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Cerebrospinal fluid inflammatory markers in patients with *Listeria monocytogenes* meningitis

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ABSTRACT

Background: Listeria monocytogenes meningitis is the third most common cause of bacterial meningitis and is associated with high rates of mortality and unfavorable outcome.
Methods: We analyzed 101 cytokines, chemokines and complement factors in CSF of adult patients with Listeria meningitis included in a prospective cohort study and compared these biomarkers between Listeria meningitis patients and negative controls, and between Listeria meningitis patients with a favorable and an unfavorable outcome.
Results: CSF was available from 26 of 62 (42%) Listeria meningitis patients and 19 negative controls. Fifteen (58%) Listeria meningitis patients had an unfavorable outcome. In Listeria meningitis CSF levels of 51 biomarkers were significantly elevated compared to negative controls after Bonferroni correction. The 11 most significantly elevated ($P < .01$) biomarkers of unfavorable outcome in Listeria meningitis were markers of T-cell activation (sIL-2Rα, sCD40L and IL-1), interferon-related (IFN-α2, IL-18, CX3CL1, CCL20), markers of complement activation (C3a), and endothelial growth factor related (VEGF, CXCL7).
Conclusions: Our data suggest that T-cell activation, complement activation, interferon- and endothelial growth factor production are important in the immune response to Listeria meningitis, and thereby influence outcome.
General significance: Our study provides target pathways for further studies in the pathophysiology of Listeria meningitis.

1. Introduction

Bacterial meningitis is caused by *Streptococcus pneumoniae* and *Neisseria meningitidis* in 85% of cases [1,2]. *Listeria monocytogenes* is the third most common cause of bacterial meningitis (identified in 6% of cases) and is associated with high mortality and rate of unfavorable outcome [3,4]. Listeria meningitis is predominantly found in elderly and immunocompromised patients, but also occurs in previous healthy adults [5,6]. In an explorative study we assess the associations between cerebrospinal fluid (CSF) inflammatory markers and outcome in Listeria meningitis.

2. Materials and methods

We identified adults (>16 years of age) who had Listeria meningitis established by positive CSF culture and were listed in the database of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) from March 2006 to April 2012. This laboratory receives CSF isolates from approximately 85% of all patients with bacterial meningitis in the Netherlands. Patients or their legal representatives received written information concerning the study and were asked to give written informed consent for participation. Patients with negative CSF cultures, hospital-acquired bacterial meningitis, or neurosurgical devices, and those within 1 month following neurosurgical procedure or neurotrauma, were excluded. The study was approved by the Medical Ethics Committee of the Academic Medical Center, University of Amsterdam, the Netherlands.

Online case record forms were used to collect data on clinical features, complications, treatment and outcome in adults with meningitis. Patients with an altered immune status owing to splenectomy, diabetes mellitus, cancer, alcoholism or the use of immunosuppressive drugs were considered immunocompromised, as were patients infected with HIV. Neurological examination was performed at discharge and outcome was scored according to the Glasgow Outcome Scale. This measurement scale is well validated, with scores varying from 1 (death) to 5 (good recovery) [7]. A favorable outcome was defined

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as a score of 5, and an unfavorable outcome was defined as a score of 1–4.

In patients with Listeria meningitis, residual CSF from the diagnostic lumbar puncture was collected, centrifuged, and supernatant was aliquoted and stored at −80 °C until analysis. Control CSF samples were collected from 19 patients in whom a lumbar puncture was performed to rule out sub-arachnoid hemorrhage in patients eventually diagnosed with benign thunderclap headache. All these CSF samples had normal leukocyte count, total protein level and glucose concentration (Appendix A1). CSF cytokine, chemokine and complement factor concentrations were determined with Luminex technology using a Milliplex MAP assay, Millipore, Billerica, MA, USA. For complement component C3a, iC3b, C5a and sC5b-9 levels the Microvue C3a, iC3b, C5a and sC5b-9 Quidel ELISA kits, Quidel, San Diego, USA were used. CSF complement component levels of C1q and MBL were measured using the Hycult ELISA kits, Hycult Biotech, Uden, the Netherlands and PAI-2 was measured using the USCN ELISA kit, USCN Life Sciences, Hubei, China.

Cytokine, chemokine and complement factor levels were compared between negative controls and patients with Listeria meningitis and subsequently between patients with Listeria meningitis with an unfavorable outcome and those with a favorable outcome. The Mann–Whitney *U*-test was used to examine associations between cytokine, chemokine and complement factor levels between groups. All statistical tests were two-tailed, and for differences between negative controls and patients a Bonferroni corrected p-Value below <.00051 was considered significant. In the comparison between patients with a favorable and unfavorable outcome the limited number of patients prevented correction for multiple testing. Therefore, we described biomarkers with P value < .01. All statistical analyses were performed with SPSS version 19.0.

3. Results

From March 2006 to April 2012, 62 patients with Listeria meningitis were identified of 1032 included episodes of bacterial meningitis (6%). CSF was available for 26 patients (42%), with median age of 68 years (interquartile range [IQR] 57–74). Patients for whom CSF was available had higher scores on the Glasgow Coma Scale on admission compared to those in whom CSF was not available (GCS 14 [IQR 11–15] *vs.* 11 [IQR 10–14], P = .03); other clinical and laboratory characteristics and outcome were comparable between patients with and without CSF available.

Table 1 shows the clinical and laboratory characteristics of 26 patients with CSF available. The majority was male (77%) and 15 patients (58%) had an immunocompromised state. Three patients (13%) were not immunocompromised and were 50 years old or younger. Median CSF leukocyte count was 766 cells/mL (interquartile range [IQR] 255–2047), protein level of 2.4 g/L (IQR 1.8–3.8) and CSF to blood glucose ratio of 0.27 (IQR 0.16–0.39). Adjunctive dexamethasone treatment was started with or before the first dose of antibiotics in 14 patients (54%) according to the standard regimen of 10 mg every 6 h for 4 days. Data on diagnostic sequence of cranial CT, lumbar puncture and initiation of treatment were available for 20 patients. In 19 patients a cranial CT was made prior to the lumbar puncture, and in only 6 the antibiotic treatment was initiated before the CT. Fifteen patients (58%) had an unfavorable outcome and 6 (23%) died.

Because of limited amounts of cerebrospinal fluid not all assays could be performed for all patients: sufficient CSF to assess 46 analytes was available from all 26 patients and 19 controls. The other 55 analytes were tested in 19–25 patients and 13–18 controls (Appendix A2). Out of 101 analytes, 10 (10%) were below the lower limit of detection in all patient and control samples (IL-9, CCL1, CCL21, IL-33, LIF, TPO, TSLP, MMP-12, MMP-13 and MMP-7). For 21 analytes (21%) the protein concentration was below the lower limit of detection in all negative control samples, but detectable concentrations were present in Listeria meningitis patients. Compared to negative controls, 51 of the 101 (50%) cytokines, chemokines and complement factors were significantly elevated in patients with Listeria meningitis after Bonferroni correction (<0.00051) for multiple testing.

Table 1
Clinical and laboratory characteristics on admission of 26 adults with community-acquired *L. monocytogenes* meningitis, 2006–2012 cohort [a].

Variable	Frequency
Median age in years (IQR)	68 (57–74)
Male sex	20 (77)
Predisposing factors	
Immunocompromised	15 (58)
Pre-treated with antibiotics	3 (12)
Triad of fever, neck stiffness, and change in mental status	8 (31)
Indexes of CSF inflammation	
WBC count (cells/μL)	766 (225–2047)
<1000 cells/mL	15 (58)
Protein level (g/L)	2.4 (1.8–3.8)
CSF to blood glucose ratio	0.27 (0.16–0.39)
Dexamethasone treatment	14 (54)
Neurological complications	19 (73)
Systemic complications	15 (58)
Unfavorable outcome	15 (58)
Death	6 (23)

[a] Continuous data are presented as medians (interquartile range), dichotomous data are presented as n (%)

We identified vascular endothelial growth factor (VEGF; P = .001, Appendix A3, Fig. 1), and (Appendix A4) complement component C3a (P = .002), soluble interleukin receptor antagonist a2 (sIL-2Rα; P = .002), chemokine (C–X–C motif) ligand 7 (CXCL7; P = .003), CX3CL1 (P = .003), CCL11 (P = .005), interferon alpha2 (IFN-α2; P = .005), chemokine (C–C motif) ligand 20 (CCL20; P = .007), interleukin 12 subunit p40 (IL-12p40; P = .008), soluble CD40 ligand (sCD40L; P = .008) and interleukin 18 (IL-18; P = .008) as the top 11 cytokines, chemokines and complement factors with the strongest association (P < .01) with unfavorable outcome in patients with Listeria meningitis.

No differences in CSF biomarker levels were identified when comparing patients in whom the lumbar puncture was performed before or after parenteral antibiotics.

4. Discussion

In our explorative study we assessed 101 cytokines, chemokines and complement factors and found that VEGF, C3a, sIL-2Rα, CXCL7, CX3CL1, CCL11, IFN-α2, CCL20, IL-12p40, IL-18, and sCD40L were most significantly different between Listeria meningitis patients with an unfavorable and those with a favorable outcome. These differences in protein levels may reflect important pathways in the pathophysiology of Listeria meningitis contributing to disease severity. VEGF, sIL-2Rα, C3a and IFN-α2 have previously been identified to be involved in the host defense mechanisms against *L. monocytogenes* [8–12].

VEGF is a growth factor and an angiogenic cytokine which is expressed intracellularly in several cell types, such as macrophages, inside which *L. monocytogenes* replicate [8,13]. *In vitro* experiments have previously shown that VEGF levels in RAW264.7 macrophage-like cells were elevated following stimulation with heat-killed *L. monocytogenes* [9]. Furthermore, an *in vivo* model showed elevated expression of VEGF in splenic macrophages in mice with *L. monocytogenes* compared to negative controls [14]. This production of VEGF is induced by CXCL7 [14], which was also elevated in patients with an unfavorable outcome. VEGF has been shown to induce endothelial changes during bacterial meningitis in patients and disrupt the blood brain barrier in a rat model [15]; thereby increasing brain inflammation and oedema. Elevated VEGF levels, mediated by CXCL7, could result in increased brain damage in patients explaining the increased rate of unfavorable outcome.

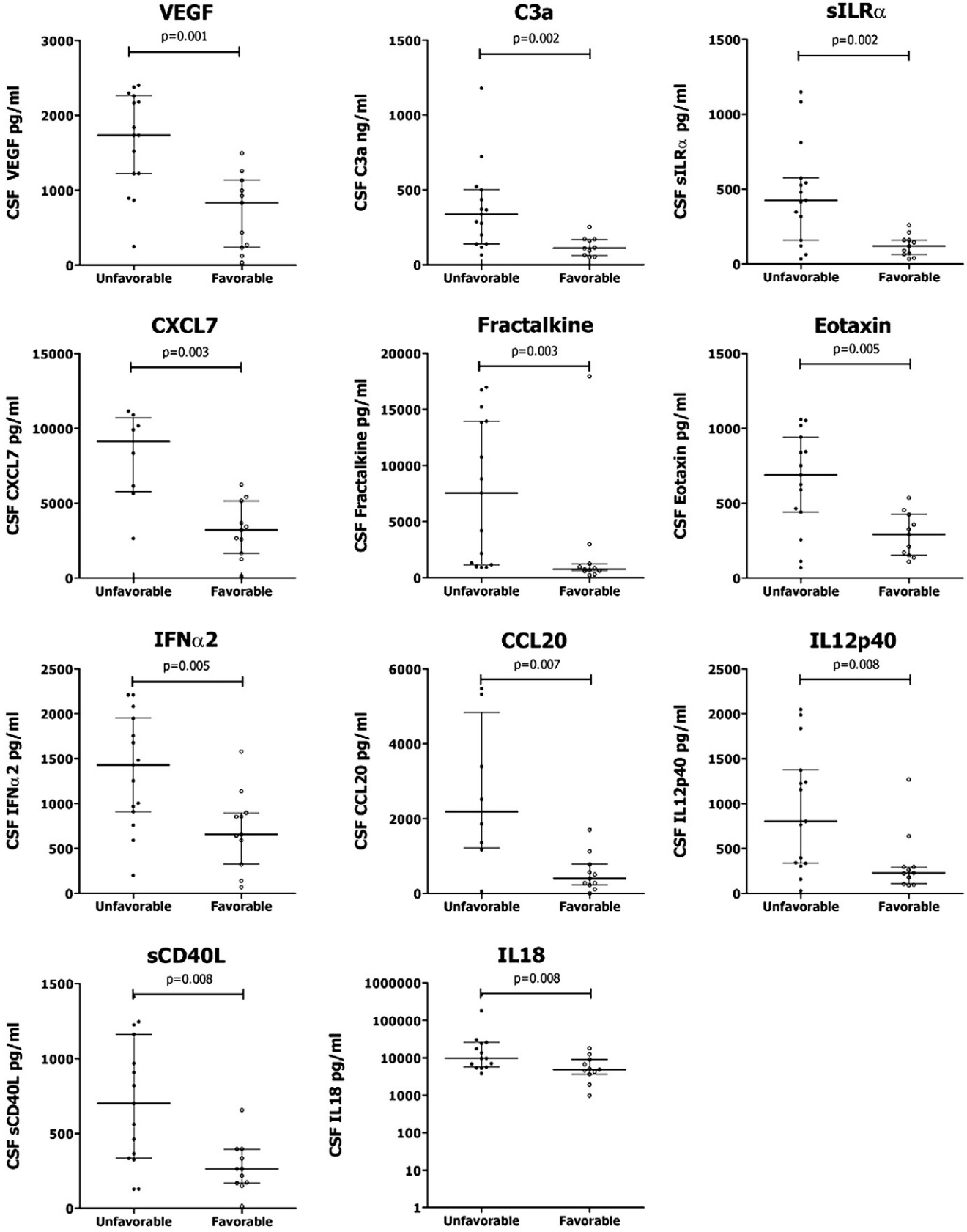

Fig. 1. Levels of top 11 cytokines, chemokines and complement factors with strongest association with unfavorable outcome in patients with Listeria meningitis. Footnote: Dots indicate individual values, bars are medians and whiskers indicate interquartile range.

C3a is an anaphylatoxin, causing attraction of phagocytic cells, recruits antibodies and initiates the adaptive immune response [16]. A study on the role of the complement in *L. monocytogenes* infections showed that listerial opsonization mainly depended on the classical complement pathway [17]. Following opsonization, activation of the final common pathway results in conversion of C3 to C3a and C3b [10,16]. The degree of complement activation has previously been shown to influence outcome in pneumococcal meningitis by aggravating brain damage [18]. In a pneumococcal meningitis mouse model inhibition of complement factor 5 was shown to improve outcome [18]. The elevated C3a levels in patients with unfavorable outcome suggest that complement activation is detrimental in Listeria meningitis as well. Whether complement inhibition is a successful strategy in Listeria meningitis is an interesting topic for experimental studies.

Several proteins that were elevated in patients with an unfavorable outcome were involved in T-cell activation. We observed elevated levels of sIL-2Rα, sCD40L and IL-12p40 in patients with an unfavorable outcome indicating a strong T-cell activation, which has been shown to be crucial in the immune response to *L. monocytogenes* in mouse models [19]. Activated T-cells express high affinity receptors for interleukin-2, which plays an important role during proliferation of T-lymphocytes. This IL-2-receptor alpha is also released in a soluble form (sIL-2Ra), which is considered to be an important marker of continuous T-cell activation [20,21]. Another protein in the activation of T-cells which was elevated in patients with an unfavorable outcome is sCD40L, which is a member of the TNF family and results in T-cell priming following infection with *L. monocytogenes* [22]. IL-12p40 is important for sustaining a sufficient number of memory/effector Th1 cells to mediate long-term protection to intracellular pathogens, and acts on T-cells and natural killer cells, which are both essential to combat *L. monocytogenes* infection [23].

Other proteins that were identified to be elevated in patients with unfavorable outcome were interferon (IFN-α2) or interferon-related (IL-18, CX3CL1, CCL20). Type 1 interferons, which include interferon alpha, have been shown to have a deleterious effect during *L. monocytogenes* infection by decreasing the viability of infected macrophages [23,24]. The increased levels of IFN-α2 in patients with an unfavorable outcome concur with these experimental studies. IFN-γ on the other hand is essential during *L. monocytogenes* infection, and provides early protection against the pathogen [23]. IL-18 a pro-inflammatory cytokine that stimulates IFN-γ production in T-helper type 1 cells and NK cells [25]. CX3CL1 and CCL20 are chemoattractants that are produced following stimulation by IFN-γ and result in the recruitment of monocytes, NK cells and T-cells [26]. The higher levels of IL-18, CX3CL1 and CCL20 were not paralleled by significantly elevated levels of IFN-γ in patients with an unfavorable outcome.

CCL11 (Eotaxin) is an eosinophil specific chemokine assumed to be involved in eosinophilic inflammatory diseases. CCL11 production is not related to interferons and does not influence T-cell activation [27]. Its role in *L. monocytogenes* infection has not been studied, and it is unclear how the elevated levels of CCL11 in patients with an unfavorable outcome must be interpreted.

Our study has several limitations. First, only patients with a positive CSF culture for *L. monocytogenes* were included in this study. Negative CSF culture results occur in 11%–30% of patients with bacterial meningitis, and this percentage may be higher in patients with Listeria meningitis [1,6,28,29]. Therefore, these patients are underrepresented in our cohort. Second, as shown in Table 1, for the 26 patients we analyzed their cytokine levels had a higher Glasgow Coma Scale compared to 36 patients for whom no CSF was available. This implies a selection towards less severe diseases in the studied population compared to the general population of Listeria meningitis patients. Third, the number of patients is insufficient to correct for multiple testing in the analysis for outcome or perform multivariate analyses. Therefore, the results must be interpreted with caution, as type 1 errors may occur due to the high number of variables tested, and validation of our results in other cohorts is warranted. Fourth, the number of patients is insufficient to analyze if the expression of biomarkers was influenced in subcategories such as the immune state of the patients, or patients with systemic of neurological complications. Finally, it remains unclear whether the identified proteins have a causative role in the development of unfavorable outcome or merely reflect increased disease severity. Despite these limitations, this study on CSF characteristics culture confirmed that Listeria meningitis is the largest series so far and gives insight in the pathophysiology of the disease.

5. Conclusions

Our data suggest that VEGF production (VEGF, CXCL7), complement activation (C3a), T-lymphocyte activation (sIL-2Rα, sCD40L, IL-12p40), and interferon production (IFN-α2, IL-18, CCL20, CX3CL1) are the most important elements of the immune response during Listeria meningitis, and may influence outcome. These data may give leads to further investigation in the pathophysiology of Listeria meningitis.

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Conflicts of interest

All authors report no conflicts of interest.

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Appendix A1

Clinical and laboratory characteristics of 26 patients in whom cytokine levels were analyzed in CSF *vs.* 36 patients in whom cytokine levels were not analyzed in CSF. All 62 adults had community-acquired Listeria meningitis and were included in the prospective cohort study between 2006 and 2012[a].

Variable	Patients with CSF collection for analysis N = 26 (%)	Patients without CSF collection for analysis N = 36 (%)	p-Value
Median age in years (IQR)	68 (57–74)	73 (62–77)	0.34
Male sex	20 (77)	19 (53)	0.05
Immunocompromised	15 (58)	29 (81)	0.05
Duration of symptoms <24 h	8 (31)	12 (33)	0.83
Glasgow Coma Scale (IQR)	14 (11–15)	11 (10–14)	0.03
<14 (indicating change in mental status)	13 (50)	25 (69)	0.12
≤8 (indicating coma)	1 (4)	4 (11)	0.39
Indexes of CSF inflammation			
WBC count (cells/mL)	766 (225–2047)	636 (367–1498)	0.89
<1000 cells/mL	15 (58)	24 (67)	0.47
Protein level (g/L)	2.4 (1.8–3.8)	2.6 (1.7–3.5)	0.99
CSF to blood glucose ratio	0.27 (0.16–0.39)	0.24 (0.06–0.34)	0.44
Dexamethasone treatment	14 (54)	19 (53)	0.92
Unfavorable outcome	15 (58)	23 (64)	0.62
Death	6 (23)	16 (44)	0.08

[a] Continuous data are presented as medians (interquartile range), dichotomous data are presented as n (%).

Appendix A2

Out of 101 cytokines, chemokines and complement factors, 73 were significantly elevated compared to negative controls. 51 were significantly elevated after Bonferroni correction.

Variable	N (LM/NC)[a]	*L. monocytogenes* meningitis Median level (IQR)	Negative controls Median level (IQR)	p-Value
EGF	26/19	151 pg/ml (62–313)	81 pg/ml (45–126)	0.012
CCL11	26/19	448 pg/ml (200–773)	81 pg/ml (28–126)	<0.0001[b]
FGF2	26/19	962 pg/ml (584–1387)	484 pg/ml (136–731)	<0.0001[b]
Flt3-Lig	26/19	250 pg/ml (98–290)	71 pg/ml (41–133)	0.002
CX3CL1	26/19	1.3 ng/ml (0.8–12)	0.5 ng/ml (0.4–0.7)	<0.0001[b]
GCSF	26/19	11.3 ng/ml (5.4–16.5)	0.06 ng/ml (0.04–0.1)	<0.0001[b]
GMCSF	26/19	269 pg/ml (119–1054)	18 pg/ml (9–31)	<0.0001[b]
CXCL1	26/19	2.2 ng/ml (1.3–5.0)	0.06 ng/ml (0.02–0.09)	<0.0001[b]
IFN-α2	26/19	938 pg/ml (628–1602)	67 pg/ml (7–125)	<0.0001[b]
IL-1a	26/19	454 pg/ml (319–2420)	18 pg/ml (11–21)	<0.0001[b]
IL-1b	26/19	189 pg/ml (91–492)	4 pg/ml (2–6)	<0.0001[b]
IL-1ra	26/19	2.3 ng/ml (982–4305)	0.02 ng/ml (0.008–0.03)	<0.0001[b]
IL-2	26/19	36 pg/ml (25–94)	4 pg/ml (3–7)	<0.0001[b]
IL-3	26/19	15 pg/ml (8–31)	15 pg/ml (7–29)	0.696
IL-4	26/19	70 pg/ml (35–101)	82 pg/ml (25–116)	0.836
IL-5	26/19	10 pg/ml (6–15)	3.8 pg/ml (1.7–7.4)	0.001
IL-6	26/19	58 ng/ml (26–89)	0.01 ng/ml (0.009–0.03)	<0.0001[b]
IL-7	26/19	163 pg/ml (95–1251)	92 pg/ml (25–139)	0.002
IL-8	26/19	11 ng/ml (5.6–19)	0.02 ng/ml (0.02–0.04)	<0.0001[b]
IL-9	26/19	75 pg/ml (29–132)	42 pg/ml (22–83)	0.118
IL-10	26/19	408 pg/ml (171–1080)	3.7 pg/ml (1.8–6.4)	<0.0001[b]
IL-12p40	26/19	338 pg/ml (213–1228)	69 pg/ml (12–103)	<0.0001[b]
IL-12p70	26/19	141 pg/ml (77–207)	19 pg/ml (13–29)	<0.0001[b]
IL-13	26/19	31 pg/ml (12–55)	11 pg/ml (5–28)	0.015
IL-15	26/19	53 pg/ml (25–83)	22 pg/ml (15–34)	0.005
IL-17	26/19	38 pg/ml (18–95)	4.8 pg/ml (3.2–5.3)	<0.0001[b]
CCL2	26/19	17 ng/ml (5.7–69)	0.5 ng/ml (0.4–0.6)	<0.0001[b]
CCL7	26/19	951 pg/ml (489–3556)	114 pg/ml (59–167)	<0.0001[b]
CCL22	26/19	951 pg/ml (362–1232)	707 pg/ml (230–883)	0.093
CCL3	26/19	209 pg/ml (126–520)	125 pg/ml (51–142)	0.001
CCL4	26/19	1.5 ng/ml (0.6–4.2)	0.6 ng/ml (0.3–0.8)	<0.0001[b]
PDGFABBB	26/19	158 pg/ml (58–333)	73 pg/ml (48–123)	0.066
CCL5	26/19	64 pg/ml (27–202)	20 pg/ml (10–29)	0.002
sCD40L	26/19	381 pg/ml (206–841)	76 pg/ml (50–104)	<0.0001[b]
sIL-2Rα	26/19	186 pg/ml (85–491)	55 pg/ml (24–96)	<0.0001[b]
TGFα	26/19	17 pg/ml (7–32)	16 pg/ml (10–26)	0.597
TNFα	26/19	319 pg/ml (212–639)	50 pg/ml (32–61)	<0.0001[b]
TNFβ	26/19	43 pg/ml (20–441)	15 pg/ml (10–24)	0.001
VEGF	26/19	1.2 ng/ml (0.7–1.9)	0.5 ng/ml (0.4–0.7)	<0.0001[b]
IL-18	26/19	6.9 ng/ml (4.8–18)	0.7 ng/ml (0.4–0.9)	<0.0001[b]
vWFAg	17/12	3 pg/ml (2–9)	0.4 pg/ml (0.11–0.51)	<0.0001[b]
Fibrinog\en	17/12	0.55 pg/ml (0.2–0.6)	0.05 pg/ml (0.03–0.06)	<0.0001[b]
TAFI	17/12	1.3 pg/ml (0.8–2.5)	0.38 pg/ml (0.11–0.54)	0.002
CCL8	19/18	7.8 ng/ml (4.1–35)	0.1 ng/ml (0.09–0.2)	<0.0001[b]
CCL13	19/18	301 pg/ml (103–392)	333 pg/ml (200–436)	0.313
ENA-78	19/18	470 pg/ml (378–701)	577 pg/ml (271–733)	0.775
CXCL12	19/18	2.3 ng/ml (1163–3824)	2.0 ng/ml (0.9–3.2)	0.538
CXCL13	19/18	16 pg/ml (8–29)	2.9 pg/ml (0.6–6.5)	<0.0001[b]
CCL1	19/18	28 pg/ml (10–57)	47 pg/ml (29–65)	0.092
IL-16	19/18	247 pg/ml (64–46)	280 pg/ml (176–459)	0.799
MIP-1d	19/18	501 pg/ml (146–710)	88 pg/ml (35–121)	<0.0001[b]
CCL17	19/18	2.7 pg/ml (1.1–4.2)	1.7 pg/ml (0.7–2.5)	0.092
CCL21	19/18	698 pg/ml (374–871)	714 pg/ml (482–922)	0.558
CCL24	19/18	54 pg/ml (21–84)	24 pg/ml (10–29)	0.008
CCL26	19/18	57 pg/ml (35–144)	41 pg/ml (27–78)	0.210
CCL27	19/18	25 pg/ml (6–61)	8 pg/ml (6–12)	0.053
IL-23	19/18	233 pg/ml (144–463)	234 pg/ml (143–443)	0.893
LIF	19/18	599 pg/ml (346–734)	636 pg/ml (439–828)	0.313
TPO	19/18	932 pg/ml (513–1299)	968 pg/ml (328–1747)	0.822
TRAIL	19/18	151 pg/ml (68–229)	10 pg/ml (4–23)	<0.0001[b]
SCF	19/18	40 pg/ml (25–73)	21 pg/ml (10–27)	0.001
TSLP	19/18	73 pg/ml (52–97)	45 pg/ml (23–85)	0.16
IL-20	19/18	1.7 ng/ml (1.4–2.7)	1.5 ng/ml (0.9–2.6)	0.27
IL-21	19/18	41 pg/ml (25–67)	20 pg/ml (3–41)	0.020
IL-28A	19/18	82 pg/ml (40–108)	68 pg/ml (34–102)	0.46
IL-33	19/18	138 pg/ml (87–163)	78 pg/ml (34–148)	0.06
MCSF	19/18	28 ng/ml (13–37)	1.5 ng/ml (1.2–2.7)	<0.0001[b]
CXCL7	19/18	5.1 ng/ml (2.6–8.3)	0.5 ng/ml (0.09–0.6)	<0.0001[b]
CXCL6	19/18	258 pg/ml (164–1172)	18 pg/ml (11–25)	<0.0001[b]
CCL14a	19/18	7.5 ng/ml (4.3–13)	1.2 ng/ml (0.9–18)	<0.0001[b]
CCL19	19/18	153 pg/ml (76–303)	79 pg/ml (38–98)	0.003
CCL20	19/18	779 pg/ml (280–1857)	23 pg/ml (10–28)	<0.0001[b]
XCL1	19/18	1.3 ng/ml (0.4–1.5)	0.6 ng/ml (0.2–0.8)	0.007
IL-11	19/18	3.2 ng/ml (1.8–4.4)	2.1 ng/ml (1.3–2.7)	0.026

Appendix A2 (*continued*)

Variable	N (LM/NC)[a]	*L. monocytogenes* meningitis Median level (IQR)	Negative controls Median level (IQR)	p-Value
IL-29	19/18	3.3 ng/ml (1.5–4.3)	1.2 ng/ml (0.5–2.1)	0.004
MMP-3	26/18	23 ng/ml (10–39)	1 ng/ml (0.7–1.9)	<0.0001[b]
MMP-12	26/18	6 ng/ml (4.1–9)	7.8 ng/ml (4–13.4)	0.34
MMP-13	26/18	4.6 ng/ml (2.4–6.7)	4.9 ng/ml (1.6–8.4)	0.63
CFH	25/18	7 ng/ml (4.8–9.1)	1.1 ng/ml (0.9–1.5)	<0.0001[b]
MMP-1	25/18	683 pg/ml (216–937)	333 pg/ml (158–594)	0.030
MMP-2	25/18	17 ng/ml (7.7–32)	26 ng/ml (9.7–33)	0.46
MMP-7	25/18	10 ng/ml (4.9–16)	128 ng/ml (7.1–160)	0.56
MMP-9	25/18	387 ng/ml (120–497)	0.1 ng/ml (0.06–0.2)	<0.0001[b]
MMP-10	25/18	266 pg/ml (154–275)	114 pg/ml (65–190)	0.003
ICAM	26/19	64 pg/ml (46–121)	2.3 pg/ml (1.0-5.1)	<0.0001[b]
tPAI-1	26/19	50 ng/ml (28–83)	0.8 ng/ml (0.6–1.8)	<0.0001[b]
MIF	26/13	1.5 ng/ml (0.6-5.1)	0.8 ng/ml (0.6–1.6)	0.16
C3a	25/19	171 pg/ml (112–369)	5.8 pg/ml (4–9)	<0.0001[b]
iC3b	25/19	15 pg/ml (8–21)	3.5 pg/ml (2.7–4.7)	<0.0001[b]
C5a	18/14	3.2 pg/ml (1.1-8.8)	0 pg/ml (0–0.4)	<0.0001[b]
C5b9	18/14	415 pg/ml (95–1294)	155 pg/ml (64–394)	0.14
IFN-γ	26/19	1.1 ng/ml (2.4-19)	0.02 ng/ml (0.009–0.03)	<0.0001[b]
CXCL9	19/18	81 ng/ml (21–357)	0.12 ng/ml (0.04–0.17)	<0.0001[b]
CXCL10	26/19	488 ng/ml (485–488)	0.4 ng/ml (0.1–0.7)	<0.0001[b]
PDGFAA	26/19	78 pg/ml (54–176)	50 pg/ml (21–73)	0.001
CXCL11	19/18	15 ng/ml (4.3–25)	0.01 ng/ml (0.008–0.02)	<0.0001[b]
C3	25/18	7.6 ng/ml (0.1–12)	1.8 ng/ml (0.2–5.4)	0.008
VCAM	26/19	394 pg/ml (290–527)	42 pg/ml (34–51)	<0.0001[b]
PAI-2	26/14	240 ng/ml (168–319)	18 ng/ml (0.3–27)	<0.0001[b]
MBL	21/19	11 ng/ml (3.9–17)	3 ng/ml (2–3.8)	<0.0001[b]
C1q	21/19	152 ng/ml (118–245)	114 ng/ml (75–139)	0.009

[a] Number of Listeria meningitis (LM) patients and number of negative controls (NC) for whom enough CSF was available to measure the analyte.
[b] Significantly elevated in *L. monocytogenes* meningitis patients compared to negative controls after Bonferroni correction ($P < 0.00051$).

Appendix A3
Top 11 cytokines, chemokines and complement factors with strongest association with an unfavorable outcome in patients with Listeria meningitis.

Cytokine, chemokine or complement factor	Unfavorable outcome (n = 15) Median level (IQR)[a]	Favorable outcome (n = 11) Median level (IQR)	p-Value
VEGF	1.2 ng/ml (1.7–2.3)	0.8 ng/ml (0.2–1.1)	.001
C3a	9.1 μg/ml (5.8–10.7)	3.2 μg/ml (1.7–5.2)	.002
sIL-2Rα	338 pg/ml (139–500)	112 pg/ml (62–170)	.002
CXCL7	426 pg/ml (159–573)	119 pg/ml (64–159)	.003
CX3CL1	7.6 ng/ml (1.2–13.9)	0.8 ng/ml (0.6–1.3)	.003
CCL11	688 pg/ml (442–942)	292 pg/ml (153–425)	.005
IFN-α2	1.4 ng/ml (0.9–2.0)	0.7 ng/ml (0.3–0.9)	.005
CCL20	2.2 ng/ml (1.2–4.8)	0.4 ng/ml (0.2–0.8)	.007
IL-12p40	803 pg/ml (335–1374)	228 pg/ml (106–294)	.008
sCD40	701 pg/ml (334–1162)	264 pg/ml (168–396)	.008
IL-18	9.8 ng/ml (5.7–25.7)	4.9 ng/ml (3.7–9.1)	.008

[a] IQR = interquartile range.

Appendix A4
Level of association in 101 cytokines, chemokines and complement factors with an unfavorable outcome in 15 Listeria meningitis patients compared to 11 Listeria meningitis patients with favorable outcome.

Variable	Unfavorable outcome (n = 15) median level (IQR)[a]	Favorable outcome (n = 11) median level (IQR)	p-Value
EGF	195 pg/ml (139–566)	101 pg/ml (59–162)	0.02
CCL11	688 ng/ml (442–942)	292 pg/ml (152–425)	0.005
FGF2	1.4 ng/ml (0.9–1.6)	699 pg/ml (338–1029)	0.01
Flt3-Lig	264 ng/ml (62–321)	241 pg/ml (114–282)	0.80
CX3CL1	7.6 ng/ml (1.2–14)	761 pg/ml (608–1258)	0.003
GCSF	8.3 ng/ml (5.8–16)	14 ng/ml (4.0–18)	0.68
GMCSF	497 pg/ml (208–1191)	158 pg/ml (104–274)	0.02
CXCL1	2.7 ng/ml (1.1–9.9)	2.2 ng/ml (1.7–3.7)	0.84
IFN-α2	1.4 ng/ml (0.9–1.9)	658 pg/ml (323–897)	0.005
IL-1a	1.5 ng/ml (0.4–3.0)	383 pg/ml (317–451)	0.04
IL-1b	151 pg/ml (96–591)	206 pg/ml (71–430)	0.92
IL-1ra	2.4 ng/ml (1.9–9.9)	993 pg/ml (723–2718)	0.03
IL-2	34 pg/ml (25–98)	37 pg/ml (24–93)	0.92
IL-3	16 pg/ml (8.2–32)	14 pg/ml (6.5–31)	0.99
IL-4	70 pg/ml (35–100)	64 pg/ml (20–104)	0.76

(continued on next page)

Appendix A4 (*continued*)

Variable	Unfavorable outcome (n = 15) median level (IQR)[a]	Favorable outcome (n = 11) median level (IQR)	p-Value
IL-5	10 pg/ml (7.3–14)	10 pg/ml (5.4–21)	0.99
IL-6	50 ng/ml (28–99)	59 ng/ml (14–78)	0.57
IL-7	989 pg/ml (133–1764)	123 pg/ml (69–174)	0.04
IL-8	12 ng/ml (5.3–26)	7.2 ng/ml (6.1–16)	0.92
IL-9	102 pg/ml (67–148)	55 pg/ml (10–73)	0.01
IL-10	439 pg/ml (175–1985)	290 pg/ml (134–827)	0.33
IL-12p40	803 pg/ml (335–1374)	228 pg/ml (106–294)	0.008
IL-12p70	146 pg/ml (81–216)	98 pg/ml (53–203)	0.47
IL-13	7 pg/ml (8–48)	42 pg/ml (14–74)	0.36
IL-15	67 pg/ml (29–83)	50 pg/ml (17–90)	0.68
IL-17	43 pg/ml (16–58)	37 pg/ml (19–115)	0.99
CCL2	20 ng/ml (6–31)	7.0 ng/ml (2.7–10)	0.84
CCL7	1.1 ng/ml (0.6–7.4)	653 pg/ml (436–1944)	0.18
CCL22	15 pg/ml (248–1216)	1.2 ng/ml (0.5–1.3)	0.36
CCL3	394 pg/ml (154–663)	155 pg/ml (107–209)	0.07
CCL4	1.7 ng/ml (1–4.2)	1.0 ng/ml (0.5–5.2)	0.51
PDGFABBB	100 pg/ml (51–328)	178 pg/ml (83–336)	0.36
CCL5	98 pg/ml (23–219)	48 pg/ml (28–199)	0.76
sCD40L	701 pg/ml (334–1162)	264 pg/ml (168–396)	0.008
sIL-2Rα	426 pg/ml (159–573)	119 pg/ml (65–159)	0.002
TGFα	16.6 pg/ml (7.6–32)	20 pg/ml (3.2–33)	0.88
TNFα	331 pg/ml (242–771)	307 pg/ml (108–558)	0.33
TNFβ	272 pg/ml (28–622)	25 pg/ml (18–55)	0.04
VEGF	1.7 ng/ml (1.2–2.3)	832 pg/ml (235–1134)	0.001
IL-18	9.8 ng/ml (5.7–26)	4.9 ng/ml (3.7–9.1)	0.008
vWFAg	7.8 pg/ml (4.1–20)	2.5 pg/ml (1.3–3.9)	0.02
Fibrinog\en	0.6 pg/ml (0.5–0.6)	0.2 pg/ml (0.2–0.6)	0.03
TAFI	2.1 pg/ml (1.0–3)	1.1 pg/ml (0.5–2.4)	0.42
CCL8	20 ng/ml (7.6–63)	5.9 ng/ml (2.2–30)	0.04
CCL13	239 pg/ml (96–392)	301 pg/ml (189–397)	0.91
ENA-78	474 pg/ml (282–680)	470 pg/ml (386–740)	0.35
CXCL12	1.8 ng/ml (1.2–3.2)	3.6 ng/ml (870–4.0)	0.40
CXCL13	20 pg/ml (12–77)	16 pg/ml (7.7–29)	0.35
CCL1	47 pg/ml (16–64)	24 pg/ml (9.4–46)	0.31
IL-16	298 pg/ml (198–515)	247 pg/ml (158–464)	0.66
MIP-1d	702 pg/ml (311–737)	315 pg/ml (78–634)	0.24
CCL17	2.6 pg/ml (0.6–3.9)	3.1 pg/ml (1.7–4.7)	0.44
CCL21	613 pg/ml (140–1021)	698 pg/ml (454–871)	0.72
CCL24	75 pg/ml (19–102)	45 pg/ml (24–62)	0.24
CCL26	66 pg/ml (49–156)	57 pg/ml (15–91)	0.40
CCL27	51 pg/ml (9.6–74)	9 pg/ml (5.9–38)	0.15
IL-23	259 pg/ml (138–437)	233 pg/ml (178–467)	0.72
LIF	465 pg/ml (242–754)	637 pg/ml (475–734)	0.35
TPO	954 pg/ml (503–1291)	859 pg/ml (513–1449)	0.84
TRAIL	227 pg/ml (133–349)	83 pg/ml (44–153)	0.03
SCF	54 pg/ml (28–98)	35 pg/ml (24–54)	0.31
TSLP	71 pg/ml (27–116)	76 pg/ml (54–97)	0.78
IL-20	1.6 ng/ml (0.8–2.9)	1.8 ng/ml (1.5–2.7)	0.49
IL-21	50 pg/ml (27–96)	35 pg/ml (6.2–57)	0.44
IL-28A	110 pg/ml (88–165)	54 pg/ml (15–91)	0.002[a]
IL-33	161 pg/ml (99–184)	96 pg/ml (64–157)	0.08
MCSF	31 ng/ml (28–49)	17 ng/ml (11–34)	0.09
CXCL7	9.1 ng/ml (5.8–11)	3.2 ng/ml (1.7–5.2)	0.003
CXCL6	658 pg/ml (111–1591)	258 pg/ml (164–1171)	0.97
CCL14a	13 ng/ml (5.5–19)	6.2 ng/ml (4.3–7.6)	0.11
CCL19	130 pg/ml (62–191)	213 pg/ml (114–308)	0.27
CCL20	2.2 ng/ml (1.2–4.8)	399 pg/ml (224–779)	0.007
XCL1	675 pg/ml (362–1488)	1.4 ng/ml (0.7–1.5)	0.27
IL-11	3.8 ng/ml (1.6–5.8)	3.2 ng/ml (2.6–4.4)	0.72
IL-29	3.4 ng/ml (2.8–3.8)	1.8 pg/ml (0.9–4.9)	0.91
MMP-3	23 ng/ml (10–42)	23 ng/ml (7.9–37)	0.76
MMP-12	6.0 ng/ml (4.2–11)	6.0 ng/ml (3.8–8.9)	0.92
MMP-13	3.4 ng/ml (2.3–6.3)	5.4 ng/ml (3.2–7.8)	0.12
CFH	7.5 ng/ml (4.2–11)	6.0 ng/ml (5.4–8.0)	0.27
MMP-1	892 pg/ml (345–1296)	521 pg/ml (163–824)	0.18
MMP-2	16 ng/ml (6.2–32)	22 ng/ml (9.3–33)	0.54
MMP-7	11 ng/ml (4.7–16)	9.2 ng/ml (4.8–16)	0.61
MMP-9	412 ng/ml (103–497)	387 ng/ml (129–497)	0.65
MMP-10	266 pg/ml (258–290)	235 pg/ml (54–278)	0.13
ICAM	83 pg/ml (59–136)	52 pg/ml (43–92)	0.11
tPAI-1	50 pg/ml (28–101)	51 pg/ml (28–77)	0.92
MIF	1.3 ng/ml (0.6–5.0)	1.5 ng/ml (0.6–6.9)	0.57
C3a	338 pg/ml (139–500)	112 pg/ml (62–170)	0.002
iC3b	18.5 pg/ml (8.3–42)	10 pg/ml (3.3–16)	0.08
C5a	8 pg/ml (2.5–16)	1.4 pg/ml (0.4–5.3)	0.03
C5b9	1.3 ng/ml (0.4–1.7)	147 pg/ml (41–435)	0.006[a]
IFN-γ	15 ng/ml (5.2–41)	2.7 pg/ml (1.4–16)	0.04

Appendix A4 (*continued*)

Variable	Unfavorable outcome (n = 15) median level (IQR)[a]	Favorable outcome (n = 11) median level (IQR)	p-Value
CXCL9	392 ng/ml (62–582)	57 ng/ml (20–252)	0.09
CXCL10	488 ng/ml (487–488)	487 ng/ml (476–488)	0.84
PDGFAA	86 pg/ml (52–168)	77 pg/ml (55–213)	0.99
CXCL11	17 ng/ml (11–25)	4.8 ng/ml (2.8–18)	0.18
C3	3.4 ng/ml (0.5–8.3)	12 ng/ml (3.3–18)	0.03
VCAM	3.9 ng/ml (2.7–5.5)	3.3 ng/ml (3.0–5.3)	0.96
PAI-2	279 ng/ml (221–342)	171 ng/ml (165–301)	0.06
MBL	11.0 ng/ml (3.9–23.5)	11.9 ng/ml (5.0–14.9)	0.75
C1q	176 ng/ml (130–295)	144 ng/ml (40–215)	0.22

[a] Variable is significantly elevated in patients with Listeria meningitis with an unfavorable outcome compared to favorable outcome, but not compared to negative controls.

(See Appendix A4.)

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