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Original article

Bacterial load in cerebrospinal fluid predicts unfavourable outcome in pneumococcal meningitis: a prospective nationwide cohort study

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ABSTRACT

Objectives: The objective of this study was to determine the role of cerebrospinal fluid (CSF) bacterial load in adults with pneumococcal meningitis.
Methods: We quantified bacterial load in CSF samples from the diagnostic lumbar puncture of adults with community-acquired pneumococcal meningitis. We also measured CSF concentrations of complement component 5a (C5a), and determined associations between bacterial load, clinical characteristics, C5a and unfavourable outcome (Glasgow Outcome Scale score <5).
Results: Bacterial load was quantified in 152 CSF samples. Median age of these patients was 61 years (interquartile range [IQR] 51–68), and 69 of 152 (45%) were female. Median CSF bacterial load was 1.6×10^4 DNA copies/mL (IQR 3.4×10^3–1.2×10^5), and did not correlate with CSF white cell count nor with CSF protein concentrations. Median CSF C5a concentration was 35.8 mg/L (IQR 15.9–105.6), and was moderately correlated with CSF bacterial loads (Spearman's rho = 0.42; $p < 0001$). High bacterial loads were associated with development of complications, such as circulatory shock (OR per logarithmic increase: 2.4, 95% CI: 2.0–2.9; $p < 0.001$) and cerebrovascular complications [OR: 1.9, 95% CI: 1.6–2.3; $p < 0.001$]). High bacterial loads were also associated with unfavourable outcome (OR: 2.8, 95% CI: 2.4–3.3; $p < 0.001$) and death (OR: 3.1, 95% CI: 2.6–3.8; $p < 0.001$). In a multivariable regression model including age, immunocompromised state, extrameningeal infection focus, admission Glasgow Coma Scale score and CSF C5a concentration, CSF bacterial load remained an independent predictor of unfavourable outcome (adjusted OR: 2.5, 95% CI: 1.6–3.9; $p < 0.001$).
Discussion: High CSF bacterial load predicts the development of complications and unfavourable outcome in adults with pneumococcal meningitis. **Nora Chekrouni, Clin Microbiol Infect 2024;30:772**

Introduction

Community-acquired pneumococcal meningitis continues to cause high morbidity and mortality worldwide despite implementation of conjugate vaccines [1–4]. The pathophysiology of pneumococcal meningitis involves an interplay between bacteria and the host's immune system [3,5–8], wherein an excessive inflammatory response of the host's immune system is a crucial mechanism leading to tissue damage and unfavourable outcome [7,8]. An immune response by the host is initiated after recognition of bacterial pathogen-associated molecular patterns by pattern recognition receptors, with Toll-like receptors being the major pattern recognition receptors involved in initial sensing of pneumococci in the central nervous system [8–10]. Experimental pneumococcal meningitis models have shown that Toll-like receptors -deficiency is associated with increased bacterial loads, higher levels of tumor necrosis factor (TNF)-α, and increased disease severity [8,11,12]. The pathogen also activates the complement system, including the generation of the anaphylatoxin complement component 5a (C5a) [10,13]. C5a is a chemoattractant for immune cells, particularly neutrophils and monocytes, but can lead to detrimental effects in the central nervous system during pneumococcal meningitis [7]. In experimental pneumococcal meningitis, C5a receptor-deficient mice had lower cerebrospinal fluid (CSF) white blood cell counts and decreased brain damage compared

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with wild-type mice [13]. In patients with pneumococcal and meningococcal sepsis, high bacterial loads in blood have been associated with more severe disease and the development of sequelae [14–16]. Bacterial loads in CSF in bacterial meningitis have been related with outcome in two small paediatric studies [16,17], whereas another study in 151 Malawian adults did not describe such association [18,19]. Here, we studied the association between CSF pneumococcal load and outcome in adults with community-acquired meningitis included in a prospective nationwide study, and determined the prognostic accuracy of CSF bacterial load. Furthermore, we studied the interaction between CSF pneumococcal load and C5a concentration in these patients.

Methods

The MeninGene is an ongoing nationwide prospective cohort study conducted in the Netherlands with the goal of identifying host and pathogen factors influencing the risk and outcome of bacterial meningitis [1,20]. In summary, patients aged 16 years or older with community-acquired bacterial meningitis are prospectively included following a report from the Netherlands Reference Laboratory for Bacterial Meningitis or a notification from the treating physician. Written informed consent is obtained from participating patients or their legal representatives.

Data on patients' baseline characteristics, clinical course, treatment and (neurological) outcome were prospectively collected with a secured online case record form. Neurological examination at discharge was assessed using the Glasgow Outcome Scale (GOS) score, ranging from a score of 1 = death to a score of 5 = mild or no disability. A favourable outcome was defined as a GOS score of 5, and an unfavourable outcome was defined as a GOS score of 1–4. Categorical variables are expressed as counts and proportions, and continuous variables are expressed as median with interquartile range (IQR).

After withdrawal of CSF for diagnostic purposes, leftover samples of included patients were kept at −80°C until analysis. For this study, CSF samples collected between August 2020 and November 2022 were selected. Patients were included if they either had a positive CSF culture for *Streptococcus pneumoniae,* or if the CSF showed at least one individual finding predictive of bacterial meningitis according to the Spanos criteria (CSF glucose <1.9 mmol/L, CSF serum glucose ratio <0.23, protein concentration >2.20 g/L, white cell count >2000 cells/mm^3 or CSF neutrophil count >1180 cells/mm^3) [21] in combination with a positive blood culture, CSF antigen or CSF PCR for *S. pneumoniae*. Patients who developed meningitis while in hospital or within 1 week after discharge, after head trauma or neurosurgery in the previous month, or with a neurosurgical device *in situ* were excluded.

To extract DNA from the CSF samples 200 μL of CSF was centrifuged (10 minutes, 4500 × g), and the pellet was resuspended and treated with a lysozyme/lysostaphin digestion buffer (20 mM Tris.Cl pH 8.0, 2 mM EDTA, 1.2% Triton-X-100, 100 uL 10 × Lysozyme and 10 uL 10 × Lysostaphin) (Sigma-Aldrich, USA). DNA was then extracted using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) according to the protocol described in the instructions of the manufacturer. As a positive control we used the *S. pneumoniae* ATCC 6303 strain. DNA from the positive control strain was isolated after overnight grown on blood agar plates, and the cells were treated with the same digestion buffer as described above. After treatment, DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). Extracted DNA was quantified using Qubit 4 Fluorometers (ThermoFisher, The Netherlands). The sample concentration after DNA extraction was taken into account when calculating bacterial load. DNA extraction was performed in a separate room to avoid contamination. Extracted DNA was stored at −20°C until further analysis. Real-time PCRs were performed using the CFX96 Real-time PCR System (BIO-RAD, the Netherlands). Quantification of the *S. pneumoniae* bacterial load was performed using a primer targeting the autolysin gene (*lytA)* as described before [22], which has been shown to be specific for *S. pneumoniae* and provides accurate quantification. External standard curves were created using genomic DNA extracted from *S. pneumoniae* ATCC 6303. Each assay was performed in triplicate, and standard precautions were taken to prevent carryover contamination.

Pneumococcal capsular serotyping was performed by the Netherlands Reference Laboratory for Bacterial Meningitis using co-agglutination and capsular swelling (Quellung reaction) with specific antisera (Statens Serum Institute, Denmark). C5a was measured in stored CSF (supernatant) using ELISA manufactured by Quidel.

Bacterial load is shown as DNA copies/mL. As the normal distribution was not met (Shapiro–Wilk test $p < 0.01$), associations between bacterial load and blood counts, CSF cell counts or CSF C5a concentration were examined using the Spearman rank correlation coefficient. Bacterial load between groups was compared using the Mann–Whitney U test or the Kruskal–Wallis test. Multivariable binary logistic regression was performed to assess the prognostic value of bacterial load for unfavourable outcome adjusted for predefined risk factors, providing ORs and 95% CIs. The assumptions of linearity between a continuous variable and the (log odds of the) outcome and homoscedasticity was assessed with the Hosmer–Lemeshow goodness of fit test and visual inspection. In the absence of a linear relationship, the continuous variable was log-transformed (in the case of bacterial load) or categorized. Missing data (4.3% of total values) were imputed using multiple imputation, by combining five imputed data sets based on all available prognostic factors, under the assumption that missing values were missing at random. We estimated both univariable and multivariable ORs corrected for all other variables in the model, and combined the coefficients of 60 rounds of imputation to obtain the final estimates for the multivariable model. All tests were two-tailed, and a p value < 0.05 was considered significant. Statistical analysis was conducted using IBM SPSS Statistics Data Editor (v.26).

Results

Between August 2020 and November 2022, 221 adult episodes with pneumococcal meningitis were included in the MeninGene study, of whom leftover CSF was available from 152 (69%) episodes, occurring in 152 patients (Fig. 1). Characteristics between patients with CSF available or not were similar (Table S1). The median age of the 152 patients was 61 years (IQR 51–68), and 69 of 152 patients (45%) were female (Table 1). Almost half of patients (74 of 151, 49%) had an otitis or sinusitis and 14 of 150 patients (9%) received antibiotics pre-admission. An immunocompromised state, defined as a history of diabetes mellitus, active cancer, splenectomy, HIV, alcoholism or the use of immunosuppressive drugs, was present in 59 of 152 patients (39%). Headache occurred in 98 of 117 patients (84%), neck stiffness in 84 of 122 patients (69%), fever in 100 of 146 patients (69%) and an altered mental status (defined by a Glasgow Coma Scale score below 14) in 118 of 150 patients (76%). In 47 of 136 patients (35%) the classic triad of fever, neck stiffness and a change in mental status was present.

A lumbar puncture was performed in all patients, and this was done within 24 hours of presentation in 150 of 152 patients (99%). CSF gram stain was positive in 90 of 125 patients (72%), CSF culture was positive in 112 of 152 patients (74%) and blood culture was positive in 119 of 137 patients (87%). Cranial computed tomography (CT) or magnetic resonance imaging (MRI) was performed on admission in 143 (94%) patients, and was abnormal in 86 patients

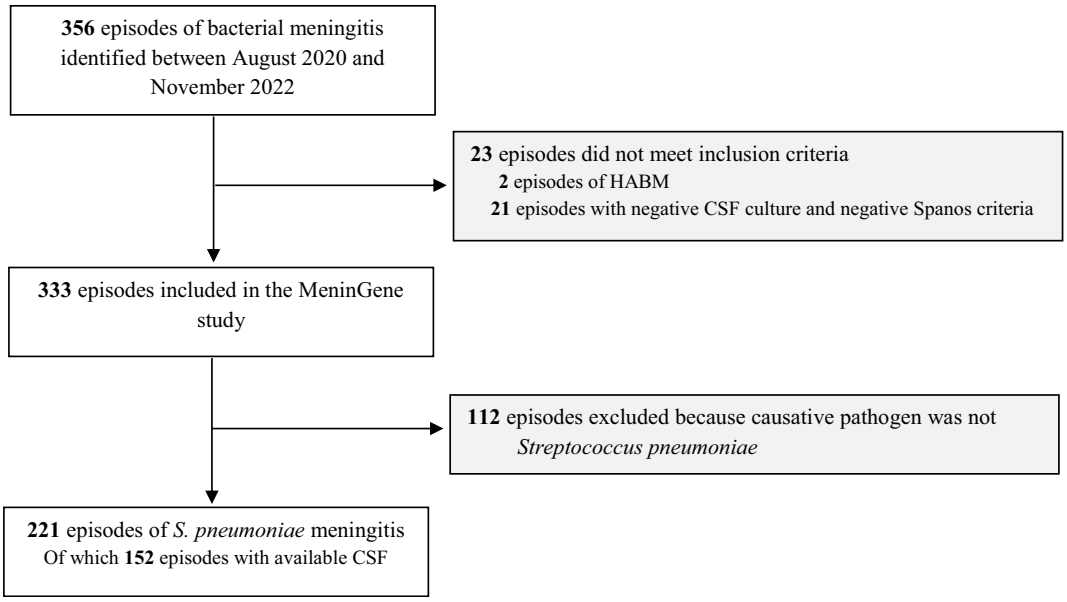

Fig. 1. Flowchart with selection of episodes included in the bacterial load cohort. CSF, cerebrospinal fluid; HABM, hospital-acquired bacterial meningitis.

(60%). Initial antibiotic treatment included a combination of amoxicillin with a third-generation cephalosporin in 126 (83%) of 152 patients. Monotherapy was started with a third-generation cephalosporin in 23 (15%) of 152 patients. Adjunctive dexamethasone was administered for 136 (92%) of 148 assessed patients, and was started before or together with the first dose of parenteral antibiotics in the standard dosage (10 mg intravenously, every 6 hours for 4 days) in all patients. Complications occurred in a high proportion of patients: circulatory shock in 17 (11%) of 148 patients, respiratory failure in 34 (23%) of 149 patients, cerebrovascular complications in 25 (17%) of 144 patients and focal neurological deficits in 43 (30%) of 145 patients. An unfavourable outcome occurred in 57 of 152 patients (37%) and 23 of 152 patients (15%) died. Of the survivors, 35% (45 of 129 patients) had focal neurological deficits upon discharge.

The median bacterial load in CSF of the 152 patients was 1.6×10^4 DNA copies/mL (IQR 3.4×10^3–1.2×10^5). Bacterial load was lower in patients with antibiotic pre-treatment (4.4×10^3 [IQR 1.9×10^3–2.0×10^4] vs. 2.4×10^4 [IQR 3.5×10^3–1.6×10^5]; $p = 0.016$) and in those with an otitis or sinusitis as infection focus (8.6×10^3 [IQR 2.5×10^3–6.7×10^4] vs. 4.4×10^4 [IQR 5.2×10^3–3.6×10^5]; $p = 0.005$). Bacterial load was higher in immunocompromised patients (3.5×10^4 [IQR 4.3×10^3–4.2×10^5] vs. 1.3×10^4 [IQR 2.6×10^3–7.5×10^4]; $p = 0.025$) and in those with a positive CSF culture (4.0×10^4 [IQR 4.9×10^3–2.3×10^5] vs. 4.5×10^3 [IQR 2.4×10^3–2.0×10^4]; $p < 0.001$) (Fig. 2). Bacterial load did not correlate with age (Spearman's rho [ρ] = −0.08), pneumococcal serotype (p 0.65), time of symptoms duration (<24 hours symptoms; p 0.37), blood parameters of inflammation (C-reactive protein [Spearman's $\rho = 0.14$], leukocyte count in blood [Spearman's $\rho = -0.13$], thrombocyte count in blood [Spearman's $\rho = -0.16$]), CSF white cell count (Spearman's $\rho = -0.20$), or CSF protein (Spearman's $\rho = 0.13$; all $p > 0.05$; Figs. S1 and S2). The median concentration of C5a in CSF in the 152 patients was 35.8 mg/L (IQR 15.9–105.6), and showed to be moderately correlated with bacterial load in CSF (Fig. 2; Spearman's $\rho = 0.42$; $p < 0.001$).

High bacterial load was associated with the development of circulatory shock (OR: 2.4 per logarithmic increase in bacterial load, 95% CI: 2.0–2.9; $p < 0.001$) and cerebrovascular complications (OR: 1.9, 95% CI: 1.6–2.3; $p < 0.001$). Bacterial load was also associated with an increased risk of unfavourable outcome (OR: 2.8 per logarithmic increase, 95% CI: 2.4–3.3; $p < 0.001$; Table 2) and death (OR: 3.1 per log increase, 95% CI: 2.6–3.8; $p < 0.001$). Patients with an unfavourable outcome had a 14-fold higher bacterial load as compared with those with a favourable outcome (9.4×10^4 [IQR 1.5×10^4–6.8×10^5] vs. 6.8×10^3 [IQR 1.9×10^3–4.8×10^4]; $p < 0.001$, Fig. 3). Non-survivors had a 45-fold higher bacterial load compared with survivors (5.5×10^5 [IQR 4.9×10^4–1.7×10^6] vs. 1.2×10^4 [IQR 2.6×10^3–7.5×10^4]; $p < 0.001$). C5a concentration in CSF was also associated with an increased risk of unfavourable outcome (OR: 3.0 per logarithmic increase, 95% CI: 2.3–3.9; $p < 0.001$). In a multivariable logistic regression model including age, immunocompromised state, presence of an extrameningeal infection focus, admission Glasgow Coma Scale (GCS) score, and CSF C5a concentration, the predictive effect of bacterial load on unfavourable outcome remained robust (adjusted OR: 2.5 per logarithmic increase, 95% CI: 1.6–3.9; $p < 0.001$). Neither categorization nor selection of variables influenced the results of the multivariable model (Tables S2 and S3).

Discussion

Our study shows that in patients with pneumococcal meningitis high bacterial load in CSF predicts unfavourable outcome and death. Animal studies of pneumococcal meningitis previously revealed a connection between high CSF bacterial loads, an insufficient response of CSF leukocytes, and the onset of intracranial complications [11,23]. Earlier smaller studies in children and adults also suggested an association between CSF bacterial loads and outcome in bacterial meningitis [16,17,24]. In our current study, CSF bacterial load was the strongest predictor for unfavourable outcome in our multivariate analysis, including previously identified prognostic factors such as advanced age, immunocompromised state and admission Glasgow Coma Scale score [1,25]. Our findings align with observations in patients with pneumococcal pneumonia, where the bacterial load in the blood was also found to be associated with both complications as well as the likelihood of death [26]. Our results differ from those of a Malawian study involving 152 adults with pneumococcal meningitis [18]. In that study, no significant correlation was observed between bacterial load and

Table 1
Baseline and clinical characteristics of bacterial load cohort ($N = 152$)

Characteristic	Data	Characteristic	Data
Age[a] (y)	61 (51–68)	Indices of CSF inflammation	
Female sex	69/152 (45%)	Opening pressure[k] (cm water)	45 (33–50)
Symptoms <24 h	68/147 (46%)	Protein[l] (g/L)	4.9 (3.2–6.5)
Recurrent meningitis	6/150 (4%)	CSF/serum glucose ratio[m]	0.03 (0.01–0.16)
Known CSF leak	8/152 (5%)	White cell count[n] (per mm^3)	2657 (859–7100)
Remote head trauma	9/150 (6%)	<100/mm^3	16/150 (11%)
Extrameningeal focus of infection	90/152 (59%)	100–999/mm^3	27/150 (18%)
Otitis or sinusitis	74/151 (49%)	999–9999/mm^3	83/150 (55%)
Pneumonia	19/150 (13%)	≥10 000/mm^3	24/150 (16%)
Endocarditis	4/150 (3%)	Blood and CSF culture	
Antibiotics pre-admission	14/150 (9%)	Positive blood culture	119/137 (87%)
Immunocompromised state	59/152 (39%)	Positive CSF gram stain	90/125 (72%)
Cancer	10/150 (7%)	Positive CSF culture	112/152 (74%)
Diabetes	27/150 (18%)	Radiological examination	
Alcoholism	16/147 (11%)	Abnormal brain CT or MRI	86/143 (60%)
Immunosuppressive treatment	17/151 (11%)	Initial treatment	
Splenectomy	2/151 (1%)	Amoxicillin + third-general cephalosporin	126/152 (83%)
Clinical signs and symptoms		Monotherapy	24/152 (16%)
Median temperature[b]	38.8 (37.9–39.4)	Adjunctive dexamethasone therapy	136/148 (92%)
Fever (>38°C)	100/146 (69%)	Clinical course	
Nausea	61/119 (51%)	Circulatory shock	17/148 (11%)
Headache	98/117 (84%)	Respiratory failure	34/149 (23%)
Neck stiffness	84/122 (69%)	Seizures	29/149 (20%)
Glasgow Coma Scale Score[c]	11 (8–13)	Pneumonia	21/148 (14%)
Altered mental status (GCS <14)	118/150 (78%)	Cerebrovascular accident	25/144 (17%)
Coma (GCS ≤8)	38/150 (25%)	Sinus thrombosis	8/149 (5%)
Classic triad[d]	47/136 (35%)	Glasgow Outcome Score	
Rash	3/118 (3%)	1 (death)	23/152 (15%)
Aphasia, monoparesis or hemiparesis	42/126 (33%)	2 (vegetative state)	1/152 (1%)
Seizures	17/143 (12%)	3 (severe disability)	5/152 (3%)
Cranial nerve palsies	13/129 (10%)	4 (moderate disability)	28/152 (18%)
Heart rate[e] (beats/min)	99 (85–113)	5 (mild or no disability)	95/152 (63%)
Systolic blood pressure[f] (mmHg)	146 (127–162)	Neurological sequelae at discharge	
Diastolic blood pressure[g] (mmHg)	80 (70–92)	Hearing impairment	55/112 (49%)
Blood chemical tests		Cognitive impairment	31/114 (27%)
C-reactive protein[h] (mg/L)	189 (85–301)	Cranial nerve palsy	5/121 (4%)
Thrombocyte count[i] (per μL)	224 (160–283)	Focal neurological deficits	45/129 (35%)
Leukocyte count[j] (per μL)	17 (11–25)	Mono- or hemiparesis	42/126 (33%)
		Aphasia	3/121 (3%)

Data are median (IQR) or n/N (%).
CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale score.
[a] Age known in all patients.
[b] Temperature known for 146 patients.
[c] Glasgow Coma Scale Score known for 150 patients.
[d] Classic triad is defined as fever, neck stiffness and altered mental status (GCS <14).
[e] Heart rate is known for 147 patients.
[f] Systolic blood pressure known for 150 patients.
[g] Diastolic blood pressure known for 149 patients.
[h] C-reactive protein known for 151 patients.
[i] Thrombocyte count in blood known for 148 patients.
[j] Leukocyte count in blood known for 148 patients.
[k] Opening pressure known for 79 patients.
[l] Protein known for 151 patients.
[m] CSF/serum glucose ratio known for 148 patients.
[n] White cell count in CSF known for 151 patients.

survival. This difference may be explained by the high prevalence of HIV infection among the included Malawian patients (81%), indicating severe immunocompromise in this setting. Consequently, Malawian patients had markedly higher median CSF bacterial load and lower median CSF white cell counts compared with those in our cohort.

The importance of CSF bacterial loads on outcome can be explained through various mechanisms. First, a high bacterial burden has been described to overwhelm the immune system's capacity to control pathogens, leading to an intense inflammatory response and increased tissue damage [27,28]. The interplay between pathogen load and host response in infectious diseases is increasingly acknowledged in infectious diseases [5,14,15,27]. Our prior research showed that the C5a contributes to poor disease outcome in humans and mice with pneumococcal meningitis [7,13]. Adjuvant treatment with dexamethasone plus anti-C5 antibodies improved outcome of mice with pneumococcal meningitis in a randomized controlled experimental trial [29]. We now show that CSF C5a concentrations were associated with CSF bacterial loads and with unfavourable outcome. However, the observation that CSF bacterial load was only moderately associated with CSF C5a concentration implies that at least part of the variation in the C5a concentration is not directly dependent on the bacterial stimulus itself, but rather that host genetic factors also influence the severity of the immune response. Indeed, a functional common non-synonymous C5 single nucleotide polymorphism (SNP) (rs17611) has previously been associated with unfavourable disease outcome in pneumococcal meningitis [13]. Human genetic factors could

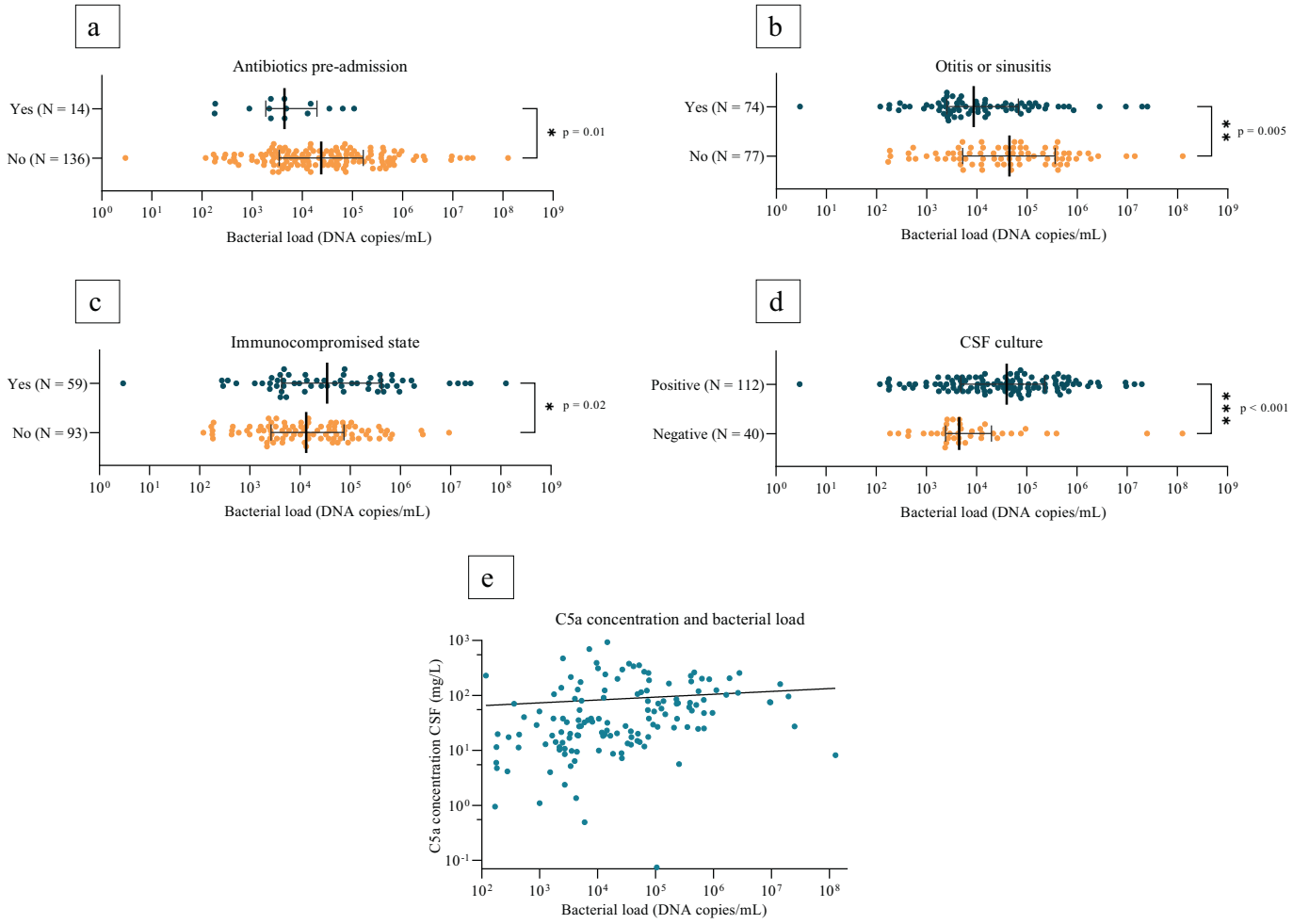

Fig. 2. Comparison of bacterial load per subgroup; antibiotics administration pre-admission (a), otitis or sinusitis as extrameningeal infection focus (b), immunocompromised state (c), and positive cerebrospinal fluid culture (d). Correlation of bacterial load in cerebrospinal fluid with complement C5a concentration in cerebrospinal fluid, Spearman's rho = 0.42, $p < 0.001$ (e).

explain about half of the variation of pneumococcal meningitis in susceptibility or outcome, which is a relatively large genetic contribution of human genetic variation compared with other infectious diseases [20].

Second, bacterial loads may impact the efficacy of antimicrobial treatments. However, in pneumococcal meningitis, adverse neurologic outcomes are generally due to inappropriate antimicrobial treatment, as CSF cultures were shown to become sterile within 6 hours of initiating antibiotic therapy [30].

Lastly, individual variability plays a role, as people differ in their ability to handle specific bacterial loads based on factors such as overall health, immune function, and underlying conditions. Indeed, we show that immunocompromised patients had significant higher bacterial loads, whereas patients that received antibiotics pre-treatment had lower bacterial loads. However, we did not observe any relationship, neither linear nor U-shaped, between CSF parameters of inflammation (such as white cell count) and bacterial load. In our regression model bacterial load even remained a robust predictor for unfavourable outcome after correcting for (low) CSF white cell count, which itself has previously been described as a strong predictor for unfavourable outcome [31]. Although speculative, our finding that bacterial load predicts outcome even when corrected for several patient and baseline characteristics may suggest the possibility of a subgroup among patients exhibiting an unrecognized relative immunocompromised state, leading to increased bacterial growth in the CSF compartment, ultimately contributing to an unfavourable disease outcome.

Our study has several limitations. First, bacterial load was measured only in pneumococcal meningitis patients with CSF available from the diagnostic lumbar puncture, which could introduce bias. However, baseline characteristics between episodes with and without CSF were similar, indicating a limited influence of selection bias. Second, symptom duration in our cohort is only described dichotomously (e.g. longer or shorter than 24 hours). The exact timing between onset of disease and hospital admission is not known, which is mostly due to the difficulty of categorizing prodromal disease and exact onset of bacterial meningitis. Third, the precise timing of antibiotics administration in regards to the lumbar puncture is not known. As treatment should be administered as soon as possible, antibiotics in some cases might precede the lumbar puncture, for instance in the cases where brain imaging is indicated before lumbar puncture can be performed [32]. However, as we use a highly sensitive and specific PCR assay that can detect both viable as well as non-viable bacteria, the influence of antibiotic administration timing on our results is expected to be limited. Fourth, we used multiple imputations to account for missing data, which is a widely accepted approach, but could potentially introduce bias if used incorrectly. However, as different iterations yielded similar results (Tables S2 and S3), the validity of this approach was ensured. Furthermore, due to our sample size,

Table 2
Predictors for an unfavourable outcome in the bacterial load cohort ($N = 152$)

	Favourable outcome ($N = 95$)	Unfavourable outcome ($N = 57$)	Univariable OR unfavourable outcome (95% CI)	Multivariable OR unfavourable outcome (95% CI)	p value multivariable analysis
Bacterial load[a]	6.8×10^3 (1.9×10^3–4.8×10^4)	9.4×10^4 (1.5×10^4–6.8×10^5)	2.8 (2.4–3.3)[h]	2.5 (1.6–3.9)	<0.001
C5a CSF [b] (mg/L)	23.9 (11.6–68.3)	78.7 (27.2–197.1)	1.03 (1.01–1.04)[h]	1.3 (0.6–2.7)	0.49
Age[c] (y)	61 (48–67)	61 (52–70)	1.02 (1.01–1.03)[h]	1.02 (0.99–1.06)	0.19
Antibiotics pre-admission	9/94 (10%)	5/56 (9%)	1 (0.6–1.5)	–	–
Otitis, sinusitis or pneumonia	62/95 (65%)	26/57 (45%)	0.5 (0.3–0.6)[h]	0.6 (0.2–1.5)	0.27
Immunocompromised state	27/95 (28%)	32/57 (56%)	3.2 (2.4–4.3)[h]	2.8 (1.2–6.5)	0.02
Rash	1/76 (1%)	2/42 (5%)	3.3 (1.4–7.5)[h]	–	–
Heart rate[d] (per 10 bpm)	96 (88–110)	101 (80–120)	1.09 (1.02–1.2)[i]	–	–
Glasgow Coma Scale score[e]	12 (10–14)	9 (6–12)	0.8 (0.7–0.8)[h]	0.8 (0.7–0.9)	0.017
C-Reactive protein[f] (mg/L)	150 (77–238)	252 (150–365)	1.05 (1.04–1.06)[h]	–	–
Leukocytes CSF[g] (per μL)	3317 (1495–8458)	1703 (113–6320)			
<100	4/95 (4%)	12/56 (21%)	7.2 (2.1–24.4)[i]	–	–
100–999	14/95 (15%)	13/56 (23%)	2.2 (0.9–5.4)	–	–
1000–10 000	59/95 (62%)	25/56 (45%)	Reference	–	–
>10 000	18/95 (19%)	6/56 (11%)	0.8 (0.3–2.4)	–	–
Positive blood culture	73/87 (84%)	46/50 (92%)	2.1 (1.3–3.3)[h]	–	–

Data are median (interquartile range) or n/N (%). The multivariable analysis used an imputed data set with 60 imputation rounds, all variables in the table were entered in the multivariable logistic regression model simultaneously.
CSF, cerebrospinal fluid.
[a] OR is for each increase in logarithm of bacterial load (DNA copies/mL).
[b] C5a concentration in CSF is known for 88 patients with a favourable outcome and 53 patients with an unfavourable outcome; OR is for each increase in logarithm of C5a.
[c] Age is known for all patients.
[d] Heart rate is known for 93 patients with a favourable outcome and 54 patients with an unfavourable outcome; OR is per increase in 10 heart beats per minute.
[e] Glasgow Coma Score known for 94 patients with a favourable outcome and 56 patients with an unfavourable outcome.
[f] C-reactive protein is known for 95 patients with a favourable outcome and 56 patients with an unfavourable outcome; OR is for each 10 mg/L increase.
[g] White cell count in CSF in known for 95 patients with a favourable outcome and 56 patients with an unfavourable outcome.
[h] Univariable OR p value < 0.001.
[i] Univariable OR p value < 0.05.

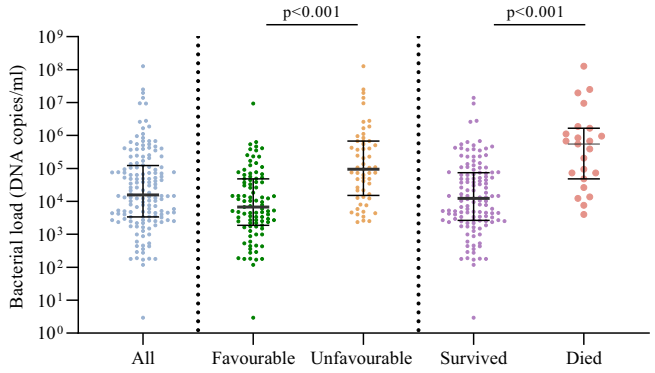

Fig. 3. Comparison of bacterial load in total cohort ($N = 152$), and for different outcome groups. Bacterial load in patients with unfavourable ($N = 57$) vs. favourable ($N = 95$) outcome (respectively 9.4×10^4 [IQR 1.5×10^4–6.8×10^5] vs. 6.8×10^3 [IQR 1.9×10^3–4.8×10^4]; $p < 0.001$). Bacterial load in deceased ($N = 23$) vs. alive ($N = 129$) patients (respectively 5.5×10^5 [IQR 4.9×10^4–1.7×10^6] vs. 1.2×10^4 [IQR 2.6×10^3–7.5×10^4]; $p < 0.001$).

we could add only a limited number of predefined predictors into the multivariable model (1 per 10 outcome events). Therefore, for our final multivariable model we initially added all univariate significant variables, and then eliminated variables based on their contribution to the overall fit of the model. Neither categorization nor selection of variables was found to introduce bias in the multivariable model (Tables S2 and S3). Finally, we do not know whether the identified association of bacterial load with poor outcome is a sign of residual confounding, e.g. due to unidentified immunocompromise, or if there is a direct causal role of bacterial load in poor outcome.

In conclusion, high CSF bacterial loads predict unfavourable outcome and death in adults with pneumococcal meningitis. After correcting for other well-defined predictors of outcome, such as advanced age and immunocompromised state, the predictive effect of CSF bacterial load remained robust as the most substantial predictor of unfavourable outcome. Furthermore, our study confirms that high CSF C5a concentrations are associated with unfavourable disease outcome. Our finding that bacterial load predicts outcome even when corrected for several patient and baseline characteristics, including CSF C5a concentration, may suggest the possibility of a subgroup among patients exhibiting an unrecognized relative immunocompromised state, leading to increased bacterial growth in the CSF compartment, ultimately contributing to an unfavourable disease outcome.

Author contributions

NC contributed to data collection, data analysis, data interpretation and writing the first draft of the manuscript. ACdCC contributed to study design, data collection, and critique to the report. TMvS contributed to data collection and critique to the report. MCB and DvdB contributed to study design, data interpretation, review and critique of the report.

Transparency declaration

The authors declare that they have no conflicts of interest.

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Data availability

The data that support the findings of this study are available on request from any qualified investigator from the corresponding author [DvdB].

Ethics approval

This study was approved by Medical Ethical Review Committee of the Amsterdam UMC (number METC 2013_043). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Participant consent

Written informed consent was obtained from all participants or their representatives.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2024.03.012.

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