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Cerebrospinal fluid complement activation in patients with pneumococcal and meningococcal meningitis

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Summary *Background:* Recent research into the treatment of bacterial meningitis has examined the innate immune system, specifically the complement system, as a potential target for adjuvant therapy. However, the effects of blocking the complement system may be pathogen dependent.
Methods: We measured cerebrospinal fluid (CSF) levels of complement components C1q, C3a, iC3b, C5a, sC5b-9, CFH and MBL in 310 patients with pneumococcal and meningococcal meningitis from a prospective nationwide cohort study. The CSF complement component levels were successfully determined for between 289 (93%) and 307 (99%) patients, depending on available volumes of stored CSF.
Results: Complement factors C1q and MBL as well as common complement pathway factors C3a, iC3b, C5a, sC5b-9 and complement regulator CFH were all elevated in patients with bacterial meningitis as compared to the controls. CSF levels of complement components C5a and sC5b-9 were higher in patients with pneumococcal meningitis compared to those with meningococcal meningitis. After correction for age, immunocompromised state and level of consciousness, the CSF concentrations of C5a and sC5b-9 remained different between causative microorganisms ($P = 0.006$ and $P = 0.016$ respectively). In pneumococcal meningitis high C5a and C5b-9 levels are associated with the occurrence of systemic complications,

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unfavorable outcome and death, whereas an inverse relationship between C5b-9 levels and mortality is observed in meningococcal meningitis.
Conclusions: Our study shows striking variations in complement activation depending on the pathogen responsible for the bacterial meningitis. In pneumococcal meningitis, high CSF complement levels were a strong indicator of disease severity and mortality, however in meningococcal meningitis, an inverse relationship between sC5b-9 and mortality was observed.

Introduction

Bacterial meningitis remains an important cause of mortality and morbidity worldwide, despite the implementation of antibiotic therapy, adjunctive dexamethasone treatment and childhood vaccination strategies.[1–3] The most common causative organisms are *Streptococcus pneumonia* and *Neisseria meningitidis*, which together account for 85% of all cases of bacterial meningitis in Europe and the United States.[2] The rates of mortality caused by these bacterial infections are 26% and 9% respectively, and of those patients who survive, up to half suffer from neurological sequelae, including hearing loss, cognitive impairment and focal neurological deficits.[2]

Recent studies have looked at the innate immune system, specifically the complement system, as a potential target for adjunctive therapy.[4] Complement factor 5 (C5), a principle component of the common complement pathway, was investigated as a novel potential target in pneumococcal meningitis.[5] Murine studies showed that mice with pneumococcal meningitis with adjuvant treatment with antibodies against C5 had no mortality, and significantly less histopathological damage and cerebralspinal fluid (CSF) inflammation as compared to mice treated with adjuvant dexamethasone or isotype antibodies.[5] The subsequent question is whether adjuvant treatment with antibodies against C5 will also be beneficial for patients with meningococcal meningitis.[6] Currently no reproducible murine meningococcal meningitis model is available. Genetic studies have shown that complement deficiencies in the common complement pathway predispose to meningococcal meningitis,[7] warranting caution in complete blocking complement as it may increase severity of meningococcal disease.[6] Evaluation of complement activation patterns in pneumococcal and meningococcal infection may provide insight in the role of complement in bacterial meningitis due to these pathogens and whether complement inhibition has potential as an adjuvant treatment.

We investigated patterns of complement activation in CSF of bacterial meningitis patients included in a prospective nation-wide cohort study. We discuss the differences in complement activation between causative pathogens, the potential consequences for future treatments and directions for further study.

Methods

We included bacterial meningitis patients older than 16 years of age with positive CSF cultures and who were identified by the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) between March 2006 and June 2010. The NRLBM receives bacterial isolates from approximately 85% of bacterial meningitis patients in the Netherlands and provided the names of the hospitals where patients with bacterial meningitis had been admitted 2–6 days previously. The treating physician was contacted, and written informed consent was obtained from all participating patients or their legally authorized representatives. We did not include patients with hospital-acquired bacterial meningitis, those with a neurosurgical device (CSF drain) or negative CSF cultures. CSF samples from 18 patients without bacterial meningitis, in whom a subarachnoidal hemorrhage was excluded by CSF examination, served as negative controls.

CSF of participating patients was obtained by lumbar puncture and stored at −80 °C. Complement levels were determined as follows: C3 using Luminex technology with Milliplex MAP kit (Millipore corp, St. Charles, MO, USA); C3a, iC3b, C5a, sC5b-9, CFH by ELISA from Microvue Quidel, (Quidel, San Diego, CA, USA); C1q and MBL by ELISA (Hycult biotech, Uden, the Netherlands). Secured online case-record forms (CRFs) were used to collect patient data including medical history, symptoms and signs on admission, treatment, complications, and outcome. Neurological complications were defined as impairment of consciousness, seizures, or focal neurological abnormalities. Systemic complications were defined as cardiorespiratory failure or need for mechanical ventilation.

The clinical outcome was graded at discharge using the validated Glasgow Outcome Scale (GOS),[8] a 5 point scale in which a score of 1 indicates death, and a score of 5 indicates mild or no disability (the patient is able to return to work or school). We defined a favorable outcome as a GOS score of 5, and unfavorable outcome as a score of 1–4. The study was approved by the medical ethical review committee of the Academic Medical Centre, Amsterdam, the Netherlands.

Continuous data are presented as medians and interquartile ranges (IQR). Differences in complement levels between different patient groups were compared using a Mann–Whitney *U*-test for continuous variables and a Chi-square test or Fisher's exact test regarding dichotomous variables. The main analysis was performed using non-parametric tests (Mann–Whitney *U*). Logistic regression analysis was used to correct for possible confounders such as age, immunocompromised state and level of consciousness at time of presentation determined by the Glasgow Coma Scale (GCS) score. Strength of relationships between continuous variables was assessed by Spearman's correlation tests. All statistical tests were 2-tailed, and a p-value of <0.05 was considered to be significant. All analyses were executed using SPSS software, version 19.0.