Explaining sex differences in risk of bloodstream infections using mediation analysis in the population-based HUNT study in Norway

Randi Marie Mohus1,2,*, Lise T. Gustad1,3,4, Anne-Sofie Furberg5,6, Martine Kjølberg Moen1,2, Kristin Vardheim Liyanarachi1,7, Åsa Askim2, Signe E. Åsberg1, Andrew T. DeWan1,8, Tormod Rogne1,8, Gunnar Skov Simonsen5,9,10, Tom Ivar Lund Nilsen1,7,11, Bjørn Olav Åsvold12,13, Jan Kristian Damås1,7,14 & Erik Solligård1,2

Previous studies indicate sex differences in incidence and severity of bloodstream infections (BSI). We examined the effect of sex on risk of BSI, BSI mortality, and BSI caused by the most common infecting bacteria. Using causal mediation analyses, we assessed if this effect is mediated by health behaviours (smoking, alcohol consumption), education, cardiovascular risk factors (systolic blood pressure, non-HDL cholesterol, body mass index) and selected comorbidities. This prospective study included 64,040 participants (46.8% men) in the population-based HUNT2 Survey (1995–1997) linked with hospital records in incident BSI. During median follow-up of 15.2 years, 1840 (2.9%) participants (51.3% men) experienced a BSI and 396 (0.6%) died (56.6% men). Men had 41% higher risk of first-time BSI (95% confidence interval (CI), 28–54%) than women. Together, health behaviours, education, cardiovascular risk factors and comorbidities mediated 34% of the excess risk of BSI observed in men. The HR of BSI mortality was 1.87 (95% CI 1.53–2.28), for BSI due to *S. aureus* 2.09 (1.28–2.54), *S. pneumoniae* 1.36 (1.05–1.76), *E. coli* 0.97 (0.84–1.13) in men vs women. This study shows that men have higher risk of BSI and BSI mortality than women. One-third of this effect was mediated by potential modifiable risk factors for incident BSI.

Bloodstream infection (BSI) is a major global burden and may lead to sepsis which constitutes up to 60% of the global mortality burden1,2. The risk of acquiring BSI depends on the bacterial virulence, host characteristics, geographical location, and biological factors1,3–8. Epidemiological studies indicate a male predominance in BSI

1Gemini Center for Sepsis Research, Institute of Circulation and Medical Imaging, NTNU, Norwegian University of Science and Technology, Trondheim, Norway. 2Clinic of Anesthesia and Intensive Care, St. Olavs Hospital, Trondheim University Hospital, Torgarden, Postboks 3250, 7006 Trondheim, Norway. 3Nord-Trøndelag Hospital Trust, Levanger, Norway. 4Faculty of Health Sciences, Nord University, Levanger, Norway. 5Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway. 6Department of Infectious Diseases, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway. 7Department of Chronic Disease Epidemiology and Center for Perinatal, Pediatric and Environmental Epidemiology, Yale School of Public Health, New Haven, CT, USA. 8Research Group for Host-Microbe Interaction, Faculty of Health Sciences, UiT – The Arctic University of Norway, Tromsø, Norway. 9Norwegian Institute of Public Health, Oslo, Norway. 10Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Trondheim, Norway. 11Department of Public Health and Nursing, K.G. Jebsen Center for Genetic Epidemiology, NTNU, Norwegian University of Science and Technology, Trondheim, Norway. 12Department of Enderocrinology, Clinic of Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway. 13Department of Clinical and Molecular Medicine, Centre of Molecular Inflammation Research, NTNU, Norwegian University of Science and Technology, Trondheim, Norway. *email: randi.m.mohus@ntnu.no
and sepsis. Nevertheless former studies on sex differences in incidence and mortality of BSI and sepsis have given conflicting results with increased risk in women\(^1\), increased risk in men\(^6\,^9\), increased mortality in women\(^10\), or increased mortality in men\(^11\,^12\). Importantly, disparities in immune function between sexes may arise from differences in biological characteristics such as anatomy and hormonal status, medical conditions, health behaviours, lifestyle, and exposure to different pathogens\(^13\,^14\). Most previous studies on sex differences in BSI and sepsis have been performed in small and selected cohorts, mainly from the intensive care unit (ICU)\(^5\,^10\,^11\) and there are limited population-based studies\(^9\,^13\,^16\) which better account for selection bias\(^17\). Studies on severe infections and sepsis tend to adjust for sex in their analyses\(^18\) but the mechanisms behind the observed sex differences are unexplored\(^19\). Little is known whether conditions that are known to increase BSI risk, like health behaviours\(^4\), cardiovascular disease risk factors or comorbidity\(^20\,^21\), contribute to the observed difference in risk of BSI between men and women. Such knowledge may help identify targets for intervention to reduce BSI and sepsis risk.

To assess the impact of sex as a risk factor for first-time BSI, BSI mortality, and BSI caused by the most common infecting bacteria, *Staphylococcus (S.) aureus*, *Streptococcus (S.) pneumoniae* and *Escherichia (E.) coli* we used data from the Norwegian HUNT study linked with prospectively recorded BSI episodes. Further, we examined if sex differences in health behaviours and education attainment, cardiovascular risk factors and selected comorbidities, which reflect known risk factors for BSI\(^20\,^21\), may contribute to the observed sex difference in risk of first-time BSI. We applied sequential mediation analysis\(^22\) using inverse-odds weighting\(^23\) to explore their potential mediating effect on the associations between sex and BSI.

**Methods**

**Study population.** The HUNT Study is a population-based health study conducted in the Nord-Trøndelag region in Norway and consists of four consecutive surveys inviting the total adult population approximately every 10th year. The second survey (HUNT2, 1995–1997), invited all adult inhabitants \(\geq 20\) years \(n = 93,898\) to a clinical examination and a comprehensive self-report of health-related topics. Of these, 65,237 (69%) chose to participate. The HUNT study database is regularly updated with information on date of migration and death from the National Registry. More details on the HUNT study are published elsewhere\(^24\). For the purpose of the present study, we excluded 47 (0.07%) participants who had a prior positive blood culture and 1150 (1.8%) who migrated or died before start of follow-up. A total of 64,040 participants were eligible for analyses (Supplemental Fig. S1).

**Measures.** The exposure is sex as registered in the National Registry. The two main outcomes were first-time BSI and BSI mortality. The participants were followed for incident BSI by linkage to the Nord-Trøndelag Hospital Trust (HNT HF) Sepsis Registry using the personal identification number of Norwegian citizens\(^25\). All BSIs were confirmed at the microbiology laboratories at Levanger Hospital which provided all microbiology services in the Nord-Trøndelag region or at St. Olavs Hospital. Details about HNT HF sepsis registry are included in the supplemental material. We defined BSI mortality as death occurring within 30 days after detection of any BSI. In secondary analyses we assessed first-time BSI caused by the most common bacteria *E. coli*, *S. aureus* and *S. pneumoniae*, and performed age-stratified analyses.

Mediators are variables that are causally located between exposure and outcome variables, and that partly explain the effect of the exposure on outcome\(^22\,^26\). Mediation analysis can assess direct and indirect effects and estimate the proportion of the total effect that works through the mediator of interest (i.e. proportion mediated)\(^27\). We used three distinct sets of mediators measured at inclusion to HUNT2; (1) health behaviours (smoking and alcohol use) and educational attainment; (2) cardiovascular risk factors (body mass index (BMI, kg/m\(^2\)), systolic blood pressure (mmHg) and non-high-density lipoprotein cholesterol (non-HDL cholesterol, mmol/L); (3) comorbidities defined by self-report of cardiovascular disease (history of myocardial infarction, angina pectoris, and/or stroke), diabetes, cancer history, lung disease (asthma or chronic obstructive pulmonary disease) and standardised measurements of kidney function (estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m\(^2\)). The three sets of mediators reflect known risk factors for BSI\(^20\,^21\). For some of the included mediators there are reports of sex differences in prevalence, pathophysiology and outcomes\(^28\,^29\). The proposed diagram for the relationship between sex and risk of BSI is shown in Fig. 1. The aim of the analysis was to examine to which extent sex differences in risk of BSI may be related to these mediating factors. Details about the measurements and categorisation of mediators are included in the Supplemental Material.

**Statistical analyses.** We used Cox proportional hazard regression to estimate the hazard ratios (HRs) with 95% confidence intervals (95% CI) of a first-time BSI and of BSI mortality in men compared to women. Attained age was used as the time scale. Start of follow-up was defined by the availability of data in the sepsis registry. For patients referred to St. Olavs hospital, the tertiary referral centre, BSI information was included depending on their primary hospital. Participants contributed person-years from inclusion date in HUNT2 except for participants having Namsos as their primary hospital, they contributed from 1 September 1999.

In the analysis of BSI risk, participants were followed until their first BSI. For BSI-mortality participants were followed until death within 30 days of any BSI episode. For both analyses participants were censored at time of migration out of Nord-Trøndelag, death of all causes, or end of follow-up set to 31 December 2011, whichever occurred first. The proportional hazards assumption was examined by visual inspection of log–log plots and tests of Schoenfeld residuals. Using Stata *stcompadj*, we estimated cumulative incidence and mortality from start of follow-up to first-time BSI and BSI mortality, accounting for death by all causes as a competing risk and we provide cumulative incidence and cumulative mortality curves to illustrate changes during follow-up. As supplemental analyses we assessed subhazard ratios taking death as a competing event into account for first-time BSI and BSI mortality.
In secondary analyses we estimated hazard ratios and cumulative incidence of first-time BSI caused by the most common infecting bacteria. Further, we conducted age-stratified analyses on risk of first-time BSI; < 50, 50 to < 65, 65 to 79, and ≥ 80 years to address whether menopause affects women's risk of BSI, and to assess sex differences in BSI risk with advancing age. To examine the associations between the mediators included in Model 2 and risk of first-time BSI, we conducted sensitivity analyses using Cox regression for BMI, systolic blood pressure and non-HDL cholesterol.

For the mediation analysis we used an inverse odds weighting (IOW) procedure. IOW is a counterfactual method that enables a decomposition of the total effect of the exposure (sex) on the outcome (first-time BSI) into a natural direct effect (NDE) from exposure on outcome, and a natural indirect effect (NIE) through multiple mediators. The method accommodates multiple mediators simultaneously and is robust to unmeasured common causes of the mediators.

The inverse odds weights were obtained by regressing the exposure on all mediators of interest with age as a covariate. In our analysis, the total effect is interpreted as the total association between sex and first-time BSI, the NIE is the proportion of excess BSI risk in men mediated by the risk factors, whereas the NDE is the proportion of excess BSI risk in men not associated with these factors. The proportion mediated is the percent of the total association that is mediated through the risk factors. We did not estimate the NIE of individual mediators separately as it may not be appropriate when the mediators affect each other or when single mediator-outcome confounders may be affected by exposure. Instead, we estimated the NIE with a sequential approach using three models. In model 1, we assessed education attainment and health behaviours (smoking and alcohol use); in model 2, we added the cardiovascular risk factors (BMI, systolic blood pressure and non-HDL cholesterol) to address potential preclinical disease; and in model 3, the selected comorbidities (cardiovascular, diabetes, cancer history, lung, and kidney disease) were included to the complete set of mediators. This approach assumes that the cardiovascular risk factors and further the comorbidities are causal descendants of the health behaviours and educational attainment. The sequential approach further implies that model 3 reflects the best interpretation of the mediation analyses as all mediators and age are included.

We performed bootstrapping based on 1000 replications to derive percentile-based CIs for all mediation parameters, and the NDE and NIE are presented as HRs with 95% CIs. The proportion mediated on the log scale was calculated using the formula \((\ln \text{HR}_{\text{NIE}} / \ln \text{HR}_{\text{TOTAL}})\). All statistical analyses were performed using Stata version 17.0. A detailed description of the IOW analyses is included in Supplemental Table S1.

**Ethical approval.** The study was approved by the Regional Committee for Medical and Health Research Ethics of Central Norway (REK no 2012/153 and REK no 94135), and by the HUNT data access committee. Participation in HUNT 2 was voluntary, and informed written consent to data collection and linking their data to other registers was obtained from all participants. All methods were performed in accordance with the Declaration of Helsinki.
**Results**

**Population characteristics.** During a median follow-up of 15.2 years (IQR 12.3–15.5 years), among 64,040 participants (46.8% men), 1840 (2.9%) experienced a BSI and 396 (0.6%) died within 30 days after a BSI episode. The median age at inclusion was similar for both sexes. Both men and women who experienced a BSI were older (median age at inclusion 67.4 and 68.0 respectively), and they had a higher comorbidity burden than participants who did not have a BSI during follow-up (Table 1).

| Table 1. Baseline characteristics of the study population at inclusion in HUNT2, n = 64,040. BSI bloodstream infection, n numbers, IQR interquartile range, BMI body mass index, HDL high-density lipoprotein. 1Percentage of total first-time BSI in both sexes. 2BSI mortality was defined as all-cause mortality within 30 days after a BSI. Percentage of BSI mortality on both sexes. 3History of myocardial infarction, angina pectoris and/or stroke. 4History of chronic obstructive pulmonary disease or asthma. |

|                        | Men     | Women   |
|------------------------|---------|---------|
| Total population n (%) | 29,962  | 34,087  |
| First-time BSI n (%) 2 | 943     | 897     |
| BSI mortality n (%) 2  | 224     | 172     |
| Age (mean, IQR)        | 48.6 (36.5–62.9) | 48.7 (36.2–64.2) |

| Smoking                |         |         |
|------------------------|---------|---------|
| Current (%)            | 8334 (27.8) | 9726 (28.5) |
| Prior (%)              | 9422 (31.4) | 6516 (19.1) |
| Never (%)              | 10,668 (35.6) | 15,230 (44.7) |

| Alcohol use            |         |         |
|------------------------|---------|---------|
| <1 unit/2 weeks (%)    | 8448 (28.2) | 16,069 (47.3) |
| 1–7 units/2 weeks (%) | 14,258 (47.6) | 14,484 (42.6) |
| 8–14 units/2 weeks (%)| 4602 (15.4) | 1861 (5.5) |
| ≥15 units/2 weeks (%) | 1643 (5.5) | 272 (0.8) |

| Education              |         |         |
|------------------------|---------|---------|
| <10 years (%)          | 20,625 (68.9) | 22,259 (65.3) |
| 10–12 years (%)        | 2302 (7.7) | 3436 (10.1) |
| >12 years (%)          | 5650 (18.9) | 6412 (18.8) |

| BMI (kg/m²)            |         |         |
|------------------------|---------|---------|
| <18.5 (%)              | 118 (3.9) | 349 (1.0) |
| 18.5–24.9 (%)          | 10,498 (35.0) | 14,736 (43.2) |
| 25–29.9 (%)            | 14,757 (49.3) | 12,345 (36.2) |
| 30–34.9 (%)            | 3674 (12.3) | 4640 (13.6) |
| 35–39.9 (%)            | 496 (1.7) | 1236 (3.6) |
| ≥40 (%)                | 74 (0.3) | 344 (1.0) |

| Systolic blood pressure (mmHg) median (IQR) |         |         |
|---------------------------------------------|---------|---------|
| 137 (127–150)                               | 131 (118–149) |

| Non-HDL cholesterol (mmol/L) median (IQR)   |         |         |
|---------------------------------------------|---------|---------|
| 4.5 (3.7–5.3)                               | 4.3 (3.5–5.3) |

| Comorbidities                             |         |         |
|-------------------------------------------|---------|---------|
| Cardiovascular disease 3 (%)              | 2918 (9.7) | 2014 (5.9) |
| Chronic kidney disease (%)                | 979 (3.3) | 1802 (5.3) |
| Diabetes (%)                              | 895 (3.1) | 970 (2.9) |
| Cancer history (%)                        | 878 (2.8) | 1413 (4.1) |
| Chronic lung disease 4 (%)                | 1183 (4.0) | 1011 (3.0) |

**Risk of BSI and BSI mortality.** Men were more likely to experience a first-time BSI and to die from a BSI compared to women. Men had 1.41 (HR, 95% CI 1.28–1.54) times the risk of first-time BSI, and had 1.87 (HR, 95% CI 1.53–2.28) times the risk of dying from a BSI (Table 2) compared with women. In analyses by the most common infecting bacteria, men had a 2.09-fold (HR, 95% CI 1.28–2.54) risk of BSI caused by *S. aureus*, and 1.36 (HR, 95% CI 1.05–1.76) increased risk of *S. pneumonia*. The corresponding result for *E. coli* did not show higher risk in men with HR of 0.97 (95% CI 0.84–1.13) (Table 3).

The above findings are illustrated by graphing the age-adjusted cumulative incidence of first-time BSI and cumulative BSI mortality in Fig. 2. The cumulative incidence of BSI was higher among men than women after the first five years of follow-up. For BSI mortality the sex difference was apparent after the first 2.5 years of follow-up and during follow-up the sex differences in mortality increased. The subhazards obtained for first-time BSI and BSI mortality, provide the same direction of the associations as the Cox regression analyses (Supplemental Table S2). We additionally present cumulative incidence curves for the three most common infecting bacteria in Fig. 3A–C. Men had higher cumulative incidence of *S. aureus*, especially after the first seven years of follow-up, whereas *E. coli* had higher cumulative incidence among women.
The population-based design ensures that all BSI occurring in residents of a defined geographical area are included, which is an advantage over ICU cohorts. Our results are supported by one study including 1051 patients, showing that men had higher risk of BSI. Like our study they described that BSI incidence increases by age, and men had twice the rate of S. aureus BSI. Another population-based study comprising 9266 patients with BSI admitted to ICU found that male sex is a risk factor for BSI. A recent study restricted to persons aged ≥ 65, found that men were at increased risk of BSI compared to females (incidence rate ratio 1.44, 95% CI 1.32–1.59).

Table 2. Associations of sex with risk of bloodstream infection and BSI mortality. BSI bloodstream infection, HRs hazard ratios, 95% CI 95% confidence intervals, No. numbers. Cox regression analyses were adjusted with age as the underlying scale. BSI mortality was defined as all-cause mortality within 30 days after a bloodstream infection.

|                  | Years at risk | No. BSI | HR  | 95% CI      | Years at risk | No. BSI deaths | HR  | 95% CI      |
|------------------|---------------|---------|-----|-------------|---------------|----------------|-----|-------------|
| Women            | 436,758       | 897     | 1.0 | Reference   | 472,012       | 172            | 1.0 | Reference   |
| Men              | 373,915       | 943     | 1.41| 1.28–1.54   | 404,723       | 224            | 1.87| 1.53–2.28   |

Table 3. Associations of sex with risk of bloodstream infections caused by the most common bacteria. BSI bloodstream infection, HRs hazard ratios, 95% CI 95% confidence intervals. Cox regression analyses were adjusted for age as the underlying scale.

|                  | Years at risk | No. BSI | HR  | 95% CI      | No. BSI | HR  | 95% CI      | No. BSI | HR  | 95% CI      |
|------------------|---------------|---------|-----|-------------|---------|-----|-------------|---------|-----|-------------|
| Women            | 436,758       | 83     | 1.0 | Reference   | 113     | 1.0 | Reference   | 399     | 1.0 | Reference   |
| Men              | 373,915       | 129    | 2.09| 1.28–2.54   | 119     | 1.36| 1.05–1.76   | 285     | 0.97| 0.84–1.13   |

Mediation analyses. In Table 4 we present the total effect, the natural direct and indirect effects of sex on risk of first-time BSI. Compared with women men had an estimated HR of 1.40 (95% CI 1.24–1.55) for first-time BSI. Behavioural risk factors and education mediated 10% (model 1), after adding the cardiovascular risk factors the proportion mediated was reduced to 5% (model 2), whereas the whole set of mediators, including comorbidities, jointly mediated 34% of the total effect (model 3).

To examine the reduction in proportion mediated from 10 to 5% in model 2, in sensitivity analyses, we observed an increased risk of BSI in persons with low BMI (< 18.5) and in persons with increasing BMI compared to the normal BMI group (18.5–24.4). For systolic blood pressure we did not observe any risk difference, and for non-HDL cholesterol the HR suggested a protective effect but with imprecise estimates (Supplemental Table S4).

Discussion
In this large Norwegian population-based study with a follow-up of more than 15 years, male sex was associated with 41% higher risk of BSI and 87% higher risk of dying from a BSI. An estimated 34% of the increased risk of BSI in men was mediated by known BSI risk factors. We additionally found that men had 2.09 times the risk of BSI caused by S. aureus compared to women. These findings add weight to the observed male preponderance in severe infections and point out modifiable BSI risk factors that are targets for preventive measures to reduce the burden of BSI.

There are few population-based studies on sex differences in the epidemiology of BSI and to our knowledge, no previous studies have performed mediation analysis to explain the sex differences of BSI. We used the IOW method which is known to be robust using multiple mediators en bloc and the rich baseline information from HUNT2 allowed us to implement mediation analyses in a time-to-event context. The sequential approach enabled us to examine if the observed excess risk in men was mediated through different known risk factors for BSI. For many medical conditions men and women differ regarding incidence, the underlying pathophysiology and responses to therapy. For health behaviours, more men reported smoking, and they reported higher alcohol use. We also observed higher prevalence of obesity among women in HUNT2. Adding cardiovascular risk factors to health behaviours and education, the proportion mediated lowered from 10 to 5%, which indicates some interactions or common pathways for these mediators. This result might be due to some of the mediators considered in model 2 being more frequent or harmful in women, or the mediators might reduce the risk of BSI. The complete model with all mediators included accommodates the assumptions required, and most likely reflects the best modelling of the associations explaining 34% of the excess BSI risk in men. Interventions to reduce modifiable risk factors in the population will likely reduce the burden of BSI, particularly in older men.
and the sex difference was most pronounced in the oldest patients, similar to our results\textsuperscript{15}. On the other hand, the Global Burden of Disease Study found that age-standardised sepsis incidence was higher among women, while sepsis-related mortality was higher among men\textsuperscript{1}. This study included results from 195 countries and comprised all age groups. They found higher sepsis incidence in low-income countries, and the pattern of sepsis incidence and mortality varied according to location, which is not directly comparable to our study population.

We identified higher BSI mortality in men which is in line with a recent study of infection-related death in UK Biobank\textsuperscript{12}. Conversely, some ICU studies report higher sepsis-related mortality in women\textsuperscript{10,29}. A recent meta-analysis evaluating the associations between sex and mortality in critically ill adults showed inconclusive results\textsuperscript{30}. This conflicting evidence concerning sex differences in mortality is most likely due to the heterogeneity of BSI and sepsis depending on the aetiology and the cohort studied\textsuperscript{17}, but may also be affected by sex differences in immune responses\textsuperscript{13,14,32} and differences in treatment\textsuperscript{10,18}. 

Figure 2. Sex differences in cumulative incidence and mortality of BSI. Age-adjusted sex difference in cumulative incidence of BSI (A), and in cumulative mortality (B), estimated for age 49.99 (the mean age of the total population). Note: due to the variation in incidence of different outcomes the scale of the Y-axis is not uniform across the panels.
Figure 3. Sex differences in cumulative incidence of BSI caused by the most common bacteria. Age-adjusted sex difference in cumulative incidence of *S. aureus* (A), *S. pneumoniae* (B), and *E. coli* (C), estimated for age 49.99 (the mean age of the total population). Note: due to the variation in incidence of different bacteria the scale of the Y-axis is not uniform across the panels.
mortality. Second, investigating BSI is dependent on clinician’s suspicion and decision to submit blood cultures during the clinical course after detection of a BSI. Therefore, we did not perform mediation analyses on risk of BSI mortality.

Mediators, are more prone to possible mediator misclassification. This could lead to underestimation of the mediating effect. Forth, the subjective assessment of some mediators, and dichotomised outcomes, could have changed during the 16 years of follow-up. This potential misclassification would most likely lead to an underestimation of the risk of BSI. Moreover, sex dependent hospital admissions. Third, the mediators were only measured once at inclusion to HUNT2, and the data were sex specific. In our study, the incidence of BSI was higher in men, which is a risk factor for invasive S. aureus infections. Other studies show that testosterone levels and use of hormone contraceptives among females alter nasal colonization, indicating that sex hormones affect the immune response to S. aureus. The higher prevalence of S. aureus colonization in men is of particular interest, as preventive measures like eradication or temporary suppression could lower the risk of invasive infections which is especially important in hospitalized patients.

In a sensitivity analysis we found that the sex differences in BSI risk are evident after predicted age of menopause indicating that alterations in both innate and adaptive immune functions with age may be sex specific. Aging is associated with chronic inflammation and a generally reduced immune function. Sex hormone levels in men and women change with age. Women face an abrupt decline during menopause, whereas men have a steady decline from second decade of life. As in former studies, our study points out that advancing age is a risk factor for developing and dying from BSI and elderly men are at particular risk.

Major strengths of our study include its large size, the population-based design, long-term follow-up and linkage to microbiological records which represent the gold standard to identify BSI within a population. Our definition of BSI as a laboratory verified positive blood culture, excluding blood cultures solely with microorganisms associated with skin contamination, ascertains the accuracy of the outcome studied. In addition, reviews of medical records of patients with S. aureus and S. pneumoniae BSI in this cohort showed that ~98% met the 2001 sepsis criteria. We were able to study BSI incidence and BSI mortality in a large population without the potential referral bias seen in single institution studies of BSI. The complete ascertainment of all BSI, together with the rich baseline measurements of known BSI risk factors in HUNT2, allows for an accurate estimation of incidence and mortality in the population, with the potential of risk factor identification and mediation analyses.

There are some limitations of our study that merit attention. First, we lack information on immunosuppressive medication which are known risk factors for BSI and BSI mortality. We did not have information on the clinical course after detection of a BSI. Therefore, we did not perform mediation analyses on risk of BSI mortality as in-hospital factors such as correct and timely antibiotics and resuscitation measures strongly influence mortality. Second, investigating BSI is dependent on clinician’s suspicion and decision to submit blood cultures during the testing, with the chance of some undetected cases. Further, we cannot rule out if the clinical presentation of infections is different in men and women, and that this could result in disproportionate blood culture sampling depending on sex. Another concern would be if the clinical presentation of infection led to disproportionate and sex dependent hospital admissions. Third, the mediators were only measured once at inclusion to HUNT2 and could have changed during the 16 years of follow-up. This potential misclassification would most likely lead to underestimation of the mediating effect. Forth, the subjective assessment of some mediators, and dichotomised mediators, are more prone to possible mediator misclassification. This could lead to underestimation of the risk of BSI.

### Table 4. Mediation of the associations between sex and BSI by behavioural risk factors, educational attainment, cardiovascular risk factors and comorbidities. BSI bloodstream infection, HRs hazard ratios, 95% CI 95% confidence intervals. Smoking, alcohol use and educational attainment at baseline. Systolic blood pressure, non-high-density lipoprotein cholesterol and Body Mass Index. Cardiovascular disease, chronic kidney disease, diabetes, history of cancer, or chronic lung disease. Percentile-based bootstrap CIs are reported. Estimates are adjusted for age as a covariate. Proportion mediated: (In HRNIE/ln HR_{TOTAL}).

| Table 4 | Mediation by behavioural risk factors and education | Risk of first-time BSI |
|---------|---------------------------------------------------|------------------------|
|         | Model 1                                           | HRs (95% CI)           |
|         | Total effect                                      | 1.40 (1.24–1.55)       |
|         | Natural direct effect (NDE)                       | 1.36 (1.18–1.57)       |
|         | Natural indirect effect (NIE)                     | 1.04 (0.97–1.07)       |
|         | Proportion mediated                              | 10%                    |
|         | Model 2                                           | HRs (95% CI)           |
|         | Total effect                                      | 1.40 (1.24–1.55)       |
|         | Natural direct effect (NDE)                       | 1.38 (1.19–1.58)       |
|         | Natural indirect effect (NIE)                     | 1.02 (0.92–1.07)       |
|         | Proportion mediated                              | 5%                     |
|         | Model 3                                           | HRs (95% CI)           |
|         | Total effect                                      | 1.40 (1.24–1.55)       |
|         | Natural direct effect (NDE)                       | 1.25 (1.05–1.47)       |
|         | Natural indirect effect (NIE)                     | 1.12 (1.02–1.17)       |
|         | Proportion mediated                              | 34%                    |
indirect effect and overestimation of the direct effect. Fifth, we were not able to assess the mediators individually, as this could have violated the model assumptions.

Despite these limitations, our study provides a foundational observation of the existing sex differences in BSI epidemiology and adds important information for clinicians, researchers, and policymakers concerning BSIs. Our results suggest that sex disparities in BSI cannot be explained fully by the mediating factors investigated. Sex affects the shape of immune responses attributed to genetic, hormonal, and environmental factors. The human X-chromosome encodes a number of critical genes involved in the regulation of immune functions. It is clear that sex extensively influences the host immune responses, but this sexual dimorphism is underappreciated, and sex bias is a major challenge in clinical trials. Sex hormones act as important modulators of immune functions and responses; testosterone and progesterone are immunosuppressive, while oestradiol is immunoenhancing. Few human studies have investigated sex hormones’ effects in severe infections and sepsis. Interestingly, in covid-19 where men are more prone to a severe course, the use of androgens in men has shown promising results on severity. Furthermore, in a review of health records in post-menopausal women with regular use of oestradiol, the fatality risk of covid-19 is reduced by more than 50%.

Future perspectives of our results include the need for targeted research on how these sex differences could be addressed to achieve a longer and healthier life for both men and women. Additional work should focus on how health behaviours, education level, cardiovascular risk factors and comorbidities play a role in the sex disparities seen in severe infectious diseases. Knowledge of mediating factors together with recognition of sex differences in severe infections are important for public health leaders, researchers, and clinicians as it can inform preventive actions and identify individuals especially at risk.

Conclusion
Our study has shown that men have an increased risk of BSI and BSI mortality. Using mediation analyses we estimated that 34% of the increased risk of BSI is mediated through known BSI risk factors. As BSI represents an important global burden of disease, our study serves as a catalyst for additional investigations by establishing the presence of sex differences and mediating risk factors. This will potentially lead to targeted management strategies to prevent BSI and sepsis in both men and women.

Data availability
Data is available from the authors upon reasonable request and by application to HUNT Research Centre. https://hunt-db.medisin.ntnu.no/hunt-db/.

Code availability
A detailed description of the IOW analyses is included in Supplemental Material.

Received: 7 December 2021; Accepted: 12 May 2022
Published online: 19 May 2022

References
1. Rudd, K. E. et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. Lancet 395, 200–211 (2020).
2. Prescott, H. C., Osterholzer, J. J., Langa, K. M., Angus, D. C. & Iwashyna, T. J. Late mortality after sepsis: Propensity matched cohort study. BMJ 353, 12375 (2016).
3. Reddick, L. E. & Alto, N. M. Bacteria fighting back: How pathogens target and subvert the host innate immune system. Mol. Cell 54, 321–328 (2014).
4. Paulsen, J. et al. Associations of obesity and lifestyle with the risk and mortality of bloodstream infection in a general population: A 15-year follow-up of 64,027 individuals in the HUNT study. Int. J. Epidemiol. 46, 1573–1581 (2017).
5. Mohus, R. M. et al. Association of iron status with the risk of bloodstream infections: Results from the prospective population-based HUNT Study in Norway. Intens. Care Med. 44, 1276–1283 (2018).
6. Esper, A. M. et al. The role of infection and comorbidity: Factors that influence disparities in sepsis. Crit. Care Med. 34, 2576–2582 (2006).
7. Askim, A. et al. Anxiety and depression symptoms in a general population and future risk of bloodstream infection: The HUNT study. Psychosom. Med. 80, 673–679 (2018).
8. Moore, J. X. et al. Community characteristics and regional variations in sepsis. Int. J. Epidemiol. 46, 1607–1617 (2017).
9. Laupland, K. B. et al. Severe bloodstream infections: A population-based assessment. Crit. Care Med. 32, 992–997 (2004).
10. Pietropaoli, A. P., Glance, L. G., Oakes, D. & Fisher, S. G. Gender differences in mortality in patients with severe sepsis or septic shock. Gender Med. 7, 422–437 (2010).
11. Adrie, C. et al. Influence of gender on the outcome of severe sepsis: A reappraisal. Chest 132, 1786–1793 (2007).
12. Drozd, M. et al. Non-communicable disease, sociodemographic factors, and risk of death from infection: A UK Biobank observational cohort study. Lancet Infect. Dis. 21, 1184–1191 (2021).
13. Vázquez-Martínez, E. R., García-Gómez, E., Camacho-Arroyo, I. & González-Pedrajo, B. Sexual dimorphism in bacterial infections. Biol. Sex Differ. 9, 27 (2018).
14. Klein, S. I. & Flanagan, K. L. Sex differences in immune responses. Nat. Rev. Immunol. 16, 626–638 (2016).
15. Uslan, D. Z. et al. Age- and sex-associated trends in bloodstream infection: A population-based study in Olmsted County, Minnesota. Arch. Intern. Med. 167, 834–839 (2007).
16. Laupland, K. B., Pasquill, K., Steele, L. & Parfitt, E. C. Burden of bloodstream infection in older persons: A population-based study. BMC Geriatr. 21, 31 (2021).
17. Laupland, K. B. Defining the epidemiology of bloodstream infections: The “gold standard” of population-based assessment. Epidemiol. Infect. 141, 2149–2157 (2013).
18. Maurais-Jarvis, F. et al. Sex and gender: Modifiers of health, disease, and medicine. Lancet 396, 565–582 (2020).
19. World Health Organization. Addressing Sex and Gender in Epidemic-Prone Infectious Diseases (World Health Organization, 2007).
20. Laupland, K. B. et al. Population-based risk factors for community-onset bloodstream infections. Eur. J. Clin. Microbiol. Infect. Dis. 39, 753–758 (2020).
21. Lindström, A.-C., Eriksson, M., Märtensson, J., Oldner, A. & Larsson, E. Nationwide case–control study of risk factors and outcomes for community-acquired sepsis. Sci. Rep. https://doi.org/10.1038/s41598-021-94558-x (2021).
22. VanderWeele, T. & Vansteelandt, S. Mediation analysis with multiple mediators. Epidemiol. Methods 2, 95–115 (2014).
23. Nguyen, Q. C., Oospak, T. L., Schmidt, N. M., Glymour, M. M. & Tchetgen Tchetgen, E. J. Practical guidance for conducting mediation analysis with multiple mediators using inverse odds ratio weighting. Am. J. Epidemiol. 181, 349–356 (2015).
24. Kroksård, S. et al. Cohort profile: The HUNT study, Norway. Int. J. Epidemiol. 42, 968–977 (2012).
25. Mehil, A. et al. Burden of bloodstream infection in an area of mid-Norway 2002–2013: A prospective population-based observational study. BMC Infect. Dis. 17, 205 (2017).
26. Lee, H. et al. A guideline for reporting mediation analyses of randomized trials and observational studies: The AGReMA statement. JAMA 326, 1045–1056 (2021).
27. Lee, H., Herbert, R. D. & McAuley, I. H. Mediation analysis. JAMA 321, 697–698 (2019).
28. Gerdis, E. & Regitz-Zagrosek, V. Sex differences in cardiometabolic disorders. Nat. Med. 25, 1657–1666 (2019).
29. Mauvais-Jarvis, F. Epidemiology of gender differences in diabetes and obesity. In Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity (ed. Mauvais-Jarvis, F.) 3–8 (Springer, 2017).
30. Antequeru, A. et al. Sex as a prognostic factor for mortality in critically ill adults with sepsis: a systematic review and meta-analysis. BMJ Open. 11 (9), e048982. https://doi.org/10.1136/bmjopen-2021-048982 (2021).
31. Preacher, K. J. & Hayes, A. F. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891 (2008).
32. Libert, C., Dejager, L. & Pinheiro, I. The X chromosome in immune functions: When a chromosome makes the difference. Nat. Rev. Immunol. 10, 594–604 (2010).
33. Paulsen, J. et al. Epidemiology and outcome of Staphylococcus aureus bloodstream infection and sepsis in a Norwegian county 1996–2011: An observational study. BMC Infect. Dis. 15, 116 (2015).
34. Sakr, A., Brégeon, F., Mége, J. L., Rolain, J. M. & Blin, O. Staphylococcus aureus nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Front. Microbiol. 9, 2419 (2018).
35. Stensd, D. B. et al. Circulating sex-steroids and Staphylococcus aureus nasal carriage in a general female population. Eur. J. Endocrinol. 184, 337–346 (2021).
36. Stensd, D. B. et al. Hormonal contraceptive use and Staphylococcus aureus nasal and throat carriage in a Norwegian youth population. PLoS ONE 14, e0218311 (2019).
37. Liu, Z. et al. Nasal decontamination for the prevention of surgical site infection in Staphylococcus aureus carriers. Cochrane Database Syst. Rev. 5, 012462 (2017).
38. Griefing-Kröll, C., Berger, P., Lepperding, G. & Grubeck-Loebenstein, B. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. Aging Cell 14, 309–321 (2015).
39. Askim, A. et al. Epidemiology and outcome of sepsis in adult patients with Streptococcus pneumoniae infection in a Norwegian county 1993–2011: An observational study. BMC Infect. Dis. 16, 223 (2016).
40. Tolsma, V. et al. Sepsis severe or septic shock: Outcome according to immune status and immunodeficiency profile. Chest 146, 1205–1213 (2014).
41. Kim, D. et al. Surviving sepsis campaign: International guidelines for management of sepsis and septic shock 2021. Intens. Care Med. 47, 1181 (2021).
42. Cadegiani, F. A., McCoy, J., Gustavo Wambier, C. & Goren, A. Early antiandrogen therapy with dutasteride reduces viral shedding, inflammatory responses, and time-to-remission in males with COVID-19: A randomized, double-blind, placebo-controlled interventional trial (EAT-DUTA AndroCoV Trial—Biochemical). Cureus 13, e13047 (2021).
43. Seeland, U. et al. Evidence for treatment with estradiol for women with SARS-CoV-2 infection. BMC Med. 18, 369 (2020).

Acknowledgements

The Trondelag Health Study (the HUNT study) is a collaboration of the HUNT Research Centre (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Trondelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. The authors sincerely thank Arne Mehl for his extensive and thorough work with the HNT HF sepsis registry, the microbiology departments of Levanger, Namssos and St. Olavs hospitals for providing microbial data, and the Department for Research at Nord-Trondelag Hospital Trust for assistance with data linkage.

Author contributions

R.M.M., L.T.G., M.K.M., K.V.L., S.E.Å., T.N., J.K.D. and E.S. conceived and designed the study. T.N., B.O.Å., L.G., A.T.D., T.R., J.K.D. and E.S. supervised the study. R.M.M., B.O.Å., A.A. and L.T.G. contributed in the acquisition of data for statistical analyses. R.M.M. analyzed the data, prepared tables, and figures. All authors interpreted the results, drafted the manuscript, contributed to the discussions, and revised the manuscript critically for important intellectual content. All authors have read and approved the final version of manuscript and agreed to be accountable for all aspects of the work.

Funding

N/A. This work was supported by a Grant from The Liaison Committee for Education, Research, and Innovation in Central Norway.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-12569-8.

Correspondence and requests for materials should be addressed to R.M.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
