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Cohort study exploring the association of cerebrospinal fluid metalloprotease levels and Microbiological characteristics to cerebral vasculitis complication in Pneumococcal meningitis

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Cerebrovascular complications are frequent in pneumococcal meningitis and are associated with poor functional outcomes. Among these complications, the incidence of cerebral vasculitis (CV) has been increasingly reported, but neither its pathogenesis nor its relationship with cortisone treatment have been conclusively established. We wanted to describe cerebrospinal fluid (CSF) metalloprotease (MMP) levels, which are linked to cerebral damage and vasculitis (MMP-2, MMP-9, and the antagonist TIMP-1), and differences in microbiological serotypes or virulence factors that could be associated to the development of this complication. A prospective multicenter cohort study was performed from January 2019 to August 2022. All adult patients diagnosed with pneumococcal meningitis and for whom CSF samples from the initial lumbar puncture were available were included and followed up for six months after discharge. *Streptococcus pneumoniae* strains isolated from CSF or blood were assessed including whole genome sequencing and CSF levels of MMP-2, MMP-9, and TIMP-1 were measured. CV developed in three of 21 patients (14.3%). The serotypes of those who developed CV were 3, 9 N, and 35 F, with no microbiological differences with respect to the non-CV group. The CV group had higher CSF levels of MMP-9 (13.2 vs. 9.8 ng/L) and TIMP-1 (699 vs. 318 ng/L), but lower CSF levels of MMP-2 (5689 vs. 10,484 ng/L) compared with the non-CV group. Although no patients with CV died, they had worse clinical outcomes than the non-CV group. CV is a frequent complication of pneumococcal meningitis that may be associated with worse outcomes. No differences in microbiological serotypes or virulence factors were detected. Further analyses should be carried out to confirm whether CSF MMP levels may be markers of CV development.

Keywords Pneumococcal meningitis, Cerebral vasculitis, Metalloproteases, CSF

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Streptococcus pneumoniae meningitis is the main causative pathogen of bacterial meningitis in Europe (53%)[1]. Dexamethasone as an adjunctive treatment before antibiotic administration has been shownto reduce mortality[2], probably by diminishing the inflammation provoked by bacterial lysis in cerebrospinal fluid (CSF). Although there is some controversy surrounding the possible reductions in global[3]or neurologic-specific[4]mortality, the use of dexamethasone has been extended, and multiple cohort studieshave shown a 15% reduction in global mortality, including death from neurological causes[5–7]. In spite of this improvement, however, morbidity remains high and leads to impaired functioning at discharge in more than half of survivors. Classically, these high morbidity rates were associated with intracranial complications such as seizures, abscesses, hydrocephalus, and cerebrovascular issues, but advances in supportive care and techniques have reduced the impact of these conditions[6,8].

Vascular damage remains a key mechanism of brain damage in pneumococcal meningitis, being present in 97% of histopathological studies after death[9]. Likewise, cerebral vasculopathy assessed by Doppler ultrasound was found in 40% of the patients with pneumococcal meningitis[6]. Cerebrovascular complications also remain more common in pneumococcal meningitis than in other etiologies (29.4%)[10].

In more recent series, the most common cerebrovascular complications are reported to be focal ischemia (12–19%), intracranial hemorrhage (3.6%), venous thrombosis (1–3.6%) and cerebrovascular vasculopathy, also termed cerebral vasculitis (CV) (1.7–24%)[5,6,11,12]. The wide variability in the incidence of CV is explained by the recent recognition of this complication and the indirect method of diagnosis, as it is defined by clinical worsening and compatible imaging results (multiple infarcts) without cerebral histopathology. Recent studies not only show a higher incidence of CV but also an association with a worse prognosis[6,11,12], yet many questions about the pathogenesis of CV remain unresolved. Presumed risk factors include a higher bacterial load, longer disease duration, less CSF inflammation, and corticosteroid treatment[11].

Information regarding the role of corticosteroids in the development of CV is controversial. Concerns regarding corticosteroid treatment were first raised after clinical reports of cases with delayed cerebral vasculopathy after corticosteroid withdrawal[13–16]. Some cohort studies have found a higher incidence of CV in patients receiving dexamethasone[5,17]but others have reported no such increase[11,12]. Moreover, there is no consensus regarding the preferred treatment for CV associated with pneumococcal meningitis, although corticosteroids are among the most frequently used agents in this scenario[18–21].

At present, no data are available on the association of bacterial serotypes or virulence factors with CV, yet these variables, in combination with host characteristics, determine the degree of pneumococcal invasiveness and virulence[22,23]. Some extracellular matrix metalloproteases (MMPs) participate in the pathogenesis of brain damage in bacterial meningitis. In clinical and animal studies, MMP-2, MMP-9 and the antagonist metallopeptidase inhibitor 1 (TIMP-1) have been shown to cause inflammation and breakdown of the blood–brain barrier[24], leading to vasculitis and worse neurological outcomes[25–27]. Experimental studies in animal models have demonstrated the relationship between MMP-9 and inflammatory activity in the CSF, as well as its down-regulation by adjuvant dexamethasone treatment[28]. Similarly, in clinical studies of tuberculous meningitis MMP-9 has been linked to brain damage, disruption of the blood–brain barrier[29], and a higher CSF neutrophil count, while dexamethasone treatment reduced CSF MMP-9 concentrations[30]. In rat models of either pneumococcal[31]or meningococcal[32]meningitis, inflammation, brain injury, and mortality were reduced with the use of inhibitors of these MMPs, and higher levels of MMP-9 were associated with vasculitis in coccidioidal meningitis[33]. Finally, other studies have evaluated the predictive value of these markers; in childhood bacterial meningitis, for example, a higher MMP-9 concentration predicted mortality whereas a higher TIMP-1 concentration correlated with sequelae but not mortality[34]. The concentrations of MMP-9 and TIMP-1 in the CSF even pointed to their potential as new markers of bacterial meningitis[35].

The present study aimed to describe factors possibly associated with CV as a complication of pneumococcal meningitis, focusing on MMP levels in the CSF of the initial lumbar puncture and the microbiological serotypes and virulence factors of *S. pneumoniae* isolates.

Materials and methods

Setting and participants

We designed a multicenter, prospective, observational cohort study at three university hospitals in Catalonia, Spain: Hospital Universitari de Bellvitge, Hospital Universitari Parc Taulí and Hospital Universitari Mútua de Terrassa. All patients aged over 16 years old diagnosed with pneumococcal meningitis admitted from January 2019 to August 2022 were evaluated for inclusion and recruited prospectively as soon as the microbiological diagnosis was available. Only patients with sufficient remaining CSF after confirmation of a pneumococcal etiology were included.

Definitions

Compatible clinical signs and elevated inflammatory markers such as proteins and leukocytes in the CSF defined meningitis. Pneumococcal etiology was defined by CSF Gram stain or culture, blood culture, CSF pneumococcal antigen, polymerase chain reaction positive for *S. pneumoniae* in CSF or pneumococcal antigen in urine.

Cases of CV were defined as clinical worsening with new-onset neurological symptoms and/or fever or lack of improvement after 72 h of adequate antimicrobial treatment (use of increased doses to achieve sufficient CSF levels and sensitivity to the antimicrobial of the isolate) and corticosteroid treatment (6 mg or 10 mg every six hours for two or four days respectively). This assessment was conducted by the treating physicians and corroborated by the principal investigators. Diagnosis also required compatible radiological signs on cranial computed tomography or magnetic resonance imaging either with angiography (CTA/MRA) or without (CT/MRI), such as multiple infarctions or hemorrhages in different vascular territories or different states of evolution.

Clinical outcomes were assessed using the Glasgow Outcome Score (GOS)[36] at discharge and after three and six months, using the following ratings: 5, good recovery; 4, moderate disability; 3, severe disability; 2, persistent vegetative state; and 1, death. The same principal investigators conducted the assessment unblinded. Cause of mortality was classified as early neurological (before 48 h of admission), late neurological or early septic (before 48 h), or late (due to any cause). Septic cause was defined as related to sepsis by hemodynamic failure or respiratory distress.

Informed consent and ethics

Patients or direct relatives (if it was not possible to ask the patient) were asked to provide written informed consent prior to inclusion in the study. This study received approval from the Ethics Committee at each participating center, with the main center's approval documented under expedient number PR158/18 by the "Comitè d'Ètica de la Investigació amb Medicaments" at Bellvitge University Hospital. The research adhered to the ethical standards of the Declaration of Helsinki.

Data collection and follow-up

CSF samples were obtained routinely by lumbar puncture in patients with suspected meningitis. The excess CSF not used for these determinations was saved for further analysis when required. The research team recorded all epidemiological, laboratory, and clinical data prospectively; however, this data collection did not interfere with diagnostic examinations or treatment. Patients were treated following the existing standard of care, using dexamethasone and antibiotic at the discretion of the treating physician. If CV was clinically suspected, a cranial image was performed (either CT or MRI, with or without angiography, in accordance with regular practice). The patient was followed for up to six months after discharge or until death with regular appointments at the outpatient clinic at three and six months.

Sample analysis

To perform MMP determinations, the research team collected CSF remaining from the initial lumbar puncture, which was preserved at −80 °C until biochemical analysis at the reference laboratory at the end of the study. CSF concentrations of the different markers were measured using commercially available enzyme-linked immunosorbent assays (ELISA) kits obtained from R&D systems for TIMP-1 and from ElabScience for MMP-2 and MMP-9.

Regarding microbiological tests, clinical samples were processed following standard procedures. Strains of *S. pneumoniae*were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and optochin susceptibility. Antibiotic susceptibility was tested by microdilution following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[37]. Serotype was determined by Quellung reaction, dot blot, and/or capsular sequence typing at the Spanish Pneumococcal Reference Laboratory. All available isolates underwent whole genome sequencing on the Illumina platform. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) and quantified with the QuantiFluor dsDNA System (Promega). Libraries were prepared with the Nextera XT kit and paired-end sequenced (2 × 300 base-pairs) on the MiSeq platform (Illumina). Read quality analysis and assembly was performed with the INNUca v4.2 pipeline (github.com/B-UMMI/INNUca). Multilocus sequence typing (MLST), in silico serotyping, and antibiotic resistance were determined using Pathogenwatch (pathogen.watch/) and reads were deposited at the European Nucleotide Archive (PRJEB61664).

Statistical analysis

We used an electronic case report form housed in REDCap, a secure web-based software platform, to collect and store study data[38]. Cohort characteristics are presented as the number of cases and percentages for categorical variables or as the median and interquartile range (IQR) for continuous variables. A 95% confidence interval was calculated to express the uncertainty of our estimates. All analyses were performed with a two-sided significance level of 0.05 in R software version 4.1.0[39].

Results

A total of 22 patients from three centers were included, as shown in Fig. 1.

Patient characteristics are summarized in Table 1.

Although the cohort had few comorbidities, almost a quarter had immunosuppression. All centers provided similar treatment with appropriate empirical antibiotics that included in 95,2% (20/21) of patients third-generation cephalosporins (ceftriaxone or cefotaxime) with or without ampicillin and vancomycin. Targeted therapy consisted in third-generation cephalosporins in 90,5% (19/21) cases, and antibiotic were maintained a median of 11 days. Only two of the isolates were not fully susceptible to cephalosporines, with a reported MIC of 1 and 2, but were successfully treated by high dose of cefotaxime as described elsewhere[40]All patients received adjunctive dexamethasone, except for one who was admitted with pneumococcal pneumonia and received a late diagnosis of meningitis. Dexamethasone treatment comprised an initial bolus of 12 mg followed by 4 mg every six hours for 48 h in 20/21 (95.2%) patients, with one patient receiving 10 mg every six hours for 96 h. Of the 18 patients without seizures until admission, 16 (88.9%) received antiseizure drug prophylaxis following our internal protocol as published elsewhere[41].

In all, 15 of the 22 patients (68.2%) required ICU admission and 13 (59.1%) required orotracheal intubation. The level of consciousness improved after a median of one day (IQR 1–2.75). Fever resolved in almost all patients (95.5%) after a mean of one day, but one patient presented persistent fever after 72 h and another presented reappearance of fever.

Neurological and non-neurological complications during admission are described in Table 2.

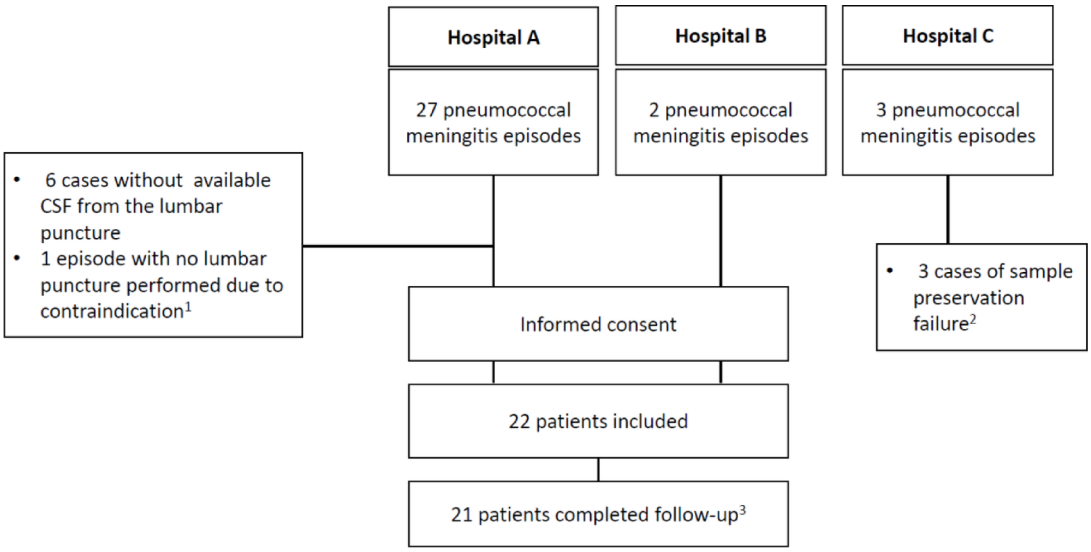

Fig. 1. Inclusion flow-chart. [1]Due to intravascular disseminated coagulopathy with spontaneous hemorrhages, no lumbar puncture was performed during the first days of treatment. [2]Due to issues in the freezing chain, these samples could not be processed. [3] One patient was not assessed for the development of vasculitis due to a lack of clinical information and follow-up resulting from the decision to limit diagnostic and therapeutic efforts, given the patient's advanced age, comorbidities, and severe condition.

Neurological complications occurred from day 1 to day 14 of admission, half of them developing after dexamethasone discontinuation and only one after antibiotic discontinuation. Diagnostic imaging was performed in all but one case of neurological complication (11/12), the one omission being due to therapeutic/diagnostic effort limitation of the patient. CT scan without angiography, performed in seven patients on median day 8 (IQR 7.5–8.5) after admission, did not detect CV; however, CTA performed in two patients at a median of day 19.5 (IQR 16.8–22.2) detected one case with vascular stenosis/ectasia. MRI was performed in eight patients on median day 8 (IQR 6.5–12.5), revealing punctate white matter hyperintense lesions on T2 and FLAIR sequences in three patients and infarcts in five, of whom three had multiple lesions in different vascular territories (two bilateral) and one showed different states of evolution. Other findings were cerebral edema and meningeal reinforcement. No hemorrhages were found. Based on diagnostic, clinical and radiological criteria, three of 21 patients (14.3%) were diagnosed with CV.

During admission, three of 22 patients (13.6%) died: one due to early sepsis (< 48 h of admission) and two attributed to late neurological complications. At discharge, six of 19 survivors presented sequelae, including one with cranial nerve palsy, three with deafness, two with neurological focal deficits, and one with a change in character. Twelve of the 22 patients (54.6%) presented a GOS of 5 at discharge. Follow-up was completed in 18 of 19 survivors; at one month after discharge, one more patient had a GOS of 5 points (13/18; 72.2%), while at six months 15/18 (83.3%) had achieved full recovery.

Differences in the main risk factors for CV and non-CV cases are shown in Table 3; however, none of them achieved statistical significance.

Figure 2: Phylogenetic tree of isolates causing pneumococcal meningitis at the Hospital Universitari de Bellvitge (2019–2022). Isolates of patients with vasculitis post-pneumococcal meningitis are marked in bold. Metadata related to year of isolation, sex, age group, source serotype and clonal complex are represented. Antibiotic susceptibility is represented in green (susceptible), yellow (intermediate) and red (resistance) under EUCAST meningeal breakpoints. PEN: Penicillin, AMX: Amoxicillin, CTX: Cefotaxime, TET: Tetracycline, ERY: Erythromycin, CLI: Clindamycin and CHL: Chloramphenicol. Acquired resistance genes for tetracycline and erythromycin are indicated. (*) In this isolate vasculitis could not be determined. (**) New ST profile.

Although there was a high diversity of serotypes among pneumococci causing meningitis, serotype 3 accounted for five cases (29.4%), most of them related to the globally disseminated clone CC180 which is associated with the rise of this serotype in Europe. Cases that developed CV had serotype 3, 9 N, and 35 F, but the small number meant that no comparisons could be made between the CV and non-CV groups.

More than half (12/22) of the patients had received at least one dose of the pneumococcal vaccine. Almost all had received at least one dose of the 23 V-polysaccharide, which covered all but five of the 17 isolated serotypes. Two out of these 12 had previously received the 13 V-conjugated vaccine in the sequential protocol. Only four patients had received the last dose of the vaccine within the last five years.

Of the overall cohort, six patients received COVID-19 vaccination, two of whom developed nosocomial COVID-19 infection prior to discharge but none at admission. Two of the patients who developed cerebral vasculitis had not received any COVID-19 vaccine, and only one had three doses prior to admission.

Treatment for the CV cases mainly involved extending the dexamethasone regimen to a median of 14 days (IQR 8–17.5) compared with the two days (IQR 2–3) required for the non-CV cases. Although none of the

Basal characteristics	
Age, years (median, IQR)	60.5 (53.0–68.5)
Male (n/N, %)	15/22 (68.2%)
Any pneumococcal vaccination before episode (n/N, %)	12/22 (54,1%)
Charlson score (median, IQR)	0 (0–2)
Previous meningitis episode (n/N, %)	2/22 (9.1%)
Immunosuppression[1] (n/N, %)	5/22 (22,7%)
Known predisposing factor[2] (n/N, %)	13/22 (59,1%)
Clinical characteristics at admission	
Time since symptom onset, hours (median, IQR)	30 (12–72)
Antibiotic treatment before admission (n/N, %) Duration, days (median, IQR)	8/22 (36,4%) 1.5 (1–2.25)
Fever at any time (n/N, %)	15/22 (68,2%)
Hypotension (n/N, %)	2/22 (18,2%)
Shock (n/N, %)	4/22 (14,3%)
Headache (n/N, %)	11/21 (52,4%)
Nuchal rigidity (n/N, %)	9/22 (52,9%)
Nausea or vomiting (n/N, %)	7/22 (31,8%)
Altered consciousness (n/N, %)	20/22 (90,9%)
Glasgow score (median, IQR)	10 (8–14)
Seizures before admission (n/N, %)	4/22 (18,2%)
Focal neurological deficit at admission (n/N, %)	5/22 (22,7%)
Laboratory test at admission	
Blood leukocytes, cel/mm3 (median, IQR)	18,900 (13875–23600)
Lactate, mmol/L (median, IQR)	2.76 (1.88–5.73)
CRP, mg/L (median, IQR)	179 (84.8–369)
Creatinine, µmol/L (median, IQR)	75.6 (59–114)
CSF leukocytes, cel/mm3 (median, IQR) % neutrophils (median, IQR)	3550 (800–6426) 90.5% (87–95)
CSF glucose, mmol/L (median, IQR)	0.11 (0.11–2)
CSF proteins, g/L (median, IQR)	5.2 (4–8.1)
Metalloprotease levels	
MMP-2, ng/mL (median, IQR)	11.5 (8.04–15.1)
MMP-9, ng/mL (median, IQR)	9482 (3734–13515)
TIMP-1, ng/mL (median, IQR)	430 (284–966)
Microbiology	
Positive blood cultures (n/N, %)	16/21 (72,7%)
CSF positive Gram stain (n/N, %)	15/21 (68,2%)
CSF positive culture (n/N, %)	14/20 (63,6%)
CSF positive antigen (n/N, %)	20/22 (90,9%)
CSF positive protein chain reaction (n/N, %)	4/21 (19%)
Susceptibility to penicillin[3] (n/N, %)	16/18 (83,3%)
Susceptibility to cefotaxime[4] (n/N, %)	16/18 (83,3%)
S pneumoniae serotype, (n) (*N* = 17)	3 (4); 9 N (2); 19 A (2), 22 F (2), 35 F (2); 7 C (1) 15 A (1), 15B (1), 19 F (1), 35B (1)
Radiology at admission	
Cranial CT scan before lumbar puncture (n/N, %)	19/22 (86,4%)
Abnormal cranial CT scan[5] (n/N, %)	4/19 (21,1%)

Table 1. Cohort main characteristics (*N* = 22). CRP: C reactive protein, CSF: cerebrospinal fluid, CT scan: computed tomography scan, EUCAST: European Committee on Antimicrobial Susceptibility Testing. [1]Immunosuppression was caused by splenectomy in three cases, biological treatment in one case and corticoid treatment in another. [2]The predisposing factors identified were: seven cases of acute otitis media, two cases of CSF leakage, two pneumonia, one chronic otitis media and one chronic sinusitis. [3]According to the EUCAST definition, *S. pneumoniae* strains were considered penicillin-susceptible with MICs ≤ 0.06 mg/L. [4]According to the EUCAST definition *S. pneumoniae* strains were considered cefotaxime-susceptible with MICs ≤ 0.5 mg/L. [5]The CT scan abnormalities were three cases of old strokes and one cerebral edema.

Non-neurological complications (*n*/*N*, %)	15/22 (68,2%)
Septic shock	4/22 (18,2%)
Respiratory failure	7/22 (31,8%)
Heart failure	5/22 (22,7%)
Renal failure	5/22 (22,7%)
Worsening liver function	2/22 (9,1%)
Multiorgan failure	3/22 (13,6%)
Arthritis	1/22 (4,6%)
Phlebitis	1/22 (4,6%)
Urinary tract nosocomial infection	1/22 (4,6%)
Neurological symptoms[1] (n/N, %)	12/22 (54,5%)
Headache	3/22 (13,6%)
Hemiparesis	3/22 (13,6%)
Cranial palsy	1/22 (4,6%)
Seizure	3/22 (13,6%)
Deterioration of level of consciousness to GCS < 8	5/22 (22,7%)
Another focal neurological deficit (n/N, %)	3/22 (13,6%)

Table 2. Complications during admission. GCS: Glasgow Coma Score. [1]Only for new onset of symptoms not present on admission.

	No CV (18/21)	CV (3/21)
Time since symptoms onset to admission, hours (median, IQR)	24 (12–72)	30 (21–39)
MMP-9 ng/mL (median, IQR)	9.8 (7.95–15.4)	13.2 (11.4–13.3)
MMP-2 ng/mL (median, IQR)	10,484 (4139–13515)	5689 (4354–7666)
TIMP-1 ng/mL (median, IQR)	318 (284–966)	699 (488–1944)
CSF proteins (median, IQR)	5.2 (4–7)	4.4 (3.7–6.7)
CSF positive gram stain (n/N, %)	11/17 (64.7%)	3/3 (100%)
CSF leukocytes (median, IQR)	3668 (1496–7846)	1058 (831–1285)
% neutrophils in CSF (median, IQR)	91.1% (88.2–95)	74.8% (64.4–85.1)
Adjuvant dexamethasone treatment (n/N, %)	17/18 (94.4%)	3/3 (100%)

Table 3. Possible cerebral vasculitis risk factors. CV: cerebral vasculitis, CSF: cerebrospinal fluid. None of the items achieved statistical significance.

patients with CV died, they presented lower GOS at discharge (GOS 4 in two cases and GOS 2 in one) than the non-CV group (12/16 [75%] survivors with GOS 5). After six months, one patient with CV achieved a GOS of 5, but there was no change in the other two patients.

Comparing MMP levels between survivors, MMP-9 and TIMP-1 were higher in patients with sequelae (13.3 [IQR 10.5–14.7] ng/mL and 853 [IQR 389–2644] ng/mL respectively) than in those without (10.6 [IQR 7.95–15.4] and 331 [IQR 294–844] ng/mL respectively), though the difference was not statistically significant ($p = 0.687$ and $p = 0.182$ respectively). In addition, MMP-2 levels were lower in patients with sequelae (7666 [IQR 3686-11,800] ng/mL) than in those without (11,648 [IQR 7308-13,630] ng/mL) again without achieving statistical significance ($p = 0.687$).

Discussion

The main findings of our cohort study were the confirmation that neurological complications are frequent, appearing in more than 50% of patients, and the recording of CV in more than 14% of patients. Although the sample size was insufficient to identify risk factors related to CV development, our results are consistent with those of previous publications suggesting that the risk of CV increases with longer times from symptom onset to admission, low neutrophil CSF counts, and a higher number of positive CSF gram stains[11].

Our results do not enable us to draw conclusions regarding the influence of COVID-19 or its vaccination on the incidence of vasculitis.

MMP levels were also in agreement with the figures reported in the literature, showing higher MMP-9 and TIMP-1 levels in patients with sequelae. Previous evidence in children with bacterial meningitis of multiple etiologies (*N. meningitidis*, *H. influenzae*, *S. pneumoniae*) showed the correlation of higher levels of MMP-9 and TIMP-1 in the first lumbar puncture with poor neurological outcome, a finding not observed with MMP-2 levels that in some studies was reported as constitutively expressed in CSF[27,34]. A relationship between higher levels of MMP-9 and vasculitis due to coccidioidal meningitis has also been suggested in animal models[33]. We cannot

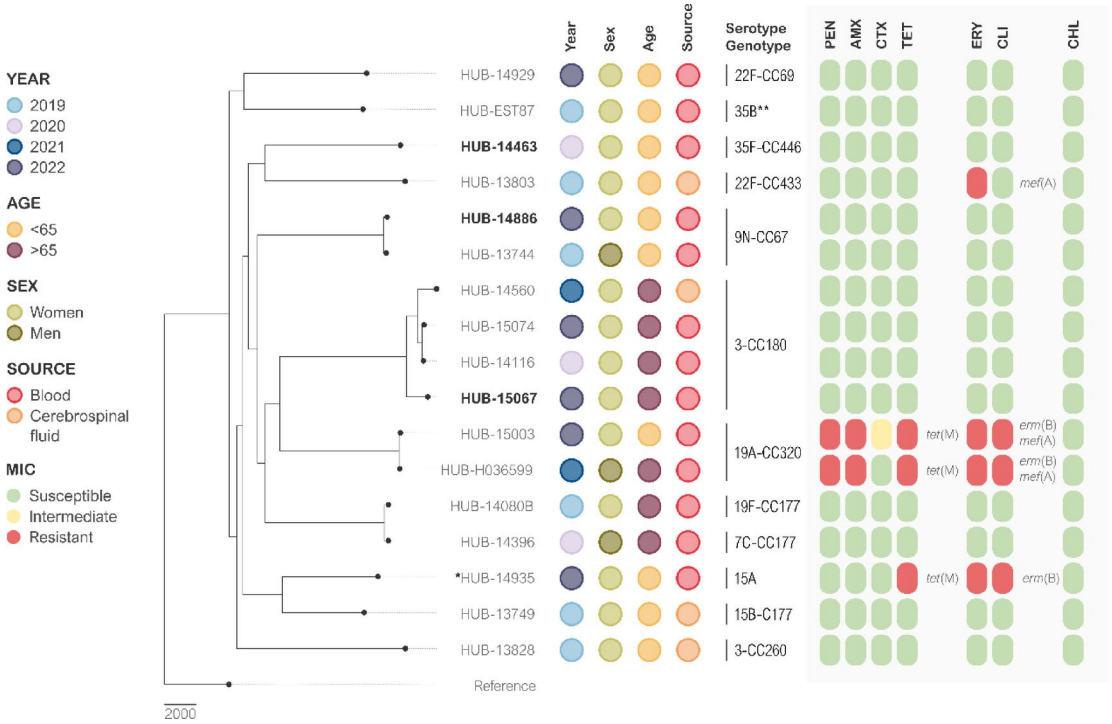

Fig. 2. Summarizes the phylogenetic tree and associated metadata of sequenced isolates, including antibiotic susceptibility and acquired resistant genes. It includes 17 of the 22 patients diagnosed by culture in the Hospital Universitari de Bellvitge.

conclude that differences in MMP levels are associated with CV, but our preliminary results should encourage further research in this field. A larger cohort could detect early markers of this severe complication and allow the design of treatments aimed at reducing its severity and frequency.

Microbiological analysis did not find any patterns in meningitis complicated with CV: that is, no phylogenetic relationship, serotype predominance, virulence factor, or antibiotic susceptibility. The high genetic diversity of pneumococci, together with the small sample size, meant that we could not rule out the possible role of microbial characteristics in the genesis of CV as a complication. It is well known that serotype 3 is the predominant cause of severe meningitis.

Most physicians followed the dexamethasone regimen proposed by Cabellos et al.[7], using a lower dose for a shorter duration than that proposed in a clinical trial[2]which found a lower incidence of CV than in other cohorts recently described that received conventional doses of dexamethasone (14.3% vs. 29.2%)[12]. This contradicts the hypothesis suggested by Koelman et al.[5] were intensified and prolonged corticosteroid treatment regimen should be evaluated to prevent cerebral vasculopathy. Further analyses of different doses of dexamethasone and the incidence of CV are now warranted.

The main limitation of our study is the small sample size and the low number of cases of CV. During the SARS-CoV-2 pandemic, the incidence of pneumococcal meningitis was low due to lock-down and mask-wearing precautions; therefore, despite extending the study period, it was not possible to include more patients and thus achieve adequate statistical power. Nevertheless, the study is the first to include MMP levels in adults with pneumococcal meningitis and to assess their relationship with CV based on previous reports in other etiologies of meningitis[29,30,32,33], experimental models[25,26,28,31], children[27,34], or with scarce representation of pneumococcal etiology[34]. To our knowledge, this is the first attempt to assess the microbiological phylogenetic relationships or virulence factors related to CV. One significant limitation is the lack of blinding among the principal investigators assessing the clinical outcomes. This was particularly challenging to implement because some treating doctors also served as the principal investigators.We cannot rule out the presence of selection bias, which may have prevented us from recruiting patients with extreme severity (e.g., patients for whom lumbar puncture was contraindicated due to altered coagulation and spontaneous hemorrhages). We aimed to control for detection bias by ensuring that the same investigators followed the entire cohort at each hospital. Although the loss of follow-up was minimal, the possibility of attrition bias cannot be dismissed, as we were unable to conduct adequate follow-up in a patient with extreme severity in whom diagnostic and therapeutic efforts were limited.

Conclusions

CV is a frequent complication of pneumococcal meningitis; in this series, it occurred in 14.3% of cases. MMP levels in the CSF could represent a potential marker of this complication, but further studies with larger samples

are needed to test this hypothesis. Indeed, the small sample in the current study prevents us from drawing any conclusions about the relation between CV and either pneumococcal serotypes or virulence factors.

Data availability

Sequence data supporting this study's findings have been deposited in the European Nucleotide Archive (PRJEB61664).

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Author contributions
CC designed and supervised the study. EV, L B-P, LG, and CC obtained informed consent, followed the patient, and collected the data. P A-R did the laboratory analysis. A G-D and CA did the microbiological analysis. LG wrote the main manuscript and tables. A G-D and CA designed Figure 2. All the authors reviewed the manuscript.

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Competing interests
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