Table S1. Classification of different diseases into the 4 groups AIND, NID, NPL, and NIND.

Autoimmune (inflammatory/rheumatic) neurological diseases (AIND; n = 147)

- Multiple sclerosis (n = 73)
  - Relapsing-remitting type (n = 61)
  - Secondary progressive type (n = 6)
  - Primary progressive type (n = 6)
- Clinically isolated syndrome (n = 3)
- Isolated optic neuritis (n = 18)
- Neuromyelitis optica spectrum disease (n = 4)
  - Aquaporin-4-antibody positive (n = 3)
  - Antibody-negative (n = 1)
- Autoimmune (limbic) encephalitis (n = 14)
- Paraneoplastic syndrome (other than limbic encephalitis) (n = 11)
  - Sensory neuronopathy (n = 5)
  - Sensorimotor neuropathy (n = 1)
  - Cerebellar degeneration (n = 6)
- CLIPPERS (n = 1)
- Sjögren's syndrome (neurological) (n = 1)
- CNS-vasculitis (n = 8)
  - Primary angiitis of the central nervous system (PACNS) (n = 4)
  - Parainfectious in case of hepatitis C (n = 1)
  - Unknown origin (n = 2)
  - Rheumatic with anti-nuclear antibodies (Sm) (n = 1)
- Neuro-Behçet's disease (n = 3)
  - Parenchymal type (n = 2)
  - Non-parenchymal type (n = 1)
- Neurosarcoidosis (n = 10)

Neuroinfectious diseases (NID; n = 273)

Bacterial (n = 79)
- Neuroborreliosis (n = 61)
  - Definitive (n = 61)
- Bacterial meningitis (n = 7)
  - Staphylococcus aureus (n = 2)
  - Streptococcus pneumoniae (n = 2)
  - Staphylococcus epidermidis (n = 1)
  - Streptococcus agalactiae (n = 1)
  - Mycobacterium tuberculosis (n = 1)
- Neurosyphilis (n = 10)
  - Definitive (n = 5)
  - Probable (n = 5)
    - Incl. HIV (n = 2)

Fungal/parasitic (n = 7)
- Aspergillosis with CNS-involvement (n = 2)
- Neuroschistosomiasis (n = 1)
- Cryptococcosis with CNS-involvement (n = 4)
  - Incl. neurosarcoidosis (n = 3)
  - Incl. HIV (n = 1)

Viral (n = 87)
- Varicella-zoster virus (n = 49)
- Herpes-simplex virus type 1 (n = 12)
- Epstein-Barr virus (n = 2)
- Tick-borne meningoencephalitis virus (n = 24)

Unknown pathogen (n = 101)
- Neuroinfectious diseases of unknown pathogen (n = 101)

CNS-neoplasia (NPL; n = 49)

B-cell non-Hodgkin lymphoma (n = 21; Primary CNS lymphoma (n = 16); Secondary CNS lymphoma (n = 9))

- Diffuse large cell B-cell lymphoma (n = 14)
- Waldenstrom's macroglobulinaemia (n = 5)
- Follicular B-cell lymphoma (n = 2)
- Marginal zone lymphoma (n = 1)
- B-cell chronic lymphocytic leukemia (n = 2)
- No histology (n = 2)

T-cell non-Hodgkin lymphoma (n = 2; Secondary CNS lymphoma (n = 2))

- Cutaneous T-cell lymphoma (mycosis fungoides; n = 1)
- Primary intestinal epitheliotropic T-cell lymphoma (n = 1)

Solid brain tumor (n = 21)

- Meningioma (n = 3)
- Pilocytic astrocytoma (n = 1)
- Anaplastic astrocytoma (n = 1)
- Diffuse astrocytoma (n = 1)
- Glioblastoma (n = 12)
- Oligodendroglioma (WHO-grade II; n = 1)
- Ganglioglioma (n = 1)
- No histology (n = 1)

Non-inflammatory neurological diseases (NIND; n = 208)

Primary headache disorders (n = 19), idiopathic intracranial hypertension (n = 3), rheumatological diseases without CNS involvement (n = 11), Parkinsons disease (n = 3), somatoform disorders (n = 17), non-inflammatory sensomotoric polyneuropathy (n = 16), restless legs syndrome (n = 1), epilepsy/first epileptic seizure (n = 23), stroke/transitory ischemic attack (n = 13), myasthenia gravis (n = 3), amyotrophic lateral sclerosis (n = 10), Huntington's chorea (n = 3), myositis/myalgia (n = 5), Bell's palsy (n = 12), transitory global amnesia (n = 1), Alzheimer's disease (n = 2), vascular dementia (n = 4), primary progressive aphasia (n = 1), lumbago/lumboischialgia (n = 11), spinal canal stenosis (n = 2), myelo-dysplastic syndrome (n = 2), cranial nerve palsies (n = 6), Phelan McDermid syndrome (n = 8), fatigue syndrome (n = 10), psychiatric diseases (n = 4), systemic borreliosis (n = 3), dural arteriovenous fistula (n = 1), hypokalemic periodic paralysis (n = 1), neuralgic shoulder amyotrophy (n = 3),

normal pressure hydrocephalus (n = 1), spinal muscular atrophy type 1/3 (n = 3), stiff-person syndrome (n = 3), multiple system atrophy (n = 2), giant cell arteritis (n = 1)

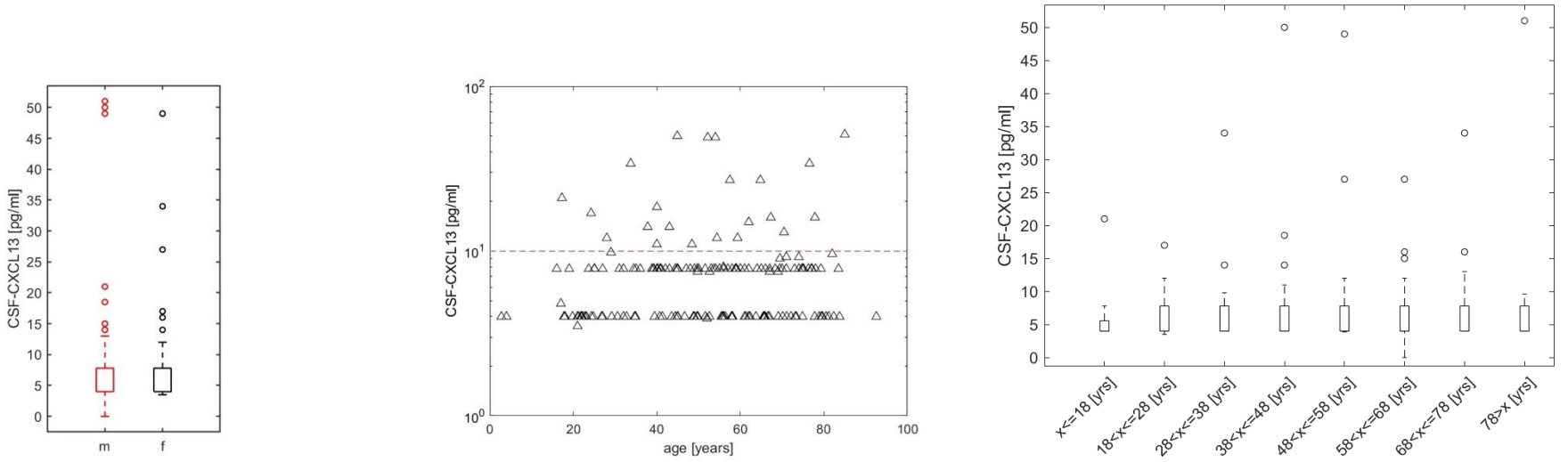
Patients of the Biobank of the Department of Neurology, University Hospital of Ulm, who had received a lumbar puncture from July 2009 to January 2023 were categorized into the 4 groups using medical chart review.

Table S2. Differences in routine CSF findings and CSF-CXCL13 in the four groups.

Parameter	**NIND-NID**	**NIND-AIND**	**NIND-NPL**	**NID-AIND**	**NID-NPL**	**AIND-NPL**
CSF-CXCL13	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p = 0.275**	**p = 0.134**
Leucocyte cell count	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p < 0.001**	**p = 0.043** p > 0.05*
QAlb	**p < 0.001**	p = 0.631	**p < 0.001**	**p < 0.001**	**p = 0.024** p > 0.05*	**p = 0.001**
Total protein	**p < 0.001**	p = 0.105	**p = 0.008**	**p < 0.001**	p = 0.133	p = 0.156

Mann-Whitney U test and the more precise Dunn's post hoc test* were used for multiple comparisons between the 4 groups. P-values below 0.05 were considered significant according to Dunn's test. All values are rounded. QAlb: CSF-albumin/serum-albumin (albumin ratio), CSF: cerebrospinal fluid, AIND: autoimmune inflammatory neurological diseases, NPL: CNS-neoplasia, NIND: non-inflammatory neurological diseases; NID: neuroinfectious diseases.

Figure S1. Analyses of CSF-CXCL13 and gender or age in the NIND group.



a) **b)** **c)**

Figure S1. a) The patients of the NIND group were divided according to gender (m: masculine and f: feminine). The Mann-Whitney U test was used to compare the values of CSF-CXCL13 in the two groups. No significant difference was detected ($p = 0.384$). **b)** Correlation analyses between CSF-CXCL13 and age in the NIND group were determined using Spearman's rank coefficient (r). The dotted lines represent the cut-off for CSF-CXCL13 for non-inflammatory controls (<10 pg/ml) in red. No significant correlation was obtained for CSF-CXCL13 and age ($r = 0.078$). **c)** The patients of the NIND group were grouped by age in steps of ten (yrs: years). Patients below the age of 18 and above 78 were summarized. The Kruskal-Wallis test was used to compare the values of CSF-CXCL13 in the different age-groups. No significant difference was found ($p = 0.156$).

Figure S2. CSF-CXCL13 value and CSF-specific oligoclonal IgG bands and intrathecal IgM synthesis

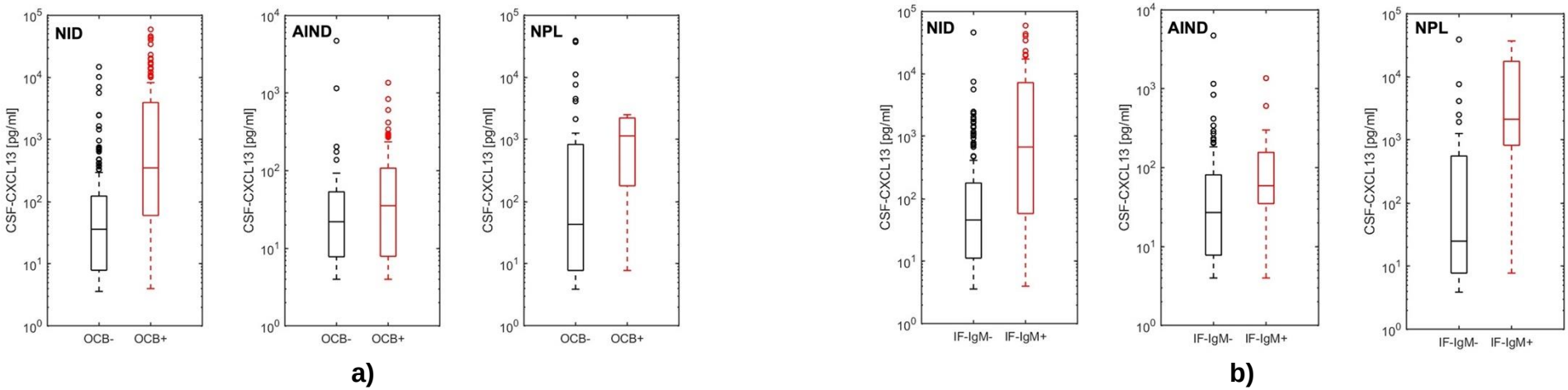


Figure S2. The patients of the three different groups NID, AIND, and NPL were grouped **a)** according to CSF-specific oligoclonal IgG bands (OCB+: OCB present and OCB-: OCB absent) and **b)** intrathecal IgM synthesis (IF-IgM+: intrathecal IgM synthesis present and IF-IgM-: intrathecal IgM synthesis absent). The Mann-Whitney U test was applied for possible significant differences regarding CSF-CXCL13 between each positive and negative status. Patients of the NID group with CSF-specific oligoclonal IgG bands and all groups with intrathecal IgM synthesis had significantly higher CSF-CXCL13 values. **a)** NID: $p < 0.001$, AIND: $p = 0.273$, NPL: $p = 0.307$ and **b)** NID: $p < 0.001$, AIND: $p = 0.001$, NPL: $p = 0.034$. NID: neuroinfectious diseases, AIND: autoimmune (inflammatory) diseases, NPL: CNS-neoplasia, IF-IgM: intrathecal fraction of IgM (=intrathecal IgM synthesis), OCB: CSF-specific oligoclonal IgG bands.

Figure S3. Classification of MS/CIS/ON/NMOSD patients according to clinical and radiographic activity and location of the lesions with contrast enhancement in MRI.

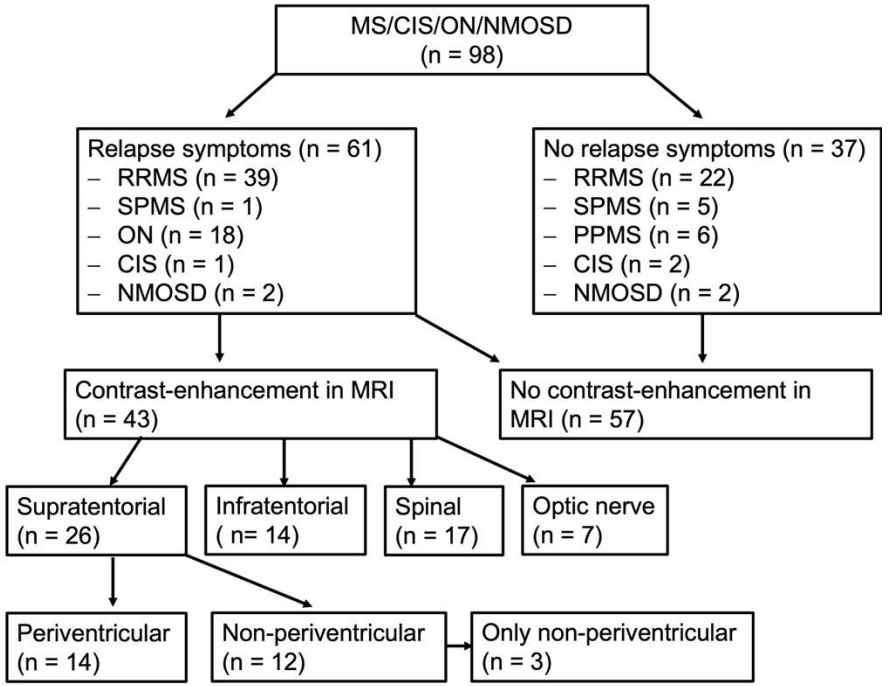


Figure S3. Ninety-eight patients with multiple sclerosis (MS), clinically isolated syndrome (CIS), optic neuritis (ON), and neuromyelitis optica spectrum disease (NMOSD) were grouped according to clinical and radiographic activity by location.

Table S3. Calculation of CSF/serum ratios and indices for CXCL13 in various neurological conditions.

Disease	S-CXCL13 median (range) [pg/ml]	CSF-CXCL13 median (range) [pg/ml]	QAlb median (range)	Q-CXCL13 = CSF-CXCL13/S-CXCL13 median (range)	I-CXCL13 = QCXCL13/ QAlb median (range)
LNB	73.0 (21 - 304)	1932 (142 - 17200)	13.6×10^{-3} (3.0×10^{-3} - 36.2×10^{-3})	24.08 (0.08 - 148.32)	1747.03 (7.9 - 12864.4)
BCL	50.1 (28 - 217)	695 (7.8 - 2500)	12.4×10^{-3} (5.6×10^{-3} - 127.1×10^{-3})	8.36 (0.11 - 24.35)	734.6 (6.16 - 3676)
Viral/I-UP/F/P/B-CNS-D	63 (7.8 - 160)	36.5 (4 - 227)	11.8×10^{-3} (4.4×10^{-3} - 28.4×10^{-3})	0.51 (0.03 - 3.22)	47.96 (0.5 - 254.68)
MS/ON/CIS/NMOSD; all	59.5 (7.8 - 158)	23.5 (7.8 - 119)	5.46×10^{-3} (2.51×10^{-3} - 12.7×10^{-3})	0.39 (0.03 - 1.73)	67.65 (4.82 - 255.02)
MS/ON/CIS/NMOSD; intact B-CSF-B	61 (7.8 - 108)	17.5 (7.8 - 97)	5.27×10^{-3} (2.51×10^{-3} - 8.8×10^{-3})	0.28 (0.03 - 1.43)	67.65 (4.82 - 295.11)
AIE/PNS	75 (21 - 137)	65 (7.8 - 209)	8.8×10^{-3} (3.44×10^{-3} - 20.8×10^{-3})	0.72 (0.17 - 5.77)	98.63 (9.33 - 359.48)
NIND	64 (7.8 - 191)	7.8 (-)	6.8×10^{-3} (2.87×10^{-3} - 15.3×10^{-3})	0.13 (0.008 - 0.33)	18.9 (1.48 - 83.01)

The ratio of CSF-CXCL13 and serum-CXCL13 (Q-CXCL13) was formed to calculate the CXCL13 index (I-CXCL13) in a further step using QAlb. Values are indicated as medians and range. QAlb: CSF-albumin/serum-albumin (albumin ratio); LNB: Lyme neuroborreliosis, BCL: primary/secondary CNS B cell lymphoma, I-UP: neuroinfectious diseases of unknown pathogens, F: fungal, P: parasitic, B-CNS-D: bacterial CNS disease, MS: multiple sclerosis, ON: optic neuritis, CIS: clinically isolated syndrome, NMOSD:

neuromyelitis optica spectrum disease, B-CSF-B: blood CSF barrier, AIE: autoimmune encephalitis, PNS: paraneoplastic syndrome, NIND: non-inflammatory neurological disease.

Figure S4. ROC curve analyses to determine a cut-off value for CSF-CXCL13, Q-CXCL13, and I-CXCL13 for neuroborreliosis.

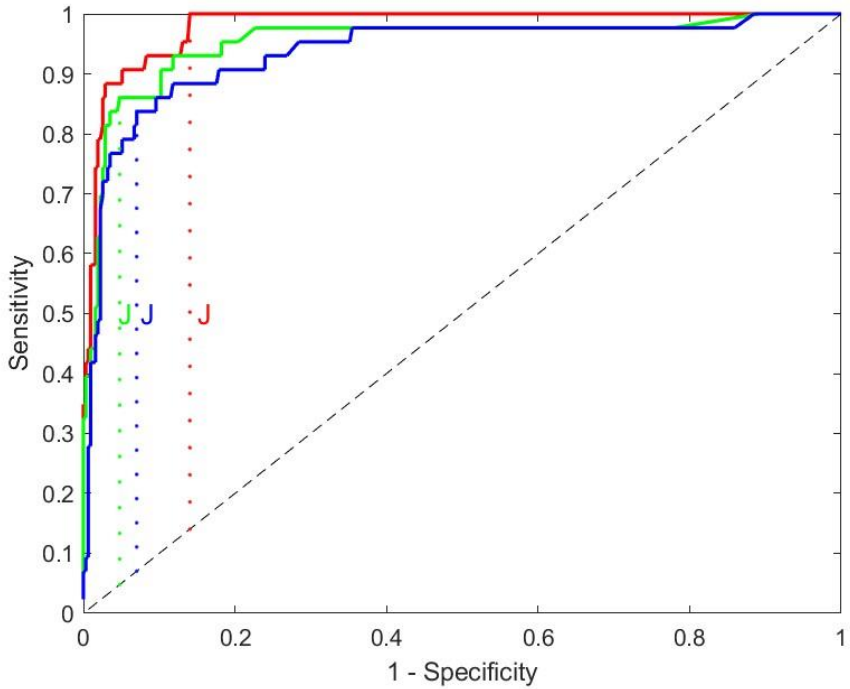


Figure S4. After calculation of a CSF-CXCL13/serum-CXCL13 ratio (Q-CXCL13) and an index (I-CXCL13 = Q-CXCL13/QAlb,) we performed receiver operating characteristic (ROC) curve analysis for CSF-CXCL13 (red curve), Q-CXCL13 (green curve), and I-CXCL13 (blue curve). We wanted to obtain the optimal cut-off value for these by using the Youden's index to distinguish neuroborreliosis from all other neuroinfectious and autoimmune (inflammatory) diseases as well as non-inflammatory controls with the highest sensitivity and specificity. We also wanted to investigate if CSF-CXCL13 could be used without regarding the corresponding serum value for calculating

ratio and index, respectively. We only had serum-CXCL13 values from 315 patients of the non-LNB group and from 43 LNB-patients. The cut-off after optimization using Youden's index (J; black dotted line) was 126.88 pg/ml for CSF-CXCL13 (red curve), 5.73 for Q-CXCL13 (green curve), and 0.36 for I-CXCL13 (blue curve). Sensitivity, specificity, the area under the curve (AUC), and the Youden's index are indicated in Table S5.

Table S4. Correlation analyses of CXCL13 and routine CSF findings in neuroborreliosis and MS/ON/CIS/NMOSD.

Parameter	**Neuroborreliosis (Spearman's r; p-value)**	**MS/ON/CIS/NMOSD (Spearman's r; p-value)**
Leucocyte count [/µl]	r = 0.426	r = 0.538
Lymphocyte count [/µl]	r = 0.471	r = 0.483
Monocyte count [/µl]	r = 0.329	r = 0.021
Plasma cell count [/µl]	r = 0.508	r = 0.531
QAlb (CSF/S)	r = 0.446	r = 0.185
CSF-specific oligoclonal IgG bands (+)	p = 0.022*	p = 0.036*
Intrathecal IgM synthesis (+)	p < 0.001*	p = 0.006*

Correlation analyses between CXCL13 and the routine CSF parameters were determined using Spearman's rank coefficient (r) in neuroborreliosis and MS/ON/CIS/NMOSD. Mann-Whitney U test* was used to compare CSF-CXCL13 levels if CSF-specific oligoclonal IgG bands or intrathecal IgM synthesis were present or absent. All values are rounded and values <0.05 were considered significant. QAlb: CSF-albumin/serum-albumin (albumin ratio), CSF: cerebrospinal fluid.

Table S5. Parameters of different ROC-analyses for neuroborreliosis using CSF-CXCL13, Q-CXCL13, and I-CXCL13.

	Cut-off	Sensitivity	Specificity	AUC	Youden‘s index
CSF-CXCL13 [pg/ml]	127	100%	85.94%	0.98	0.86
CSF-CXCL13/serum-CXCL13 (= Q-CXCL13)	5.73	86.05%	95.21%	0.95	0.81
Q-CXCL13/QAlb (= I-CXCL13)	0.36	83.72%	92.97%	0.94	0.77

After calculation of a CSF-CXCL13/serum-CXCL13 ratio (Q-CXCL13) and an index (I-CXCL13 = Q-CXCL13/QAlb), we performed receiver operating characteristic (ROC) curve analysis for each (see Figure S4). Calculated cut-offs, sensitivity, specificity, the area under the ROC curve (AUC), and the Youden’s index are shown here. The area under the ROC curve (AUC) indicating the test performance was highest for CSF-CXCL13 (0.98). Therefore, calculations with CSF-CXCL13 alone might be sufficient.
We only considered patients with measurement of CSF-CXCL13 and serum-CXCL13 in these ROC-analyses (n = 315 for the non-LNB group and n = 43 for LNB-patients).

Table S6. CSF-CXCL13 cut-offs for neuroborreliosis from the literature implemented in our patient cohort.

Reference [no.]	Total individual count (n)	LNB count for ROC-analysis (n) def./prob.	Non-LNB cohort	Method, manufacturer	Proposed cut-off [pg/ml], [ng/g total protein]*	Sensitivity (≙ TPR) [%]; for our data set	Specificity (≙ 1-FPR) [%]; for our data set	Youden's index (J); for our data set
Hytönen et al., 2014 [13]	366	38 (def. + prob.)	Viral CNS-inf., NL, MS, non-infl. co.	ELISA, Quantikine	415	91.94	95.94	0.88
Schmidt et al., 2011 [8]	205	27 (def.)	Viral/bacterial/fungal CNS-inf. (M/E), MS, prim./sec. CNS-lymphoma, non-infl. co.	ELISA, Quantikine	1229	67.74	97.88	0.66
van Burgel et al., 2011 [36]	268	58 (def. + prob.)	Viral/bacterial/fungal CNS-inf., MS/ADEM, HIV, non-infl. co.	ELISA, Quantikine; ELISA, Quantikine	250	93.55	92.40	0.86
Wagner et al., 2018 [10]	459	20 (def.)	Viral/bacterial CNS-inf., MS, stroke, limbic encephalitis, non-infl. co., intracranial malignancies/TU	ELISA, Euroimmun	93.83	100.00	82.51	0.83
Markowicz et al., 2018 [31]	100	25 (def.)	TBE, aseptic M/ME (undefined pathogen)	recomBead CXCL13, Mikrogen; ELISA, Euroimmun	131 259	100.00 93.65	86.22 92.52	0.86 0.86

Rupprecht et al., 2018 [17]	Meta-analysis (18 studies; 2944 individuals)	-	-	ELISA, Quantikine; ELISA, Euroimmun	91 (cross-sectional studies) 164 (case-control studies)	100.00 95.16	82.12 87.63	0.82 0.83
Remy et al., 2017 [21]	185	53 (def.)	Viral CNS-inf., non-infl. co.	ELISA, Quantikine	55	100.00	75.09	0.75
Henningsson et al., 2018 [26]	191	No ROC-analysis	Viral CNS-inf., stroke, non-infl. co.	recomBead CXCL13, Mikrogen	160	96.77	87.63	0.84
Cerar et al., 2013 [23]	174	46 (def. + prob.)	TBE, MS, non-infl. co.	ELISA, Quantikine	18.9	100.00	56.89	0.57
Tjernberg et al., 2011 [35]	261	124 (def.)	Head/neck pain, CNP, radiculitis	ELISA, Quantikine	142	100.00	86.75	0.87
Wutte et al., 2011 [37]	75	No ROC-analysis	Viral meningitis, CNP, systemic borreliosis, non-infl. co.	ELISA, Quantikine	0 500	100.00 88.71	0 96.64	0 0.85
Ljøstadt et al., 2008 [30]	116	No ROC-analysis	Viral/bacterial CNS-inf. (M/E), MS, non-infl. co.	ELISA, Quantikine	125 500	100.00 88.71	85.87 96.64	0.86 0.85
Knudtzen et al., 2020 [27]	619	37 (def.)	Viral/bacterial CNS-inf. (M/E), NL, NS, cer. malignancies incl. prim. CNS-lymphoma, non-infl. co.	ELISA, Euroimmun	49	100.00	71.02	0.71

Henningsson et al., 2016 [38]	132	35 (def.)	Non-infl. co.	ELISA, Quantikine; recomBead CXCL13, Mikrogen;	56 158	100.00 96.77	75.27 87.63	0.75 0.84
Lintner et al., 2020 [29]	1410	29 (def.)	Viral/bacterial/fungal CNS-inf., MS, intracranial malignancies/TU, non-infl. co.	ELISA, Euroimmun	55.5	100.00	75.27	0.75
Pìcha et al., 2016 [33]	244	74 (def. + prob.)	Aseptic CNS-inf., Bell‘s palsy, peripheral neuropathy, non-infl. co.	ELISA, Quantikine	29	100.00	62.01	0.62
Barstadt et al., 2020 [22]	195	77 (def. + prob.)	Aseptic meningitis/encephalitis, non-infl. co.	Multiplex bead assay, Millipore corp.	213	95.16	90.63	0.86
Eckman et al., 2021 [24]	162	19 (def. + prob.)	Viral/fungal CNS-inf. (M/E), NL, KRY, MS, non-infl. co.	Multiplex bead assay, Thermo Fisher Sci.	1726	62.90	98.59	0.61
van Gorkom et al., 2021 [25]	156	15 (def. + prob.)	Viral/bacterial/fungal CNS-inf., TBC, NL, MS, non-infl. co.	ELISA, Quantikine; recomBead CXCL13, Mikrogen	85.9 252.2	100.00 93.55	80.74 92.40	0.81 0.86

Leth et al., 2022 [28]	130	31 (def.)	Viral/bacterial CNS-inf., non-infl. co.	Bead-based assay, Bio-Rad	50.7	100.00	72.44	0.72
Senel et al., 2010 [9]	126	28 (def.)	Viral/bacterial CNS-inf. (M/E), non-infl. co.	ELISA, Quantikine	337*	77.05	93.57	0.71

The cut-offs from the literature, either determined by ROC analyses or set as in-house cut-off, were implemented in our data set and sensitivity (TPR) and specificity (1-FPR) including Youden's index J were calculated (last three columns of the table). One cut-off (proposed by Senel et al., 2010 [9]) is given as ng/g total protein to compensate for differences in CSF protein. For the implementation of this cut-off in our cohort, the CSF-CXCL13 values were also given in ng/g total protein. Def.: definitive, prob.: probable, TPR: true positivity rate, FPR: false positivity rate, MS: multiple sclerosis, NL: neurosyphilis, TBC: tuberculosis, M/E: meningitis/encephalitis, non-infl. co: non-inflammatory controls, ADEM: acute demyelinating encephalomyelitis, HIV: human immunodeficiency virus, TU: tumor, KRY: cryptococcosis, TBE: tick-borne meningoencephalitis, CNP: cranial nerve palsies.