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Short Communication

Cerebrospinal fluid inflammatory markers to differentiate between neonatal bacterial meningitis and sepsis: A prospective study of diagnostic accuracy

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ABSTRACT

Objectives: We evaluated the diagnostic accuracy of cerebrospinal fluid (CSF) inflammatory markers for diagnosing bacterial meningitis in neonates with sepsis and/or meningitis.
Methods: Cases were identified from a prospective multicenter study including patients aged 0-3 months with Group B Streptococcal (GBS) or *Escherichia coli* culture positive sepsis/meningitis. CSF CXCL10, MDC, IL-6, IL-8, IL-10, TNF- α, MIF, IL-1RA, CXCL13, IL-1β, CRP and procalcitonin concentrations were measured with Luminex technology.
Results: In 61/373 patients (17%) residual CSF from the lumbar puncture was available, of whom 16 (26%) had definitive meningitis, 15 (25%) probable meningitis and 30 (49%) had sepsis. All biomarkers were detectable in CSF and showed significantly higher concentrations in definitive meningitis versus sepsis patients and six biomarkers in probable meningitis versus sepsis patients. Discrimination between definitive meningitis and sepsis was excellent for IL-1RA (area under the receiver operating characteristic curve [AUC] 0.93), TNF-α (AUC 0.92), CXCL10 (AUC 0.90), IL-1β (AUC 0.92), IL-6 (AUC 0.94), IL-10 (AUC 0.93) and a combination of IL-1RA, TNF-α, CXCL-10 and CSF leukocyte count (AUC 0.95). CSF leukocyte count remained the predictor with the highest diagnostic accuracy (AUC 0.96).
Conclusion: CSF inflammatory markers can be used to differentiate between neonatal sepsis and meningitis.

1. Introduction

Neonatal and infant meningitis and sepsis are associated with substantial mortality and long-term morbidity [1]. Early detection of meningitis in suspected sepsis patients is important because of the difference in empiric antibiotic regimen, dosage and duration of antibiotic treatment. Also, it influences the need for monitoring of complications during hospitalization, as well as the risk of long-term neurodevelopmental impairment [2]. The interpretation of CSF parameters in neonates can be difficult due to considerable overlap of CSF characteristics between sepsis and meningitis [3]. We performed a study on the diagnostic accuracy of CSF inflammatory markers for diagnosing bacterial meningitis in neonates with sepsis and/or meningitis.

2. Methods

2.1. Study population

We identified neonates aged 0-3 months who had GBS or *Escherichia coli* culture positive sepsis or meningitis between Jan

Abbreviations: AUC, area under the curve; BM, bacterial meningitis; GBS, group-b-streptococcus; CI, confidence interval; CNS, central nervous system; CSF, cerebrospinal fluid; IQR, interquartile range; NOGBS, The Netherlands observational study on GBS disease, bacterial virulence and protective serology; NRLBM, Netherlands Reference Laboratory for Bacterial Meningitis; ROC, receiver-operating characteristic.

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Details on the NOGBS study group can be found in the supplementary material.

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Table 1
Baseline characteristics[e]

Clinical variables	Definitive meningitis, n = 16	Probable meningitis, n = 15	Sepsis, n = 30
Postnatal age (days), median (IQR)	6 (3-11)	5 (0-6)	7 (0-24)
Female, n (%)	9/16 (56)	7/15 (47)	10/30 (33)
Birthweight, grams	2898 (2112-3458)	2720 (1130-3340)	2490 (1345-3520)
Gestational age, weeks			
<28	0 (0)	4 (27)	4 (13)
28-32	4 (25)	3 (20)	8 (27)
32-37	5 (31)	2 (13)	7 (23)
>37	7 (44)	6 (40)	11 (37)
Early onset sepsis, n (%)	7/16 (44)	8/15 (53)	14/30 (47)
Fever, n (%)	8/16 (50)	4/13 (31)	8/28 (29)
Feeding problems, n (%)[c]	11/16 (69)[a]	5/14 (36)	10/30 (33)
Behavioural changes, n (%)	12/15 (80)	8/13 (62)	16/27 (59)
Temperature, median (IQR)[c]	38.2 (37.7-38.4)[a]	37.5 (36.9-37.7)	37.4 (36.8-37.8)
Heart rate, median (IQR)[c]	185 (160-200)	162 (135-173)	166 (146-180)
Neck stiffness, n (%)	4/16 (25)	1/15 (7)	3/30 (10)
Bulging fontanel, n (%)	13/16 (81)	13/15 (87)	22/30 (73)
AVPU score, n (%)‡			
Normal (Alert)	7/12 (58)	11/13 (85)	15/24 (63)
Abnormal (Verbal, Pain or Unresponsive)	5/12 (42)	2/13 (15)	9/24 (38)
Outcome, n (%)			
Death	2/16 (13)	1/15 (7)	0/30 (0)
Hearing loss or deafness	0/16 (0)	0/15 (0)	0/30 (0)
Laboratory variables in blood			
CRP (mg/L)¶	61 (14-107)[a]	26 (6-48)	20 (7-43)
Leukocyte count ([e]10^9/L)	8 (3-18)	10 (7-13)	11 (4-14)
Thrombocyte count ([e]10^9/L)†,[c]	200 (153-264)[a]	252 (210-300)	286 (232-394)
Blood culture, n (%)[c]			
E. coli	5 (31)	7 (47)	5(17)
GBS	10 (62)	8 (53)	25 (83)
Negative	1 (6)	0 (0)	0 (0)
Laboratory variables in CSF			
CSF leukocyte count (cells/mm^3)††[,d]	4177 (324-8444)[b]	44 (22-96)[b]	4 (3-7)
CSF glucose (mg/dL)‡[,c]	0.90 (0.20-2.28)[a]	2.35 (2.03-2.79)	2.90 (2.35-3.58)
CSF:blood glucose ratio‡[,c]	0.13 (0.06-0.43)[a]	0.59 (0.32-0.66)	0.59 (0.42-0.87)
CSF protein (g/L)ϕ[,c]	2.61 (1.39-4.81)[b]	1.29 (0.93-1.81)	0.92 (0.64-1.22)
CSF culture, n (%)†[,b,d]			
E. coli	3 (19)		
GBS	9 (56)		
Negative	3 (19)		
Other[f]	1 (6)		

CRP = C-reactive protein; CSF = cerebrospinal fluid; *E. Coli* = *Escherichia coli;* GBS = Group B Streptococcus; IQR = interquartile range.
[a] Significantly different compared to sepsis cases ($P<0.05$).
[b] Significantly different compared to sepsis cases ($P<0.001$).
[c] Significantly different across all groups ($P<0.05$).
[d] Significantly different across all groups ($P<0.001$).
[e] Data reported as no. of patients/no. (%) of patients in which these data were available or median (IQR).
[f] CSF culture was positive for *Staphylococcus aureus*.¶Data available in 60 patients, † data available in 57 patients, †† data available in 58 patients, ‡ data available in 49 patients, ϕ data available in 51 patients.

1, 2018, and May 1, 2023, and were included in a consecutive prospective study in 45 Dutch hospitals. We included patients who had a minimum of 80 µl of residual CSF from the diagnostic lumbar puncture. Written informed consent for participation was obtained from patient caregivers. The study was approved by the Medical Ethics Committee of the Academic Medical Center, University of Amsterdam, the Netherlands (protocol no. NL-63,123.018.17, approved on October 12th, 2017).

Sepsis was defined as a positive blood culture. Definitive meningitis was defined as a positive CSF culture or a negative CSF culture but with a CSF leukocyte count of >2000 cells/mm^3 [4]. Possible meningitis was defined as a negative CSF culture with a CSF leukocyte count of 16-2000 cells/mm^3 ($\leq$ 28 days of age) or 10-2000 cells/mm^3 (>28 days of age) with a positive GBS or *E. coli* blood culture [5].

2.2. Procedures

Supernatant of the CSF was stored at −20 °C within 6 hours after lumbar puncture in most cases and aliquoted and stored at −80 °C within 1 month until further analysis. Biomarker concentrations in CSF were measured using a Human Luminex Discovery Custom Assay kit on a Luminex platform, according to manufacturer's instructions. The index test consisted of concentrations of a selection of 12 cytokines and acute phase reactants in CSF: C-reactive protein (CRP), C-X-C Motif Chemokine Ligand 10 (CXCL 10), C–C motif chemokine 22 (CCL22/MDC), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), Tumor Necrosis Factor-alpha (TNF-α), Macrophage migration inhibitory factor (MIF), Interleukin-1 Receptor Antagonist (IL-1RA), C-X-C Motif Chemokine Ligand 13 (CXCL13) and interleukin-1 beta (IL-1β). This selection was based on previous literature [6].

2.3. Statistical analysis

The Wilcoxon Rank Sum Test was applied to compare continuous data that were not normally distributed and the Fisher's Exact test to compare categorical data.

The area under the curve (AUC) of the receiver operator characteristic (ROC) curve with 95% confidence intervals (CI) was used

to evaluate diagnostic accuracy of the biomarkers. An AUC value of ≥ 0.90 was considered excellent discrimination, between 0.80-0.90 as good discrimination, 0.70-0.80 fair discrimination and 0.60-0.70 poor discrimination [7]. The optimal cut-off and associated sensitivity and specificity for diagnosis of meningitis was calculated by using the Youden Index. Multivariable least absolute shrinkage and selection operator (LASSO) logistic regression analysis was performed to determine the predictive value of the combination of the biomarkers in addition to CSF leukocyte count.

3. Results

In total 483 episodes were included in the NOGBS cohort between 2018 and 2023. A lumbar puncture was performed in 353 of 483 (73%) of these episodes, and 292 (83%) episodes were excluded due to insufficient amounts of CSF, leaving 61 patients (17%) for the current study.

Median age at disease onset overall was 6 days (IQR 0-19 days) and 26 patients were female (43%). A total of 30 (49%) patients were diagnosed with sepsis without meningitis and 31 (51%) were diagnosed with meningitis (Table 1). The diagnosis of meningitis was definitive in 16 of 31 (52%) and probable in 15 (48%) patients.

The median leukocyte count was higher in the definitive meningitis group (4177 cells/mm^3 IQR 324-8444), as compared with the probable meningitis (44 cells/mm^3 [IQR 22-96]; $P<0.001$) and sepsis group (4 cells/mm^3 [IQR 3-7]; $P<0.001$, Table 1).

All evaluated inflammatory markers had higher concentrations in the definitive meningitis group compared to the sepsis group. Concentrations of IL-1RA, MIF, TNF-α, CXCL10 and IL-10 were higher in the probable meningitis group as compared to the sepsis group (Table 2). Discrimination between definitive meningitis and sepsis was excellent for inflammatory markers IL-1RA, TNF-α, CXCL10, IL-1β, IL-6 and IL-10 (Table 2). IL-6 showed the highest discrimination with an AUC of 0.94 (95% CI 0.85-1.00), which came closest to the AUC of CSF leukocyte count of 0.96 (95% CI 0.89-1.00). None of the biomarkers showed excellent or good discrimination between probable meningitis and sepsis diagnosis, although MIF showed a moderate discrimination (AUC 0.78; 95% CI 0.68-0.96).

The cut-off of IL-6 with the highest combination of sensitivity and specificity (Youden index 0.874) for diagnosis of definitive meningitis versus sepsis was 2721 pg/mL (sensitivity 81%, 95% CI 63-100%, specificity 100%, 95% CI 100-100%). The cut-off that had a 100% sensitivity (95% CI 100-100%) was 9.1 pg/mL (Youden index 0.075), with a specificity of 67% (95% CI 48-83%). When using LASSO regression a model with IL-1RA, TNF-α, CXCL10 and CSF leukocyte count showed an AUC of 0.95 (95% CI 0.87-1.00).

4. Discussion

Our study of diagnostic accuracy showed that CSF inflammatory markers CXCL10, MDC, IL-6, IL-8, IL-10, TNF-α, MIF, IL-1RA, CXCL13, IL-1β, CRP and procalcitonin can be used to differentiate between neonatal sepsis and definite meningitis. IL-6 showed the highest discrimination between definitive meningitis and sepsis. This was comparable to the discriminative value of CSF leukocyte count. A combination of IL-1RA, TNF-α, CXCL10 and CSF leukocyte showed excellent discrimination.

Previous studies showed comparable results and also showed that elevated concentrations of IL-6 in CSF in neonates had excellent discrimination between culture proven bacterial meningitis and sepsis patients, however using different cut-offs for IL-6 [8]. Point-of care tests for cytokines, including IL-6, are available but

Table 2
Concentrations and area-under the ROC curves (AUC) of inflammatory markers in CSF[f]

Inflammatory marker CSF[e]	Definitive meningitis (def. men.), n = 16	Probable meningitis (prob. men.), n = 15	Sepsis, n = 30[g]	Youden index[h]	Cut-off[h]	Sensitivity[h]	Specificity[h]	AUC def. men. vs. sepsis (95% CI)	AUC prob. men. vs. sepsis (95% CI)
CRP[2]	54.10 (19.4-54.10)[a]	9.36 (1.31-54.10)	4.03 (0.79-48.01)[c]	0.481	54.10	0.69	0.79	0.76 (0.61-0.90)	0.61 (0.43-0.79)
CCL22/MDC	173 (138-197)[b]	67 (38-88)	48 (14-67)[d]	0.744	123.2	0.81	0.93	0.88 (0.76-1)	0.64 (0.47-0.81)
CXCL13	485 (287-774)[b]	117 (54-244)	57 (51-95)[d]	0.778	276.2	0.81	0.97	0.88 (0.74-1)	0.68 (0.50-0.86)
IL-1RA	6730 (6676-6730)[b]	1399 (645-6118)[a]	294 (183-876)[d]	0.778	6516	0.81	0.97	0.93 (0.85-1)	0.75 (0.59-0.90)
IL-8/CXCL-8§	2075 (848-2282)[b]	383 (194-1170)	180 (113-472)[d]	0.668	616.7	0.88	0.79	0.86 (0.75-0.97)	0.65 (0.47-0.83)
MIF[2]	72.68 (35.79-236.3)[b]	44.14 (15.05-111.5)[a]	13.12 (9.64-18.57)[d]	0.647	42.73	0.75	0.90	0.81 (0.67-0.97)	0.78 (0.63-0.93)
TNF-α	512 (68-2539)[b]	6 (4-10)[a]	2 (2-5)[d]	0.813	51.45	0.81	1.0	0.92 (0.82-1)	0.74 (0.59-0.89)
CXCL10¶	1054 (1054-1054)[b]	350 (100-558)[a]	77 (46-231)[d]	0.778	1054	0.81	0.97	0.90 (0.79-1)	0.69 (0.50-0.88)
IL-1β	160 (76-6828)[b]	9 (4-28)	4 (4-12)[d]	0.778	45.76	0.81	0.97	0.92 (0.83-1)	0.65 (0.49-0.82)
IL-6†	2721 (2721-2721)[b]	25 (11-137)	3 (2-87)[d]	0.813	2721	0.91	1.0	0.94 (0.88-1)	0.68 (0.51-0.85)
IL-10	501 (83-1902)[b]	36 (14-43)[a]	11 (6-31)[d]	0.778	73.97	0.81	0.97	0.93 (0.82-1)	0.72 (0.55-0.89)
Procalcitonin	749 (429-1290)	744 (426-1182)	585 (439-911)	0.224	850.2	0.50	0.72	0.52 (0.33-0.71)	0.55 (0.36-0.74)

CSF = cerebrospinal fluid; Def. men = definitive meningitis; Prob. Men. = probable meningitis; CRP = C-reactive protein; CXCL10 = C-X-C Motif Chemokine Ligand 10; CCL22/MDC = C-C motif chemokine 22; IQR = interquartile range; CXCL13= C-X-C Motif Chemokine Ligand 13; IL-1β = interleukin-1 beta; IL-1RA = Interleukin-1 Receptor Antagonist; IL-6 = interleukin-6; IL-8 = interleukin-8; IL-10 = interleukin-10; MIF = Macrophage migration inhibitory factor; TNF-α = Tumor Necrosis Factor-alpha.

[a] Significantly different compared to sepsis cases ($P<0.05$).
[b] Significantly different compared to sepsis cases ($P<0.001$).
[c] Significantly different across all groups ($P<0.05$).
[d] Significantly different across all groups ($P<0.001$).
[e] Concentrations are in pg/mL and represented as median (IQR). [2] CRP and MIF concentrations are in µg/mL and represented as median (IQR).
[f] Concentrations are medians (interquartile ranges).
[g] In one sepsis patient only CRP was investigated due to less available CSF.
[h] Youden index, cut-off, sensitivity and specificity for the comparison definitive meningitis versus sepsis.§, ¶ and † are described in the Supplementary results.

are usually developed for blood and can only detect one cytokine at a time, limiting the clinical use for neonatal bacterial meningitis.

Introducing several biomarkers that have undergone limited prior evaluation (CXCL10, CCL22/MDC, IL-8, IL-10, MIF, IL-1RA, CXCL13 and IL-1β) did not identify a biomarker that could discriminate better between meningitis and sepsis without meningitis cases when compared to CSF leukocyte count. Moreover, other diagnostic tools, such as prediction models, that combine clinical and laboratory variables to predict bacterial meningitis, did not perform better than CSF leukocyte count alone [9].

Future research should therefore be two-fold. Firstly, to improve identification of neonatal meningitis cases, these (individual and combined) biomarkers should be investigated in a larger cohort, with emphasis on culture negative meningitis and neonates with low or normal CSF leukocyte count. This group carries the highest diagnostic uncertainty and could benefit most from new and timely diagnostic markers. Furthermore, novel biomarkers warrant investigation for their potential to distinguish between neonatal sepsis and meningitis.

Our study has limitations. First, residual CSF was only available for a subgroup of cases. This may potentially lead to selection bias. However, clinical and laboratory characteristics of patients with and without CSF available were comparable, suggesting a limited selection bias.

Another limitation was that the concentrations of IL-6, IL-8, and CXCL10 might in fact have been higher, as the median and interquartile ranges of these biomarkers in the definitive meningitis group consistently exceeded the predefined limit of the assay. Due to the simultaneous measurement of multiple cytokines in a single sample, we were unable to customize dilution for each specific cytokine.

To conclude, biomarkers IL-1RA, TNF-α, CXCL10, IL-1β, IL-6 and IL-10 showed excellent discrimination between definitive meningitis and sepsis patients. IL-6 showed the highest discrimination. A combination of IL-1RA, TNF-α, CXCL10 and CSF leukocyte showed excellent discrimination.

Access to data

Requests to share the review protocol or anonymous individual patient data for scientific research can be made to the corresponding author.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approval

We have read and complied with the policy of the journal on ethical consent.

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Author contributions

NSG, SEO, DvdB, MCB and MWB contributed to the study design. LS, DHV, MWB and the study-group contributed to the data-collection. NSG, LS, DHV, MWB and the study-group accessed and verified the original data from the manuscript. NSG, SEO, MCB and MWB analysed an interpreted the data. NSG prepared the first draft of the manuscript and created the figures and tables. All authors contributed to data interpretation and writing. NSG, DvdB, MCB and MWB had final responsibility for the decision to submit for publication. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.02.013.

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