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Cerebrospinal fluid sex steroid hormones in bacterial meningitis

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Abstract

Unfavorable outcome in bacterial meningitis is related to excessive inflammation and higher inflammatory markers have been reported in female than in male patients. Sex steroid hormones have immunomodulatory properties and can be found in the cerebrospinal fluid (CSF); however, their actions have not been studied in bacterial meningitis. We investigated the association between CSF sex steroid hormone levels and inflammatory parameters, disease severity, and outcome in pneumococcal meningitis. We identified adults with culture-proven pneumococcal meningitis in a prospective cohort study (2006–2014). We measured estradiol and testosterone in CSF using liquid chromatography-tandem mass spectrometry and sex hormone-binding globulin (SHBG) using an enzyme-linked immunoassay. Hormone levels were compared according to outcome, which was graded using the Glasgow Outcome Scale (a score of 5 indicating favorable, 1–4 unfavorable outcome). Correlation analysis was used to measure the association between hormone levels and inflammatory cytokines, chemokines, and complement factors as well as severity of illness, as measured by the Glasgow Coma Scale and the Dutch Meningitis Risk Score. We included 60 patients: 20 men, 20 premenopausal (<50 years), and 20 postmenopausal (>50 years) women. Twenty-one (35%) patients had an unfavorable outcome and 11 (18%) died. Cases with an unfavorable outcome exhibited higher estradiol (median 14.0 vs 5.0 pmol/L, $P = .04$) and lower SHBG (0.40 vs 1.0 nmol/L, $P = .03$) levels compared with those with a favorable outcome. Estradiol was positively correlated with C-reactive protein ($R = 0.42$, $P = .001$), CSF protein ($R = 0.33$, $P = .01$), and proinflammatory cytokine levels. CSF concentrations of the sex steroid hormone estradiol were associated with outcome and CSF inflammation. Understanding the dose and time-dependent interaction between sex steroid hormones and the inflammatory response in bacterial meningitis represents an important and understudied topic.

Abbreviations: CSF = cerebrospinal fluid, CXCL = [C-X-C motif], E2 = 17β-estradiol, ICAM-1 = soluble intercellular adhesion molecule-1, IL = interleukin, LLOQ = lower limit of quantification, MMP = matrix metalloproteinase, SHBG = sex hormone-binding globulin, TAFI = thrombin-activatable fibrinolysis inhibitor, TNF = tumor necrosis factor.

Keywords: bacterial meningitis, cerebrospinal fluid, inflammation, pneumococcal disease, sex hormones

1. Introduction

Bacterial meningitis is a life-threatening infection resulting from bacterial invasion of the meninges.[1] *Streptococcus pneumoniae* is the most common cause and is associated with high case fatality rates and long-term sequelae.[2] Unfavorable outcome is largely driven by an excessive inflammatory reaction in the subarachnoid space,[3] which is the rationale for treatment with adjunctive corticosteroids.[4,5]

Females exhibit stronger immune responses than males,[6] and higher inflammatory markers have been found in women than in men with bacterial meningitis.[7] In a previous study, male sex was found to be an independent predictor of poor prognosis.[7] Furthermore, a post hoc analysis of a large European trial of dexamethasone versus placebo suggested a potential difference in the magnitude of treatment effect—with a larger benefit in women.[8] Together, these findings raise the hypothesis that sex-based differences in outcome could be related to varying degrees of inflammation and susceptibility to anti-inflammatory treatment.

Sex steroid hormones exert their influence in many biological processes besides the hypothalamic-pituitary-gonadal axis and have immunomodulatory properties as well.[9,10] Estrogen provides a protective effect in bacterial infections

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by enhancing cell-mediated and humoral immunity,[6,11] but also has anti-inflammatory qualities in the setting of excessive inflammation.[11,12] Moreover, mounting evidence supports a neuroprotective effect.[13,14] Testosterone, on the other hand, is widely considered to have an immunosuppressive effect.[6,11] Sex hormone-binding globulin (SHBG) binds androgens and estrogens and is responsible for their delivery to sex hormone-responsive tissues.[15]

Sex hormones can be found in the cerebrospinal fluid (CSF), as circulating sex steroids are able cross the blood-brain barrier. In addition, the central nervous system is capable of synthesizing neuroactive steroids de novo[16–18] and increasing evidence shows sex steroids from neural origin are involved in a variety of non-reproductive functions.[19] Nevertheless, their actions have not been investigated in bacterial meningitis.

In this study, we examine the association between CSF levels of 17β-estradiol (E2), testosterone, SHBG, and outcome in a cohort of adult men and women with community-acquired pneumococcal meningitis, and correlate them with inflammation markers and indicators of disease severity.

2. Materials and Methods

2.1. Study population and procedures

We identified patients over 16 years old with pneumococcal meningitis (defined as *S pneumoniae* cultured in the CSF) included in a nationwide prospective cohort study in the Netherlands from January 1, 2006 to July 1, 2014. Detailed methods of the MeninGene study have been published elsewhere.[2] The study was approved by all appropriate ethics committees and all included patients or their legal representants gave written informed consent for participation.

Exclusion criteria for the MeninGene study were as follows: episodes of hospital-acquired meningitis, defined as meningitis occurring during hospitalization or within 1 week of discharge; patients who experienced head trauma or neurosurgery in the month prior to the meningitis episode; those with a neurosurgical device; and cases with missing outcome. For this study, we further excluded episodes with missing patient sex, as well as cases of pregnancy, breastfeeding, and use of exogenous sex hormones or anti-hormonal drugs (apart from hormonal contraception), when that information was available.

In order to study the effect of sex and menopausal status, patients were split up according to age and sex into 3 groups: males (all ages), premenopausal (defined as women under 50 years) and postmenopausal (over 50 years) females. Each of these groups was subdivided according to outcome (favorable vs unfavorable). For feasibility reasons, a convenience sample of sixty cases (20 men, 20 premenopausal, and 20 postmenopausal women) with sufficient leftover CSF from the diagnostic lumbar puncture was selected at random from each of these groups in numbers approximating the rate of unfavorable outcome for that group in the whole cohort, in order to get a representative sample. Because our goal was to study inflammation, only cases for whom inflammatory cytokine and chemokine measurements were available were considered. If there was an insufficient number of patients meeting this condition, then patients without cytokine measurements were selected at random for inclusion in each group.

Comprehensive data on patient history, medication, symptoms and signs on admission, laboratory results, treatment, complications, and outcome were available for all patients. We calculated illness severity using the Glasgow Coma Scale and the Dutch Meningitis Risk Score, a validated bedside risk score based on routinely collected data from which risk of an adverse outcome can be predicted.[20] Outcome was graded according to the Glasgow Outcome Scale, as assessed by the patient's physician at discharge. A score of 1 on this scale indicates death; 2, a vegetative state; 3, severe disability; 4, moderate disability; and 5, mild or no disability. A favorable outcome was defined as a score of 5, and an unfavorable outcome as a score of 1 to 4.

2.2. CSF collection, storage, and hormone measurements

We used leftover CSF from the diagnostic lumbar puncture to measure sex steroid hormone levels. CSF was centrifuged and the supernatant stored at −80°C until analysis, with no extra procedures performed. Levels of E2 and total testosterone were measured using liquid chromatography-tandem mass spectrometry as described before.[21,22] and SHBG levels were determined using an enzyme-linked immunoassay (Architect, Abbott Diagnostics, USA). The lower limit of quantification (LLOQ) was 10 pmol/L for E2, 0.1 nmol/L for testosterone and 0.1 nmol/L for SHBG. Furthermore, extensive laboratory results (including blood and CSF inflammatory parameters) were available for correlation purposes. We also had access to prior measurements of cytokines, chemokines, and complement factors, including: chemokine [C-C motif] ligand (CCL) 1 through 5, CCL7, CCL8, CCL11, CCL13, CCL14a, CCL15, CCL17, CCL19 through 22, CCL24, CCL26, CCL27, chemokine [C-X-C motif] ligand (CXCL) 1, CXCL5 through 7, CXCL9 through 13, chemokine [C motif] ligand 1 (XCL1), chemokine [C-3X-C motif] ligand 1 (CX3CL1), interleukin (IL)-1-α, IL-1-β, IL-1 receptor antagonist, IL-2 through IL-11, IL-12p40, IL12p70, IL-13, IL-15 to IL-18, IL-20, IL-21, IL-23, IL-28A, IL-29, IL-33, interferon (IFN)-α2, IFN-γ, leukemia inhibitory factor, thrombopoietin, tumor necrosis factor (TNF)-related apoptosis-inducing ligand, stem cell factor, thymic stromal lymphopoietin, macrophage colony-stimulating factor, epidermal growth factor, fibroblast growth factor-2, FMS-like tyrosine kinase-3 ligand, granulocyte colony-stimulating factor, granulocyte macrophage-colony stimulating factor, platelet derived growth factor-AA, platelet derived growth factor-AB/BB, soluble cluster of differentiation 40 ligand, soluble IL-2 receptor alpha (sCD25), transforming growth factor alpha, TNF-α, TNF-β, vascular endothelial growth factor, soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1 (ICAM-1), matrix metalloproteinase (MMP)-1 through 3, MMP-7, MMP-9, MMP-10, MMP-12, MMP-13, macrophage migration inhibitory factor, von Willebrand factor antigen, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, thrombin-activatable fibrinolysis inhibitor, fibrinogen, complement factor H, C3, C3a, iC3b, C5a, and C5b9.

2.3. Statistical analysis

For the purpose of statistical analysis, a value of half the LLOQ was attributed to hormone measurements below that limit. In addition, we performed pre-specified sensitivity analyses excluding these cases. Continuous variables are expressed as median and interquartile range and were compared with the Mann–Whitney *U* test (for comparisons involving 2 groups) or the Kruskal–Wallis *H* test (for 3 or more groups). We used the χ^2 or the Fisher exact test, as appropriate, to study categorical variables and Spearman's rank correlation was used for correlation analyses. We examined nonlinearity using visual inspection. We adjusted for possible confounders and tested hormone-dexamethasone interactions using logistic regression. All tests were 2-tailed and a *P* value under. 05 was considered statistically significant. For analyses involving a large number of cytokines, chemokines, and complement factors, a Bonferroni correction was applied.

Analyses were conducted using IBM SPSS Statistics for Windows, version 26.0 (Armonk, NY: IBM Corp., 2017). Plots were designed using the ggplot2 package in R (version 4.0.3, R Core Team, 2020).

3. Results

We included 60 patients in the study: 20 males, 20 premenopausal, and 20 postmenopausal women. Clinical characteristics were comparable between groups (Table 1). The exception was age, which significantly differed between the 3 groups, as determined by the study design, but not between the 2 sexes overall (median 65 [47–70] years in males vs 51 [38–70] in women of all ages, $P = .29$). Twenty-one (35%) patients had an unfavorable outcome and 11 (18%) died. Fifty-one (85%) of the 60 patients received adjunctive dexamethasone treatment according to established guidelines, with no significant differences between the groups or between sexes. Initial antibiotic treatment included a combination of amoxicillin and a third-generation cephalosporin in 7/19 (37%) episodes in men, 3/16 (19%) in premenopausal and 7/19 (37%) in postmenopausal women. Monotherapy was started with a third-generation cephalosporin in 0/19 (0%) episodes in males, 5/16 (31%) in premenopausal and 3/19 (16%) in postmenopausal females. Monotherapy with either penicillin or amoxicillin occurred in 6/19 (32%), 4/16 (25%) and 8/19 (42%) episodes, respectively. Other regimens were used in the remaining patients.

Sex steroid hormone measurements were successful in all 60 patients (Table 2). Testosterone differed between the groups (median 0.24 nmol/L in males, 0.05 in premenopausal and 0.13 in postmenopausal females, $P < .001$), whereas E2 and SHBG were comparable between groups.

When compared with cases with a favorable outcome (Table 3), those with an unfavorable outcome exhibited higher E2 (median 14.0 vs 5.0 pmol/L, $P = .04$) and lower SHBG levels (0.40 vs 1.0 nmol/L, $P = .03$). This trend was seen in all 3 groups (Fig. 1A and B). These associations persisted after correcting for age and sex (adjusted $P = .02$ for E2 and $P = .01$ for SHGB) and for CSF protein levels (adjusted $P = .03$ for E2, $P = .02$ for SHBG) and in the sensitivity analysis excluding cases below the LLOQ for each hormone from the analysis ($P = .02$ for E2, $P = .004$ for SHBG). There was no difference in testosterone levels between cases with an unfavorable *vs* those with a favorable outcome, both overall or within the groups (Fig. 1C), either with or without including the cases below the LLOQ. We also found no differences in hormone levels between patients who died and those who did not, although after excluding cases below the LLOQ, CSF E2 was significantly higher in patients who died (median 45.2 [22.3–50.5] vs 15.0 [11.0–20.6], $P = .003$). There was no interaction between any of the hormones and dexamethasone.

We found patients with an altered consciousness (defined as a score below 15 on the Glasgow Coma Scale) on admission to have higher testosterone levels than those with a normal mental status (0.14 [0.05–0.23] vs 0.05 [0.05–0.11] nmol/L, $P = .03$). No such difference was seen for E2 (10.0 [5.0–17.2] vs 7.5 [5.0–15.2] pmol/L, $P = .61$) or SHBG (0.65 [0.30–1.5] vs 0.50 [0.40–1.2] nmol/L, $P = .92$).

E2 levels were moderately positively correlated with C-reactive protein ($R = 0.42$, $P = .001$) and CSF protein levels ($R = 0.33$, $P = .01$) and weakly negatively correlated with the CSF:blood glucose ratio ($R = -0.29$, $P = .03$). We found no correlation between hormone levels and the Dutch Meningitis Risk Score and the hormones were not correlated with each other.

Cytokine, chemokine, and complement factor measurements were available for 52 patients. After correcting for multiple testing, E2 was moderately positively correlated with IL-18 ($R = 0.50$, $P = 1.3 \times 10^{-4}$), CCL7 ($R = 0.53$, $P = 5.3 \times 10^{-5}$), CXCL9 ($R = 0.52$, $P = 1.1 \times 10^{-4}$), ICAM-1 ($R = 0.58$, $P = 5.7 \times 10^{-6}$), and fibrinogen ($R = 0.63$, $P = 1.2 \times 10^{-6}$). SHBG was moderately positively correlated with CCL14a ($R = 0.60$, $P = 3 \times 10^{-6}$). There was no correlation between testosterone

Table 1

Baseline characteristics of the study groups (n = 60).

		Women	
Characteristic*	**Men (n = 20)**	**Premenopausal (n = 20)**	**Postmenopausal (n = 20)**
Age, yr	65 (48–70)	38 (34–45)	70 (60–73)
Recurrent meningitis	1 (5)	4 (20)	1 (5)
Predisposing conditions			
Otitis or sinusitis	8 (40)	7 (37)	11 (55)
Immunocompromise†	6 (30)	8 (40)	5 (25)
GCS score	9 (8–11)	12 (10–15)	10 (8–12)
<14 (altered mental status)	18 (90)	11 (55)	17 (85)
Dutch meningitis risk score‡	28 (27–33)	26 (20–29)	30 (24–34)
Unfavorable outcome	8 (40)	5 (25)	8 (40)
Death	4 (20)	2 (10)	5 (25)
Serum inflammation markers§			
Leukocyte count (per mm^3)	16,650 (11,350–22,980)	20,850 (14,250–26,520)	22,150 (14,550–24,770)
C-reactive protein (mg/L)	182 (59–325)	207 (39–305)	220 (166–348)
ESR (mm/h)	46 (19–61)	49 (47–58)	42 (25–59)
Indices of CSF inflammation‖			
White cell count (per mm^3)	4105 (1547–7422)	2600 (955–7422)	3109 (1297–11,784)
Protein (g/L)	4.2 (2.9–5.8)	4.6 (2.3–6.0)	4.4 (2.4–6.4)
CSF:blood glucose ratio	0.05 (0–0.26)	0.04 (0–0.26)	0.04 (0–0.18)

CSF = cerebrospinal fluid, ESR = erythrocyte sedimentation rate, GCS = Glasgow Coma Scale.

* Value is n (%) or median (interquartile range). Percentages may not add up to 100% due to rounding.

† Patients with a history of splenectomy, diabetes mellitus, alcoholism, human immunodeficiency virus infection, or immunosuppressive treatment were considered to be immunocompromised.

‡ The Dutch Meningitis Risk Score can range from 0 to 65, with associated risk estimates for an unfavorable outcome varying between 3.2 and 96%, respectively. It could be calculated in 49 cases (17 males, 15 premenopausal and 17 postmenopausal females).

§ Blood leukocyte count was measured in all patients, CRP in 57 (19 males, 20 premenopausal and 18 postmenopausal females), ESR in 30 (10 men, 5 premenopausal and 15 postmenopausal women).

‖ CSF white cell count was available in 59 patients (all but one postmenopausal female) and CSF protein was measured in 59 cases (all except one premenopausal female).

Table 2

Cerebrospinal fluid hormone levels in men, pre and postmenopausal women.

		Women		
Hormone—median (IQR)	**Men (n = 20)**	**Premenopausal (n = 20)**	**Postmenopausal (n = 20)**	***P* value***
Estradiol (pmol/L)†	5.0 (5.0–11.3)	10.5 (5.0–18.5)	10.5 (5.0–17.9)	.31
Testosterone (nmol/L)‡	0.24 (0.16–0.31)	0.05 (0.05–0.05)	0.13 (0.05–0.20)	<.001
SHBG (nmol/L)§	0.65 (0.40–1.05)	0.45 (0.30–1.25)	1.10 (0.38–1.50)	.52

IQR = interquartile range, SHBG = sex hormone-binding globulin.

* A *P* value <.05 was considered to be statistically significant.

† Twenty-nine (48%) cases were under the lower limit of quantification of 10 pmol/L for estradiol (12 males, 9 premenopausal and 8 postmenopausal women).

‡ Twenty-eight (47%) cases were below the lower limit of quantification of 0.10 nmol/L for testosterone (four males, 17 premenopausal and 7 postmenopausal women).

§ Three (5%) cases were under the lower limit of quantification of 0.10 nmol/L for SHBG (one in each group).

Table 3

Cerebrospinal fluid hormone levels according to outcome and death status.

Hormone—median (IQR)	**Favorable outcome (n = 39)**	**Unfavorable outcome (n = 21)**	***P* value***
Estradiol (pmol/L)†	5.0 (5.0–13.5)	14.0 (5.0–24.6)	.045
Testosterone (nmol/L)‡	0.05 (0.05–0.18)	0.18 (0.05–0.20)	.14
SHBG (nmol/L)§	1.0 (0.40–2.1)	0.40 (0.20–0.80)	.03
	Alive (n = 49)	**Deceased (n = 11)**	
Estradiol (pmol/L)	10.0 (5.0–15.0)	5.0 (5.0–34.9)	.49
Testosterone (nmol/L)	0.05 (0.05–0.20)	0.18 (0.08–0.21)	.24
SHBG (nmol/L)	0.70 (0.30 vs 1.40)	0.50 (0.40–0.90)	.48

IQR = interquartile range, SHBG = sex hormone-binding globulin.

* A *P* value <.05 was considered to be statistically significant.

† Twenty-nine (48%) cases were under the lower limit of quantification of 10 pmol/L for estradiol (21 with a favorable outcome).

‡ Twenty-eight (47%) cases were below the lower limit of quantification of 0.10 nmol/L for testosterone (22 with a favorable outcome).

§ Three (5%) cases were under the lower limit of quantification of 0.10 nmol/L for SHBG (all with a favorable outcome).

and any of the cytokines, chemokines, and complement factors measured.

4. Discussion

In this exploratory study, higher CSF E2 concentrations were associated with an unfavorable outcome, as well as with higher serum and CSF markers of inflammation and levels of proinflammatory cytokines and chemokines. These include a neutrophil-chemoattractant (CCL7), a chemokine involved in immune cell migration, differentiation, and activation (CXCL9), and a proinflammatory cytokine of the IL-1 family (IL-18), as well as an adhesion molecule involved in leukocyte recruitment and endothelial transmigration (ICAM-1) and a protein involved in coagulation and fibrinolysis that plays a role in vascular inflammation and endothelial dysfunction (fibrinogen).

While robust immune responses are essential for bacterial clearance, in pneumococcal meningitis unfavorable outcome is associated with a stronger inflammatory reaction to the highly immunogenic compounds released by the bacteria.[3] Estrogen has a complex role in inflammation.[12] It enhances both cell-mediated and humoral immunity and can augment or dampen innate signaling pathways depending on the context, estrogen concentrations, and time.[12,23]

On the one hand, it promotes the production of pro-inflammatory cytokines in response to toll-like receptor ligands,[24] which results in females having a stronger immune response to infection compared with males. Furthermore, E2 at low physiologic concentrations stimulates a Th1-type response, enhances cell-mediated immunity,[11,25] and promotes type I interferon innate pathways leading to pro-inflammatory cytokine production.[23]

On the other hand, higher estrogen concentrations promote a shift to Th2-cell and humoral responses, inhibiting pro-inflammatory and stimulating anti-inflammatory pathways.[11,25] In many infections, estrogen has an anti-inflammatory effect that is protective against tissue damage. For example, in experimental pneumococcal pneumonia, E2 increased several critical components of regulatory T-cell function, accelerating resolution of lung inflammation.[26] Furthermore, under pathological conditions, aromatase (an enzyme which converts androgens into estrogens) is upregulated in the central nervous system,[19] where estrogen is considered to have anti-inflammatory actions.[13,14]

The effects of estrogen are also time-dependent,[12] enhancing the immune response in certain acute conditions, such as trauma[27] or sepsis,[28] while having an anti-inflammatory action similar to that of glucocorticoids—with reduced expression of transcription factors involved in the inflammatory response and reduced recruitment of neutrophils by decreasing the production of IL, chemokines, and adhesion molecules—in some chronic diseases like Crohn's disease or arthritis.[29,30]

Due to the correlative nature of our analysis, we cannot establish causal relationships between CSF E2, inflammation, and outcome. While it is possible that, in the context of acute bacterial meningitis, E2 promotes pro-inflammatory signaling pathways, it is also plausible that E2 levels are elevated as a reaction to inflammation, either as a biomarker or as part of a compensatory response. We did not find a difference in E2 levels between groups, which could mean that this occurs regardless of patient sex or menopausal status.

SHBG levels were positively correlated with CSF protein levels but not with other markers of serum or CSF inflammation, probably reflecting SHBG leakage into the CSF due to blood-brain barrier breakdown in the context of meningitis.[31]

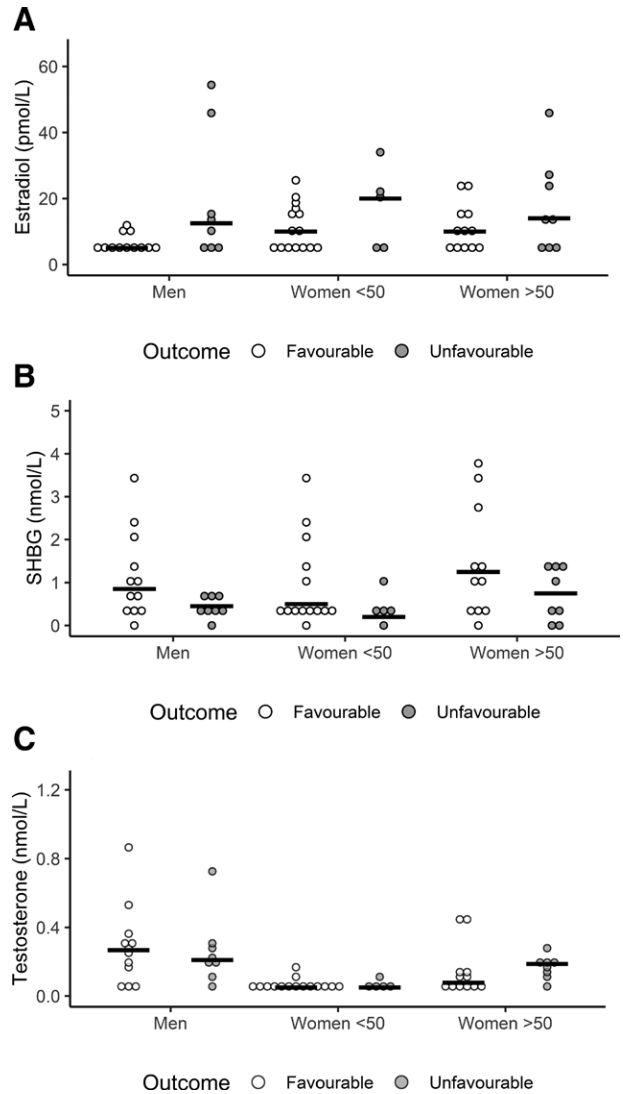

Figure 1. Distribution of estradiol (A), sex hormone-binding globulin (B), and testosterone (C) levels in cases with a favorable (white dots) and unfavorable (gray dots) outcome in men (n = 20), premenopausal (n = 20) and postmenopausal (n = 20) women. Each dot represents a case; black lines represent the median level in each group.

In addition, low SHBG levels were associated with an unfavorable outcome. This could be a result of its relationship with E2, since lower SHBG levels would be associated with a decrease in the SHBG-bound fraction and an increase in bioavailable E2,[32] although we did not find a significant correlation between the 2 hormones.

An important limitation of our study was that there was no systematic collection of data regarding hormonal treatment, pregnancy, or menopausal status. Furthermore, in premenopausal women the menstrual cycle phase was unknown and these measurements may not be representative of sex steroid hormone concentrations throughout the menstrual cycle. E2 in particular, has important cyclical variations throughout the menstrual cycle and our inability to account for this may have biased the results.

Our study had several other limitations. The sample size was relatively modest and the use of a convenience sample may lead to potential bias and lack of power to identify significant differences. Although our cases were part of a large prospective cohort study, all the samples were from the initial lumbar puncture, thus not allowing for longitudinal comparisons and we could not contrast the CSF results with blood measurements. Moreover, due to the blood-brain barrier dysfunction associated with meningitis, leakage could account for some of the results, although the fact that we obtained similar results after accounting for protein levels argues against it. In addition, in many samples, E2 and testosterone levels were below the LLOQ, and we do not know the free E2 and testosterone levels, which could be biologically more relevant than the bound concentrations.

The exploratory nature of our study does not allow us to draw definitive conclusions about the causal relationships between CSF sex steroid hormones, inflammation, and outcome. Nevertheless, our results show that sex steroid hormones are associated with disease severity and outcome in pneumococcal meningitis, and suggest that this effect could be mediated by the patient's inflammatory response. Understanding the dose and time-dependent interaction between sex steroid hormones and the inflammatory response in infectious disease represents an important and understudied topic.

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