nerve palsies.

The main strength of our study is the large number of patients with complete data and the complete follow-up. Another is the cross-sectional study design including all patients in the study period with a CXCL13 test performed. This includes a large number of patients with a broad range of other diagnoses than LNB. It also includes both adult and paediatric patients. As there were no significant differences in mean CXCL13 between adult and paediatric patients, and due to the small size of the paediatric cohort, we chose to analyse the group as a whole.
Incorrect handling of samples prior to reaching the laboratory represents a potential bias, as this could lead to a reduction in CXCL13 concentrations. However, the complete access to data from the laboratory information system and patient charts providing an overview on both pre-analytic and

analytic handling of samples reduces this risk. Other study limitations are the retrospective design and the low number of patients having a second lumbar puncture performed. A larger number could have strengthened the conclusion regarding CXCL13s role in early LNB. *Bb* PCR was not available at our hospital at the time of this study. Even though the sensitivity of *Bb* PCR is low, one or more positive samples could have strengthened our results [1,2]. Our study was performed in a European setting where *Borrelia garinii* is the predominant cause of LNB [2]. Whether the assay performs as well in an American setting where *Borrelia burgdorferi* sensu stricto is the dominant genospecies remains to be resolved.

In conclusion, CXCL13 has a high sensitivity and specificity and can be a useful additional diagnostic test in treatment-naive patients suspected of LNB. CXCL13 can be used to monitor treatment response in LNB patients.

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Table 1. Characteristics and cerebrospinal fluid CXCL13 levels in 619 patients.

		All patients (n=619)	Definite LNB (n=51)	Possible LNB (n=14)	Prior LNB (n=8)	No LNB (n=546)
Sex	Female (%)	347 (56.1 %)	30 (58.8 %)	6 (42.9 %)	1 (12.5 %)	310 (56.8 %)
Age	Years, median (iqr)	48 (iqr28)	57 (iqr24)	57.5 (iqr22)	57.5 (iqr56.5)	46 (iqr26)
CXCL13	Mean (95 % CI, range)	347.3 (179.1-515.5, 9-35.400)	3753.4[a,b] (1916.8-5590.1, 9-35.400)	588.1[a,b] (195.7-980.4, 63-2550)	9.5[b] (8.3-10.7, 9-13)	27.9[b] (12.5-43.4, 9-2840)
Proposed optimal CXCL13 cut-off	CXCL13 > 49.5	74 (12.0 %)	42 (82.4 %)	14 (100 %)	0 (0 %)	18 (3.3 %)

[a]p=0.110 between definite and possible LNB [b] p<0.001 between definite/possible LNB and no/prior LNB

LNB = Lyme neuroborreliosis

Table 2. Final diagnosis in 18 patients without Lyme neuroborreliosis and a CSF-CXCL13 > 49.5 ng/L.

Diagnosis	Number of patients	CSF-CXCL13 (ng/L)	CSF white blood count (x 106/L)	CSF *Bb* AI	Main symptoms	Symptom duration at lumbar puncture (days)
Infection						
Neurosyphilis	2	84 412	6 58	neg neg	Impaired hearing Sensitivity disturbance	122 122
Varicella zoster encephalitis	2	459 83	- 393	neg neg	Headache, confusion Fascial nerve palsy, headache	5 1
Rickettsiosis (Rickettsia typhi)	1	188	25	neg	Fatigue, arthralgia	17
Unspecified viral meningitis	1	1840	600	neg	Headache, visual disturbance	26
Acute HIV-infection syndrome	1	97	38	neg	Radicular pain, fever, sensitivity disturbance	24
Unspecified meningitis	1	122	190	neg	Fever, neck stiffness, headache	6
Malignancy						
Cerebral metastasis from malignant neoplasm of lung	1	54	53	neg	Fascial nerve palsy, dizziness	9
Meningeal carcinomatosis, neuroendocrine tumor	1	60	44	neg	Fascial nerve palsy	4
Diffuse large B-cell	1	2840	180	neg	Headache, sensitivity	123

lymphoma					disturbance, fever	
Acute lymphoblastic leukaemia	1	96	4851	-	Radicular pain, peripheral paresis, sensitivity disturbance	60
Miscellaneous						
Neurosarcoidosis	1	100	32	neg	Paresis n.abduscens, n.fascialis, n.recurrens	185
Necrotizing cerebral vasculitis	1	400	11	neg	Headache	18
Encephalo-myelitis	1	135	65	neg	Confusion, fever	16
Acute disseminated encephalomyelitis (ADEM)	1	188	143	neg	Fascial nerve palsy, sensitivity disturbance, peripheral paresis	6
Cerebral infarction (in patient with malignant melanoma)	1	107	23	neg	Fascial nerve palsy, headache	7
Rheumatoid meningitis	1	2569	170	neg	Headache, balance disturbance	116

Bb AI = *Borrelia burgdorferi* Antibody Index; CSF = cerebrospinal fluid

Table 3. CSF-CXCL13 results in different patient groups, based on diagnosis given at end of disease course.

Diagnosis	Number of patients	CSF-CXCL13, ng/L Mean (SD)	CSF-CXCL13, ng/L Median, iqr
Lyme neuroborreliosis	65	3071.7 (5927.0)	690 (2660)
Other neuroinfections	44	83.4 (286.1)	9 (26)
Malignancies	10	316.4 (887.2)	39 (51)
Demyelinating disorders	19	21.1 (40.9)	9 (0)
Autoimmune disorders	10	313.2 (802.0)	9 (91)
Bell's palsy	44	10.2 (4.0)	9 (0)
"Unspecified symptom from nervous system"	82	11.6 (17.0)	9 (0)
Observation on suspicion of Lyme neuroborreliosis	144	9.1 (0.4)	9 (0)
Other miscellaneous diagnoses	201	11.9 (16.1)	9 (0)

Table 4. Cerebrospinal fluid CXCL13 results in 36 patients with repeated measurements.

	CXCL13, first measurement (ng/L)	Days from first to second measurement	CXCL13, second measurement (ng/L)	Days from second to third measurement	CXCL13, third measurement (ng/L)
Definite LNB group, n=10					
	3.910	191	< 10	-	-
	7.900	639	< 10	-	-
	711	39	133	21	< 10
	12.200	2	501	-	-
	1.250	8	90	-	-
	1.270	11	< 10	-	-
	153	9	46	-	-
	6.100	26	< 10	-	-
	< 10[a]	9	< 10	-	-
	< 10[a]	202	< 10	-	-
Possible LNB group, n=3				-	-
	161	42	< 10	-	-
	901	175	< 10	-	-
	160	7	136	-	-
No LNB group, n=23				-	-
	34[a]	327	27	-	-
	188	18	< 10	-	-

	2.840	30	1.714	-	-
	2.569	23	3.580	-	-
	13	32	< 10	-	-
	21	301	44	-	-
	1.840	9	21	55	< 10
	13	52	< 10	-	-
	< 10	19	23	-	-
	135	10	387	28	< 10
(n: 11-23)[b]	< 10	8-1.071	< 10	-	-

[a] received antibiotic treatment before measurement [b] 4/13 received antibiotic treatment before measurement

LNB = Lyme neuroborreliosis

Fig 1 Flow chart of inclusion of patients in the study and into the different study groups

CSF = cerebrospinal fluid; *Bb* AI = *Borrelia burgdorferi* Antibody Index

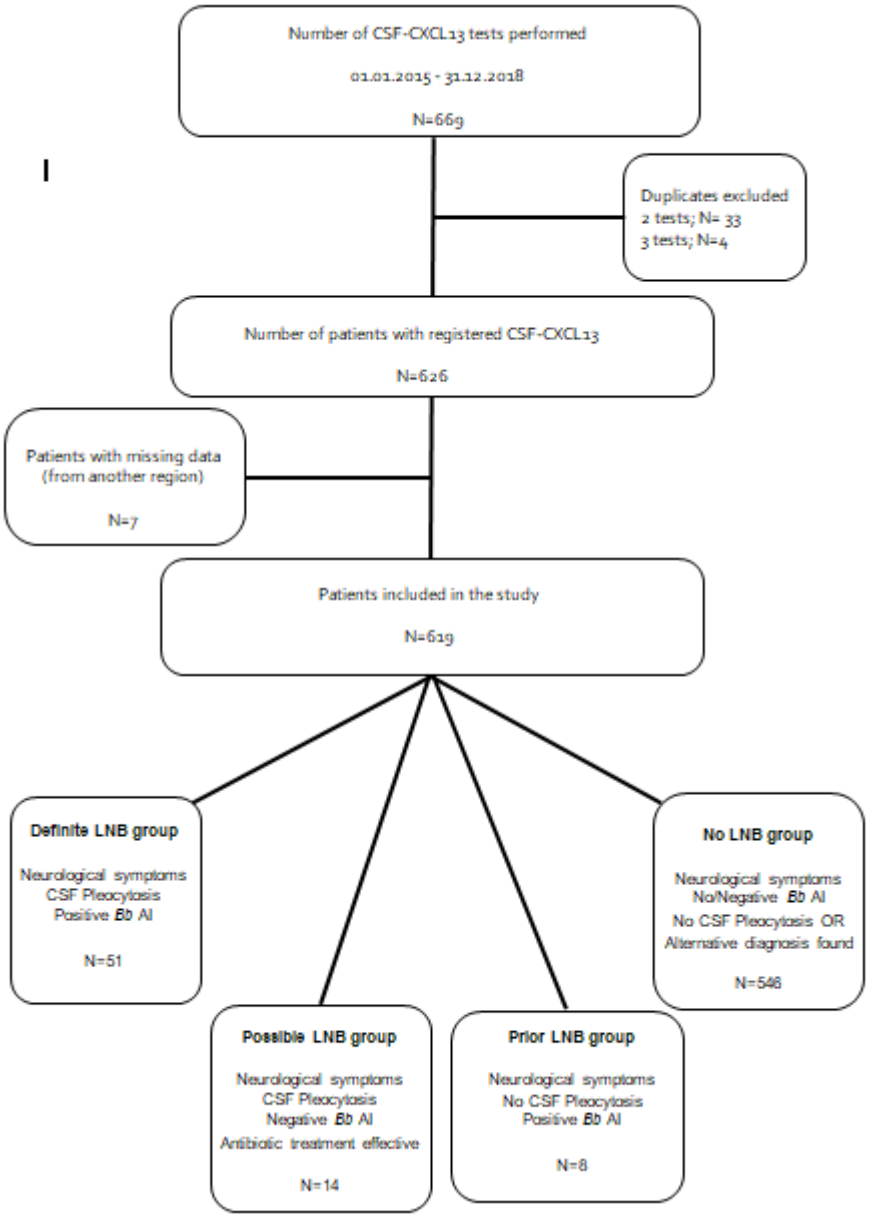

Fig 2 ROC-curve displaying cerebrospinal fluid CXCL13 values in 37 patients with definite Lyme neuroborreliosis not receiving antibiotic therapy before lumbar puncture. AUC 0.99 [95%CI 0.98-1.00]. Figure created using STATA version 15

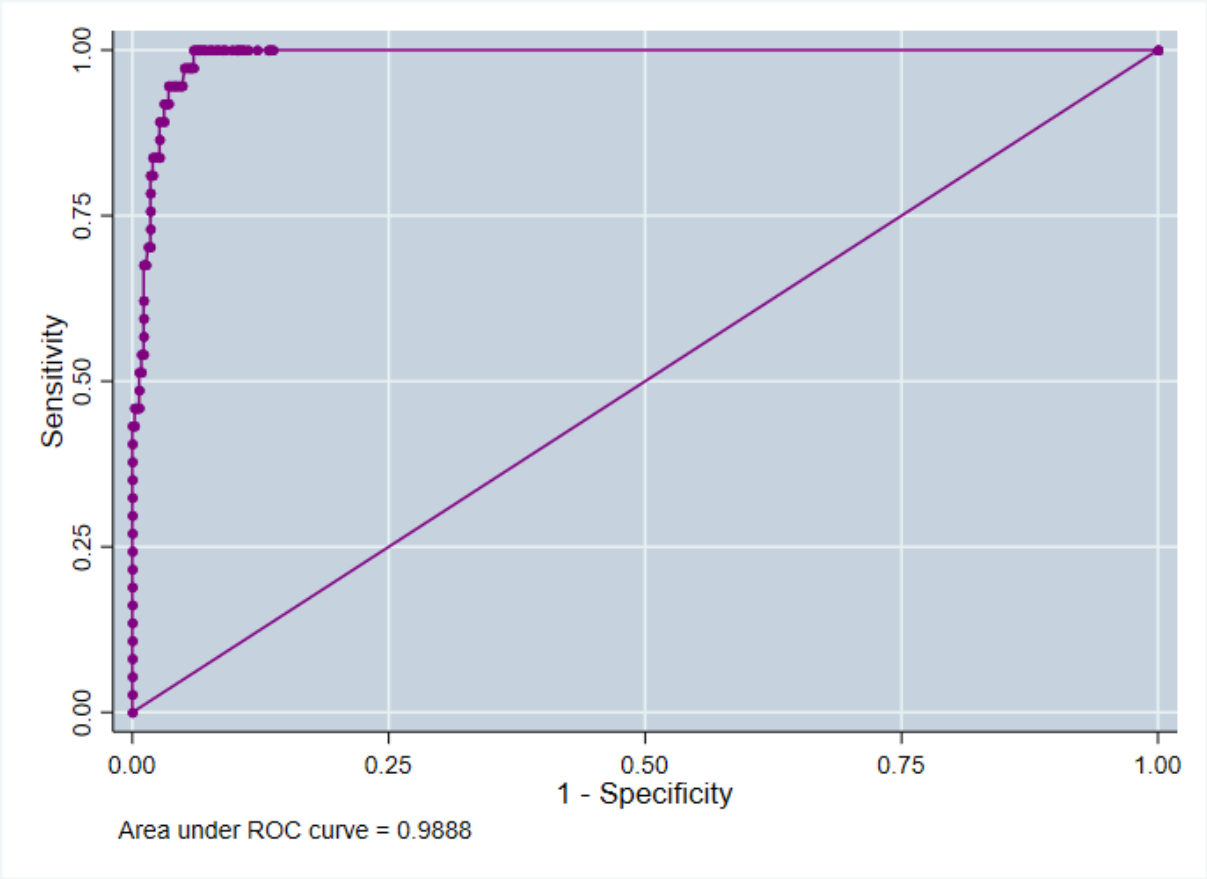

Fig 3 Percentage of patients with a CSF-CXCL13 > 49 ng/L in four different patient groups consisting of in all 489 patients suspected of LNB with no prior antibiotic treatment
LNB = Lyme neuroborreliosis.