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SEX AND GENDER DIFFERENCES IN BACTERIAL MENINGITIS

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CHAPTER 1

INTRODUCTION

Bacterial meningitis is a life-threatening infection of the central nervous system resulting from bacterial invasion and subsequent inflammation of the subarachnoid space. It can be acquired in the community or in the hospital due to invasive procedures or head trauma.[1] It can be caused by a wide range of pathogens, and clinical severity and case fatality rates vary with host factors, causative pathogens, and country income.[1]

Streptococcus pneumoniae, *Neisseria meningitidis*, and *Haemophilus influenzae* type b are the main causative pathogens globally.[2] Large-scale immunisation programs against these bacteria have substantially reduced the incidence of bacterial meningitis worldwide.[2-4] Besides individual protection, these vaccines have also proved effective in preventing nasopharyngeal carriage and transmission, resulting in herd protection of the unvaccinated population.[5]

S. pneumoniae has the highest case fatality rates and currently causes around 70% of cases in the Netherlands.[6] Several conjugate vaccines targeting specific pneumococcal capsular serotypes have been developed and implemented in routine paediatric immunisation schedules,[4] as young children are the key transmitters of *S. pneumoniae* in the population. The seven-valent pneumococcal conjugate vaccine (PCV7) was first introduced in the Netherlands in 2006 and contained capsular polysaccharides from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, which previously caused the majority of meningitis cases. The 10-valent pneumococcal conjugate vaccine (PCV10), additionally covering serotypes 1, 5, and 7F, was subsequently introduced in 2011. Widespread use of these vaccines has greatly reduced the burden of meningitis caused by vaccine serotypes.[4] However, replacement with non-vaccine serotypes has been observed in varying degrees, limiting their overall effect.[7]

Experimental studies have demonstrated that unfavourable outcome in bacterial meningitis is largely driven by an excessive host inflammatory reaction in the subarachnoid space.[8] A large multicentre European randomised controlled trial of dexamethasone *vs* placebo showed treatment with dexamethasone improves outcome in adults with community-acquired bacterial meningitis.[9] Several meta-analyses and implementation studies have corroborated these findings and support the use of corticosteroids in high-income countries,[2,10] and dexamethasone has become an established adjunctive treatment for community-acquired bacterial meningitis.

Despite these efforts in vaccination and management, the burden of bacterial meningitis remains unacceptably high, causing steep mortality rates, disabling many survivors, and taking a significant social and economic toll.[11,12] Further improvements in the outcome of bacterial meningitis are essential; besides the development of new preventive measures, they are likely to come from a better understanding of host factors that determine disease pathology.

From an evolutionary standpoint, sex is one of the most well-conserved characteristics in biology,[13] yet one of the most underappreciated. The terms sex and gender are often used interchangeably, but they are not the same. Sex is a biological variable determined by sex chromosomes, reproductive organs, and sex hormones.[14] Gender, on the other hand, refers to socially constructed characteristics, including norms, behaviours, and roles associated with each sex.[15] Sex and gender often interact with each other, and both are important determinants of infectious diseases. Sex determines physiologic and anatomical differences that influence susceptibility, pathophysiology, pathogen recognition and clearance, disease transmission, and response to treatment of bacterial diseases, whereas gender affects exposure to pathogens, health-seeking behaviour, access to healthcare, and adherence to treatment recommendations.[16]

Most infections have a male predisposition, with men more often and more severely affected by a variety of bacterial, viral, parasitic, and fungal diseases.[17] This sexual dimorphism is noted in the pathophysiology, epidemiology, clinical presentation, disease severity, response to treatment, and prognosis. The reasons for this bias are manifold and include both biological and behavioural factors. It is rooted in genetic differences that provide a survival advantage to females, such as the presence of two X chromosomes.[18] Immune function markedly differs by sex, partly due to the effect of sex steroid hormones. Women generally have stronger immune responses to self and foreign antigens than men, resulting in a better response to infection and vaccination but also making them more susceptible to autoimmune and inflammatory diseases.[16] Oestrogen typically enhances humoral and cell-mediated immune responses, whereas testosterone has a suppressive effect.[19]

Nevertheless, little is known about the influence of patient sex in bacterial meningitis. A study in children with acute bacterial meningitis found the clinical presentation to vary with patient sex.[20] Other studies in children have identified male sex as an independent predictor of adverse outcome,[21,22] neurological sequelae,[23] and cognitive or behavioural problems.[24] In adults, information is mostly lacking, although a few studies found no association between sex and outcome in univariable analyses.[25-29]

AIM AND OUTLINE OF THIS THESIS

This thesis aims to study sex-based differences in the aetiology, epidemiology, clinical manifestations, response to treatment, and outcome in community-acquired bacterial meningitis and to investigate their underlying pathophysiology, particularly concerning the role of inflammation and sex steroid hormones.

Chapter 2 gives an overview of sex and gender differences in bacterial infections. We review the literature to describe how genetic, anatomical, immunological, and hormonal factors affect sex bias in bacterial diseases and how gender-related behaviours interact with pathobiology.

Chapter 3 focuses on bacterial meningitis and examines sex-based differences in clinical features, laboratory findings, causative pathogens, disease severity, and outcome in 1,412 episodes of adult community-acquired bacterial meningitis included in a prospective nationwide cohort study.

Using the same cohort, in **chapter 4**, we compare pneumococcal serotype distribution and incidence trends in men and women with pneumococcal meningitis in the Netherlands following the nationwide implementation of paediatric conjugate vaccines.

In order to investigate whether patient sex influences the response to anti-inflammatory treatment, in **chapter 5**, we perform a *post hoc* analysis of a well-known European randomised controlled trial of dexamethasone *vs* placebo in adults with community-acquired bacterial meningitis.

Sex steroids have immunomodulatory properties, and their different concentrations influence the inflammatory response to infectious diseases. **Chapter 6** examines the association between cerebrospinal fluid levels of oestradiol, testosterone, and sex hormone-binding globulin and markers of inflammation, disease severity, and outcome in pneumococcal meningitis.

This thesis concludes with a general discussion in **chapter 7**.

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CHAPTER 2

SEX AND GENDER DIFFERENCES IN BACTERIAL INFECTIONS

Sara P. Dias, Matthijs C. Brouwer, Diederik van de Beek
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ABSTRACT

There is growing awareness of the importance of sex and gender in medicine and research. Women typically have stronger immune responses to self and foreign antigens than men, resulting in sex-based differences in autoimmunity and infectious diseases. In both animals and humans, males are generally more susceptible to bacterial infections than females. At the same time, gender differences in health-seeking behaviour, quality of healthcare, and adherence to treatment recommendations have been reported. This review explores our current understanding of differences between males and females in bacterial diseases. We describe how genetic, immunological, hormonal, and anatomical factors interact to influence sex-based differences in pathophysiology, epidemiology, clinical presentation, disease severity, and prognosis, and how gender roles affect the behaviour of patients and providers in the healthcare system.

INTRODUCTION

Patient sex is an important determinant in health and disease and infectious diseases are no exception. Biological sex (defined by sex chromosome complement, sex steroid hormones, and reproductive organs) has been shown to influence susceptibility to infection, pathophysiology, immune responses, clinical presentation, disease severity, and response to treatment and vaccination.[1] On the other hand, gender roles (referring to characteristics that are socially constructed) and social norms can influence risk factors and exposure to infection, determine health-seeking behaviours, and impact therapeutic decisions.[2] **Figure 1** describes how sex and gender interact to influence differences in bacterial infections.

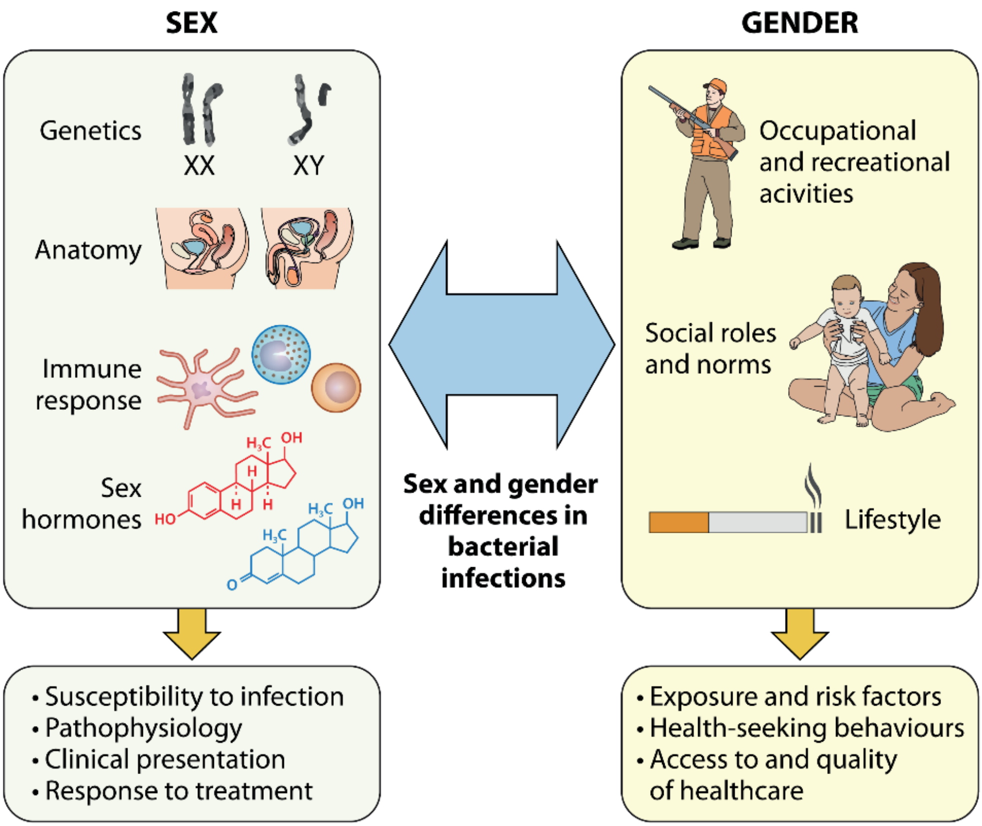

Figure 1: Interaction between sex and gender in bacterial diseases.

In this review, we discuss the current knowledge on sex-based differences in bacterial infections, focusing on genetic, anatomical, immunological, hormonal, and behavioural influences and on the epidemiology, pathophysiology, clinical presentation, resolution, and prognosis of selected bacterial diseases.

GENETIC FACTORS

Sex differences begin at conception, with the formation of an embryo carrying XX or XY chromosomes. This establishes a lifelong inequality between male and female cells in the expression of genes encoded in the X and Y chromosomes.

The X chromosome is home to around 1,100 genes and harbours several genes that regulate immune function, such as interleukin-1 (IL-1) receptor-associated kinase 1 (IRAK1), IL-2 receptor-γ chain, IL-3 receptor-α chain, IL-9 receptor, Toll-like receptor 7 (TLR7) and 8, and FOXP3.[3]

Females have two X chromosomes and one of them is randomly silenced in each cell to avoid gene overdosage.[4] This X chromosome inactivation, however, is only partial, with up to one-third of genes escaping silencing.[4] These are often expressed at higher levels in females and can be associated with sex-specific susceptibility to infection and autoimmunity. For example, TLR7 has been shown to escape chromosome X inactivation in immune cells, increasing the risk of autoimmune disease.[5]

Furthermore, because the same chromosome is not expressed in each cell, random inactivation leads to female cell mosaicism, which also provides a survival advantage.[6] Males, on the contrary, have only a single copy of their X chromosome genes, making them vulnerable to X-linked mutations. This is exemplified by X-linked primary immunodeficiencies, which make affected males susceptible to recurrent bacterial, fungal, and viral infections.[7]

In addition to evading the harmful effects of these mutations, females benefit from the added diversity when facing new immune challenges, such as invading pathogens.[8] The X chromosome is also rich in microRNAs when compared with the Y chromosome, many of which are known to affect immunity.[9] For example, miRNA-223, located in the X chromosome, controls susceptibility to tuberculosis (TB) by regulating lung neutrophil recruitment and its deletion renders mice highly susceptible to infection.[10]

The Y chromosome has the least number of genes out of all nuclear chromosomes and is significantly shorter than the X chromosome. The notion that its function is restricted to sex determination and spermatogenesis has recently been challenged by the discovery of multiple genes with extra-gonadal expression, with evidence suggesting that the Y chromosome influences immune responses in males.[11] For instance, a murine Y chromosome long arm deletion is associated with deficiencies in B cell and natural killer (NK) cell development, although the precise molecular mechanisms behind this are unclear.[12]

IMMUNE RESPONSE

In general, females have stronger innate and adaptive immune responses than males (**figure 2**).[13] These allow better pathogen clearance and response to vaccination, but also make females more prone to inflammatory and autoimmune diseases.

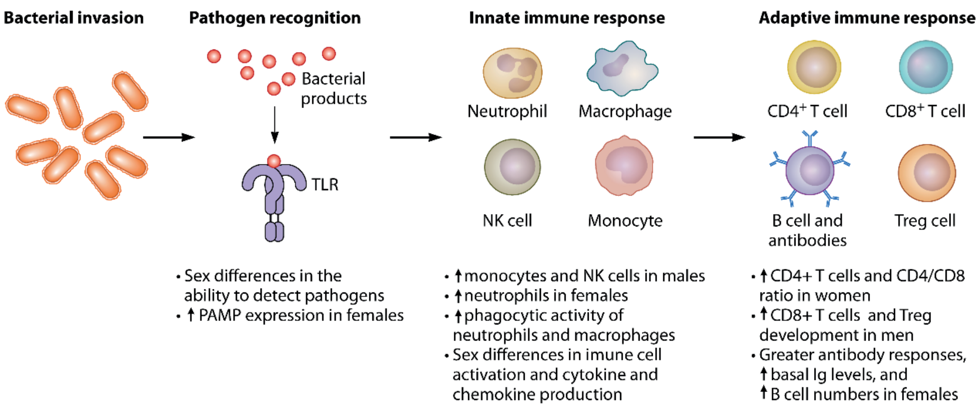

Figure 2: Sex differences in immune responses associated with bacterial infection. Ig, immunoglobulin; PAMP, pathogen-associated molecular patterns; TLR, toll-like receptor.

The innate immune system is the first line of immunological defence. There are sex-specific differences in the number and relative distribution of innate immune cells. Males have higher proportions of circulating monocytes[14] and NK cell counts,[15] whereas females have higher neutrophil counts in the peripheral blood.[16] Antigen-presenting cells (APCs) from females are more efficient in initiating a secondary response from primed lymphocytes than APCs from males, and the responsiveness of female cells to alloantigens is superior to that of males.[17]

In an *ex vivo* study, males had a stronger monocyte-derived cytokine response, such as IL-1β, tumour necrosis factor alpha (TNF-α), and IL-6 production in response to lipopolysaccharide (LPS), although these differences disappeared after accounting for differences in monocyte concentration.[18] Conversely, genes in the type I interferon (IFN) pathway are upregulated in females compared with males, which promotes enhanced responses to TLR agonists.[19]

Furthermore, there are sex differences in the ability to detect pathogens as females have higher expression of pathogen-associated molecular pattern receptors than males.[20] Compared with males, female rodent-derived resident macrophages express higher TLR2 and TLR3 levels and are more efficient at phagocytosis and bacterial killing, while also limiting excessive cytokine production and neutrophil recruitment.[21] Bone marrow-derived macrophages from female mice have a significant increase in TLR8 expression compared to male-derived cells. In addition, TLR7 expression is higher in leukocytes from women.[22] On the other hand, neutrophils from human males express higher levels of TLR4 and these are increased following activation with LPS, resulting in greater pro-inflammatory cytokine production, which may underlie increased susceptibility to endotoxic shock.[23]

Sex also impacts lymphocyte subset distribution. Females have higher absolute and relative CD4+ T cell numbers and higher CD4/CD8 ratios than males, while males have a higher percentage of CD8+ T cells.[25] Sex also influences the development of regulatory T (Treg) cells, which are higher in the peripheral blood of males.[26] In addition, there are sex differences in humoral immunity. Adult females have greater antibody responses, higher B cell numbers,[15] higher IgM and IgG levels, and lower IgA, compared with males.[27,28] In children, B cell numbers and IgG and IgM levels are comparable between sexes, but females have lower IgA levels.[27]

SEX STEROID HORMONES

Sex steroid hormones have immunomodulatory properties and changes in their levels over the life course influence the susceptibility and response to infectious diseases. These differences begin *in utero* with the formation of the testes in male embryos. Once formed, they begin to secrete androgens that cause masculinization and lead to the early development of androgen-dependent sex differences in immunity.[29-31] After puberty, concentrations of oestrogens and progesterone (P4) in females and androgens in males rise significantly. During this period, there is generally a male bias in infectious diseases, with males more frequently and more severely affected by bacterial, viral, and parasitic infections, whereas females are more affected by autoimmune disease.[32] Differences are also evident during pregnancy, when an increase in the levels of oestrogen and, in particular, P4 promote a state of immune tolerance, making pregnant women more susceptible to many infectious diseases.[33] With menopause, oestrogen and P4 levels drop rapidly in women, while a gradual decline in androgen levels is observed in aging males.[1]

Sex steroids can influence immune responses by binding to specific receptors expressed in immune cells, including lymphocytes, macrophages, and dendritic cells (DCs),[13] and can also have a direct effect over bacterial metabolism, growth, and expression of virulence factors.[34]

Oestrogen

Oestrogens are present in both sexes, but are highest in females of reproductive age. The principal endogenous biologically active oestrogens are oestrone (E1), oestradiol (E2), and oestriol (E3), the latter being the main pregnancy oestrogen.[35] In females, levels vary during the menstrual cycle. They are low before puberty and after menopause and high during pregnancy.

Oestrogen receptors (ERs) are ubiquitous in the immune system, and oestrogen signals through two different nuclear receptors: ER alpha (ERα) and ER beta (ERβ).[36] Expression of ERs is influenced by age and sex. Monocytes from premenopausal women express ERα at lower levels than monocytes from men and postmenopausal women, whereas no difference was found in ERβ.[37] Furthermore, monocytes from men and postmenopausal women contain significantly more ERα compared with ERβ, suggesting that monocytes from these two groups may respond similarly to oestrogens.[37] On the other hand, ER expression is similar in T or B cells and in plasmacytoid DCs from both sexes.[37,38] *In vitro*, ERα signalling stimulates differentiation of DCs from monocytes, which produce pro-inflammatory cytokines in response to TLR stimulation. ER signalling also promotes the TLR-driven production of type I interferons (IFNs) in mouse plasmacytoid DCs *in vivo*.[39] In humans, treatment of postmenopausal women with E2 markedly enhances production of IFN-α by plasmacytoid DCs.[40]

E2 can augment or dampen immune signalling pathways and enhances both cell-mediated and humoral immunity in a concentration-dependent fashion. Low E2 concentrations promote a Th1-type response, boost cell-mediated immunity, and stimulate type I IFN responses and production of pro-inflammatory cytokines and chemokines, including IL-1β, IL-6, and TNF-α.[41,42] E2 at high concentrations promotes Th2-type and humoral responses, inhibits pro-inflammatory pathways and promotes production of anti-inflammatory cytokines, such as IL-4 and IL-10.[41,42] Numbers of antibody-secreting cells have been reported to be significantly higher during the peri-ovulatory period in female rhesus macaques.[43] Treatment of mice with physiological levels of oestrogen results in retention of high-affinity autoreactive B cells, interfering with tolerance induction.[44] On the other hand, E2 increases immunoglobulin class-switch recombination and somatic hypermutation in germinal centres. These changes lead to an improved response to vaccination but also an increased propensity to autoimmune diseases in women.[45]

E2 stimulates the expansion of Treg cells[46] which are higher during the follicular phase of the menstrual cycle.[47] Oestrogens also reduce the proliferation of immature T lymphocytes and induce thymic involution in mice.[48]

Oestrogen has been reported to have a protective effect in several infections, such as *Vibrio vulnificus*, which mostly affects males. In a rat model, ovariectomy was associated with increased mortality, and oestrogen replacement decreased mortality in both gonadectomised sexes.[49] In contrast, in females with cystic fibrosis, oestrogen induces conversion of *Pseudomonas aeruginosa* into the more virulent mucoid form, and the majority of infectious exacerbations occurs at times of high circulating E2 levels.[50]

Progesterone

P4 is produced by the corpus luteum in the ovaries during the menstrual cycle and by the placenta during pregnancy. Progesterone receptors (PRs) are present in a variety of cell types, including immune cells such as NK cells, macrophages, DCs, and T cells.[51] There are sex differences in PR expression, which can explain sex-based disparities in immune responses. For instance, the expression of PR is higher in female than in male DCs, which could justify the differential suppressive effect of P4 on these cells in female *vs* male rats.[33,52]

P4 modulates the immune system in order to achieve a successful pregnancy. Increased maternal P4 levels promote a Th2-dominant cytokine phenotype,[53,54] causing an increase in anti-inflammatory cytokines such as IL-4, IL-5, and IL-10[55-57] and a decrease in pro-inflammatory cytokines, such as TNF-α, IFN-γ, and IL-1β.[58,59] P4 also increases the number of Treg cells and inhibits Th17 cells.[60,61] P4 inhibits maturation of DCs and DC-mediated proliferation of T cells, favouring immature DCs that have a tolerogenic phenotype.[58] This state of immune tolerance, while avoiding fetal rejection, makes the susceptibility and severity of many infections higher during pregnancy.[33] Pregnant women are much more susceptible to *Listeria monocytogenes* infection than similarly-aged healthy adults,[62] and P4 increases susceptibility to *Chlamydia trachomatis* in female rats.[63] In contrast, P4 at high doses inhibits the growth of *Neisseria gonorrhoeae* and *N. meningitidis*[64] and the germination of *Clostridioides difficile* spores.[65]

Androgens

Androgens occur in higher concentrations in post-pubertal males than in females.[13] Testosterone is the principal androgen secreted from the testes in males and in small quantities from the ovaries in females. The androgen receptor works as a steroid hormone–activated transcription factor, which signals through ligand-dependent and independent signalling pathways.[66] Both testosterone and its metabolite, dihydrotestosterone (DHT), generally have suppressive effects on both humoral and cellular immune responses, leading to decreased T and B cell proliferation and decreased immunoglobulin and cytokine production.[3]

DHT-treated female mice produce more IL-10 and less IL-12 than untreated female mice and DHT can act on CD4+ T lymphocytes to increase IL-10 gene expression via androgen receptor signalling,[67] thereby promoting an anti-inflammatory response. Treatment of LPS or TNF-α-stimulated human endothelial cells with testosterone controls the inflammatory response mediated by NF-κB.[68] In males with symptomatic androgen deficiencies, treatment with testosterone lowers pro-inflammatory cytokines (such TNF-α, IL-1β, and IL-6) and increases anti-inflammatory cytokines (such as IL-10).[69] Testosterone deficiency in males is associated with increased CD4+ counts and CD4/CD8 ratios, higher immunoglobulin levels, and increased B cell numbers compared with controls, and these changes are reversed by hormonal replacement.[70,71]

In mice, testosterone decreases the expression of TLR4 in macrophages.[72] Testosterone suppresses uropathogenic *E. coli* (UPEC) invasion and colonization by inhibiting the JAK/STAT1 signalling pathway in a prostatitis cell model,[73,74] and also inhibits the expression of pro-inflammatory IL-1β, IL-6, and IL-8 cytokines.[74] Male patients with TB show impaired production of gonadal androgens with lower levels of testosterone when compared with healthy controls.[75]

GENDER

Gender-related occupational and recreational activities can affect exposure to pathogens. Women are more likely to assume caretaking roles, making them more exposed to childhood diseases.[76] On the other hand, men wash their hands less often than women.[77] Occupational exposure to animals plays a role in male bias in brucellosis[78] and Q fever,[79] while male-predominant mine-related silicosis is a risk factor for TB.[80]

Access to care also differs between men and women. In some countries, there is a parental preference for boys over girls. Studies in Bangladesh have shown parents are more likely to bring their male children to the hospital for pneumonia or diarrhoea than their female counterparts, and girls have a longer delay to diagnosis, a more severe illness on admission, and higher in-hospital mortality.[81,82] In adults, sociocultural and religious norms can also constrain access to healthcare, and poverty and stigma are important factors in limiting access to care for women in low-income countries. Furthermore, men consistently use more intensive care unit (ICU) resources and are more likely to be admitted to an ICU and receive advanced life-supporting measures than women.[83]

SEX AND GENDER DIFFERENCES IN BACTERIAL DISEASES

Many bacterial pathogens exhibit a sex preference and most of them show a male bias (**table 1**). **Figure 3** summarises differences in the incidence and severity of bacterial diseases across different organ systems.

Gastrointestinal tract infections

Bacterial gastrointestinal infections are a leading cause of illness and death globally and are generally more common and more severe in males.[84] This is partly explained by behavioural differences as men are more likely than women to practice food-handling, preparation, and consumption behaviours that carry a high risk of foodborne diseases.[85] Furthermore, differences in the immune response place males at higher risk of poor outcomes, and sex hormones also play an important role.

Table 1. Sex bias by specific bacterial species.

Bacterial species	Bias	Sex and gender-based risk factors	References
Escherichia coli	Female	Food consumption and handling practices, anatomical differences	247
Streptococcus pneumoniae	Male	Smoking, alcohol use	129
Legionella pneumophila	Male	Smoking, travel	133-136
Mycobacterium tuberculosis	Male	Occupational (e.g., mining), smoking, travel	143
Clostridioides difficile	Female	Antibiotic prescription, exposure to infants	97,98,101
Campylobacter spp	Male	Food-handling practices	248,249
Helicobacter pylori	Male	Smoking, low oestrogen	87,88
Listeria monocytogenes	Young women, elderly men	Pregnancy, waning cellular immunity	62,109,110
Leptospira spp	Male	Working outdoors or with animals	250,251
Francisella tularensis	Male	Outdoor activities, contact with animals	252
Borrelia burgdorferi	Male predominance in the U.S.A., female in Europe	Outdoor activities	222-225
Coxiella burnettii	Male	Contact with animals	79,234,235
Brucella spp	Male	Contact with animals, food consumption habits	78,253
Chlamydia trachomatis	Female	Screening bias	182,183
Neisseria gonorrhoeae	Male	High-risk sexual behaviours	183,191,254
Treponema pallidum	Male	High-risk sexual behaviours	145,254,255

Helicobacter pylori

Helicobacter pylori infection is highly prevalent worldwide and is the strongest risk factor for stomach cancer.[86] Infection has a slight male bias[87,88] and males exhibit more severe inflammation, atrophy, and intestinal metaplasia scores compared with females.[89] Gastric cancer is twice as common in men as in women.[86]

Epidemiological evidence and animal studies suggest a protective effect of female sex hormones, namely oestrogen. A longer fertility window and use of oral contraceptives or hormone replacement therapy are associated with a lower risk of gastric cancer.[90] Transgenic hypergastrinemic mice infected with *H. pylori* develop gastric carcinoma in a male-predominant fashion[91] and oestrogen supplementation, but not castration, attenuates gastric lesions.[92] Ovariectomised mice develop significantly more severe *H. pylori*-induced gastritis and gastric cancer, and E2 supplementation has a protective effect.[93]

On the other hand, female sex is associated with clarithromycin and metronidazole resistance[94] and *H. pylori* eradication failure.[95]

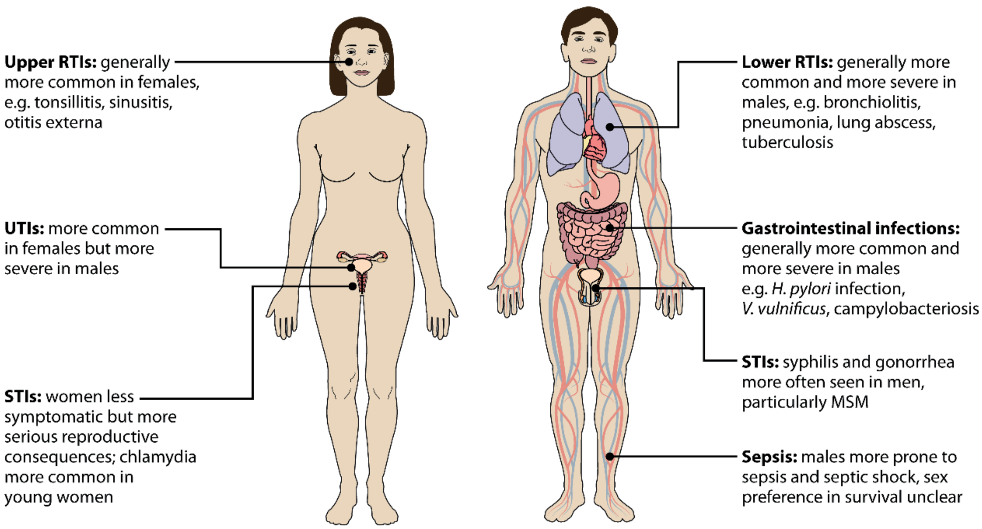

Figure 3: Sex and gender bias in bacterial infections.
Abbreviations: MSM, men who have sex with men; RTIs, respiratory tract infections; STIs, sexual transmitted infections; UTIs, urinary tract infections

Clostridioides difficile

C. difficile is a major cause of healthcare-associated infection, although community-acquired cases are increasingly reported.[96] *C. difficile* infection (CDI) is more common in females, who account for 55-60% of cases.[97,98] In the United States, females account for 67% of community-acquired CDI,[99] potentially due to more frequent antibiotic prescription in women.[100] In addition, traditional gender roles result in women generally having more exposure to infants, which is a risk factor for community-acquired CDI.[99] Women also account for the majority of hospital-acquired cases and are responsible for 55% of healthcare-associated CDI in Europe.[101] Furthermore, females have an increased risk of recurrent CDI[102] and severe cases have been reported in pregnant and peripartum women.[103,104] On the other hand, male sex is an independent predictor of mortality.[105]

Listeriosis

L. monocytogenes is a foodborne pathogen that can cause septicaemia and meningitis as well as fetal infection or abortion in pregnant women.[106] Pregnant women are about 20 times more likely to contract this infection compared to the general population[62] due to suppressed cellular immunity and the placental tropism of *L. monocytogenes*.[106] In pregnant women and mice, increased P4 weakens CD8+ T memory cell-mediated IFN-γ responses, which are crucial to host defence against listerial infection.[59] Treatment of female mice with E2 decreased IL-12,TNF-α, and IFN-γ and increased IL-4 and IL-10 expression.[107] Oestrogen also depressed monocyte and lymphocyte accumulation at infective foci and increased mortality of female mice.[108]

Incidence rates of invasive listeriosis are higher in females than in males during reproductive years (likely reflecting pregnancy-related listeriosis). In contrast, in older age groups, rates are 2–4 times higher in males,[109,110] with similar case fatality rates.[109] In mice, however, infection with *L. monocytogenes* led to significantly higher lethality rates and bacterial numbers in females compared with males.[111]

Respiratory tract infections

Generally, males are more susceptible to respiratory tract infections (RTIs) and have a more severe disease course and higher mortality compared with females. Males are more affected by lower RTIs, such as pneumonia, bronchiolitis, or lung abscess, while females more often develop upper RTIs, such as sinusitis, tonsillitis, and otitis externa.[112] However, there are some exceptions. Males are more often affected by otitis media[113] and mastoiditis,[114] whereas pertussis has higher incidence rates in females.[115]

Anatomical factors can explain some of these differences. For instance, peripheral airways are narrower in the first year of life in males, which may predispose them to lower RTIs.[116] On the other hand, after puberty, males have significantly larger central airway luminal areas than females, independently of height.[117] This could explain why in cystic fibrosis (which affects prepubescent males and females equally), post-pubescent females have an increased rate and severity of exacerbations and have a more rapid decline in lung function after colonization with *P. aeruginosa* compared with males.[118] It has also been suggested that females have smaller ostia making them more susceptible to sinus obstruction and infection.[119]

Otitis media

Middle ear infections are a leading cause of medical visits and antibiotic prescription in infants and preschool aged children. Acute otitis media is more common in boys than in girls,[113,120] and children with more severe disease are more often males.[121] Studies also show that male sex is a risk factor for recurrent infection,[113] as well as a predictor of chronic otitis media.[122]

The reasons for these differences are not well understood, however it has been proposed that abnormal pneumatisation of the mastoid process (with a smaller mastoid cell air system in boys compared with girls), could result in more frequent and severe ear infections in male children.[123]

Pneumonia

Pneumonia is a leading cause of hospitalization and death worldwide, and all types of bacterial pneumonia are more common in males.[124] In community-acquired pneumonia, male sex is significantly associated with hospitalization and death, with males 1.3 times more likely to die compared with females.[125,126] Community-acquired pneumonia is also more common in boys than in girls,[127] and male sex is associated with bacteraemia in children.[128]

Streptococcus pneumoniae is the most common bacterial pathogen in both sexes. Pneumococcal pneumonia and invasive pneumococcal disease are more frequent in males compared with females,[129] and male sex is associated with mortality in bacteraemic pneumococcal pneumonia.[130] Although older (over 50 years) females generally have lower antibody responses to pneumococcal vaccines than males,[131] the 23–valent pneumococcal vaccine is more effective to prevent hospitalizations caused by *S. pneumoniae* in women.[132] Legionellosis is also more frequently notified in males, with male:female ratios of 1.7–5 reported in Europe, the U.S., Australia, and Japan.[133-136]

Hospital-acquired pneumonia is also more common in men,[124,137] and male sex is a risk factor for aspiration pneumonia in older patients.[138] Furthermore, males are 1.6 times more likely to develop ventilator-associated pneumonia,[139] although women have a more severe disease and higher mortality.[124,140]

Animal models suggest sex hormones are involved in pneumonia caused by different pathogens. In some instances, oestrogen appears to have a protective role. In a mouse model of pneumococcal pneumonia, E2 promoted control of macrophage inflammatory activity and resolution of lung inflammation.[141] In contrast, in a murine model of *Acinetobacter baumannii* pneumonia, female mice were more susceptible to infection, and treatment of male mice with E2 increased their susceptibility.[142]

Tuberculosis

TB is the leading cause of death from a bacterial disease among adults worldwide. TB rates are significantly higher in men than in women. According to the World Health Organization, men accounted for 56% of all TB cases in 2020 *vs* 33% in women, with children accounting for the remaining 11%.[143] The reasons for this bias, however, are not entirely clear. It has been proposed that it could result from systematic underreporting and underdiagnosis of TB in women. Women may be less likely to seek appropriate medical care[144] and present difficulties in diagnostic testing, such as poorer-quality sputum samples.[145] In addition, men undergo chest imaging sooner and are more likely to have a sputum smear sample performed.[146] However, male bias persists when survey prevalence, rather than notification rates, are analysed,[143] and male predominance is seen even in low-burden countries where differences in access to healthcare should be negligible.[80]

Both gender and sex-related factors play a role. Men have more social contacts and more often participate in activities that place them at higher risk for TB, such as travelling, smoking, drinking, spending time in settings conducive to transmission (e.g., bars), and engaging in hazardous careers (e.g., mining).[80] However, other risk factors, such as household contacts and human immunodeficiency virus (HIV) infection, are not male-biased.

Both human and animal studies have shown protective immune responses against *Mycobacterium tuberculosis* are largely mediated by CD4+ Th1 cells, which secrete IFN-γ, and this response is mediated by IL-12.[147] However, excessive inflammation can exacerbate lung infection and lead to early death. In a mouse model, elevated *M. tuberculosis* loads in males were associated with an early exaggerated pulmonary inflammatory response resulting in accelerated disease progression and increased mortality.[148] B cells also play a role, and smaller B cell follicles have been reported in male compared with female lungs in mice and are associated with greater disease progression.[149]

Male bias does not appear until puberty, suggesting a role for sex steroid hormones. Female and castrated male mice express significant higher TNF-α, IFN-γ, and IL-12 than non-castrated males,[150] and their treatment with testosterone increases susceptibility to *M. intracellulare* and *M. marinum* infection.[151] Conversely, oestrogen appears to have a protective role, as ovariectomised mice have a higher susceptibility to *M. avium*, which is lessened by treatment with E2.[152] This is paralleled in humans by postmenopausal women, who are more susceptible to *M. avium* complex disease.[153]

Certain X-linked gene mutations and polymorphisms confer increased risk of TB. Mutations in CYBB result in X-linked chronic granulomatous disease in males and increase susceptibility to mycobacterial disease,[154] and TLR8 polymorphisms are linked to susceptibility to tuberculosis in males.[155]

From a clinical standpoint, women are usually less symptomatic than males. Men are more likely to be smokers, have more comorbidities, and present with haemoptysis, weight loss, and pleural effusion.[156] Men also have more advanced radiological findings than women[157] and begin treatment earlier[146] and in a prospective observational study, male sex was associated with worse treatment outcomes.[156] Males also have higher treatment dropout rates[158] and are at higher risk of recurrence.[159]

For unknown reasons, female sex is a risk factor for developing extra-pulmonary TB; studies in the United States[160] and in Nepal[161] found women to be 1.7 times more likely to develop extra-pulmonary TB relative to males. In addition, a prospective cohort study in eight countries showed significantly more women than men had extensively drug-resistant TB.[162]

Urinary tract infections

Urinary tract infections (UTIs) also exhibit a sex-based preference, with a bias towards women. Aetiology is influenced by patient sex, as *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus agalactiae* are more frequently isolated in females than in males, while the opposite is true for *Enterococcus faecalis*, *Proteus mirabilis*, and *P. aeruginosa*.[163] The most common causative agent in both sexes is UPEC.[164]

Male UTI shows a bimodal distribution at the extremes of age, whereas the burden of infection in women is durable over a lifetime.[164] During the first few months of life, the incidence of UTI in boys exceeds that in girls,[165] but afterwards females of all ages are more prone to UTIs than males, and around half of all women will experience at least one UTI during their lifetime.[166] This gap significantly decreases with age as the incidence of benign prostatic hyperplasia, urinary retention, and incontinence increases in the male population.

The increased female susceptibility to UTI is due to several factors. The female urethra is shorter than its male counterpart, which has been proposed to make it easier for ascending bacteria to reach the bladder.[167] Physical proximity of the urethral opening to the rectum and vagina is another important risk factor as it can lead to colonization of the periurethral mucosa with enteric bacteria,[168] and vaginal dysbiosis is associated with an increased risk of UTI.[169] A dryer environment at the urethral opening and the anti-bacterial properties of prostate secretions are additional protective features in men.[170]

While more common in women, UTIs are more persistent and have a higher morbidity and risk of complications in men. Other organs, namely the prostate, are often involved[170] and male UTIs are usually treated with antibiotics for a longer period compared with female UTIs.[171] In UPEC-infected mice, more males than females are unable to clear bacteria and remain chronically infected, and male mice more frequently develop advanced pyelonephritis and kidney abscesses compared with females.[172,173] Furthermore, there is a strong and rapid increase in pro-inflammatory cytokine expression in female mice that is not observed in males and there is a larger infiltration by immune cells,[172,173] which may contribute to better bacterial clearance.

Sex hormones are also likely involved. Treatment of UPEC-infected female mice with testosterone leads to persistent bacteriuria and chronic cystitis,[172] and castrated male mice have a significantly lower bacterial burden than sham-operated controls.[173] After menopause, a decrease in oestrogen levels contributes to physiologic and structural changes that increase the risk of UTI in postmenopausal women, such as reduced urinary flow, increased postvoid residual volume, and incontinence[174] along with a rise in vaginal pH, loss of commensal lactobacilli, and increased vaginal colonization by enteric organisms.[167] Furthermore, randomised controlled trials have shown vaginal oestrogen administration reduces UTI recurrence rates in postmenopausal women.[175]

Sexually transmitted infections

Despite being curable, bacterial sexually transmitted infections (STIs) are associated with a significant burden of disease. STI-related morbidity disproportionately affects women, with important implications for women of reproductive age.

In many societies, more restrictive sociocultural norms regarding sexual behaviour in women may limit their sexual freedom, restrict their access to information, and reduce their ability to practice safe sexual behaviours.[176] Male-to-female transmission of STIs is also thought to be more efficient than female-to-male transmission, possibly due to retention of the infected ejaculate within the vagina and greater tissue injury during intercourse.[177]

In addition, STIs are more often asymptomatic in women than in men. Undiagnosed and untreated STIs can result in important long-term reproductive complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility.[178,179] Furthermore, infections in pregnant women are associated with maternal morbidity as well as adverse fetal and perinatal outcomes.[180]

Chlamydia

Chlamydia is the most common bacterial STI globally.[181] Persons between 15 and 24 years report the highest infection rates, and young women are twice as affected as men,[182,183] although this partly reflects screening programs that primarily target women.

The infection is asymptomatic in a large proportion of cases in both sexes, especially in women,[184] but if left untreated can cause severe damage, particularly to the female reproductive tract, and chlamydia is an important cause of PID.[178] In men, urethritis can be complicated by epididymitis and male infertility.[185] *C. trachomatis* is the most common genitourinary trigger of reactive arthritis, and Chlamydia-induced arthritis is most often seen in men.[186]

The mechanisms by which sex hormones affect *C. trachomatis* infection are not entirely clear. The likelihood of developing chlamydial or gonococcal salpingitis has been reported to be highest during the oestrogen-dominant proliferative phase of the menstrual cycle,[187] and a positive correlation was shown between chlamydial load and E2 levels in women.[188] *In vitro* studies have also demonstrated oestrogen enhances chlamydial adherence and intracellular development.[189] In contrast, other studies have found increased detection of *C. trachomatis* during the secretory phase when P4 is higher.[190]

Gonorrhoea

Gonorrhoea is the second most common bacterial STI[181] and rates of reported infections continue to increase, particularly among men. Rates are highest among adolescents and young adults, and men – especially men who have sex with men (MSM) – are currently more often affected than women in high-income countries.[183,191] In 2018, the male-to-female ratio was 3.2 in Europe and 1.4 in the United States.[183,191]

Urethritis is the most common manifestation of gonococcal infection in men, whereas the endocervical canal is the primary infection site in women.[192] Most women show no symptoms of infection[184] while males are often symptomatic.[193] Rectal gonorrhoea occurs in both sexes and is usually asymptomatic in women, whereas cases in MSM can be associated with complaints of overt proctitis.[194] Complications in men include epididymitis, infertility, prostatitis, and seminal vesiculitis.[192] Similarly to chlamydial infection, PID is the main complication of gonorrhoea in women.[178] Disseminated gonococcal infection is the most common systemic complication in both sexes, and probably occurs more frequently in women.[192] Oestrogen likely plays a role, as E2-treated mice show an enhanced susceptibility to disseminated gonococcal infection.[195]

The molecular mechanisms used by the gonococcus to initiate infection, and the resulting inflammatory response, also differ between sexes. In men, interaction with the urethral epithelial cells triggers the release of pro-inflammatory cytokines, promoting an inflammatory response and contributing to the symptomatic nature of gonococcal disease in men.[196] Similarly, ascending gonococcal infection of the uterus and fallopian tubes also results in inflammation. In contrast, gonococcal cervicitis is mostly asymptomatic, as the gonococcus is able to evade host immune function by subverting the alternative pathway of complement and does not elicit strong immune responses during uncomplicated genital infections in women.[196]

Emergence of gonococcal antimicrobial resistance is a major public health threat, and a study found men infected with *N. gonorrhoeae* had a 4-fold higher expression of gonococcal antimicrobial resistance genes compared with women,[197] which could have implications for sex-specific treatment.

Sepsis

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection[198] and is a major global health problem.[199] If not identified and treated promptly, it can lead to septic shock, multiple organ failure, and death.[198] The most common source is the respiratory tract in males and the urinary tract in females.[200,201] Most studies report higher rates of sepsis and septic shock in males, who account for 55 to 64% of cases,[202-204] and male sex has been identified as a predictor of sepsis after trauma[205] and surgery.[206] Experimental studies have consistently shown a survival advantage and a protective effect of sex hormones in females. In humans, however, reports on sex and mortality have shown conflicting results. Some have found higher mortality in women,[201,207] others in men,[200,208] whereas others reported no differences.[209,210]

In animal models, oestrogen exerts a protective effect by maintaining adequate immune responses and cardiac function. Ovariectomised females show depressed macrophage and splenocyte functions after trauma-haemorrhage, which are associated with significantly increased mortality from subsequent sepsis,[211,212] and addition of E2 normalises immune functional capacities.[212] Sepsis-induced cardiac dysfunction is also less pronounced in female than in male mice.[213] In contrast, in humans, circulating E2 levels are increased in non-survivors compared with survivors.[214] Testosterone levels are generally low in male patients with sepsis,[215] and androgen depletion appears to be protective in animals. Testosterone receptor blockade after trauma-haemorrhage in male mice restores the depressed immune functions and improves survival following subsequent sepsis.[216] Males also show an inappropriate inflammatory response to sepsis and produce significantly higher levels of pro-inflammatory cytokines (including TNF-α, IL-6, IL-8, IL-1β, and procalcitonin) following endotoxemia induction or sepsis than females,[23,217,218] which could render them more susceptible to septic shock.

Sex-based differences in healthcare have also been reported. Women experience significantly longer delays to initial antibiotic administration than men,[219,220] and in a nationwide cohort study, a complete 1h emergency department sepsis care bundle was fulfilled 38% more often in men.[220] In the UCI, women are less likely to receive deep venous thrombosis prophylaxis, haemodialysis catheters, invasive mechanical ventilation,[201,207] or vasopressor support,[209] and have a shorter length of stay[208] compared with men.

Other infections

Lyme borreliosis

Lyme borreliosis is the most common human vector-borne infection in Europe and the United States.[221] It has a slight male predominance in the United States, around 57% of cases being male,[222] presumably due to higher occupational risks and outdoor recreational activities. However, in Europe, 55-60% of affected patients are female.[223-225] Furthermore, females have been reported to attract 33% more tick bites than males, despite spending less time outdoors.[226]

One study in Sweden found erythema migrans in women was less likely to have the classic "bull's eye" appearance, and the duration from treatment until disappearance of the lesion was significantly longer in women than men.[226]

In the United States, 70% of patients with Lyme carditis and 60% of cases of Lyme arthritis are male.[222] A retrospective study in Slovenia reported 75% of patients with Lyme arthritis to be men, and men accounted for 60% of cases of neuroborreliosis.[227] Acrodermatitis chronica atrophicans, a late cutaneous manifestation of Lyme disease, was more common in females, who represented nearly 70% of cases.[227] Women may also be at higher risk of developing post-treatment Lyme disease syndrome.[228] Reinfection rates are higher in women, particularly postmenopausal women,[229] which could be due to falling oestrogen levels and differences in the immune response.[230]

Sex also impacts diagnosis, as the recommended two-tier testing is male biased. The magnitude of enzyme-linked immunosorbent assay (ELISA) and IgG serologic responses is larger in men,[231] and men have on average six reactive bands on the IgG immunoblot, whereas women have only four.[232] Current Centers for Disease Control and Prevention criteria require five bands for a positive test, likely underestimating the true number of female cases.[232]

Q fever

Q fever is a zoonosis caused by *Coxiella burnetii*.[233] Seroprevalence is higher in men, a study in Australia reporting a male-to-female ratio around 1.6.[79] An even greater proportion of men are diagnosed with the disease (sex ratio of 2–5),[234,235] suggesting men more often develop symptomatic Q fever than women.[236] In contrast, boys and girls are almost equally represented,[233] suggesting sex hormones could be involved.

In *C. burnetii*-infected mice, bacterial load and granuloma numbers were lower in intact females than males and ovariectomised females, and treatment with E2 reduced bacterial load and granuloma numbers in ovariectomised mice.[237] P4 inhibits *C. burnetii* replication in infected placenta-derived cells,[238] and bacterial loads increase towards parturition[239] when P4 levels decrease. However, both animals and humans exhibit an increased risk of persistent infection and unfavourable outcomes during pregnancy,[233] likely due to impaired cellular immunity.

Meningitis

Bacterial meningitis is an infection of the membranes that cover the brain and spinal cord caused by a bacterial pathogen. *S. pneumoniae*, *N. meningitidis*, and *Haemophilus influenzae* are the most frequently isolated bacteria. Some studies have reported similar rates of bacterial meningitis in men and women while others have found a slight male bias.[240-243] Male sex has been identified as a predictor of poor outcome in children[244,245] and adults,[240,243] despite females having a higher disease severity and higher inflammation markers on admission.[240] This may be in part related to a better female response to anti-inflammatory treatment with corticosteroids.[246] Sex steroid hormones may also play a role.

CONCLUSIONS

Many bacterial infections exhibit sex and gender differences in pathophysiology, incidence, clinical presentation, disease course, response to treatment, and outcome. Both biological and gender factors come into play and their recognition is essential to improving patient care. Behavioural differences play an important role in the exposure to pathogens, whereas sex differences in the immune response are directly influenced by sex chromosome complement and concentrations of sex steroid hormones.

Nevertheless, these observations have not been systematically integrated into research practices or resulted in changes to medical guidelines, which are mostly not sex-specific. This needs to change, and funding agencies and medical journals should promote scientific research that is sex-conscious and provides sex-disaggregated data. Incorporating implementation science methods to translate existing evidence into sex-specific guidelines is essential to promote an improved and more personalised patient care.

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CHAPTER 3

SEX-BASED DIFFERENCES IN ADULTS WITH COMMUNITY-ACQUIRED BACTERIAL MENINGITIS: A PROSPECTIVE COHORT STUDY

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ABSTRACT

Objectives: To investigate sex-based differences in clinical features, causative pathogens, outcome and treatment of adult community-acquired meningitis.

Methods: From January 2006 to July 2014, adults with community-acquired bacterial meningitis were prospectively evaluated in a nationwide cohort study in the Netherlands. Sex was analysed along with known predictors of unfavourable outcome using logistic regression.

Results: We evaluated 1412 episodes of meningitis, 707 (50%) in males. Men more often presented with a history of remote head injury (41/667 [6%] *vs* 14/658 [2%] females, p=0.0002) or alcoholism (61/652 [9%] *vs* 21/660 [3%] females, p<0.0001). Neck stiffness was less common in males (453/651 [70%] *vs* 524/671 [78%] females, p=0.0004). Despite greater illness severity, women were less likely to receive treatment in an intensive care unit (odds ratio [OR] 0.72 95% CI 0.58–0.89, p=0.003) or mechanical ventilation (OR 0.67 95% CI 0.54–0.85, p=0.001). Female patients exhibited higher serum inflammatory parameters than males (median C-reactive protein 211 *vs* 171, p=0.0001; median erythrocyte sedimentation rate 48 *vs* 33, p<0.0001). Corticosteroids improved prognosis in both sexes, but absolute risk reduction was higher in women (20% vs 15%, p=0.001), although we found no significant interaction between sex and dexamethasone (p=0.38). In the multivariable analysis, male sex was an independent predictor of unfavourable outcome (OR 1.34, 95% CI 1.03–1.75, p=0.03) and death (OR 1.47, 95% CI 1.04–2.07, p=0.03).

Conclusions: Male sex is an independent risk factor for adverse outcome. It is possible that sex-based differences in immune reaction could determine a distinct response to corticosteroids.

INTRODUCTION

Sex-based differences in infectious diseases have been increasingly recognised, with men and women showing differences in susceptibility to pathogens,[1] carriage rates,[2,3] immune responses to illness[4] and vaccination,[5] manifestations and severity of disease,[4-6] and response to treatment.[7] The underlying mechanisms are likely multifactorial, and involve a complex interplay between social, behavioural, and biological influences.[8] Females exhibit more robust cell-mediated and humoral immune responses to antigenic challenges, such as infection and vaccination, compared with males,[4] which leads to a lower susceptibility to many infectious diseases.[1] This enhanced immune response, however, can also have deleterious effects due to an excessive inflammatory reaction.[4,8]

Bacterial meningitis is an important health problem worldwide, and despite advances in prevention and treatment, it remains a significant cause of morbidity and mortality in adults.[9] Experimental animal models have shown outcome is related to severity of inflammation in the subarachnoid space,[10] which is the rationale for treatment with corticosteroids. A randomised clinical trial and meta-analyses showed dexamethasone improves outcome of adults with community-acquired bacterial meningitis.[11-14]

Clinical features and prognostic factors of bacterial meningitis have been described previously,[15,16] but the impact of patient sex remains largely unknown. We performed an exploratory study prospectively investigating sex-based differences in adults with community-acquired bacterial meningitis.

METHODS

Study population and procedures

In a nationwide prospective cohort study, we identified adults (defined as patients over 16 years) with bacterial meningitis listed in the database of the Netherlands Reference Laboratory for Bacterial Meningitis from January 1st 2006 to July 1st 2014. This laboratory receives cerebrospinal fluid (CSF) isolates from around 85% of patients with bacterial meningitis in the Netherlands (population 17 million).[17] This cohort is included in an ongoing prospective genetic association study (MeninGene),[18] parts of which have been reported previously.[16,19-24] The laboratory provided daily updates with the names of hospitals where patients with bacterial meningitis had been admitted in the preceding two to six days and their attending physicians. In addition, physicians were informed about the study by telephone and could contact investigators 24/7 to include patients without preceding report from the laboratory. Written informed consent was obtained from all participants or their legally authorised representatives. Online case-record forms were used to collect data on patients' history, symptoms and laboratory findings on admission, clinical course, outcome, neurologic findings upon discharge, and treatment. Before the study, all Dutch neurologists received information about it, followed by periodic reminders.

Bacterial meningitis was defined as a bacterial pathogen cultured in the CSF, or the combination of a positive CSF polymerase chain reaction (PCR) or antigen test for *Streptococcus pneumoniae* or *Neisseria meningitidis* with at least one specific CSF finding predictive of bacterial meningitis (Spanos criteria: glucose level under 34 mg/dL, CSF:blood glucose ratio lower than 0.23, protein over 220 mg/dL, a white-cell count higher than 2000/mm^3 or more than 1180 polymorphonuclear leucocytes/mm^3).[25] Episodes of hospital-acquired meningitis, defined as bacterial meningitis occurring during hospitalization or within one week of discharge,[26] were excluded, as were patients with head trauma or neurosurgery in the previous month and those with a neurosurgical device or missing outcomes.

Patients with a history of splenectomy, diabetes mellitus, alcoholism, human immunodeficiency virus (HIV) infection, or immunosuppressive treatment were considered immunocompromised. Neurological examination was performed on admission and discharge. Illness severity was scored using the Dutch Meningitis Risk Score developed by Weisfelt and colleagues.[27] Systemic complications were defined as hemodynamic shock, respiratory failure, or need for intubation. Development of newly impaired consciousness during admission, focal neurologic deficits, seizures, sinus thrombosis, and cerebrovascular accidents were considered to be neurological complications. Outcome was classified using the Glasgow Outcome Scale (GOS),[28] as assessed by the patient's physician; favourable outcome was defined as a GOS score of 5, and unfavourable outcome as a score of 1–4.

Statistical analysis

Continuous variables are expressed as median (interquartile range [IQR]), and were compared using the Mann–Whitney *U* test; the Chi-Square test or Fisher exact test, as appropriate, were used to study categorical variables. Treatment effects are expressed as relative risk (RR) for treated *vs* untreated patients (with a RR<1.0 indicating a beneficial effect). Where appropriate, patients were stratified by sex and predefined age groups for analyses, and adjustments were made for known confounders based on previous studies. All statistical tests were two-tailed, with significance set at 0.05. For univariable testing, the threshold for statistical significance was adjusted to account for multiple comparisons by dividing 0.05 by the number of comparisons (61 items; α=0.0008).

We used logistic regression analysis to examine the association between sex, severity of illness, and the likelihood of unfavourable outcome. Patient sex was added to two previously published multivariable models predicting unfavourable outcome in bacterial meningitis.[16,27] Odds ratios (OR) and 95% confidence intervals (CI) were used to quantify the strength of associations. The maximum number of possible predictors was limited by a number of events per variable set at fifteen or more. Confounding was defined as a predictor that showed a statistically significant association at $p < 0.05$ with both patient sex and unfavourable outcome. Interaction of variables in the association of patient sex and outcome was evaluated by interaction terms. The linear relationship between continuous predictors and unfavourable outcome was evaluated by the Hosmer-Lemeshow goodness of fit test and inspection of locally weighted scatterplot smoothing curves. In case of a clearly non-linear relationship, continuous variables were categorised using normal ranges, when possible, to facilitate interpretation. We estimated both univariable crude OR and multivariable OR corrected for all variables in the model. All co-variables were entered simultaneously in the logistic regression model. Multiple imputation was performed for missing data in the multivariable analysis using all predictors together. Final estimates were obtained by pooling the coefficients of 60 rounds of imputation. All statistical tests were two-tailed. Analyses were performed using IBM SPSS Statistics (version 22.0; IBM Corp., Armonk, NY, USA), and imputation was done using the MICE package (version 2.25) in R version 3.2.3.

Role of the funding source

The sponsor had no role in the study design, data collection, analysis or interpretation, or writing of the report. The corresponding author had full access to all data and had final responsibility in the decision to submit for publication.

RESULTS

From January 2006 to June 2014, we identified 1412 episodes of bacterial meningitis in 1391 patients, 707 (50%) in males. Characteristics of the study population are shown in **table 1**. Median age was higher in women than in men (62 *vs* 60 years, p=0.001), although this difference was not significant after adjusting for multiple testing.

Predisposing conditions were present in similar proportion between male and female patients. A history of an immunocompromised state was significantly more common in men (211 [30%] of episodes in males *vs* 153 [22%] of episodes in females, p=0.0005), due to higher rates of alcoholism, HIV infection, and use of immunosuppressive drugs.

On admission, 13% of patients were comatose, and median Glasgow Coma Scale (GCS) score did not differ between sexes. Neck stiffness was more frequently described in women (78% *vs* 70% of men, p=0.0004), while seizures (affecting 60 of 677 [9%] men *vs* 38 of 676 [6%] women, p=0.02) and focal neurological deficits (observed in 203 of 703 (29%) men and 168 of 701 (24%) women (p=0.04) did not differ significantly between sexes after correcting for multiple testing.

Table 1. Characteristics of the study population (n=1412).

Characteristic [a]	Men (n=707)	Women (n=705)	p-value [b]
Age – years	60 (45–68)	62 (49–71)	0.001
History of meningitis	42/702 (6)	51/694 (7)	0.31
Duration of symptoms <24 hours	334/676 (49)	302/677 (45)	0.07
Seizures prior to admission	60/677 (9)	38/676 (6)	0.02
Pre-treatment with antibiotics	60/687 (9)	92/690 (13)	0.006
Predisposing condition	420/707 (59)	411/705 (58)	0.67
Otitis or sinusitis	214/705 (30)	266/699 (38)	0.002
Pneumonia	60/672 (9)	62/675 (9)	0.87
Remote head injury [c]	41/667 (6)	14/658 (2)	0.0002
Immunocompromise	211/707 (30)	153/705 (22)	0.0005
Alcoholism	61/652 (9)	21/660 (3)	<0.0001
Known HIV-positive status [d]	10/679 (2)	2/684 (0.3)	0.02
Immunosuppressive therapy	64/700 (9)	43/691 (6)	0.04
Symptoms and signs on presentation			
Headache	506/612 (83)	509/611 (83)	0.77
Nausea	335/576 (58)	378/583 (65)	0.02

Table 1. Continued

Characteristic [a]	**Men (n=707)**	**Women (n=705)**	**p-value** [b]
Neck stiffness	453/651 (70)	524/671 (78)	0.0004
Rash	54/707 (8)	62/705 (9)	0.43
Temperature [e]	38.9 (37.9–39.6)	39.0 (37.8–39.7)	0.75
Fever (≥ 38 °C)	519/694 (75)	514/697 (74)	0.66
Heart rate – beats/min [e]	99 (82–110)	100 (86–114)	0.02
Systolic blood pressure – mmHg [e]	144 (124–65)	141 (125–160)	0.48
Diastolic blood pressure – mmHg [e]	80 (70–90)	80 (68–90)	0.11
GCS score [f]	11 (9–14)	11 (9–14)	0.69
< 14 (altered mental status)	490/704 (70)	508/701 (72)	0.24
< 8 (coma)	99/704 (14)	88/701 (13)	0.40
Triad of fever, neck stiffness, altered mental status	268/670 (40)	295/677 (44)	0.18
Focal neurologic deficits	203/703 (29)	168/701 (24)	0.04
Cranial nerve palsy	68/621 (11)	41/624 (7)	0.006
Aphasia, hemiparesis or monoparesis	138/609 (23)	130/612 (21)	0.55
Dutch Meningitis Risk Score [g]	27 (19–35)	28 (21–38)	0.01

Abbreviations: GCS, Glasgow coma scale; HIV, human immunodeficiency virus.

The study included 1412 episodes of community-acquired meningitis in 1391 patients.

[a] Categorical variables are expressed as no./no. evaluated (%). Percentages may not add up to 100% due to rounding.

[b] p <0.0008 was considered to be statistically significant after correction for multiple testing.

[c] Remote head injury denotes injury occurring more than one month before onset of meningitis.

[d] The number of patients tested for HIV is unknown.

[e] Temperature was evaluated in 694 male and 697 female patients, heart rate in 683 males and 680 females, and systolic and diastolic blood pressure in 690 males and 692 females.

[f] GCS score can range from 3 to 15, with 15 indicating a normal level of consciousness, and was available for 704 male and 701 female patients.

[g] The Dutch Meningitis Risk Score is a validated bedside risk score based on routinely collected data, resulting in a nomogram from which risk for adverse outcome can be predicted; scores can range from 0–65, with associated risk estimates for an unfavorable outcome varying between 3.2 and 96%, respectively.

Lumbar puncture (LP) was performed in all patients (**table 2**). Cranial imaging was performed prior to LP in 85% of patients, in similar proportions in men and women, and there were no differences between sexes in the frequency of imaging abnormalities. CSF analysis revealed similar findings in both sexes regarding CSF inflammatory markers. Women exhibited higher values of serum acute phase reactants than men, namely C-reactive protein (median 211 *vs* 171, respectively, p=0.0001) and erythrocyte sedimentation rate (median 48 *vs* 33, respectively, p<0.0001).

Table 2. Laboratory and imaging results.

Characteristic [a]	Men (n=707)	Women (n=705)	p-value [b]
Blood chemical tests			
Erythrocyte sedimentation rate – mm/h [c]	33 (17–66)	48 (28–75)	<0.0001
C-reactive protein – mg/L [c]	171 (79–288)	211 (97–330)	0.0001
Thrombocyte count – /mm^3 [c]	191 (148–244)	206 (155–261)	0.005
Indices of CSF inflammation			
Opening pressure > 400 mm of water	141/254 (56)	112/226 (50)	0.20
CSF white cell count – /mm^3 [c]	2509 (550–7599)	2237 (513–6193)	0.07
Total protein – g/L [c]	3.8 (2.2–6)	4 (2.3–6.2)	0.71
CSF:blood glucose ratio [c]	0.06 (0–0.27)	0.03 (0–0.25)	0.09
Positive Gram stain	525/632 (83)	532/613 (87)	0.07
Positive blood culture	457/622 (74)	470/621 (76)	0.37
Causative pathogen			
S. pneumoniae	498/707 (70)	519/705 (74)	0.18
N. meningitidis	71/707 (10)	79/705 (11)	0.48
L. monocytogenes	49/707 (7)	25/705 (4)	0.004
Other	89/707 (13)	82/705 (12)	0.30
Cranial imaging on admission	616/705 (87)	589/697 (84)	0.12
Prior to lumbar puncture	491/563 (87)	446/534 (84)	0.08
Abnormal findings	270/616 (44)	264/589 (45)	0.72

Abbreviations: CSF, cerebrospinal fluid.
The study included 1412 episodes of community-acquired meningitis in 1391 patients.
[a] Continuous variables are expressed as median (interquartile range). Categorical variables are expressed as no./no. evaluated (%). Percentages may not add up to 100% due to rounding.
[b] p <0.0008 was considered to be statistically significant after correction for multiple testing.
[c] ESR was evaluated in 321 men and 335 women, CRP in 681 men and 672 women, thrombocyte count in 678 men and 667 women, CSF white cell count in 680 men and 672 women, total CSF protein in 674 men and 670 women, CSF:blood glucose ratio in 653 men and 656 women

The most frequent causative pathogens were *S. pneumoniae* and *N. meningitidis,* occurring in 1017 (72%) and 150 (11%) cases, respectively, without differences between sexes. Meningitis caused by *Listeria monocytogenes* was more common in men, accounting for 49 (7%) isolates in men and 25 (4%) in women (p=0.004). This difference was not statistically significant after correcting for multiple testing. Of note, seven of 44 (16%) episodes of Listeria meningitis in men were documented in alcoholic patients, and none of the episodes of bacterial meningitis in pregnant women were caused by *L. monocytogenes.*

As recommended by Dutch guidelines, a combination of amoxicillin and a third-generation cephalosporin was started as empiric treatment on the day of LP in 245 (38%) of 639 episodes in men, and 420 (34%) of 634 episodes in women (p=0.09). Monotherapy was started with a third-generation cephalosporin in 171 (27%) episodes in males and in 194 (31%) episodes in females (p=0.14). Monotherapy with either penicillin or amoxicillin occurred in 133 (21%) episodes in men and 126 (20%) episodes in women (p=0.73). There were no differences between men and women in the rate of antibiotic administration before LP (**table 3**). Corticosteroids were administered to 89% of cases in both sexes; a similar proportion of male and female patients was treated with adjunctive dexamethasone according to guideline recommendations (10 mg intravenously every six hours for four days),[9,29,30] which was started before or with the first dose of antibiotics to 528 of 695 (76%) men and 547 of 689 (79%) women.

Table 3. Treatment, clinical course and outcome.

Characteristic [a]	**Men (n=707)**	**Women (n=705)**	**p-value** [b]
Antibiotics prior to imaging	200/502 (40)	189/472 (40)	0.95
Adjunctive corticosteroids	618/695 (89)	616/689 (89)	0.77
Clinical course			
Neurological complications	407/508 (80)	373/471 (79)	0.72
Seizures	101/681 (15)	84/672 (12)	0.21
Systemic complications	298/695 (43)	232/687 (34)	0.0005
ICU admission or transfer	449/707 (64)	392/705 (56)	0.003
Cardiorespiratory failure	204/707 (29)	181/705 (26)	0.18
Mechanical ventilation	261/682 (38)	199/675 (30)	0.001
Outcome			
Cognitive impairment	101/478 (21)	75/497 (15)	0.01
Hearing loss	68/451 (15)	76/451 (17)	0.48
Score on GOS	5 (4–5)	5 (4–5)	0.14
Death	132/707 (19)	112/705 (16)	0.17
Unfavourable outcome	279/707 (40)	252/705 (36)	0.15
With adjunctive dexamethasone	188/528 (36)	172/547 (31)	0.15
Without adjunctive dexamethasone	84/167 (50)	73/142 (51)	0.85

Abbreviations: GOS, Glasgow Outcome Scale; ICU, intensive care unit.

The study included 1412 episodes of community-acquired meningitis in 1391 patients.

[a] Categorical variables are expressed as no./no. evaluated (%). Percentages may not add up to 100% due to rounding.

[b] p <0.0008 was considered to be statistically significant after correction for multiple testing.

During clinical course, there was no difference between male and female patients in the rate of neurological complications. Systemic complications, however, were more frequent in men, affecting 298 of 695 (43%) males compared with 232 of 687 (34%) females (p=0.0005). Despite similar rates of cardiorespiratory failure, women were less likely to receive mechanical ventilation than men (199 of 675 [30%] female *vs* 261 of 682 [38%] male patients, p=0.001). This difference was not statistically significant after correcting for multiple testing, and case fatality rates and unfavourable outcome rates were evenly distributed between sexes. Death occurred in 132 (19%) of men and 112 (16%) of women, while unfavourable outcome occurred in 279 (40%) and 252 (36%) cases, respectively.

Neurologic examination was performed at discharge in 1137 of 1168 surviving patients (97%); motor deficits were found in 74 (6%) and hearing loss in 144 of 902 (16%), with no differences between sexes. Cognitive impairment was more commonly reported in men, affecting 101 of 478 (21%) surviving men compared with 75 of 497 (15%) women (p=0.01). This difference was however not statistically significant after correcting for multiple testing.

The proportion of unfavourable outcome was lower in patients treated with adjunctive dexamethasone in accordance to guideline recommendations than in those who were not. This effect was more pronounced in women (RR 0.61, 95% CI 0.50–0.75; ARR 20%; p<0.001) than in men (RR 0.71, 95% CI 0.59–0.86; ARR 15%; p=0.001), although we did not observe a significant interaction between sex and dexamethasone (p=0.38).

In a multivariable analysis (**table 4**), male sex showed an independent association with unfavourable outcome (OR 1.34, 95% CI 1.03–1.75, p=0.03) and death (OR 1.47, 95% CI 1.04–2.07, p=0.03). The association remained significant after further correcting for *S. pneumoniae* as causative pathogen and adjunctive treatment with dexamethasone (OR for unfavourable outcome 1.32, 95% CI 1.01–1.72, p=0.04; and OR for death 1.45, 95% CI 1.02–2.05, p=0.04).

Based on the Dutch Meningitis Risk Score, illness severity at presentation was higher in women than in men (median 28 *vs* 27, respectively, p=0.01). Nevertheless, they were less likely to be admitted or transferred to an intensive care unit (ICU, univariable OR 0.72 95% CI 0.58–0.89, p=0.003). This association remained after correcting for variables associated with sepsis (pulse, systolic and diastolic blood pressure, positive blood culture, and GCS score [OR 0.68 95% CI 0.54–0.86, p=0.001]), and for all variables in **table 4** (OR 0.69 95% CI 0.54–0.89, p=0.004). The case fatality rate (111 of 449 [25%] men and 82 of 392 [21%] women, p=0.22) and unfavourable outcome rate (221 of 449 [49%] men and 184 of 392 [47%] women, p=0.56) was similar between male and female ICU patients.

Table 4. Multivariable analysis of the association between patient sex and outcome.

Characteristic	Multivariable OR for Unfavourable Outcome (95% CI)	p-value	Multivariable OR for Death (95% CI)	p-value
Male sex	1.34 (1.03–1.75)	0.03	1.47 (1.04–2.07)	0.03
Age – years	0.80 (0.61–1.05)	0.11	0.80 (0.61–1.05)	0.11
16–39 years	Ref		Ref	
40–70 years	1.55 (1.01–2.37)	0.04	1.14 (0.62–2.11)	0.67
> 70 years	3.15 (1.94–5.09)	<0.0001	3.75 (1.97–7.16)	<0.0001
Symptoms <24 hours	0.80 (0.61–1.05)	0.11	0.81 (0.56–1.16)	0.25
Seizures	1.43 (0.84–2.44)	0.19	1.62 (0.87–3.02)	0.13
Pre-treated antibiotics	1.13 (0.74–1.73)	0.58	1.21 (0.70–2.12)	0.49
Otitis or sinusitis	0.75 (0.56–1.01)	0.06	0.62 (0.41–0.93)	0.02
Pneumonia	1.39 (0.88–2.20)	0.15	1.21 (0.72–2.05)	0.47
Immunosuppressive drugs	1.00 (0.61–1.64)	1.00	0.97 (0.54–1.76)	0.93
History of splenectomy	1.08 (0.47–2.51)	0.86	0.83 (0.30–2.30)	0.72
History of cancer	1.17 (0.80–1.73)	0.42	1.04 (0.65–1.66)	0.87
Diabetes	1.36 (0.92–2.02)	0.12	1.76 (1.09–2.84)	0.02
HIV-positive	0.56 (0.13–2.46)	0.45	1.23 (0.22–6.90)	0.81
Alcoholism	1.91 (1.11–3.29)	0.02	1.28 (0.69–2.40)	0.43
Headache	0.68 (0.47–0.98)	0.04	0.77 (0.48–1.23)	0.27
Nausea	0.89 (0.66–1.20)	0.43	0.86 (0.57–1.30)	0.48
Neck stiffness	0.85 (0.58–1.25)	0.40	0.46 (0.28–0.77)	0.003
Rash	0.91 (0.55–1.51)	0.71	0.84 (0.41–1.73)	0.63
Heart rate – beats /min [a]	1.10 (1.03–1.17)	0.003	1.05 (0.97–1.14)	0.21
Diastolic blood pressure– mmHg [b]	1.02 (0.95–1.10)	0.60	0.95 (0.86–1.04)	0.27
Temperature – °C [c]	0.90 (0.80–1.01)	0.07	0.91 (0.79–1.06)	0.22
Score on GCS [d]	0.92 (0.87–0.97)	0.002	0.91 (0.85–0.97)	0.006
Triad of fever, neck stiffness and GCS <14	1.03 (0.68–1.55)	0.90	1.21 (0.70–2.01)	0.50
Aphasia, hemiparesis or monoparesis [c]	1.23 (0.82–1.85)	0.32	1.19 (0.71–1.99)	0.52
Cranial nerve palsy [c]	2.70 (1.65–4.45)	<0.0001	1.57 (0.88–2.79)	<0.13
CSF white cell count/mm^3 [e]				
<100 /mm^3	2.18 (1.37–3.46)	0.00098	3.47 (2.08–5.80)	<0.0001
100 – 999/mm^3	2.08 (1.50–2.90)	<0.0001	1.85 (1.21–2.83)	0.0043

Table 4. Continued

Characteristic	**Multivariable OR for Unfavourable Outcome (95% CI)**	**p-value**	**Multivariable OR for Death (95% CI)**	**p-value**
1000–10,000/mm^3	Ref		Ref	
>10,000 /mm^3	0.80 (0.55–1.18)	0.26	0.89 (0.52–1.54)	0.68
CSF:blood glucose ratio [e]				
<0.25	1.31 (0.65–2.66)	0.45	1.61 (0.63–4.07)	0.32
0.25–0.5	0.74 (0.35–1.56)	0.42	1.00 (0.36–2.79)	1.00
>0.5	Ref		Ref	
CSF Protein – g/L [e]	1.03 (0.99–1.08)	0.11	1.05 (1.01–1.10)	0.03
Positive blood culture	1.45 (1.03–2.05)	0.04	1.06 (0.68–1.69)	0.80
Thrombocyte count				
<150	1.27 (0.92–1.75)	0.14	1.66 (1.14–2.42)	0.009
150–450	Ref		Ref	
>450	0.47 (0.17–1.33)	0.16	2.05 (0.71–5.99)	0.18
C-reactive protein – mg/L [f]	1.03 (1.01–1.04)	<0.0001	1.03 (1.02–1.05)	<0.0001

Abbreviations: CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; HIV, human immunodeficiency virus. The study included 1412 episodes of community-acquired meningitis in 1391 patients. 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.

[a] Evaluated in 1363 episodes; odds ratio is for an increase of 10 beats/min.

[b] Evaluated in 1381 episodes; odds ratio is for a 10 mmHg increase.

[c] Evaluated in 1391 episodes.

[d] Evaluated in 1403 episodes; odds ratio is for a one-point increase.

[e] CSF white cell count was evaluated in 1352 episodes, CSF protein in 1344 episodes, and CSF:blood glucose ratio in 1309 episodes.

[f] Evaluated in 1353 episodes; odds ratio is for a 10 mg/L increase.

DISCUSSION

Our results show initial presentation of adult male patients with bacterial meningitis differs from that of female patients. Prevalence of classical signs and symptoms was similar in both sexes, apart from an increased frequency of neck stiffness in women. A recent retrospective study also found nuchal rigidity to be more common in female patients with community-acquired meningitis,[31] although the reason for this is unclear.

It is recognised that males have an increased susceptibility to several pathogens.[1] In this study, *L. monocytogenes* infection was more common in men, although this was non-significant after adjusting for multiple testing. Literature on sex distribution in Listeria meningitis is scarce and conflicting, with some studies showing a similar distribution[32] while others have reported a slight to overt male preference.[33,34] Reasons for a possibly increased incidence in men are unclear, but could potentially be due to differences in food consumption habits between men and women,[35] with men more likely to consume high-risk foods.[36] Contrary to previous articles which report most cases of invasive listeriosis in women of childbearing age to be associated with pregnancy,[37] in our study none of the episodes were pregnancy-related. In line with previous studies,[32] the majority of patients with Listeria meningitis in our cohort belonged to well-established risk groups,[38] and a substantial number of males were alcoholics.[39]

Despite treatment, rates of adverse outcome were high in our cohort, and in a multivariable analysis we found male sex to be an independent predictor of unfavourable outcome. A recent retrospective study found females with meningitis due to an urgent treatable aetiology to have worse outcomes than males.[31] However, only a small fraction of patients in this group had bacterial meningitis, which limits comparisons with our study.

Interestingly, women in our cohort had a more severe illness on admission according to a validated bacterial meningitis severity score, but this did not result in a worse outcome. We could speculate that this might be due to disparities in treatment; however, we found no differences in management that would benefit female patients. In fact, women were less likely to be admitted to an ICU and to receive mechanical ventilation than men. This has been previously reported in critically ill patients, with several studies showing women to be less likely to receive care in an ICU setting, life-saving interventions, and invasive measures of care.[40,41] In some studies, these differences were attenuated after adjusting for confounders,[40] but in our cohort, they persisted even after making such adjustments. Reasons for this bias are largely unknown, but it has been hypothesised different social contexts and gender roles may influence decisions regarding aggressiveness of end-of-life care.[41] In contrast to previous studies, there was no increased fatality rate or worse outcome among female ICU patients, so it is possible these findings might be explained by differences in eligibility, contraindications for treatment, or other unmeasured clinical factors. This apparent discrepancy between severity of illness and outcome in women could therefore be related to a more benign clinical course or better response to treatment despite an initially more severe disease, and point to the need for risk scores that include patient sex.

Other potential mechanisms for better outcome in female patients are biological differences and their role in immune response. Women in our cohort exhibited higher levels of serum inflammatory parameters when compared with males, and although we could not find a significant difference in CSF markers of inflammation, they appeared to show a superior benefit from treatment with dexamethasone. We hypothesise a stronger inflammatory response may render women more responsive to the effect of anti-inflammatory agents. Due to the observational nature of our study, evaluation of treatment effect must be carefully interpreted. Indication bias is of particular concern, but we believe it is unlikely to have been significant in our cohort, due to widespread implementation of adjunctive corticosteroid treatment, in similar proportions, to both sexes. Furthermore, the treatment benefit observed in our cohort as a whole was comparable to the one reported in the European randomised clinical trial on dexamethasone.[11]

Neuropsychological deficits are common after bacterial meningitis, affecting almost one-third of adult survivors.[42,43] Subjective cognitive impairment, as assessed by the patients' physicians at discharge was more common in male survivors in this study. The difference was not statistically significant after correcting for multiple testing; however, a study with formal neuropsychological evaluation also found male sex to be a risk factor for cognitive impairment after bacterial meningitis.[42]

Several aspects should be considered when interpreting the results of our study. First, it has several strengths, namely its prospective design and large sample, which allowed us to find significant differences while avoiding some of the methodological issues that often arise in sex and gender studies.[44] Both sexes were included prospectively in similar numbers, which are representative of the general population, and we present sex-disaggregated data and sex-based analyses of relevant characteristics. Selective non-availability of data is also unlikely to have been a problem, as average missing values were similar between the two groups. By correcting for multiple testing we have reduced the probability of false positive associations.

Our study also had several limitations. First, the majority of patients included in our cohort had positive CSF cultures. Negative CSF cultures occur in a significant number of patients with bacterial meningitis, and yield is even lower in those pre-treated with antibiotics.[45] Since more women than men were under antibiotic treatment prior to admission, this may have led to a selection bias. We tried to overcome this limitation by including patients with negative cultures but positive PCR or antigen test; these, however, represent only a small fraction of the cohort. Furthermore, only patients who underwent LP were included; this has probably led to an underrepresentation of patients in whom LP is contraindicated or might need to be postponed, which can result in negative CSF culture results.[9] No significant differences in clinical presentation, however, have been reported between patients with culture-positive and culture-negative bacterial meningitis, and despite the fact that sex-disaggregated data on this subject is lacking, there is no reason to believe it should be an important confounding factor for comparisons between sexes. In addition, analysis of the impact of cultural and behavioural influences, which can have an important role in exposure to infections, was limited, due to lack of data on these factors. Finally, due to the observational nature of the study, only routine laboratorial data were collected; it would have been useful to have more data on other blood and CSF acute phase reactants and pro-inflammatory cytokines in order to better assess the role of inflammation, as well as follow-up results to determine the temporal evolution of the inflammatory response.

Our findings show sex-based differences in adults with community-acquired bacterial meningitis regarding clinical characteristics, disease progression, and treatment. Sex is an independent predictor of outcome, with male sex associated with significantly poorer prognosis. These differences may justify the need for individualised approaches taking sex-specific host factors into account; the inclusion of sex in prognostic scores could contribute to improved outcome prediction, and an immunological sexual dimorphism may warrant different adjuvant therapies in male and female patients with bacterial meningitis. Further studies and replication in different cohorts will be necessary to confirm our results and verify their clinical significance.

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CHAPTER 4

SEX-BASED DIFFERENCES IN SEROTYPE DISTRIBUTION IN ADULTS WITH PNEUMOCOCCAL MENINGITIS

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We read with interest the study by Gounder et al. on the epidemiology of bacterial meningitis in the North American Arctic following the introduction of paediatric conjugate vaccines, which was published recently in this journal.[1] Widespread use of paediatric conjugate vaccines has changed the epidemiological landscape of bacterial meningitis; however, it remains a serious health problem worldwide.[2] In the Netherlands, there has been a significant decrease in the incidence of adult bacterial meningitis since their inclusion in childhood immunisation schedules, most sharply among *Streptococcus pneumoniae* capsular serotypes included in the seven-valent (PCV7) and ten-valent (PCV10) pneumococcal conjugate vaccines.[3] These were introduced in the Netherlands in 2006 and 2011, respectively; a 23-valent polysaccharide vaccine (PPV23) is also available and recommended for certain risk groups.[4] Similarly to what Gounder and colleagues reported, *S. pneumoniae* is currently the leading cause of bacterial meningitis in the Dutch population.[3] As pneumococcal vaccines target only a limited set of the over 90 known serotypes,[5] emergence of non-conjugate vaccine type (NVT) disease remains a concern.[6]

Pneumococcal serotypes have been associated with differences in invasiveness potential and outcome,[4] making knowledge of factors affecting serotype distribution important. A patient's sex is a key determinant of infectious diseases, influencing susceptibility to illness and response to vaccination, while social and behavioural factors may also play a role.[7] Data regarding sex-based differences in pneumococcal serotype distribution, however, are limited,[8-10] and the impact of patient sex on serotype distribution in pneumococcal meningitis remains unclear. To investigate this, we analysed serotype distribution and post-vaccination incidence trends in adult men and women with community-acquired pneumococcal meningitis in a prospective nationwide cohort study.

We included all patients with pneumococcal meningitis, identified through the Netherlands Reference Laboratory for Bacterial Meningitis between March 2006 and June 2014, for whom capsular serotype was available. All participants or their legally authorised representatives provided informed consent. Pneumococcal meningitis was defined as a positive cerebrospinal fluid culture for *S. pneumoniae*, and isolates were serotyped by Quellung reaction using specific antisera. Individual serotypes were grouped into vaccine types (i.e., those included in PCV7, PCV10, and PPV23) and NVT for analyses and compared between sexes using the Chi-Square or Fisher exact test (as appropriate), with Bonferroni correction for multiple testing. Incidence rates in men and women were calculated as the number of cases per 100,000 male or female population >16 years-old, respectively, per epidemiological year from July 2007 onward, as due to pending ethical approval in several centres this was the first year in which all Dutch hospitals participated in the study. Incidence rate ratios (IRR) and 95% confidence intervals (CI) were used to assess differences in incidence rates between men and women. Analyses were performed using IBM SPSS Statistics software (version 22.0), with two-tailed p-values below 0.05 considered statistically significant. Detailed methods of the MeninGene Study have been described previously.[3]

We identified 997 episodes of pneumococcal meningitis. Serotype was available in 928 (93%), of which 447 (48%) were male, and 46 distinct capsular types were identified (**table 1**).

Serotypes 3, 7F, and 8, in that order, were the most commonly found in men, while 7F, 3, and 22F were the most frequent in women. After correcting for multiple testing, we found no differences between sexes in serotype distribution, neither individually nor using serotype 7F as a reference (data not shown). We also found no differences between men and women in the proportion of episodes due to serotypes included in the PCV7, PCV10, or PPV23 vaccines.

Overall incidence of pneumococcal meningitis was 0.78/100,000 male and 0.85/100,000 female patients, with an IRR of 0.92 (95% CI 0.80-1.06). There was a marked decrease over the study period (**figure 1**), mainly driven by a reduction in the incidence of PCV7 serotypes in both sexes (from 0.37 in 2007 to 0.02 in 2013). Incidence of disease caused by PPV23-exclusive serotypes remained largely unchanged throughout the study period (from 0.41 in 2007 to 0.36 in 2013), with no difference between sexes. There was no absolute increase in the incidence of non-PCV10 types in either sex, although the proportion of cases caused by these types did rise, from 58% (37 of 64 cases) in 2007 to 91% (40 of 44 cases) in 2013 in men ($p<0.001$) and 42% (36 of 86 cases) in 2007 to 87% (34 of 39 cases) in 2013 in women ($p<0.001$).

In the Netherlands, coverage rates for conjugate vaccines in infants are around 95%, while adults are not routinely vaccinated.[4] As a result, sex-based differences in response to pneumococcal vaccination are unlikely to have influenced our results, as evidenced by the relative stability of disease caused by serotypes exclusive to the PPV23 during the study period. These findings are, therefore, best explained as a result of herd protection.

Table 1. Capsular pneumococcal serotypes from 447 men and 481 women with pneumococcal meningitis. Individual serotypes are presented in descending order of frequency.

Serotype	Men – no. (%)	Women – no. (%)	p-value
7F	54 (12)	56 (12)	0.82
3	59 (13)	47 (10)	0.10
8	43 (10)	35 (7)	0.19
22F	27 (6)	41 (9)	0.15
23F	21 (5)	23 (5)	1.00
19A	17 (4)	23 (5)	0.47
19F	15 (3)	19 (4)	0.64
10A	12 (3)	18 (4)	0.37
23B	15 (3)	15 (3)	0.82
4	13 (3)	15 (3)	0.86
12F	11 (2)	16 (3)	0.44
6B	11 (2)	14 (3)	0.68
1	8 (2)	15 (3)	0.20
14	8 (2)	15 (3)	0.20
18C	9 (2)	14 (3)	0.39
9N	8 (2)	13 (3)	0.35
11A	11 (2)	9 (2)	0.53
23A	10 (2)	10 (2)	0.86
28 other serotypes[a]	95 (21)	83 (17)	-
PCV7 types[b]	89 (20)	104 (22)	0.54
PCV10 types[c]	152 (34)	177 (37)	0.40
PPV23 types[d]	362 (81)	401 (83)	0.42
Total[e]	447	481	

Abbreviations: PCV7, heptavalent conjugate vaccine; PCV10, 10-valent conjugate vaccine; PPV23, 23-valent polysaccharide vaccine.

[a] Other serotypes were as follows: 6A in eight males and ten females; 24F in seven male and nine females patients; 33F in nine men and eight women; 9V in 12 men and four women; 15B in four males and 11 females; 6C in seven male and five female patients; 16F in four men and six women; 35F in seven males and three females; 15A and 17F in five male and three female patients each; 28 in three males and three females; 31 in three men and two women; 15C in four males and two females; 20 in four males; 37 in four females; 18B in one male and three females; 5 in one male and two females; 34 and 35B both in two males and one female; 22A in two males; 10B, 13, 25A, 28F and 7B in one male each; 27, 24B and 7A in one female each.

[b] 4, 6B, 9V, 14, 18C, 19F and 23F.

[c] 1, 5 and 7F + PCV7 types.

[d] 2, 3, 9N, 10A, 11A, 12F, 15B, 17F, 19A 20, 22F and 33F + PCV10 types.

[e] Percentages may not add up to 100% due to rounding

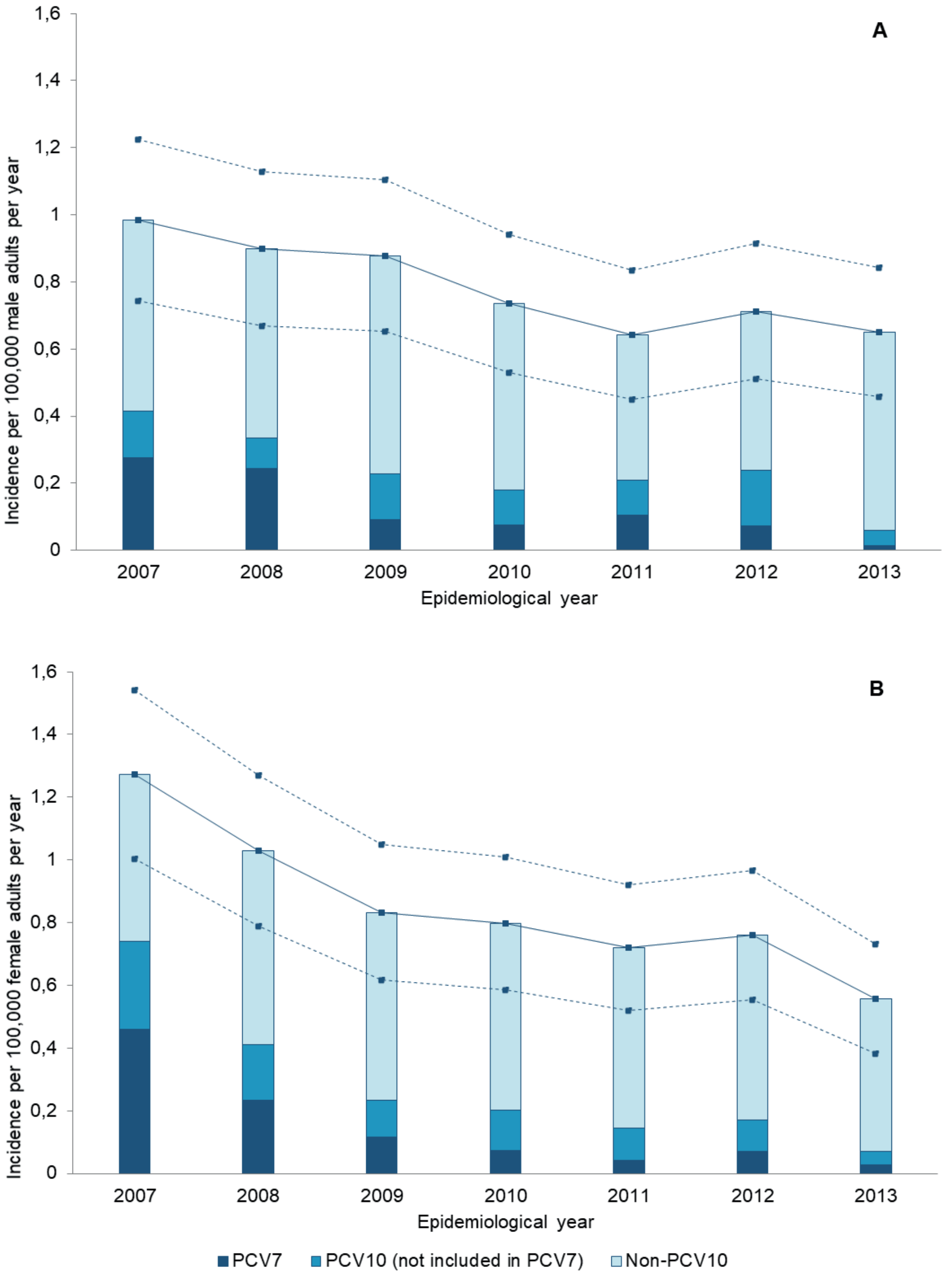

Figure 1. Total incidence of pneumococcal meningitis (solid line) with 95% confidence intervals (dotted lines) and incidence due to serotypes included in the PCV7 and PCV10 conjugate vaccines (bars) in men (A) and women (B) from 2007 to 2013. Due to pending ethical approval in several, not all patients could be included in the first months of the study, and therefore only cases from July 2007 onward were considered.

Parents are thought to often acquire pneumococci from their children, who are the primary community reservoir, and increased NVT carriage rates have been reported in parents of PCV7-vaccinated children.[10] In many societies, women are more active in childcare than men, which could favour acquisition of pneumococci, specifically NVT. However, although the female to male ratio in pneumococcal meningitis in our cohort was slightly higher when compared with non-pneumococcal cases, this was not statistically significant,[3] and there was no female preference for NVT disease.

We also found no significant sex-based differences in the distribution of individual serotypes. Literature on this topic is scarce, and studies have yielded varying results. Rodríguez et al. reported serotype 8 (not included in pneumococcal vaccines) to be more common in men, and 1 and 7F (both included in the PCV10) in women with invasive pneumococcal disease (IPD),[9] while van Mens and colleagues found increased serotype 1 IPD prevalence in young women;[10] a pre-vaccination study by Scott et al. described a female preference for serogroup 14 and 23 IPD.[8] It is possible these findings are the result of chance, natural fluctuation in serotypes, differences in methodology, or publication bias. Furthermore, none of these studies specifically investigated meningitis, and potentially the influence of sex on serotype distribution may differ between meningitis and other types of IPD.

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CHAPTER 5

SEX-BASED DIFFERENCES IN THE RESPONSE TO DEXAMETHASONE IN BACTERIAL MENINGITIS: ANALYSIS OF THE EUROPEAN DEXAMETHASONE IN ADULTHOOD BACTERIAL MENINGITIS STUDY

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ABSTRACT

Higher inflammatory markers have been found in women than men with bacterial meningitis. To investigate sex-based differences in the response to dexamethasone, we performed a *post hoc* analysis of a double-blind, randomised multicentre trial of adjunctive dexamethasone (10 mg, four times daily for four days) *vs* placebo in adults with bacterial meningitis. The primary outcome measure was the Glasgow Outcome Scale score at eight weeks and interaction tests were used to examine subgroup differences. Between June 1993-December 2001, 301 patients (56% male) were randomly assigned to a treatment group: 157 received dexamethasone and 144 placebo. Although dexamethasone reduced the risk of unfavourable outcome to a greater extent in women (relative risk [RR] 0.42, 95% confidence interval [CI] 0.21-0.86, p=0.02) than men (RR 0.79, 95% CI 0.41-1.51, p=0.55), on interaction testing (ratio of RR women:men 0.53, 95% CI 0.20-1.39, p=0.19) patient sex was not a significant modifier of the effect of dexamethasone.

INTRODUCTION

Bacterial meningitis is a serious disease, and despite advances in prevention and treatment,[1] it remains a cause of substantial morbidity and mortality worldwide.[2] Experimental models have demonstrated that outcome in bacterial meningitis is related to the severity of subarachnoid inflammation, which is improved by administration of corticosteroids, particularly dexamethasone.[3-5] Since the publication of a large multicentre European randomised controlled trial which showed treatment with corticosteroids improves outcome in adults with community-acquired bacterial meningitis,[6] dexamethasone has become an established adjunctive treatment, with several meta-analyses and implementation studies supporting its use in high-income countries.[7-9]

Patient sex influences a wide range of biological processes, including immune responses, which contribute to differences in the prevalence and pathogenesis of infectious, inflammatory, and autoimmune diseases.[10-13] In a nationwide prospective cohort study investigating sex-based differences in community-acquired bacterial meningitis, male sex was found to be independently associated with a poor prognosis, despite the fact that women had a more severe illness on admission.[14] Women in this cohort exhibited higher serum inflammatory markers and appeared to have superior benefit from treatment with corticosteroids, although no significant interaction between sex and dexamethasone was found. However, since the study took place following widespread implementation of dexamethasone use in the Netherlands, only a small portion of patients were untreated. In addition, as this was an observational study, treatment effects must be carefully interpreted.

We therefore analysed sex-based differences in outcome and treatment effect of adjunctive dexamethasone therapy using data from the European Dexamethasone in Adulthood Bacterial Meningitis trial.[6]

METHODS

We performed a *post hoc* analysis of a prospective, randomised, double-blind, multicentre trial in Europe, the enrolment for which took place between June 1993 and December 2001. Details of the study design, procedures, treatment, outcome assessment, and statistical analysis have been described previously.[6]

In brief, patients were eligible for the study if they were aged 17 years or older, had suspected meningitis in combination with cloudy cerebrospinal fluid (CSF), presence of bacteria in CSF Gram staining, or a CSF leukocyte count exceeding 1000 per cubic millimetre. Exclusion criteria were as follows: a history of hypersensitivity to β-lactams or corticosteroids; pregnancy; presence of a cerebrospinal shunt; treatment with oral or parenteral antibiotics in the preceding 48 hours; a history of active tuberculosis or fungal infection; a recent history of head trauma, neurosurgery, or peptic ulcer disease; and enrolment in another trial at the time of the study. All relevant institutional boards of participating hospitals approved the study protocol and all patients or their legally authorised representatives gave written informed consent prior to enrolment.

Patients were randomly assigned to receive either dexamethasone (10 mg every six hours intravenously for four days, before or with the first dose of antibiotics) or placebo. Investigators were blinded to treatment assignments, which were concealed except in the event of an emergency. Patients were initially treated with amoxicillin (2 g given intravenously every four hours) for seven to 10 days. Routine blood and CSF studies and cultures were performed prior to antibiotic treatment and initial regimen was maintained or changed according to aetiology and clinical response. The protocol was later amended to allow investigators to follow local guidelines for administering empirical antibiotic therapy. The primary outcome measure was the score on the Glasgow Outcome Scale at eight weeks, with a score of 5 indicating a favourable outcome and score of 1 to 4 indicating an unfavourable outcome.[15]

Analysis of outcomes was performed on an intention-to-treat basis with the use of a last observation-carried-forward procedure. Continuous variables are expressed as median (interquartile range) and were compared using the Mann-Whitney U test; the Fisher exact test was used to study categorical variables. Severity of illness was calculated based on available clinical and laboratory data using the Dutch Meningitis Risk Score.[16] Treatment effect is expressed as relative risk (RR) for treated *vs* untreated patients (with a RR<1.0 indicating a beneficial effect). Forest plots were used to display treatment effects across subgroups and were created using Review Manager (RevMan), version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). A test for interaction, as described by Altman and Bland,[17] was performed to determine whether effect sizes differed significantly between men and women, and adjustment for potentially confounding variables was performed using logistic regression analysis.

All statistical tests were two-tailed, and analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (Armonk, NY: IBM Corp.), with a p-value under 0.05 considered statistically significant.

RESULTS

In total, 301 patients were included in the trial, 157 (52%) of which were randomly assigned to receive dexamethasone. There were 89 (57%) male patients in the dexamethasone group and 80 (56%) in the placebo group. Baseline characteristics and clinical and laboratory admission findings of male and female patients included in the study can be found in **table 1**. Overall, there were no statistically significant differences between sexes regarding these features. However, within the placebo group, women were older than men (median 52 *vs* 41 years, p=0.005). Furthermore, there was a difference in the age of female patients between the two treatment groups (median 52 years for women in the placebo *vs* 44 years for women in the dexamethasone group, p=0.02).

Classic symptoms and signs of meningitis were present the majority of patients, in similar proportions in both sexes. Neck stiffness was reported in 146 of 160 (91%) males and 121 of 125 (97%) females, and 144 of 169 (85%) men and 101 of 131 (77%) women had fever. Headache was present in 94% of cases in both sexes (142 of 151 males and 106 of 113 females). An altered mental status (defined as a Glasgow Coma Scale score below 14) was found in 114 (67%) men and 84 (64%) women. Mean Glasgow Coma Scale score on admission did not differ between sexes or across treatment groups. Fifty percent of men and 45% of women exhibited the classic trial of fever, neck stiffness, and altered mental status.

Lumbar puncture was performed in all patients and CSF cultures were positive in 77% of male and 80% of female cases. *Streptococcus pneumoniae* was the most frequent pathogen in both sexes. Based on the Dutch Meningitis Risk Score,[16] there were no significant differences between sexes regarding severity of illness on admission (median 16 in men *vs* 15 in women, p=0.47).

During clinical course, impairment of consciousness was documented in 19 of 80 (24%) men in the placebo group and nine of 89 (10%) in the dexamethasone group (p=0.02), while 17 of 64 (27%) women treated with placebo and nine of 68 (13%) treated with dexamethasone developed this complication (p=0.08).

Cardiorespiratory failure was seen in 14 (18%) and 11 (12%) male patients in the placebo and dexamethasone groups, respectively (p=0.39), and in 15 (23%) women treated with placebo and five (7%) treated with dexamethasone (p=0.01). Ten (12%) male patients treated with placebo and four (4%) treated with dexamethasone exhibited seizures (p=0.09), whereas in female patients this number was seven (11%) and four (6%), respectively (p=0.36).

Table 1. Baseline characteristics and clinical and laboratory admission findings of the study population (n=301).

Characteristic [a]	**Men (n=169)**	**Women (n=132)**
Age – years (median, IQR)	42 (26-61)	48 (32-63)
Duration of symptoms before admission – hours (median, IQR)	24 (12-55)	24 (12-48)
Range	1-336	1-168
Seizures prior to admission – no. (%)	15 (9)	7 (5)
Findings on admission		
Score on the Glasgow Coma Scale (median, IQR) [b]	12 (9-14)	12 (9-14)
Coma (defined as a score under 8) – no. (%)	31 (18)	17 (13)
Papilledema – no. /no. evaluated (%)	4/91 (4)	11/84 (13)
Cranial nerve palsy – no. /no. evaluated (%)	19/121 (16)	13/96 (14)
Hemiparesis – no. /no. evaluated (%)	9/166 (5)	13/128 (10)
CSF white cell count – per mm^3 (median, IQR) [c]	4,533 (1,500-11,098)	3,333 (1,377-9,949)
CSF opening pressure – cm of water (median, IQR) [c]	38 (28-50)	30 (24-50)
Dutch Meningitis Risk Score (median, IQR) [d]	16 (6-26)	15 (9-26)
CSF culture – no. (%) [e]		
Streptococcus pneumoniae	57 (34)	51 (39)
Neisseria meningitidis	54 (32)	43 (33)
Other bacteria	18 (11)	11 (8)
Negative	38 (23)	27 (20)
Positive blood culture – no. /no. evaluated (%)	73/148 (49)	59/116 (51)

Abbreviations: CSF=cerebrospinal fluid, IQR=interquartile range

[a] Percentages may not add to 100% due to rounding

[b] Glasgow Coma Scale scores range from 3 to 14, with 14 indicating a normal level of consciousness (abnormal flexion was omitted from the scale).

[c] CSF white cell count was evaluated in 167 male and 129 female patients, opening pressure in 84 males and 73 females.

[d] The Dutch Meningitis Risk Score is a validated bedside risk score based on routinely collected data, resulting in a nomogram from which risk for adverse outcome can be predicted; scores can range from 0-65, with associated risk estimates for an unfavourable outcome varying between 3.2 and 96%, respectively.

[e] CSF culture was performed in 167 male and 132 female patients.

On eight-week follow-up, the number of patients with an unfavourable outcome was significantly lower in the dexamethasone compared with the placebo group (15 vs 25%, RR 0.59; 95% CI 0.37-0.94; p=0.03). In subgroup analyses by sex (**figure 1**), treatment with corticosteroids decreased the rate of unfavourable outcome from 31 to 13% in females (RR 0.42, 95% CI 0.21-0.86, p=0.02) and from 20 to 16% in males (RR 0.79, 95% CI 0.41-1.51, p=0.55). However, tests of interaction were not statistically significant (ratio of relative risks [RRR] women:men 0.53, 95% CI 0.20-1.39, p=0.19), and when comparing the proportion of unfavourable outcome between sexes in each treatment arm separately, we could not find a significant difference in either (p=0.13 in the placebo and p=0.82 in the dexamethasone group).

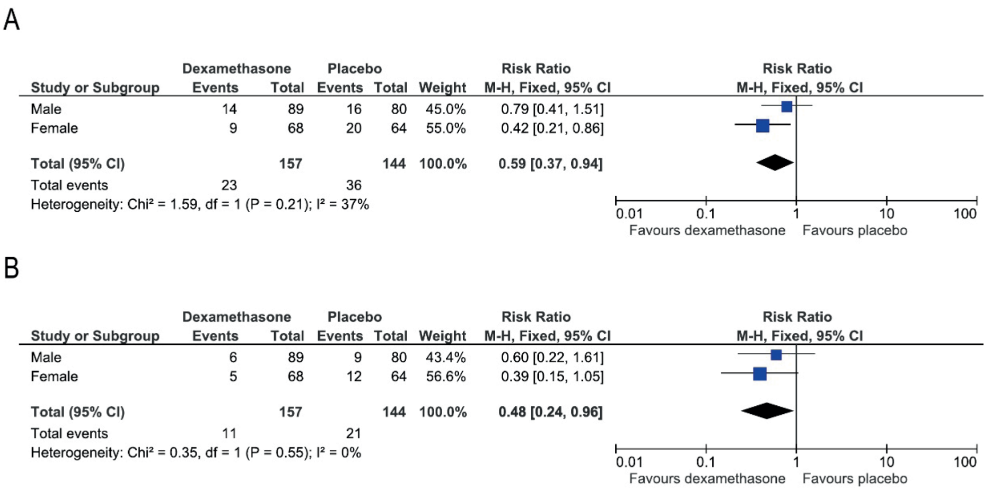

Figure 1. Risk of unfavourable outcome (A) and death (B) in male and female patients with bacterial meningitis according to treatment assignment.
Abbreviations: CI=confidence interval, M-H=Mantel-Haenszel method

In pneumococcal meningitis (**figure 2**), dexamethasone reduced the rate of unfavourable outcome in women from 56 to 17% (RR 0.30, 95% CI 0.12-0.78, p=0.008) and death from 41 to 12% (RR 0.31, 95% CI 0.10-0.97, p=0.03), whereas this reduction was less pronounced in men (from 48 to 32% unfavourable outcome, RR 0.68, 95% CI 0.35-1.29, p=0.28; and from 22 to 18% fatality rate, RR 0.81, 95% CI 0.28-2.35, p=0.32). Nevertheless, there was no statistically significant interaction between sex and dexamethasone (RRR women:men for unfavourable outcome 0.44, 95% CI 0.14-1.38, p=0.16; for death 0.55, 95% CI 0.11-2.75). Further analysis splitting female patients intro pre and postmenopausal age groups did not yield significant results, and interaction terms remained non-significant after adjusting for age using logistic regression analysis.

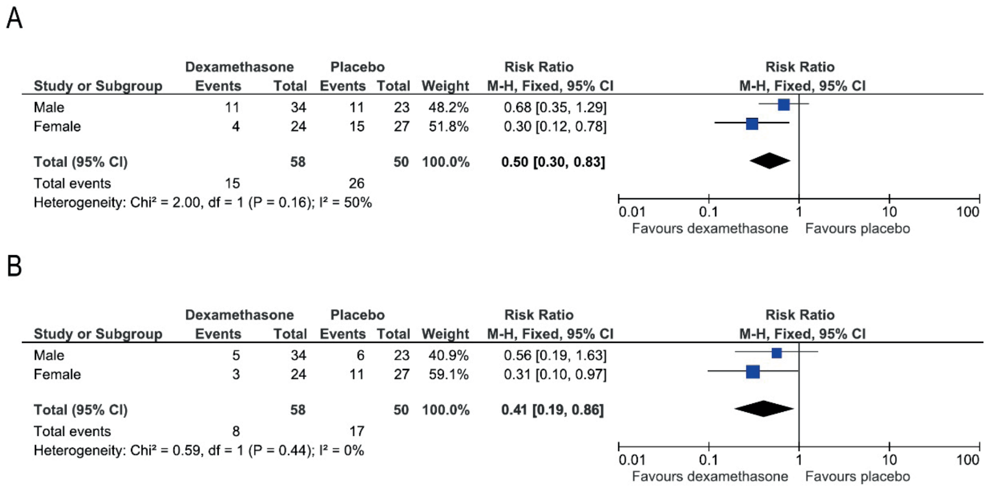

Figure 2. Risk of unfavourable outcome (A) and death (B) in male and female patients with pneumococcal meningitis according to treatment assignment.
Abbreviations: CI=confidence interval, M-H=Mantel-Haenszel method

DISCUSSION

Dexamethasone is currently used as a proven adjunctive treatment for adults with community-acquired bacterial meningitis, and should be prescribed without consideration for patient sex.[18,19] Although our analyses suggest a sex-based difference in response to dexamethasone, formal testing did not reveal a statistically significant interaction between sex and dexamethasone on the risk of unfavourable outcome or death. This is in line with a meta-analysis of individual patient data from five randomised placebo-controlled trials, which included 2,029 patients of all ages (58% male) and showed no evidence of heterogeneity between sexes in the effect of dexamethasone on death or neurological sequelae.[9]

Furthermore, unlike a previous cohort study where male sex was independently associated with unfavourable outcome,[14] in this trial a multivariable logistic regression analysis of baseline variables, as described in the original publication, identified coma on admission, hypotension, and pneumococcal meningitis, but not sex, as predictors of poor prognosis. It should be noted that the two populations are considerably different, with the younger age and higher proportion of meningococcal cases among patients included in the trial likely responsible for a lower severity of illness.

It is recognised that females exhibit more robust innate and adaptive immune responses to self and foreign antigens than males,[10,20] which is thought to be due to hormonal, genetic, and environmental influences.[10,21] Inflammation appears to be a common underlying feature of many disorders that disproportionally affect women[22] and glucocorticoids are a cornerstone in the treatment of many inflammatory disorders.[23] Men and women can also differ in drug pharmacokinetics and pharmacodynamics,[24] and clinical efficacy of glucocorticoid treatment depends on these parameters.[25-31] Consequently, it would be reasonable to expect there could be a sex-related difference in the effect of drug treatment, specifically that a stronger immune reaction might render women more responsive to anti-inflammatory therapies.

The results of our analysis are consistent with previous observational findings,[14] supporting the notion of a sex-based difference in the magnitude of the effect of dexamethasone. Although a significant effect modification could not be confirmed, this is not surprising; since the trial was powered to determine the overall effect of treatment,[6] subgroup analyses are inherently underpowered, limiting the ability to detect subgroup-treatment interactions. A large multinational randomised controlled trial would be necessary for this purpose,[7] but is unlikely to be performed. It took almost nine years to complete the European Dexamethasone Trial, and since its publication the incidence of community-acquired bacterial meningitis has gradually declined in Europe and the United States,[2,32-34] such that an even lower inclusion rate would be expected.

There are several other limitations to consider. *Post hoc* analyses must always be carefully interpreted, as they are prone to finding false results. An imbalance of prognostic factors between subgroups, negating the benefit of randomisation, was also a matter of concern, as was the possibility selection bias. To control for this, we compared the baseline characteristics of patients enrolled in the study with data from 345 men and 351 women included in a prospective nationwide cohort of adults with acute bacterial meningitis, collected between 1998 to 2002;[35] there were no significant differences between the two studies with respect to the Glasgow Coma Scale score on admission.

In conclusion, despite an apparently more pronounced risk reduction in women than in men, we did not find the effect of adjunctive dexamethasone treatment to be significantly modified by patient sex. Future drug trials should, whenever possible, be adequately powered to detect anticipated interactions, and further research into the pathogenesis of sex-based differences in inflammatory reaction in bacterial meningitis, including the role of sex steroid hormones, is needed.

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CHAPTER 6

SEX STEROID HORMONES IN BACTERIAL MENINGITIS

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ABSTRACT

Unfavourable 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 oestradiol and testosterone in the 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 favourable, 1–4 unfavourable 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 unfavourable outcome and 11 (18%) died. Cases with an unfavourable outcome exhibited higher oestradiol (median 14.0 vs 5.0 pmol/L, $p=0.04$) and lower SHBG (0.40 vs 1.0 nmol/L, $p=0.03$) levels compared with those with a favourable outcome. Oestradiol was positively correlated with C-reactive protein ($r=0.42$, $p=0.001$), CSF protein ($r=0.33$, $p=0.01$), and pro-inflammatory cytokine levels. In conclusion, CSF concentrations of the sex steroid hormone oestradiol 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.

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] Unfavourable 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 *vs* 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] Oestrogen provides a protective effect in bacterial infections 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 neuroprotective actions.[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 oestrogens 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 CNS 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-oestradiol (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.

MATERIALS AND METHODS

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 1 January 2006 to 1 July 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 one 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 three 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 (favourable *vs* unfavourable). 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 unfavourable 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 (GCS) 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 favourable outcome was defined as a score of 5, and an unfavourable outcome as a score of 1–4.

Cerebrospinal fluid 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-1RA), 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, leukaemia inhibitory factor (LIF), thrombopoietin (TPO), tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), stem cell factor (SCF), thymic stromal lymphopoietin (TSLP), macrophage colony-stimulating factor (M-CSF), epidermal growth factor (EGF), fibroblast growth factor (FGF)-2, FMS-like tyrosine kinase (Flt)-3 ligand, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF)-AA, PDGF-AB/BB, soluble cluster of differentiation 40 ligand (sCD40L), soluble IL-2 receptor alpha (sIL-2Ra; sCD25), transforming growth factor alpha (TGF-α), TNF-α, TNF-β, vascular endothelial growth factor (VEGF), soluble vascular cell adhesion molecule-1 (VCAM-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 (MIF), von Willebrand factor (vWF) antigen, plasminogen activator inhibitor (PAI)-1, PAI-2, thrombin-activatable fibrinolysis inhibitor (TAFI), fibrinogen, complement factor H, C3, C3a, iC3b, C5a, and C5b9.

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 (IQR) and were compared with the Mann-Whitney U test (for comparisons involving two groups) or the Kruskal-Wallis H test (for three 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 two-tailed and a p-value under 0.05 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).

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 three groups, as determined by the study design, but not between the two sexes overall (median 65 [47-70] years in males *vs* 51 [38-70] in women of all ages, p=0.29). Twenty-one (35%) patients had an unfavourable 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, $p<0.001$), whereas E2 and SHBG were comparable between groups.

Table 1. Baseline characteristics of the study groups (n=60).

Characteristic [a]	Men (n=20)	Women	
		Premenopausal (n=20)	Postmenopausal (n=20)
Age, years	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)	7 (37)
Immunocompromise [b]	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)	11 (55)
Dutch Meningitis Risk Score [c]	28 (27-33)	26 (20-29)	30 (24-34)
Unfavourable outcome	8 (40)	5 (25)	8 (40)
Death	4 (20)	2 (10)	5 (25)
Serum inflammation markers [d]			
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 [e]			
White cell count (per mm^3)	4,105 (1,547-7,422)	2,600 (955-7,422)	3,109 (1,297-11,784)
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)

Abbreviations: CSF, cerebrospinal fluid; ESR, erythrocyte sedimentation rate; GCS, Glasgow Coma Scale.

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

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

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

[d] 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).

[e] 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.

Hormone – median (IQR)	Men (n=20)	Women		p-value [a]
		Premenopausal (n=20)	Postmenopausal (n=20)	
Oestradiol (pmol/L) [b]	5.0 (5.0-11.3)	10.5 (5.0-18.5)	10.5 (5.0-17.9)	0.31
Testosterone (nmol/L) [c]	0.24 (0.16-0.31)	0.05 (0.05-0.05)	0.13 (0.05-0.20)	<0.001
SHBG (nmol/L) [d]	0.65 (0.40-1.05)	0.45 (0.30-1.25)	1.10 (0.38-1.50)	0.52

Abbreviations: IQR, interquartile range; SHBG, sex hormone-binding globulin.

[a] A p-value<0.05 was considered to be statistically significant.

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

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

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

When compared with cases with a favourable outcome (**table 3**), those with an unfavourable outcome exhibited higher E2 (median 14.0 *vs* 5.0 pmol/L, p=0.04) and lower SHBG levels (0.40 *vs* 1.0 nmol/L, p=0.03).

Table 3. Cerebrospinal fluid hormone levels according to outcome and death status.

Hormone – median (IQR)	Favourable outcome (n=39)	Unfavourable outcome (n=21)	p-value [a]
Oestradiol (pmol/L) [b]	5.0 (5.0-13.5)	14.0 (5.0-24.6)	0.045
Testosterone (nmol/L) [c]	0.05 (0.05-0.18)	0.18 (0.05-0.20)	0.14
SHBG (nmol/L) [d]	1.0 (0.40-2.1)	0.40 (0.20-0.80)	0.03
	Alive (n=49)	**Deceased (n=11)**	
Oestradiol (pmol/L)	10.0 (5.0-15.0)	5.0 (5.0-34.9)	0.49
Testosterone (nmol/L)	0.05 (0.05-0.20)	0.18 (0.08-0.21)	0.24
SHBG (nmol/L)	0.70 (0.30 vs 1.40)	0.50 (0.40-0.90)	0.48

Abbreviations: IQR, interquartile range; SHBG, sex hormone-binding globulin.

[a] A p-value<0.05 was considered to be statistically significant.

[b] Twenty-nine (48%) cases were under the lower limit of quantification of 10 pmol/L for oestradiol (21 with a favourable outcome).

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

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

This trend was seen in all three groups (**figures 1a** and **b**). These associations persisted after correcting for age and sex (adjusted p=0.02 for E2 and p=0.01 for SHGB) and for CSF protein levels (adjusted p=0.03 for E2, p=0.02 for SHBG) and in the sensitivity analysis excluding cases below the LLOQ for each hormone from the analysis (p=0.02 for E2, p=0.004 for SHBG). There was no difference in testosterone levels between cases with an unfavourable *vs* those with a favourable outcome, both overall or within the groups (**figure 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=0.003). 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 GCS) 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=0.03). No such difference was seen for E2 (10.0 [5.0-17.2] *vs* 7.5 [5.0-15.2] pmol/L, p=0.61) or SHBG (0.65 [0.30-1.5] *vs* 0.50 [0.40-1.2] nmol/L, p=0.92).

E2 levels were moderately positively correlated with C-reactive protein (r=0.42, p=0.001) and CSF protein levels (r=0.33, p=0.01) and weakly negatively correlated with the CSF:blood glucose ratio (r=-0.29, p=0.03). 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=1.3×10^{-4}), CCL7 (r=0.53, p=5.3×10^{-5}), CXCL9 (r=0.52, p=1.1×10^{-4}), ICAM-1 (r=0.58, p=5.7×10^{-6}), and fibrinogen (r=0.63, p=1.2×10^{-6}). SHBG was moderately positively correlated with CCL14a (r=0.60, p=3×10^{-6}). There was no correlation between testosterone and any of the cytokines, chemokines, and complement factors measured.

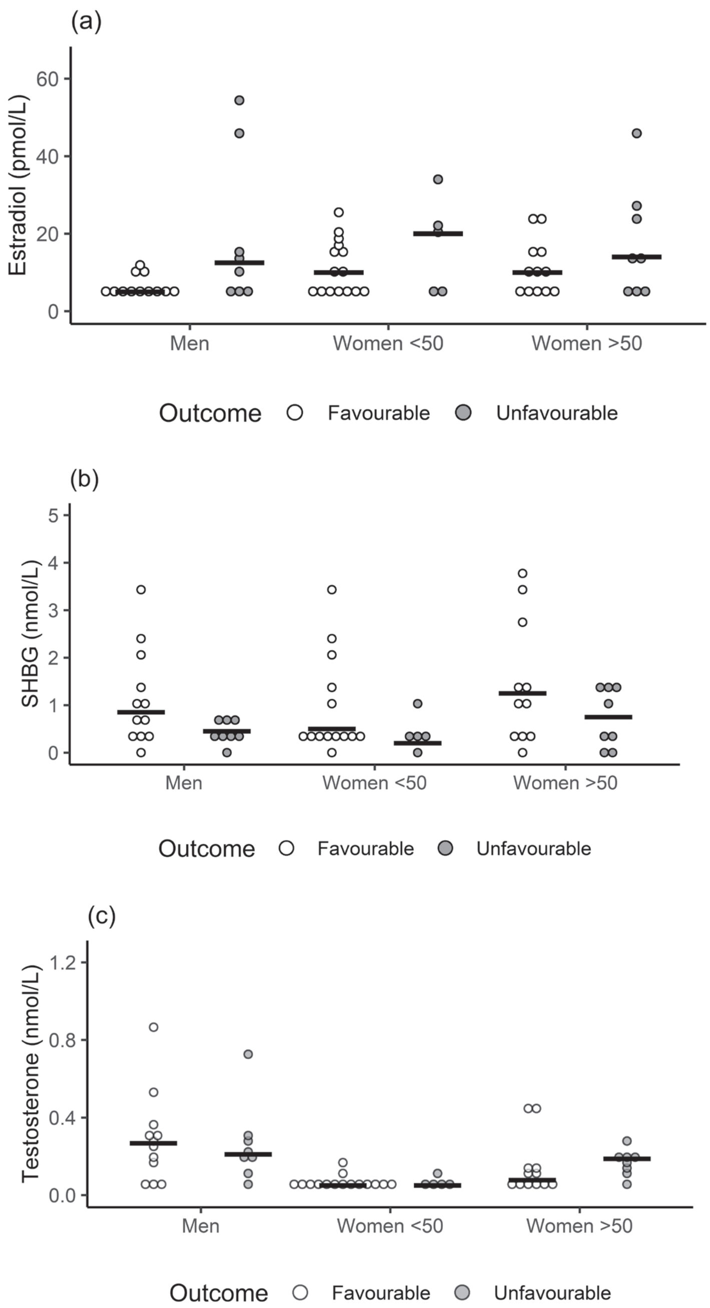

Figure 1. Distribution of oestradiol (a), sex hormone-binding globulin (b), and testosterone (c) levels in cases with a favourable (white dots) and unfavourable (grey dots) outcome in men (n=20), premenopausal (n=20), and postmenopausal (n=20) women. Each dot represents a case; black lines represent the median level in each group.

DISCUSSION

In this exploratory study, higher CSF E2 concentrations were associated with an unfavourable outcome, as well as with higher serum and CSF markers of inflammation and levels of pro-inflammatory cytokines and chemokines. These include a neutrophil-chemoattractant (CCL7), a chemokine involved in immune cell migration, differentiation, and activation (CXCL9), and a pro-inflammatory 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 unfavourable outcome is associated with a stronger inflammatory reaction to the highly immunogenic compounds released by the bacteria.[3] Oestrogen has a complex role in inflammation.[12] It enhances both cell-mediated and humoral immunity and can augment or dampen innate signalling pathways depending on the context, oestrogen 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 oestrogen concentrations promote a shift to Th2-cell and humoral responses, inhibiting pro-inflammatory and stimulating anti-inflammatory pathways.[11,25] In many infections, oestrogen 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 oestrogens) is upregulated in the central nervous system (CNS),[19] where oestrogen is considered to have anti-inflammatory actions.[13,14]

The effects of oestrogen 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 interleukins, 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 signalling 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] In addition, low SHBG levels were associated with an unfavourable 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 two 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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CHAPTER 7

DISCUSSION

INTRODUCTION

The aim of this thesis was to investigate sex-based differences in bacterial meningitis. Specifically, we aimed to study differences between men and women in the aetiology, epidemiology, clinical features, and prognosis of community-acquired bacterial meningitis, to analyse sex-based disparities in response to treatment, and to examine the role of inflammation and sex steroid hormones. We investigated these research questions in a nationwide prospective cohort study from 2006 to 2014, as well as using data from a randomised clinical trial on the use of dexamethasone in bacterial meningitis.

In this chapter, we will discuss our main results in the context of current literature, examine them from a broader perspective, and propose directions for future research.

AETIOLOGY AND EPIDEMIOLOGY

The MeninGene cohort included 1,412 episodes of community-acquired bacterial meningitis in adults (**chapter 3**), with cases equally distributed between sexes. Some studies have reported similar findings, whereas others have found a slight male preference.[1-4]

Streptococcus pneumoniae was the main causative pathogen in both men and women, followed by *Neisseria meningitidis.* Men accounted for 49% of cases with pneumococcal meningitis and 47% with meningococcal meningitis. *Listeria monocytogenes* was the third most frequent cause of bacterial meningitis in our cohort and was more common in men, who accounted for 66% of cases. Other studies have observed higher rates of Listeria meningitis in males.[3,5,6] As described in **chapter 2**, listeriosis is more common in females than in males during reproductive years, but this is likely due to pregnancy-related listeriosis,[7] and there were no pregnant women among the cases of Listeria meningitis in our study. In older age groups, which are overrepresented in our study population, the incidence is higher in males than in females.[7] Furthermore, *L. monocytogenes* infection more commonly occurs in patients with impaired cellular immunity,[8] and male patients in our cohort were more likely to be immunocompromised. This was mainly due to high rates of alcoholism, which is also more common in males in the general population[9] and is known to increase the risk of Listeria meningitis.[10]

The incidence of community-acquired bacterial meningitis decreased over the study period.[11] This is partly the result of herd protection provided by paediatric conjugate vaccines,[12-14] as reduced carriage and transmission among the vaccinated population has been shown to protect the unvaccinated population as well.[15] For pneumococcal meningitis, the introduction of these vaccines led to a marked decline in vaccine serotypes, which was observed in both sexes in our study (**chapter 4**). However, in many countries, the emergence of disease caused by non-vaccine serotypes has diminished or nullified the effect of vaccination on the overall incidence.[16]

This serotype replacement was not observed overall or in either sex in our cohort between 2006 and 2014. However, a more recent study analysing surveillance data in the Netherlands from 1988 to 2019 found a 35% increase in non-vaccine serotypes following the eradication of vaccine serotypes.[13]

We also found no differences between sexes in serotype distribution. While we could not find specific data on bacterial meningitis in the literature, a few studies have reported sex-based serotype differences in invasive pneumococcal disease. Most notably, in the Netherlands, there was a significant increase in the incidence of non-vaccine serotype invasive pneumococcal disease in young females after the introduction of PCV7, mainly due to serotype 1 disease, which was not observed in similarly aged men.[17] Serotype 1 disease was also more common in females in a study in Spain,[18] and an overall increase in serotype 1 disease was observed, as was the case in other countries.[19,20] Unfortunately, sex-disaggregated data were not reported.

CLINICAL FEATURES, RISK FACTORS, AND LABORATORY FINDINGS

We found differences between men and women regarding prior medical history and clinical presentation (**chapter 3**). Men were more likely to have a history of head injury, as described in another study,[21] which is likely due to their more common involvement in occupational and recreational activities with a high risk of injury.[22] Moreover, as mentioned in the previous section, more men than women were immunocompromised, and male sex was significantly associated with alcoholism. Other studies have reported this association between male sex and immunocompromise or alcoholism in patients with bacterial meningitis.[21,23,24] On the other hand, female patients more often presented with neck stiffness than males. This has been reported in another study,[25] although the reason is unclear.

Women also had higher serum inflammatory markers, including C-reactive protein and erythrocyte sedimentation rate, than men. This suggests that women with bacterial meningitis may have a more robust inflammatory reaction than men, even though this was not reflected in cerebrospinal fluid (CSF) inflammation markers.

OUTCOME

In a previous publication, advanced age, absence of otitis or sinusitis, alcoholism, tachycardia, a lower score on the Glasgow Coma Scale, cranial nerve palsy, a CSF white-cell count lower than 1000 cells per μL, a positive blood culture, and a high serum C-reactive protein concentration were reported to be independent predictors of an unfavourable outcome.[11] Upon adding patient sex as a predictor to this multivariable model, we identified male sex as an independent risk factor in community-acquired bacterial meningitis, with males 1.3 times more likely to have an unfavourable outcome and 1.5 times more likely to die compared with females (**chapter 3**).

Men generally have a worse outcome in infectious diseases, including bacterial diseases (**chapter 2**), so it makes sense that this would be the case with bacterial meningitis as well. Since the publication of our study, another large prospective multicentre cohort study in France, which included 533 adult patients with community-acquired bacterial meningitis, found male sex to be an independent predictor of an unfavourable outcome (adjusted odds ratio 2.1, 95% confidence interval 1.25-3.57).[4]

Some smaller retrospective studies found contrasting results. A multicentre retrospective study of 79 adults with community-acquired bacterial meningitis in Spain identified female sex as an independent predictor of a poor prognosis (odds ratio 9.93, 95% confidence interval 1.29-70.7).[26] This study, however, relied on a small sample size to adjust for multiple confounders, thus providing estimates with high levels of uncertainty, and only 48% of patients were treated with corticosteroids. Using a different methodology, a retrospective study in Denmark including 147 patients with community-acquired bacterial meningitis found female sex to be associated with long but not short-term mortality in a Cox proportional hazards regression analysis.[27] Prior studies found no relation between patient sex and outcome, although only univariable analyses were used.[6,21,28-31] Different study designs, sample sizes, inclusion criteria, main causative pathogens, and outcome measures may account for this heterogeneity between studies.

Females generally mount more robust immune responses than males during infections, contributing to greater bacterial clearance, which could partly explain the female survival advantage in bacterial meningitis. However, a heightened inflammatory reaction can also result in increased immunopathology, and in bacterial meningitis, an unfavourable outcome is related to an excessive inflammatory reaction to the highly immunogenic compounds released by bacteria.[32] Nevertheless, despite having higher inflammation markers, women in our cohort had a better outcome than men.

RESPONSE TO TREATMENT

We found no differences in management that would justify a better outcome in women. In fact, female patients less often received care in an intensive care unit (ICU) or mechanical ventilation than males (**chapter 3**). Although this difference was not statistically significant, it is in line with other studies that have reported men to be more likely to be admitted to an ICU and receive advanced life-supporting measures than women (**chapter 2**). Antibiotic prescription was similar in male and female patients. Adjunctive dexamethasone treatment was administered to 89% of cases in both sexes and was associated with a significant improvement in outcome. This effect was more prominent in women than in men, although we did not find a significant interaction between sex and dexamethasone. Because this was an observational study, however, treatment effects must be interpreted with caution.

To build on these results, we performed a *post hoc* analysis of the European dexamethasone in adulthood bacterial meningitis study (**chapter 5**) and again found a greater magnitude of treatment effect in women. We still could not find a significant effect modification, although this was likely due to lack of power.

While the use of a *post hoc* analysis precludes any firm conclusions about treatment effects, these results, together with the previous observational data, suggest that women, perhaps owing to a stronger inflammatory background, may be more susceptible to treatment with anti-inflammatory drugs, which in turn could explain their better outcome.

ROLE OF SEX STEROID HORMONES

Sex steroid hormones are known to influence the inflammatory response, and a relationship between their concentrations, the inflammatory response, and prognosis in bacterial meningitis is biologically plausible. For this reason, we investigated the role of oestradiol, sex hormone-binding globulin, and testosterone in 60 patients (20 men, 20 premenopausal, and 20 postmenopausal women) with pneumococcal meningitis and found their levels to be associated with the severity of illness, inflammation, and outcome (**chapter 6**).

More severely ill patients (with a lower score on the Glasgow Coma Scale) exhibited higher testosterone levels. Contrary to our findings, critically ill patients generally exhibit low testosterone levels due to decreased androgen production and increased aromatase-mediated conversion of androgens into oestrogens.[33] Testosterone promotes an anti-inflammatory response via androgen receptor signalling and suppresses pro-inflammatory responses. During experimental haemorrhagic shock in male rats, castration restores normal immune function[34] and administration of a testosterone blocker improves outcome following subsequent sepsis,[35] suggesting hypotestosteronaemia could be an adaptative reaction to reduce the deleterious effects of testosterone. However, this may not be the case in bacterial meningitis, and we found no association between testosterone levels and outcome in our study.

Oestrogen has bidirectional effects on the immune system and whether it exerts positive or negative regulatory effects on pro-inflammatory cytokine production depends on the immune stimulus, cell type, oestrogen concentrations, context, and time.[36,37] Oestradiol at peri-ovulatory or pregnancy concentrations has beneficial immunomodulatory and anti-inflammatory effects in humans and animal models.[37] Its actions include inhibiting the production of pro-inflammatory cytokines and stimulating the production of anti-inflammatory cytokines, generally promoting Th2-type anti-inflammatory responses.[38] On the other hand, low physiologic concentrations (such as those found in postmenopausal women) favour Th1-type responses and increase the production of pro-inflammatory cytokines.[38]

On the contrary, in our study, higher oestradiol concentrations were associated with higher levels of serum and cerebrospinal fluid (CSF) inflammatory markers, as well as a number of pro-inflammatory cytokines and chemokines. Perhaps as a result of excessive inflammation, higher levels of oestradiol were also associated with an unfavourable outcome, regardless of patient sex. In other acute illnesses, such as sepsis, high oestrogen concentrations are associated with a stronger inflammatory response and a greater likelihood of death.[39,40] In contrast, in some chronic diseases, such as multiple sclerosis, oestrogen has beneficial anti-inflammatory actions, as evidenced by its protection against experimental autoimmune encephalomyelitis and the remission of patients with multiple sclerosis in pregnancy.[41,42]

In infectious diseases, opposing actions have also been described. While in most infections, oestrogen has beneficial anti-inflammatory actions, for instance, in *Pseudomonas aeruginosa* pneumonia, it has been reported to worsen inflammation and outcome by oestrogen-mediated neutrophil dysfunction and enhancement of Th17-regulated inflammation.[43,44]

Several explanations for our findings are possible. It may be that in the context of acute bacterial meningitis, oestradiol promotes pro-inflammatory signalling pathways and is thus associated with a poor outcome. However, it is also possible that it rises in response to an excessive inflammatory reaction, either as part of a compensatory response or as a marker of inflammation.

In any case, while in line with previous findings of higher inflammatory markers in women with bacterial meningitis compared with men, our results alone do not seem to explain the better outcome observed in female patients.

FUTURE DIRECTIONS

Sex- and gender-informed medicine is a relatively young field. It is often viewed as a speciality area of interest rather than an essential consideration in biomedical research and clinical practice. Research has historically focused on male subjects, under the assumption that males and females are biologically identical. Clinical trials often excluded women of reproductive age for protective reasons, leaving many drugs untested in this group.[45] Experimental studies are also often done in male animals due to female animals' presumed higher hormonal variability.[46] When studies do include females, results are often not presented in a disaggregated manner. Although journals and funding agencies have been gradually implementing policies that require balanced inclusion and sex-disaggregated data, they are not always followed,[47] and much remains to be done.

Bacterial meningitis is no exception, and few studies on the influence of sex have been published previously. While most studies in the literature included male and female subjects, sex-disaggregated data were mostly not given. This must change, as when research fails to account for sex, erroneous conclusions may be drawn as the overall outcome and treatment data are extrapolated to each sex. Taking differences between men and women into account will undoubtedly increase our understanding of the susceptibility, clinical course, and outcome of bacterial meningitis and other infectious diseases, as well as improve their diagnosis and treatment.

Future experimental studies should investigate how sex hormones might be protective or detrimental against excessive inflammation elicited by invading pathogens. Administration of different concentrations of sex steroids to male and female animal models will no doubt provide valuable information on their true effect on the inflammatory response and outcome. It would also be interesting to examine the effect of other hormones, namely progesterone, on inflammation and outcome in bacterial meningitis. While the evidence does not currently support sex hormone modulation, therapies to modify sex steroid hormone profiles are a promising area of research and should be investigated in the future.

Furthermore, candidate genetic or inflammatory markers should be examined to determine if sex differences exist and whether they affect the outcome. New insights into sex-based host factors in the immune response to bacterial meningitis would lead to a better understanding of sex differences in the response to anti-inflammatory treatment. This could then guide the development of individualised treatment approaches and direct future sex-specific anti-inflammatory therapies. In addition, future clinical trials must be designed to have sufficient power to perform sex-disaggregated analyses.

In conclusion, much remains to be discovered about the pathogenesis of bacterial meningitis. Patient sex affects the response to treatment and outcome, which may be influenced by inflammation and sex steroid hormones. Integrating sex and gender analysis into research on bacterial meningitis is an essential step toward precision medicine and a greater understanding of disease pathophysiology.

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SUMMARY

SUMMARY

Significant progress has been made in the past decades in the prevention and management of community-acquired bacterial meningitis. There has been a substantial decline in incidence due to the widespread use of paediatric conjugate vaccines, as well as a significant improvement in outcome following the implementation of adjunctive corticosteroid treatment. Nevertheless, case fatality rates remain high and neurological sequelae occur in a substantial number of survivors, taking an important toll on healthcare systems as well as having a major socioeconomic impact. A greater understanding of disease pathophysiology is, therefore, of paramount importance.

Sex is a multidimensional biological characteristic defined on the basis of sex chromosome complement, reproductive tissues, and sex steroid hormones. It is distinct – though not independent – from gender, which refers to characteristics determined by societal and cultural factors. Both shape the susceptibility and response to infectious diseases. However, their role in bacterial meningitis is largely unknown.

The first objective of this thesis was to investigate whether there are differences between sexes in the epidemiology, clinical features, and causative pathogens and whether patient sex is a prognostic factor in community-acquired bacterial meningitis. Secondly, we aimed to study differences between men and women in response to treatment and whether sex steroid hormones play a role in the pathophysiology of sex-based differences in bacterial meningitis.

In **chapter 2**, we offer a comprehensive appraisal of the current knowledge on sex and gender differences in bacterial infections. First, we discuss how immune, hormonal, genetic, and behavioural factors contribute to the susceptibility and response to bacterial infections. In the second part, we review sex-based differences in selected bacterial diseases, including infections of the gastrointestinal, respiratory, and genitourinary tracts, sepsis, and other infections. Sex and gender differences can cause a disadvantage to males or females depending on the pathogen, but overall, there is a male bias, with men more often and more severely affected by various bacterial diseases.

In **chapter 3**, we investigate sex-based differences in adults with community-acquired bacterial meningitis regarding clinical characteristics, causative pathogens,

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treatment, and outcome. In a prospective cohort study including 1,412 episodes, cases were evenly distributed between men and women. *Listeria monocytogenes* was the third most frequent cause of bacterial meningitis (after *Streptococcus pneumoniae* and *Neisseria meningitidis*) and was more common in men. We found a history of head injury and immunocompromise, particularly alcoholism, to be more frequent in males. In a multivariable regression analysis, we identified male sex as an independent predictor of unfavourable outcome. Women had higher serum inflammatory markers and a greater risk reduction with dexamethasone treatment than men. This led us to hypothesise that a stronger inflammatory response could render women more responsive to the effect of anti-inflammatory agents, which in turn could lead to a better outcome. This hypothesis is investigated in **chapter 5**.

The introduction of large-scale immunisation programmes with conjugate vaccines has markedly reduced the incidence of bacterial meningitis. However, in pneumococcal meningitis, the decrease in disease caused by vaccine serotypes has been offset by a rise in non-vaccine serotypes. In **chapter 4**, we explore sex-based differences in pneumococcal serotype distribution and incidence trends since the introduction of paediatric pneumococcal conjugate vaccines. We found no differences in serotype distribution between 447 men and 481 women with pneumococcal meningitis or evidence of serotype replacement in either sex during the study period. We also found no differences in the magnitude of the decrease in incidence over this period, which was mainly driven by a decline in seven-valent vaccine serotypes as a result of herd protection.

To investigate whether there is a difference between men and women in the effect of corticosteroid treatment, in **chapter 5**, we perform a *post hoc* analysis of the European dexamethasone in adulthood bacterial meningitis study, a randomised controlled trial of dexamethasone *vs* placebo which included 169 men and 132 women with community-acquired bacterial meningitis. We found a difference in the magnitude of the treatment effect, which was larger in women, although there was no statistically significant effect modification.

Sex steroid hormones are known to have immunomodulatory properties, and a relation between their levels, inflammation, disease severity, and outcome in bacterial meningitis is biologically plausible. In **chapter 6**, we explore the role of sex steroid hormones in 20 men, 20 premenopausal, and 20 postmenopausal women with pneumococcal meningitis. We found CSF testosterone to be associated with

illness severity, with patients with an altered consciousness on admission having higher testosterone levels than those with normal mental status. CSF oestradiol was positively correlated with serum and CSF markers of inflammation, as well as with several pro-inflammatory cytokines and chemokines. Furthermore, higher oestrogen levels were associated with an unfavourable outcome.

SAMENVATTING

SAMENVATTING

In de afgelopen decennia is aanzienlijke vooruitgang geboekt bij de preventie en behandeling van "community-acquired" bacteriële meningitis. De incidentie is aanzienlijk gedaald dankzij het wijdverbreide gebruik van geconjugeerde vaccins voor kinderen, en de uitkomst van de ziekte is aanzienlijk verbeterd door de invoering van behandeling met corticosteroïden. Desalniettemin blijven de sterftecijfers hoog en komen neurologische restverschijnselen frequent voor, hetgeen een zware tol eist van de gezondheidszorg en een grote sociaal-economische impact heeft. Een beter begrip van de pathofysiologie van de ziekte is dan ook uitermate belangrijk.

Geslacht of sekse is een multidimensioneel biologisch kenmerk dat wordt gedefinieerd op basis van het geslachtschromosomen, -organen en -hormonen. Het verschilt - maar staat niet los van – *gender*, dat verwijst naar kenmerken die worden bepaald door maatschappelijke en culturele factoren. Beide bepalen de gevoeligheid voor en de reactie op infectieziekten. Hun rol bij bacteriële meningitis is echter grotendeels onbekend.

Het eerste doel van dit proefschrift was te onderzoeken of er verschillen zijn tussen de geslachten in de epidemiologie, klinische kenmerken en oorzakelijke pathogenen, en of het geslacht van de patiënt een prognostische factor is bij "community-acquired" bacteriële meningitis. Ten tweede wilden we verschillen tussen mannen en vrouwen in hun respons op behandeling bestuderen, en of geslachtsgebonden steroïdhormonen een rol spelen in de pathofysiologie van geslachtsgebonden verschillen in bacteriële meningitis.

In **hoofdstuk 2** wordt een uitgebreid overzicht gegeven van de huidige kennis over geslachts- en genderverschillen bij bacteriële infecties. Eerst bespreken we hoe immuun-, hormonale, genetische en gedragsfactoren bijdragen aan de gevoeligheid voor en de reactie op bacteriële infecties. In het tweede deel bespreken we geslachtsgebonden verschillen in geselecteerde bacteriële ziekten, waaronder infecties van de gastro-intestinale, respiratoire en urogenitale tractus, sepsis en andere infecties. Verschillen tussen geslacht en gender kunnen een nadeel vormen voor mannen of vrouwen, afhankelijk van de ziekteverwekker, maar over het algemeen zijn mannen ernstiger aangedaan door bacteriële ziekten.

In **hoofdstuk 3** onderzoeken we geslachtsgebonden verschillen in volwassenen met "community-acquired" bacteriële meningitis met betrekking tot klinische karakteristieken, oorzakelijke pathogenen, behandeling, en uitkomst. In een prospectieve cohortstudie met 1.412 episoden, waren er evenveel mannen als vrouwen geïncludeerd. *Listeria monocytogenes* was de derde meest voorkomende oorzaak van bacteriële meningitis (na *Streptococcus pneumoniae* en *Neisseria meningitidis*) maar kwam opvallend vaker voor bij mannen. Een doorgemaakt hoofdletsel in de voorgeschiedenis of aandoeningen leidend tot een verminderd functioneren van het immuunsysteem - in het bijzonder alcoholisme - bleken vaker voor te komen bij mannen. In een multivariabele regressieanalyse identificeerden wij het mannelijke geslacht als een onafhankelijke voorspeller van een slechte uitkomst. Vrouwen hadden hogere serum ontstekingsmarkers en een betere respons op behandeling met dexamethason dan mannen. Dit bracht ons tot de hypothese dat een sterkere ontstekingsreactie vrouwen gevoeliger zou kunnen maken voor het effect van ontstekingsremmers, wat op zijn beurt zou kunnen leiden tot een betere uitkomst. Deze hypothese werd verder onderzocht in **hoofdstuk 5**.

De invoering van grootschalige immunisatieprogramma's met conjugaatvaccins heeft de incidentie van bacteriële meningitis aanzienlijk verminderd. Echter, bij pneumokokkenmeningitis is de afname in ziekte veroorzaakt door vaccinserotypen tenietgedaan door een toename van niet-vaccinserotypen. In **hoofdstuk 4** onderzoeken we geslachtsgebonden verschillen in pneumokokkenserotypeverdeling en incidentietrends sinds de introductie van pneumokokken-conjugaatvaccins bij kinderen. Wij vonden geen verschillen in serotypeverdeling tussen 447 mannen en 481 vrouwen met pneumokokkenmeningitis of bewijs van serotypevervanging in een van beide geslachten tijdens de studieperiode. Wij vonden ook geen verschillen in de grootte van de daling van de incidentie tijdens deze periode, die hoofdzakelijk werd veroorzaakt door een daling van de serotypes van zeven-valente vaccins als gevolg van kuddebescherming.

Om te onderzoeken of er een verschil is tussen mannen en vrouwen in het effect van een behandeling met corticosteroïden, voerden wij in **hoofdstuk 5** een *post hoc* analyse uit van de *European dexamethasone in adulthood bacterial meningitis study*, een gerandomiseerde gecontroleerde trial van dexamethason versus placebo waarin 169 mannen en 132 vrouwen met "community-acquired" bacteriële meningitis werden geïncludeerd. Wij vonden een verschil in de grootte van het behandelingseffect, dat groter was bij vrouwen, hoewel er geen statistisch significant effect geobserveerd werd.

Van geslachtshormonen is bekend dat ze immunomodulerende eigenschappen hebben en een verband tussen hun niveaus, ontsteking, ziekte-ernst, en uitkomst bij bacteriële meningitis is biologisch plausibel. In **hoofdstuk 6** onderzoeken we de rol van geslachtshormonen in 20 mannen, 20 premenopauzale, en 20 postmenopauzale vrouwen met pneumokokkenmeningitis. We vonden dat de concentratie testosteron in het hersenvocht (liquor) geassocieerd was met de ernst van de ziekte, waarbij patiënten met een gedaald bewustzijn bij opname hogere testosteron concentraties hadden dan patiënten met een normaal bewustzijn. De estradiolconcentratie in liquor was positief gecorreleerd met serum en CSF markers van ontsteking, evenals met een aantal pro-inflammatoire cytokines en chemokines. Bovendien waren hogere oestrogeenspiegels geassocieerd met een slechte uitkomst.

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CURRICULUM VITAE

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Sara Dias was born on April 12, 1986, in Lisbon, Portugal. She finished secondary school in 2004 at Colégio de Santa Doroteia and obtained a medical degree from Nova Medical School in Lisbon in 2010.

After graduation, she worked for a year as a general resident at the Central Lisbon University Hospital Centre, and in 2012, she was admitted to a Neurology residency programme at the same institution. During her residency, she obtained a Master's degree in Advanced Medical Skills from the University of Barcelona. In 2015, she did a research internship at the Amsterdam UMC, where she began working with the Neuroinfections research group and started some of the work presented in this thesis. After completing her residency in 2018, she moved to Amsterdam to pursue a doctoral degree. She worked on this thesis under the supervision of Professor Diederik van de Beek and Professor Matthijs Brouwer.

Sara currently works as a neurologist at the Neurology Department of Central Lisbon University Hospital Centre. She lives in Lisbon with her husband, André.

PORTFOLIO

PORTFOLIO

1. PhD training

	Year	Workload (ECTS)
Courses		
- Dutch Language Course (INTT): A0 through B1	2018-20	10.4
- Practical Biostatistics	2019	1.1
- Bioinformatics	2019	1.1
- Computing in R	2019	0.4
- Infectious Diseases	2019	1.3
- Writing a Scientific Paper	2020	1.5
- Advanced Topics in Biostatistics	2021	2.1
- Clinical Epidemiology: Observational Epidemiology	2021	0.6
- Clinical Epidemiology: Systematic Reviews	2021	0.7
- Clinical Epidemiology: Randomized Clinical Trials	2021	0.6
Seminars, workshops and master classes		
- Weekly research meetings, Department of Neurology, Amsterdam UMC	2018-19	1.3
Presentations		
- "Sex-based differences in patients with community-acquired bacterial meningitis: a prospective nationwide cohort study", poster presentation at the 26th ECCMID	2016	0.5
- "Sex-based differences in response to corticosteroids in adults with community-acquired bacterial meningitis: analysis of data from the European Dexamethasone Trial", poster presentation at the 29th ECCMID	2019	0.5
- "Differences between sexes in the response to corticosteroids in adults with community-acquired bacterial meningitis", oral presentation at the 5th Congress of the EAN	2019	0.5
- "Differences between sexes in the response to corticosteroids in adults with community-acquired bacterial meningitis: analysis of data from the European Dexamethasone in Adulthood Bacterial Meningitis Study", poster presentation at the Amsterdam Neuroscience Annual Meeting	2019	0.5
- "Cerebrospinal fluid sex steroid hormones in pneumococcal meningitis", poster presentation at the 32nd ECCMID	2022	0.5

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(Inter)national conferences		
- Amsterdam Neuroscience Annual Meeting, 5 October 2018, Amsterdam	2018	25
- 29th ECCMID, 13-16 April 2019, Amsterdam	2019	1
- 5th Congress of the EAN, 29 June to 2 July 2 2019, Oslo	2019	1
- Amsterdam Neuroscience Annual Meeting, 4 October 2019, Amsterdam	2019	0.25
- 32nd ECCMID, 23-26 April 2022, Lisbon	2022	1
- 8th Congress of the EAN, 25-28 June 2022, Vienna	2022	1
Other		
- Presentations at weekly research meeting (4; 4 hours per presentation)	2018-19	0.6

2. Teaching		
	Year	**Workload (ECTS)**
Lecturing		
- "Central nervous system infections", Neurological Emergencies Course, Nova Medical School, Lisbon	2018	0.5
- "Bacterial meningitis: progress and challenges", 39th Conference Cycle on Infectious Diseases, Hospital de Santa Maria, Lisbon	2018	0.5
- "Central nervous system infections", Neurological Emergencies Course, Nova Medical School, Lisbon	2021	0.5
- "Antibiotic treatment of central nervous system infections", Antibiotics Course, Central Lisbon University Hospital Centre, Lisbon	2021	0.5

3. Parameters of Esteem	
	Year
Grants	
- ECCMID travel grant	2016
- EAN congress bursary	2017
- ESCMID Research Grant	2018
- EAN Research Training Fellowship	2019
- ECCMID travel grant	2019
- EAN congress bursary	2019
- EAN congress bursary	2020

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LIST OF PUBLICATIONS

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