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

Merel M. Koopmans [a], Matthijs C. Brouwer [a], Madelijn Geldhoff [a], Mercedes Valls Seron [a], Judith Houben [a], Arie van der Ende [b,c], Diederik van de Beek [a,*]

[a] Department of Neurology, Academic Medical Center, University of Amsterdam, Center for Infection and Immunity Amsterdam (CINIMA), Amsterdam, The Netherlands
[b] Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Center for Infection and Immunity Amsterdam (CINIMA), Amsterdam, The Netherlands
[c] The Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam, The Netherlands

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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

* Corresponding author at: Department of Neurology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, PO Box 22660, 1100DD Amsterdam, The Netherlands. Tel.: +31 205663842; fax: +31 205669374.
E-mail address: d.vandebeek@amc.uva.nl (D. van de Beek).

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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.