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Diagnostic accuracy of inflammatory markers in adults with suspected central nervous system infections

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SUMMARY

Objectives: We aimed to determine diagnostic accuracy of inflammatory markers in plasma and cerebrospinal fluid (CSF) for the diagnosis of central nervous system (CNS) infections and specifically bacterial meningitis.

Methods: We analyzed 12 cytokines, chemokines, and acute phase reactants in CSF and plasma of 738 patients with suspected neurological infection included in a multicenter prospective cohort. We determined diagnostic accuracy for predicting any CNS infection and bacterial meningitis.

Results: We included 738 episodes between 2017 and 2022, split into a derivation (n = 450) and validation cohort (n = 288). Of these patients, 224 (30%) were diagnosed with CNS infection, of which 81 (11%) with bacterial meningitis, 107 (14%) with viral meningitis or encephalitis, and 35 patients (5%) with another CNS infection. Diagnostic accuracy of CRP, IL-6, and Il-1β in CSF was high, especially for diagnosing bacterial meningitis. Combining these biomarkers in a multivariable model increased accuracy and provided excellent discrimination between bacterial meningitis and all other disorders (AUC = 0.99), outperforming all individual biomarkers as well as CSF leukocytes (AUC = 0.97). When applied to the population of patients with a CSF leukocyte count of 5–1000 cells/mm^3, accuracy of the model also provided a high diagnostic accuracy (AUC model = 0.97 vs. AUC CSF leukocytes = 0.80) with 100% sensitivity and 92% specificity. These results remained robust in a temporal validation cohort.

Conclusions: Inflammatory biomarkers in CSF are able to differentiate CNS infections and especially bacterial meningitis from other disorders. When these biomarkers are combined, their diagnostic accuracy exceeds that of CSF leukocytes alone and as such these markers have added value to current clinical practice.

Introduction

In patients with central nervous system (CNS) infections, rapid and accurate diagnosis is essential to start adequate treatment and improve the prognosis.[1] The diagnosis can, however, be difficult as multiple neurologic conditions and non-neurologic infections are included in the differential diagnosis. In a pilot study of 363 patients suspected of a CNS infection, we found that clinical characteristics and blood laboratory tests failed to identify those with an infection.[2] In this study, 89 (25%) patients were diagnosed with a CNS infection and 27 (7%) with bacterial meningitis. The best predictor for bacterial meningitis and CNS infections was the cerebrospinal fluid (CSF) leukocyte count, although an elevated CSF leukocyte count also occurred in up to 50% of patients with an alternate diagnosis. Thus, diagnostic uncertainty is still an issue, especially in the population of patients with an inconclusive CSF leukocyte count of 5 to 1000 cells per mm^3. Microbiological tests such as CSF culture and polymerase chain reactio (PCR) can confirm CNS infections but take time and, when negative, do not exclude the possibility of an infection.[1,3,4] Hence, there is an ongoing need for novel markers in the diagnosis of neurological infections, preferably those that can be determined in a timely fashion.

Previous studies on biomarkers of infection in patients with CNS infections have focused on distinguishing CNS infections from negative controls or culture proven bacterial meningitis from viral or

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aseptic meningitis.[5–8] In clinical practice, patients presenting with a suspected neurological infection however often have alternate diagnoses such as systemic infections or non-infectious neurological diseases.[2,9,10] They frequently show CSF changes and the CNS infections are not always culture or PCR proven.[1–4,11,12]

In this study, we aimed to identify inflammatory markers in blood and CSF that can help differentiate CNS infections in general and bacterial meningitis (as most consequential disease) from other diagnoses and distinguish bacterial from viral CNS infections in patients with suspected CNS infection. Furthermore, we determined the diagnostic accuracy of combinations of biomarkers in addition to CSF leukocyte count.

Methods

Patient inclusion and reference standard

For this study, we used data, plasma, and CSF from the I-PACE study (Improving Prognosis by using innovative methods to diAgnose Causes of Encephalitis). The I-PACE study is an ongoing multicenter prospective cohort study conducted in the Netherlands with the goal of improving diagnostics in encephalitis. All adult patients (≥16 years of age) with a suspected CNS infection who underwent CSF examination could be included. Patients eligible for inclusion were identified during morning rounds or reported to the investigators by the treating physician. Written informed consent was obtained from all participating patients or their legal representatives. Patients with recent head trauma or neurosurgery (≤1 month) and patients with neurosurgical devices were excluded. Data on patients' characteristics, ancillary investigations, and outcome were collected in secured online case record forms and the study was carried out in accordance with Dutch privacy legislation. The study was approved by the biobank ethics committee of the Amsterdam UMC, location AMC, Amsterdam, The Netherlands (number BTC AMC2014_290).

The final diagnosis of all episodes was classified into five categories; CNS infection, CNS inflammatory disease, systemic infection, other neurological disorder, and non-infectious, non-neurological disorder. The methods and rationale of this classification have been described previously.[2] Two clinicians independently categorized all episodes and inconsistencies were resolved by consultation of a third clinician (Kappa coefficient 0.64). To reflect clinical practice, this final diagnosis was considered the reference standard for the diagnostic tests.

Sample collection and index test

Residual CSF from the diagnostic lumbar puncture was collected and stored in the I-PACE biobank at −80 °C until further analysis. Plasma samples were collected as soon as possible after the lumbar puncture (always within 48 h of CSF sampling), processed and stored at −80 °C until further analysis. Patients were included in the current study if there was sufficient CSF and/or plasma available for the planned assays. The index test consisted of concentrations of 12 cytokines, chemokines, and acute phase reactants in blood and CSF: CRP, procalcitonin, CXCL-10, MDC, IL6, Il-8, IL-10, TNF-a, MIF, IL-1RA, CXCL13, IL-1B. This selection was based on previous literature on biomarkers of CNS infection. We included markers that were previously measured in CSF for the purpose of diagnosing bacterial meningitis or CNS infection in general, and that showed potential in discriminating these from other disorders. We also included markers in which positive results were yielded before, but that were not applied to a clinically relevant population.[6–8,13–17] Biomarker concentrations in CSF and plasma were measured using a Human Luminex Discovery Custom Assay kit (ref. LXSAHM, R&D Systems) on a Luminex platform, according to manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted with the use of SPSS statistical software, version 28 (SPSS, Inc), and R, version 4.2.1. We used descriptive statistics for baseline characteristics with medians and interquartile range (IQR). The area under the curve (AUC) of the receiver operator characteristics (ROC) curve was used to evaluate diagnostic accuracy of biomarkers with 95% confidence intervals (CI) in plasma and CSF. An AUC value of > 0.90 was considered excellent discrimination, between 0.80 and 0.90 as good discrimination, 0.70–0.80 as fair discrimination, 0.60–0.70 as poor discrimination, and < 0.60 was considered as no discrimination at all.[18] For all patients with available plasma and CSF samples, the blood to CSF ratio was calculated as separate diagnostic test. Multivariable LASSO logistic regression analysis was performed to determine the predictive value of the inflammatory markers' concentrations in CSF (either separate or combined) in addition to CSF leukocyte count, the previously identified best predictor of CSF infections. We assessed the linearity of the relation between all markers the outcome variable and if necessary, values were square root or $\log^{10}$ transformed.

Comparisons made were 1) CNS infections versus all other diagnoses, 2) bacterial meningitis versus all other diagnoses, and 3) bacterial meningitis versus viral meningitis/encephalitis. The analysis was performed in all patients and in a subgroup of patients with CSF leukocyte count between 5 and 1000 cells/mm^3. The rationale for the latter group was that within this group the diagnostic uncertainty is highest, compared to patients with normal leukocyte counts or those with very high leukocyte counts, who almost invariably have bacterial meningitis. Since this study is considered an exploratory diagnostic accuracy study and no prior data on the test characteristics of the index test was available, a power calculation could not be performed.

We first performed the measurements in a derivation cohort consisting of patients included between 2017–2020 and subsequently performed a temporal validation study in patients included between 2020 and 2022. This study was reported according to the Standards for Reporting Diagnostic accuracy studies (STARD) checklist.[19]

Role of the funding source

The funding source has had no involvement in study design, collection analysis or interpretation of data, writing the report, or in the decision to submit the paper for publication.

Results

Patient characteristics

From 2017 to 2022, 820 episodes with suspected CNS infection were included in the I-PACE study, which was split into the derivation (n = 532) and validation cohort (n = 288). Of 450 (85%) episodes included in the derivation cohort, a sufficient amount of either CSF or plasma was available for current analysis. CSF was available in 385 out of 450 (86%) episodes, plasma in 210 (47%). Both CSF and plasma were available for 145 (32%) episodes. Validation was performed in 288 episodes from which CSF was available. A total of 738 episodes were included in the final analysis. Clinical and laboratory features of the derivation and validation cohort were similar (Supplementary Material Table 1).

In the total cohort, the median age was 55 years (IQR 37–68) and 364 (49%) episodes occurred in women (Table 1, Supplementary Table 2). The most common presenting feature was headache in 387 of 599 episodes (65%). Fever (≥38º) occurred in 247 of 710 (34%) episodes, neck stiffness in 110 of 497 (22%), and 324 of 732 (44%) episodes presented with an altered mental status, defined as a score

Table 1
Baseline characteristics of total cohort.[a]

	All (n = 738)	CNS infection (n = 224, 30%)	Bacterial meningitis (n = 81, 11%)	Viral CNS infection (n = 107, 14%)	Other CNS infection (n = 36, 5%)	All other disorders (n = 514, 70%)
Age, years	55 (37–68)	53 (37–67)	55 (43–67)	47 (31–63)	58 (49–68)	56 (37–68)
Sex, female	364/738 (49)	112/224 (50)	43/81 (53)	58/107 (54)	11/36 (31)	252/514 (49)
Clinical presentation						
Headache	387/599 (65)	150/196 (77)	57/67 (85)	72/95 (76)	21/34 (62)	237/403 (59)
Fever (≥38ª)	247/710 (34)	101/222 (45)	50/81 (62)	44/107 (41)	7/34 (21)	136/488 (28)
Seizures	105/670 (16)	20/211 (9)	6/73 (8)	13/105 (12)	1/33 (3)	85/459 (19)
Neck stiffness	110/497 (22)	69/173 (40)	46/69 (67)	16/81 (20)	7/23 (30)	41/324 (13)
Score Glasgow coma scale[b]	15 (12–15)	15 (13–15)	13 (10–15)	15 (14–15)	15 (15–15)	15 (12–15)
Altered mental status ≤14	324/732 (44)	96/223 (43)	58/80 (73)	30/107 (28)	8/36 (22)	228/509 (45)
Coma ≤8	81/732 (11)	17/223 (8)	13/80 (16)	4/107 (4)	0/36 (0)	64/509 (13)
Blood chemistry[c]						
C-reactive protein (mg/L)	17 (3–70)	29 (7–131)	166 (76–283)	7 (2–22)	19 (5–65)	12 (2–52)
Leukocytes (×10^9/L)	9 (6–13)	10 (7–15)	17 (11–23)	8 (6–11)	9 (6–11)	8 (6–13)
CSF examination [d]						
CSF leukocytes (/mm3)	4 (1–55)	216 (44–1418)	2136 (918–6317)	96 (35–239)	44 (10–241)	2 (1–5)
CSF leukocytes > 4/mm^3	352/734 (48)	213/222 (96)	80/80 (100)	102/106 (96)	21/36 (58)	139/512 (27)
CSF leukocytes > 100/mm^3	152/734 (21)	135/222 (61)	73/80 (9)	51/106 (48)	11/36 (31)	17/512 (3)
Outcome						
Death	55/738 (8)	18/224 (8)	11/81 (14)	5/107 (5)	2/36 (5)	37/514 (7)
Unfavorable	330/738 (45)	84/224 (38)	33/81 (41)	34/107 (32)	17/36 (47)	246/514 (48)
Good recovery	408/738 (55)	140/224 (63)	48/81 (59)	73/107 (68)	19/36 (53)	268/514 (52)

[a] Data are n/N (%) or median (interquartile range).
[b] Glasgow Coma Scale score was known for 732 patients.
[c] CRP was known for 663 episodes, blood leukocytes for 714 episodes.
[d] CSF leukocytes was known for 734 episodes.

of 14 or lower on the Glasgow Coma Scale. Blood chemistry showed an elevated (> 5 mg/L) CRP in blood in 450 of 699 (64%) episodes. Blood leukocytosis (> 10.5 × 10^9/L) was present in 289 of 730 (40%) episodes. Lumbar puncture was performed in all episodes. The median CSF leukocyte count was 4/mm^3 (IQR 1–55). An elevated CSF leukocyte count (> 4/mm^3) was present in 352 out of 734 (48%) episodes and 152 (21%) episodes presented with a CSF leukocyte count over 100/mm^3. There were no episodes of bacterial meningitis with a normal CSF leukocyte count.

A diagnosis of CNS infection was made in 224 out of 738 (30%) episodes, consisting of bacterial meningitis in 81 episodes (11%) and viral meningitis or encephalitis in 107 episodes (14%). CNS inflammatory diseases were diagnosed in 76 (10%) episodes, systemic infection in 148 (20%), other neurological disorders in 263 (36%), and a non-infectious, non-neurological disease in 27 (4%) episodes (Fig. 1 and Supplementary Table 3). Outcome at discharge was known for all episodes: 408 (55%) had a favorable outcome, defined as a Glasgow Outcome Scale score of 5, and mortality was 8% (55 episodes).[20] Clinical characteristics had poor diagnostic accuracy for predicting CNS infection (Supplementary Material Table 4).

We aimed to measure 12 markers in all available CSF and plasma samples. From all 2520 measurements performed in plasma, 646 (26%) were below the lower limit of detection and 38 (2%) were above the upper limit of detection. From the 8061 CSF measurements this applied to 2107 (26%) and 490 (6%) measurements, respectively (Table 2).

Diagnostic accuracy plasma parameters

The AUC values of all biomarkers in plasma for differentiating CNS infection from all other disorders were below 0.7, indicating none to poor discrimination (Supplementary Material Table 5, Supplementary Material Fig. 1). With regard to differentiating bacterial meningitis from all other disorders only procalcitonin showed an AUC value of > 0.70 (0.71). For the differentiation between bacterial and viral CNS infection, AUC values of CRP, procalcitonin, CXCL13 and IL-6 were between 0.70 and 0.80, indicating fair discrimination. None of the biomarkers in plasma showed good or excellent discrimination (AUC > 0.80).

Diagnostic accuracy CSF parameters

The CSF leukocyte count was found to be the best predictor for CNS infection in all analyzed patients with an AUC of 0.94. Most biomarkers in CSF had an AUC value of > 0.80 for differentiating CNS infection from all other disorders, indicating good to excellent discrimination (Table 3). Similar results were found with regard to differentiating bacterial meningitis from all other disorders and differentiating bacterial from viral CNS infections.

In episodes with a CSF leukocyte count of 5–1000/mm^3, we found that CSF leukocytes remained the best predictor for CNS infection (Table 4). However, in distinguishing bacterial meningitis from other diseases, CRP (AUC 0.85), CXCL13 (AUC 0.84), IL1-Ra (AUC 0.83), IL-1β (AUC 0.86), IL-6 (AUC 0.93) and TNF-α (AUC 0.82) performed better than CSF leukocyte count (AUC 0.80). When differentiating bacterial from viral CNS infections, CSF leukocyte count showed poor discrimination (AUC 0.64). CRP (AUC 0.87), CCL22/MDC (AUC 0.73), CXCL13 (AUC 0.80), IL-8 (AUC 0.72), procalcitonin (AUC 0.74), IL-1β (AUC 0.83), IL-6 (AUC 0.87), MIF (AUC 0.73) and TNF-α (AUC 0.72) all performed better, showing fair to good discrimination.

We calculated the CSF:blood ratios of all biomarkers in 145 episodes with concentrations measured in both CSF and plasma. We found that the CSF:blood ratios did not perform better than concentrations in CSF alone (Supplementary Material Table 6).

Regression analysis

We performed LASSO regression to assess if combining individual biomarkers would increase diagnostic accuracy. For predicting CNS infection in all patients, the combination of CSF CXCL10 concentration and CSF leukocytes performed better than individual biomarkers with an AUC of 0.96 (Fig. 2a, Table 3). For distinguishing bacterial meningitis from other disorders and bacterial meningitis from viral CNS infections the combination of IL-1β and CRP showed the best combination of tests (AUCs 0.99 and 0.97), outperforming

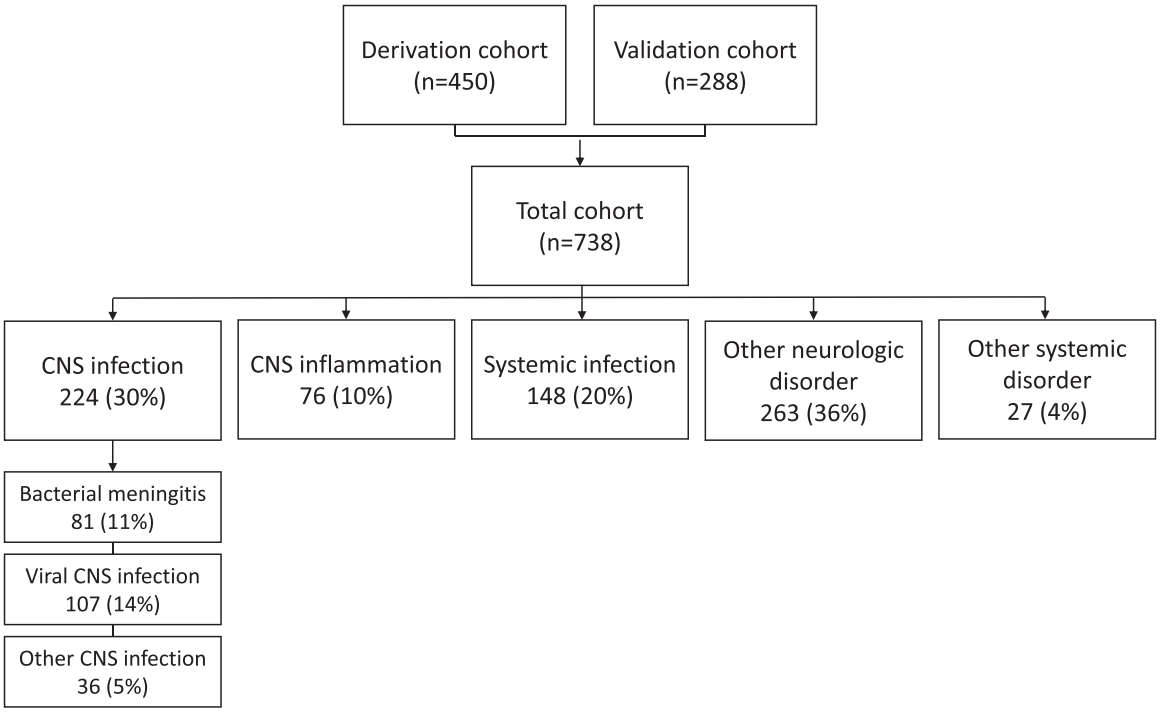

Fig. 1. Flowchart of diagnostic categories.

Table 2
Concentration of inflammatory markers in CSF in total cohort.[a]

Inflammatory marker	All (n = 673)	CNS infection (n = 197)	Bacterial meningitis (n = 71)	Viral CNS infection (n = 90)	Other CNS infection (n = 35)	All other disorders (n = 477)
CRP[b,c]	22 (3–149)	78 (9–586)	1250 (161–3192)	13 (3–6)	34 (11–168)	15 (3–84)
CCL22/MDC	105 (105–127)	136 (105–215)	188 (134–320)	117 (105–174)	105 (105–141)	105 (105–105)
CXCL13	25 (11–73)	134 (52–343)	342 (208–441)	77 (36–136)	97 (34–423)	18 (11–34)
IL-1Ra	166 (65–1686)	11,288 (1326–40,380)	40,380 (40,380–40,380)	5432 (591–32,837)	1577 (106–6755)	102 (52–241)
IL-8/CXCL8	124 (51–981)	1096 (268–4560)	4560 (1136–4560)	1047 (231–4332)	173 (72–607)	94 (42–247)
Procalcitonin	240 (169–375)	258 (180–422)	341 (224–660)	234 (169–336)	224 (182–362)	232 (164–350)
CXCL10	128 (24–2100)	2100 (1465–2100)	2100 (2100–2100)	2100 (1716–2100)	972 (106–2100)	67 (11–198)
IL-1β	10 (10–20)	32 (10–147)	397 (70–3937)	22 (10–37)	10 (10–36)	10 (10–10)
IL-6	11 (2–248)	1345 (35–5400)	5400 (5400–5400)	236 (18–1844)	35 (4–487)	5 (2–32)
Il-10	17 (8–69)	103 (61–271)	96 (55–163)	96 (18–163)	60 (17–84)	11 (8–23)
MIF[b]	5 (3–10)	9 (5–31)	25 (9–66)	7 (5–12)	6 (4–10)	4 (3–8)
TNF-α	4 (4–10)	20 (7–99)	384 (61–3065)	13 (5–26)	7 (4–21)	4 (4–4)

[a] Concentrations are in pg/mL, data are portrayed as median (interquartile range).
[b] CRP and MIF concentrations are portrayed as ng/mL.
[c] CRP was known for 658 episodes.

Table 3
AUC values of the inflammatory markers in CSF for differentiating CNS infection from other diseases, bacterial meningitis from other diseases and bacterial meningitis from viral CNS infection in the derivation cohort.

	CNS infection from other diseases		Bacterial meningitis from other diseases		Bacterial meningitis from viral CNS infection	
Inflammatory marker	AUC value	95% CI interval	AUC value	95% CI interval	AUC value	95% CI interval
CRP	0.66***	0.60–0.73	0.92***	0.87–0.97	0.92***	0.86–0.98
CCL22/MDC	0.81***	0.75–0.86	0.90***	0.83–0.96	0.81***	0.72–0.91
CXCL13	0.86***	0.81–0.91	0.95***	0.93–0.98	0.89***	0.82–0.96
IL-1Ra	0.87***	0.81–0.92	0.95***	0.93–0.98	0.79***	0.70–0.88
IL-8/CXCL8	0.78***	0.72–0.83	0.87***	0.82–0.92	0.71***	0.61–0.81
Procalcitonin	0.55	0.49–0.61	0.71***	0.63–0.80	0.76***	0.66–0.86
CXCL10	0.89***	0.85–0.93	0.90***	0.86–0.94	0.61	0.49–0.72
IL-1β	0.81***	0.75–0.86	0.93***	0.87–0.99	0.90***	0.82–0.98
IL-6	0.83***	0.78–0.88	0.98***	0.96–0.99	0.92***	0.86–0.98
Il-10	0.89***	0.85–0.93	0.93***	0.89–0.98	0.75***	0.65–0.85
MIF	0.70***	0.64–0.76	0.83***	0.75–0.91	0.78***	0.68–0.88
TNF-α	0.85***	0.80–0.91	0.96***	0.91–1.00	0.88***	0.80–0.95
CSF leukocytes	0.94***	0.92–0.96	0.97***	0.96–0.99	0.91***	0.85–0.96

*** p-value ≤0.001.

Table 4
AUC values of the inflammatory markers in CSF for differentiating CNS infection from other diseases, bacterial meningitis from other diseases and bacterial meningitis from viral CNS infections in patients with a CSF leukocyte count 5–1000/mm3 in the derivation cohort.

	CNS infection from other diseases		Bacterial meningitis from other diseases		Bacterial meningitis from viral CNS infection	
Inflammatory marker	AUC value	95% CI interval	AUC value	95% CI interval	AUC value	95% CI interval
CRP	0.47	0.38–0.57	0.85***	0.67–1.00	0.87***	0.68–1.00
CCL22/MDC	0.68***	0.59–0.77	0.80***	0.65–0.95	0.73*	0.55–0.91
CXCL13	0.71***	0.63–0.80	0.84***	0.72–0.96	0.80**	0.63–0.96
IL-1Ra	0.76***	0.67–0.84	0.83***	0.71–0.95	0.68	0.51–0.86
IL-8/CXCL8	0.61*	0.51–0.70	0.78**	0.65–0.91	0.72*	0.56–0.88
Procalcitonin	0.44	0.35–0.53	0.69*	0.55–0.82	0.74*	0.59–0.89
CXCL10	0.79***	0.71–0.86	0.71*	0.57–0.85	0.50	0.32–0.68
IL-1β	0.70***	0.61–0.78	0.86***	0.70–1.00	0.83***	0.64–1.00
IL-6	0.68***	0.59–0.77	0.93***	0.86–1.00	0.87***	0.76–0.98
Il-10	0.78***	0.70–0.86	0.77**	0.62–0.92	0.61	0.41–0.81
MIF	0.51	0.41–0.60	0.72*	0.54–0.90	0.73*	0.54–0.92
TNF-α	0.76***	0.68–0.84	0.82***	0.66–0.98	0.72*	0.53–0.91
CSF leukocytes	0.83***	0.76–0.90	0.80***	0.69–0.91	0.64	0.46–0.83

* p-value ≤0.05.
** p-value ≤0.01.
*** p-value ≤0.001.

all individual markers including CSF leukocytes (Fig. 2c and e, Table 3). In the subpopulation of patients with a CSF leukocyte count of 5–1000/mm^3, the combination of IL-1Ra, CXCL10, MIF, IL-6 and CSF leukocytes had the highest diagnostic accuracy for distinguishing CNS infections from other diagnoses (AUC of 0.91, Fig. 2b, Table 4). For distinguishing bacterial meningitis from other disorders and bacterial meningitis from viral CNS infections, combining IL-6 and CRP gave an AUC of respectively 0.99 and 0.97, again outperforming all individual markers (Fig. 2d and f, Table 4). When applying these models to a subgroup consisting of only patients with a microbiological confirmed bacterial meningitis or viral CNS infection, AUC values remained similar.

Differentiation between CNS infections and other diseases in all patients, and those with 5–1000/mm^3 CSF leukocytes with 100% sensitivity with abovementioned LASSO regression derived models, could only be achieved at the expense of a low specificity (35% and 14%) (Table 5). For the differentiation between bacterial meningitis and other diseases in all patients and those with 5–1000/mm^3 CSF

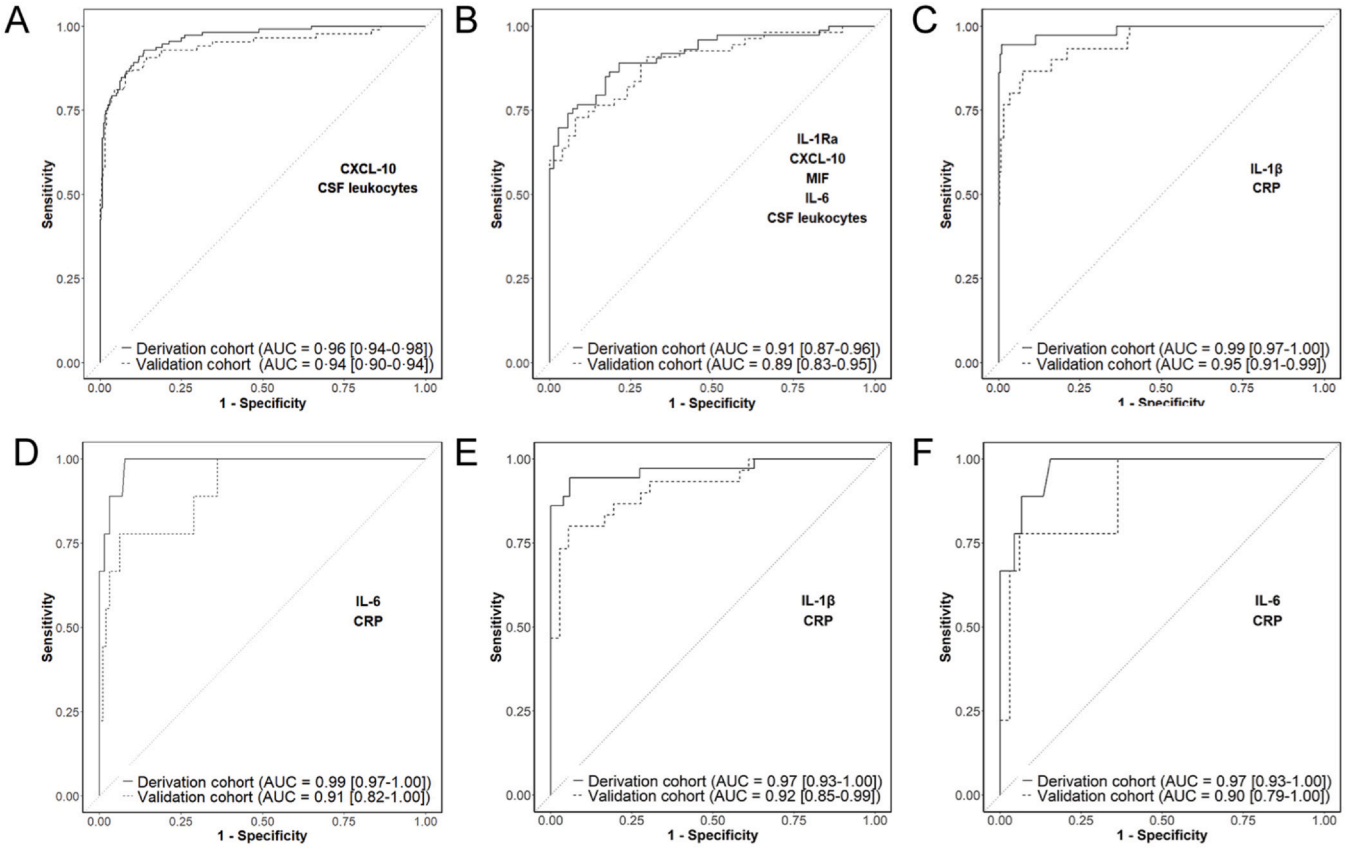

Fig. 2. ROC curves of multivariable models. A: Model 1. Differentiating CNS infection from all other diseases in the whole population. B: Model 2. Differentiating CNS infection from all other disease in the population of patients with 5–1000/mm^3 CSF leukocytes. C: Model 3. Differentiating bacterial meningitis from all other diseases in the whole population. D: Model 4. Differentiating bacterial meningitis from all other disease in the population of patients with 5–1000/mm^3 CSF leukocytes. E: Model 5. Differentiating bacterial meningitis from viral CNS infections in the whole population. F: Model 6. Differentiating bacterial meningitis from viral CNS infections in the population of patients with 5–1000/mm^3 CSF leukocytes.

Table 5
Test characteristics of regression models and CSF leukocytes in the derivation cohort.

		Cut-off value[a]	Sensitivity	Specificity		Cut-off value (cells/m^3)	Sensitivity	Specificity
CNS infection versus all other diseases whole population	**Model 1**				**CSF leukocytes**			
	Youden's index	0.14	0.93 (0.87–0.97)	0.86 (0.82–0.90)	Youden's index	12.5	0.90 (0.84–0.96)	0.90 (0.86 – 0.95)
	100% sensitivity	0.02	1.00 (1.00–1.00)	0.35 (0.29–0.41)	100% sensitivity	0	1.00 (1.00–1.00)	0.00 (0.00–0.00)
	100% specificity	0.97	0.42 (0.33–0.51)	1.00 (1.00–1.00)	100% specificity	3006	0.18 (0.12–0.25)	1.00 (1.00–1.00)
CNS infection versus all other diseases 5–1000 m^3 CSF leukocytes	**Model 2**				**CSF leukocytes**			
	Youden's index	0.71	0.74 (0.63–0.84)	0.94 (0.89–0.99)	Youden's index	24	0.81 (0.71–0.90)	0.76 (0.65–0.86)
	100% sensitivity	0.06	1.00 (1.00–1.00)	0.14 (0.07–0.23)	100% sensitivity	5	1.00 (1.00–1.00)	0.00 (0.00–0.00)
	100% specificity	0.88	0.57 (0.47–0.68)	1.00 (1.00–1.00)	100% specificity	633	0.07 (0.01–0.14)	1.00 (1.00–1.00)
Bacterial meningitis versus all other diseases whole population	**Model 3**				**CSF leukocytes**			
	Youden's index	0.27	0.94 (0.86–1.00)	0.99 (0.98–1.00)	Youden's index	108	0.90 (80–0.97)	0.90 (0.86–0.93)
	100% sensitivity	0.00	1.00 (1.00–1.00)	0.64 (0.59–0.69)	100% sensitivity	24	1.00 (1.00–1.00)	0.80 (0.75–0.84)
	100% specificity	0.64	0.86 (0.75–0.97)	1.00 (1.00–1.00)	100% specificity	3006	0.49 (0.34–0.63)	1.00 (1.00–1.00)
Bacterial meningitis versus all other diseases 5–1000 m^3 CSF leukocytes	**Model 4**				**CSF leukocytes**			
	Youden's index	0.07	1.00 (1.00–1.00)	0.92 (0.87–0.96)	Youden's index	24	1.00 (1.00–1.00)	0.51 (0.42–0.60)
	100% sensitivity	0.07	1.00 (1.00–1.00)	0.92 (0.87–0.96)	100% sensitivity	24	1.00 (1.00–1.00)	0.51 (0.42–0.60)
	100% specificity	0.76	0.67 (0.33–0.67)	1.00 (1.00–1.00)	100% specificity	933	0.09 (0.00–0.27)	1.00 (1.00–1.00)
Bacterial meningitis versus viral CNS infection whole population	**Model 5**				**CSF leukocytes**			
	Youden's index	0.37	0.94 (0.86–1.00)	0.94 (0.86–1.00)	Youden's index	932	0.76 (0.61–0.88)	0.94 (0.87–1.00)
	100% sensitivity	0.02	1.00 (1.00–1.00)	0.37 (0.25–0.51)	100% sensitivity	22	1.00 (1.00–1.00)	0.22 (0.11–0.35)
	100% specificity	0.70	0.86 (0.75–0.97)	1.00 (1.00–1.00)	100% specificity	1942	0.58 (0.44–0.73)	1.00 (1.00–1.00)
Bacterial meningitis versus viral CNS infection 5–1000 m^3 CSF leukocytes	**Model 6**				**CSF leukocytes**			
	Youden's index	0.08	1.00 (1.00–1.00)	0.84 (0.73–0.93)	Youden's index	532	0.36 (0.09–0.64)	0.92 (0.83–0.98)
	100% sensitivity	0.08	1.00 (1.00–1.00)	0.84 (0.73–0.93)	100% sensitivity	22	1.00 (1.00–1.00)	0.19 (0.08–0.29)
	100% specificity	0.79	0.67 (0.33–1.00)	1.00 (1.00–1.00)	100% specificity	933	0.09 (0.00–0.27)	1.00 (1.00–1.00)

[a] Cut-off values represent the predicted chance according to the regression model.

leukocytes, specificity was respectively 64% and 92% while maintaining 100% sensitivity. Differentiation between bacterial meningitis and viral CNS infection in both populations with a 100% sensitivity gave a specificity of 37% in the whole population and 84% in patients with a CSF leukocyte count of 5–1000/mm^3.

Validation cohort

We validated our results in 288 new CSF samples. AUC values of all models were somewhat lower compared to those in the derivation cohort, but results remained robust with excellent discrimination (AUC > 0.90, Fig. 2a-f).

Discussion

Our study shows that diagnostic accuracy of inflammatory biomarkers in CSF for the diagnosis of CNS infection and bacterial meningitis in patients suspected of a CNS infection is high. These biomarkers were able to provide incremental diagnostic value to CSF leukocyte count alone in certain populations. Implementation of measuring additional inflammatory markers in CSF in clinical practice might be possible with relative ease due to the fact that these tests (e.g. CRP, IL6) are already routinely performed in many clinical laboratories. CSF leukocytes remained the best individual predictor for differentiating CNS infection from all other disorders compared to all evaluated biomarkers. Combining CSF leukocytes with inflammatory biomarkers increased diagnostic accuracy slightly in this population. However, for the differentiation of bacterial meningitis from all other disorders and viral CNS infection specifically, we found that inflammatory biomarkers performed better than CSF leukocytes. This effect was even more pronounced in the population with a CSF leukocyte count of 5–1000/mm^3, where the diagnostic uncertainty is the highest. Diagnostic accuracy increased when biomarkers were combined, outperforming all individual biomarkers. Including CSF leukocyte count in these models did not increase accuracy. Previous studies attempting to identify markers for discriminating CNS infections often did not study the relevant population - those suspected of a CNS infection - or did not look at incremental value in addition to the currently best available diagnostic predictors. We previously showed that the diagnostic accuracy of clinical and laboratory features, except for CSF leukocytes, was low.[2] Clinical prediction models combining baseline, clinical and laboratory characteristics were found not to perform well enough to be used in clinical practice.[21]

CRP and IL-6 formed the best model to differentiate bacterial meningitis from viral CNS infection and all other disorders in the whole population. Combining CRP and IL-1β performed the best in the population of patients with a CSF leukocyte count of 5–1000/mm^3. CRP is an acute phase reactant synthesized in the liver in response to inflammation and tissue damage and levels increase rapidly during infection. IL-6 and Il-1β are considered pro-inflammatory cytokines. When pathogens are recognized by immune cells such as macrophages and monocytes, this triggers an intracellular signaling cascade which leads to the production and release of pro-inflammatory cytokines such as Il-6 and Il-1β. Previous studies suggested that both CRP, IL-6 and IL-1β are potentially helpful in the diagnosis of bacterial meningitis.[7,8,16,22–30] Sample sizes in these studies were often small and populations were not representable for clinical practice. Our study confirms that these results stay robust in a clinically representative population and after validation.

Our results show that blood inflammatory markers have low diagnostic accuracy for the diagnosis of CNS infection and bacterial meningitis. Previous studies showed that blood levels of acute phase reactants CRP and procalcitonin were helpful in differentiation of CNS infection and specifically bacterial meningitis from other disorders.[22,31,32] However, these studies were not performed in the whole population of patients with suspected CNS infection and did not include patients with systemic infections without CNS involvement, where acute phase reactants are also elevated. Our results indicate that inflammatory markers in blood have a limited diagnostic value in the diagnosis of CNS infection and these cannot be used to exclude this.

Our study has several limitations. Firstly, we collected data, CSF, and plasma prospectively but the material was frozen for later use. Some samples were frozen for multiple years and all samples underwent one additional freeze thaw cycle for the analysis. We do not know to what extent this might have influenced results, and if the results would be different when measured in fresh samples. Additionally, plasma samples in this study were obtained anywhere within 48 h after the lumbar puncture, which might complicate comparison to the CSF results. This study only included adult patients in the Netherlands. Cytokine and acute phase reactant profiles might not be the same in other populations with a different spectrum of diseases. This might influence the external validity of our results, and necessitates external validation in other populations. From previous research we know that bacterial meningitis with a normal CSF leukocyte count upon presentation occurs in approximately 2% of cases.[33] However, in our cohort there were no bacterial meningitis cases with a normal CSF leukocyte count, making it impossible to assess the performance of cytokines in this population, where it could be especially useful. Lastly, the reference standard is a clinical diagnosis based on all available information, and it could be that certain episodes are misclassified by the clinician. However, when performing a subgroup analysis on only microbiologically confirmed bacterial meningitis and viral CNS infections, results remained similar. Therefore, chances of this significantly influencing our results are small.

In conclusion, inflammatory markers in CSF have the potential to distinguish CNS infection and bacterial meningitis from other disorders. This is especially true in the population of patients where CSF leukocyte count is not conclusive and the diagnostic uncertainty is the highest. When combined, these biomarkers outperform CSF leukocytes in diagnosing bacterial meningitis and as such they might have incremental value in clinical practice.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

S.O.: methodology, data collection, data analysis, data interpretation, and writing of the original draft of the manuscript; S.S.: data collection, review and editing of the report; L.t.H.: data

collection, review and editing of the report; I.v.Z.: study design, data collection, review and editing of the report; W.M.: data collection, review and editing of the report; M.T.: data analysis, review and editing of the report; D.v.d.B.: review, editing and supervision of the report; M.B.: methodology, study design, data interpretation, review, editing and supervision of the report, and funding acquisition. All authors read and approved the final manuscript.

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Data sharing

Data protection regulations in the Netherlands do not allow sharing of individual participant data. Data sets with selected aggregated data will be shared upon request. Proposals can be directed to ipace@amsterdamumc.nl.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.01.016.

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