The incidence and prognosis of patients with bacteremia

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LIST OF PAPERS

The PhD thesis is based on the following three original research studies, which will be referred to by their roman numerals:

STUDY I:
Nielsen SL, Pedersen C, Jensen TG, Gradel KO, Kolmos HJ, Lassen AT. Decreasing incidence rates of bacteremia: a 9-year population-based study. The Journal of infection. Jul 2014;69(1):51-59.

STUDY II:
Nielsen SL, Lassen AT, Kolmos HJ, Jensen TG, Gradel KO, Pedersen C. The overall and daily risk of bacteremia during hospitalization: a 9-year multicenter cohort study [submitted]

STUDY III:
Nielsen SL, Lassen AT, Gradel KO, Jensen TG, Kolmos HJ, Hallas J, Pedersen C. Bacteremia is associated with excess long-term mortality: a 12-year population-based cohort study. The Journal of Infection. Feb 2015;70(2):111-126

INTRODUCTION

Bacteremia is associated with increased morbidity and mortality, and ranks among the top seven causes of death in North America and Europe [1]. Of great concern, the incidence rate of bacteremia has increased for decades while short-term prognosis has remained unchanged or improved only slightly [2-4]. Consequently, we are facing an increased number of bacteremia survivors for whom we know little about long-term survival and causes of death [5]. The epidemiology of bacteremia is changing with ageing of the population, shifts in healthcare, and advances in medicine such as increased use of immunosuppressive treatment, intravascular devices and invasive procedures [6]. Contemporary knowledge on the epidemiology and outcome of bacteremia is important to assess its impact on public health and is a prerequisite for any effective prevention and improvement of prognosis.

The aims of this thesis were to investigate the occurrence of bacteremia in the general population and among hospitalized patients, and to investigate long-term mortality and causes of death after bacteremia.

INTRODUCTION TO BACTERIA AND DISEASE

In humans, bacteria reside in a relationship that can be commensalistic, mutualistic or parasitic [7,8]. Most bacteria in humans live in a *commensalistic* relationship; they live off but do not help or harm the host. In a *mutualistic* relationship both the bacteria and the host benefit; the bacteria feed of the host but keep other harmful microbes from taking up residence. Examples are bacteria that live inside the mouth, nose, throat, and intestines of humans as part of the normal flora, also termed “the indigenous microbiota”. In a *parasitic* relationship the bacteria benefit while the host is harmed; the bacteria evade the host’s immune system and grow at the expense of the host. Such parasitic bacteria are potential pathogens and may colonize and invade the host, and cause disease.

On a daily bases, the body is exposed to hordes of potential pathogens. Bacteria enter the body through easily accessible sites such as broken skin, the gastrointestinal track, the respiratory system or via indwelling catheters. The transition from colonization of the host to clinical disease is complex and determined by factors not limited to microbe pathogenicity and host defense mechanisms [9,10]. Local infections may develop when bacteria manage to escape the host immune mechanisms while dissemination into the bloodstream occurs when the immune response fails to control bacterial spread.

A disseminated infection often triggers a systemic inflammatory response in the body, which in the presence of an infection is denoted sepsis. The word sepsis originates from Greek and means *“decomposition of animal or vegetable organic matter in the presence of bacteria”*, and was first encountered in Homer’s poems as a derivative of the verb form sepo, which means *“I rot”* [11]. Sepsis is defined by a range of clinical and paraclinical criteria and is categorized according to severity into sepsis, severe sepsis and septic shock with increasing mortality [12-15]. Most patients with bacteremia fulfill the criteria for sepsis [16-19] while less than 50% of patients with sepsis have bacteremia [13,20-23]. In fact, a recent Danish study by Henriksen et al. (2014) found that only 10% of hospitalized acute medical patient with sepsis of any severity had bacteremia; the occurrence of bacteremia in-

creased with sepsis severity (5% for sepsis, 12% for severe sepsis, and 38% for septic shock) [22].

INTRODUCTION TO BACTEREMIA

DEFINITION OF BACTEREMIA

Bacteremia is defined as the presence of viable bacteria in the bloodstream as evidenced by growth in blood cultures where contamination has been ruled out [24-26]. Contamination occurs when blood cultures are positive due to microorganisms not present in the bloodstream and may result from inadequate sterile technique in obtaining blood cultures [27]. Positive blood cultures with predominantly pathogenic microorganisms such as *Escherichia coli* or *Streptococcus pneumoniae* usually indicate true bacteremia; in contrast, it may be difficult to determine the significance of blood cultures with common skin contaminants [28]. In everyday clinical practice the diagnosis of bacteremia is based on all available microbiological and clinical data [26,29]. However, this approach is not feasible if data are collected retrospectively from microbiological databases or electronic surveillance systems because they often lack clinical data. Instead, computer algorithms that rely on blood culture data alone without clinical data have been developed to distinguish between true bacteremia and contamination [30-32]. Most bacteremias can be considered clinically important since blood cultures are normally drawn upon signs of infection. However, transient bacteremia without clinical symptoms may occur as a result of dental manipulation, orotracheal intubation or simply tooth brushing [33-35]. In this thesis, we use the collective term bacteremia to denote both bacteremia and fungemia (presence of fungi in the bloodstream).

CLASSIFICATION OF BACTEREMIA

Bacteremia can be classified according to place of acquisition, causative microorganism and focus of infection.

Place of acquisition

Bacteremias have traditionally been classified according to place of acquisition as either *community-acquired* or *nosocomial* [29]. Community-acquired bacteremias were those evident or incubating at the time of admission whereas nosocomial bacteremias were those occurring after admission. The differentiation was based on all available clinical information. However, many studies have used a 48-hour [26,27,36-40] or 72-hour time limit [41,42] after admission to distinguish between community-acquired and nosocomial bacteremia and such a predefined time limit has its merits. First, it facilitates comparison between studies. Second, because bacteremia databases based on retrospectively collected data often comprise thousands of bacteremias it would be extremely labor intensive to ascertain place of acquisition by detailed chart review. Also, chart review may be biased due to interobserver variance [43]. No consensus on a fixed time limit has been reached and in accordance with Leibovici et al [44], we have recently shown that no specific time limit unambiguously distinguish between community and hospital acquisition with regard to patient characteristics or causative microorganisms [45].

An increasing number of patients have frequent contacts with the healthcare system in outpatient clinics where they receive medical care such as chemotherapy or hemodialysis. In 2002, Friedman et al. acknowledged the importance of distinguishing between community-acquired bacteremia in patients with no recent healthcare contact (denoted *community-acquired* bacteremia) and in patients with recent healthcare contact (denoted *healthcare-associated* bacteremia) since these two groups of patients show important differences with respect to clinical characteristics, isolated microorganisms and outcome [38]. In short, Friedman et al.'s definitions of healthcare-association included at least one of the following: recent hospitalization, residence in a nursing home or long-term care facility, recent attendance at a hospital clinic for hemodialysis or intravenous therapy, or receipt of specialized medical service at home. Although the definitions by Friedman et al. have been widely used varying definitions are being published across the literature [2,31,39,46-48]. In summary, bacteremia can be classified according to place of acquisition as either community-acquired, healthcare-associated or nosocomial.

Causative microorganisms

Bacteremias may be classified according to the general class of microorganisms (e.g. Gram-negative rods) or specific microorganisms that have invaded the bloodstream [49]. In the Western countries, the most common causes of bacteremia among non-selected populations are *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* [2,50-55]. In the developing countries *Salmonella enterica* serotype Typhi predominates and accounts for 30% of all bacteremias [56,57]. The distribution of microorganisms is closely related to place of acquisition and focus of infection. *Escherichia coli* and *Staphylococcus aureus* are common causes of bacteremia regardless of place of acquisition. In addition *Streptococcus pneumoniae* often causes community-acquired bacteremia while coagulase-negative staphylococci, *Pseudomonas* species, *Enterococcus* species, fungi and multiple organisms (polymicrobial bacteremias) to a higher degree cause healthcare-associated and nosocomial bacteremia [2,36,52,58,59].

Focus of infection

In general, the most common foci of bacteremia are the urinary tract, lower respiratory tract and gastrointestinal tract [2,52]. However, the distribution of foci varies according to place of acquisition and isolated microorganism, which may provide clues as where to search for the focus of infection. Community-acquired bacteremias are often caused by infection of the urinary tract or the lower respiratory tract whereas healthcare-associated and nosocomial bacteremias are more often associated with catheter-related infections [38,60,61]. The focus of infection remains unknown in about 22% of bacteremia patients [62].

Knowledge on the interdependent relationship between place of acquisition, causative microorganisms and focus of infection can help clinicians search for foci and guide choice of appropriate empirical antibiotics.

THE OCCURRENCE OF BACTEREMIA

Population-based studies are commonly accepted as the optimal design for establishing the occurrence of bacteremia in a population. Population-based studies aim to ascertain all cases of bacteremia in a well-defined geographical area with a known population size where non-residents are excluded [55,63].

The first population-based study to report on the occurrence of bacteremia was conducted in Charlson County, South-Carolina, USA during 1974–1976 [64]. The authors reported an overall incidence rate of 80 per 100,000 person years (42 for community-acquired, 31 for nosocomial, and 7 for unknown). The incidence rate was highest for neonates, infants and the oldest; 84% of the

patients were registered with an underlying medical condition; the most common causative microorganisms were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella*, and *Streptococcus pneumoniae*; and the most common foci were the urinary tract (26%) and pulmonary tract (17%).

Since the 1980s, reported incidences rates of bacteremia have ranged from 95 to 215 per 100,000 person years in population-based studies [2,3,50-52,54,60,65-68] and most studies have reported an increasing trend [2,3,51,54,65,67]. The incidence is excepted to increase in the future owing to factors not limited to ageing of the population, increased longevity of patients with chronic disease, advances in provided healthcare such as immunosuppressive treatment and invasive procedures, lowered threshold for taking blood cultures and improvement in blood culture methodology [27,69]. In particular, more bacteremias are expected because of an increasing average life-expectancy since the elderly are at highest risk of bacteremia [51,52] and since the incidence has increased the most among the elderly [51]. The incidence of bacteremia has increased quite dramatically during the past decades as evidenced in studies by Madsen et al. in Denmark (102% increase during 1981–1994) [3], Rodríguez-Créixems et al. in Spain (107% increase during 1985–2006) [67], Søgaard et al. in Denmark (68% increase during 1992–2006) [2], and Skogberg et al. in Finland (14% increase during 2004–2007) [54]. This annual increase in the mentioned studies ranged between 3.5% and 5.6%.

Population-based studies have rarely distinguished between community-acquired, healthcare-associated and nosocomial bacteremia and the distribution of microorganisms and trends for these categories of bacteremia remains poorly elucidated [2,60,68]. Regardless of place of acquisition, the incidence of bacteremia increased in Northern Jutland, Denmark during 1992–2006 with the largest increase seen for healthcare-associated bacteremia (from 3 bacteremias per 100,000 person years in 1992 to 40 bacteremias per 100,000 person years in 2006) [2]. Although less pronounced, Laupland et al. confirmed this increasing trend for healthcare-associated bacteremia in Calgary, Canada during 2000–2008 [68]. In the same study, no trend was seen for community-acquired or nosocomial bacteremia. Finally, studies limited to community-acquired bacteremia without considering healthcare-association have reported either no trend or an increasing trend [53,70,71].

Why are population-based studies on the occurrence of and trend in bacteremia of importance?

First, population-based studies on the occurrence of bacteremia can be used to determine its importance in absolute terms and relative to other major health conditions. A Canadian study by Laupland et al. estimated that community-onset bacteremia affects nearly 1/1000 residents per year (which was comparable to that of stroke, myocardial infarction and major trauma) and accounted for one hospital day per 100 residents per year [53]. On a larger scale, Goto et al. estimated that nearly 2 million bacteremias and 250,000 deaths occur annually in Europe and North America [1]. Consequently, bacteremia ranked among the top seven causes of death in the included countries. Based on these numbers, Goto et al. speculated that the worldwide annual number of deaths from bacteremia may be comparable to or higher than each of human immunodeficiency virus (HIV), tuberculosis, and malaria. However, it may be more accurate to conclude that the deaths were associated with rather than caused by bacteremia as causality was not established in the study.

Second, population-based studies can be used to evaluate the impact of restructurings of healthcare and/or preventive measures on the epidemiology of bacteremia in the general population. Hospital-based studies of selected populations (e.g. nosocomial bacteremia) cannot stand alone since fewer nosocomial bacteremias may simply be a consequence of a shift in healthcare from in-hospital care to outpatient clinics [53,68,71]. Therefore, studies should report community-acquired, healthcare-associated, and nosocomial bacteremia separately as trends within acquisition groups may otherwise remain undetected.

Third, population-based studies can detect changes in the distribution of microorganisms or emerging trends in the community or healthcare setting. This is important as timely appropriate empirical antibiotic treatment reduces mortality [72,73]. As mentioned, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* have remained the predominant causative microorganisms for decades [55]. However, interesting shifts have been observed for less frequent microorganisms. Already in the 1980s, Sjöberg et al. noted that *"The reason why Pseudomonas aeruginosa and Enterococcus faecalis, two relatively antibiotic-resistant organisms, show a tendency to increase since the beginning of this decade is not known...However, if this tendency continues it will be necessary to ascertain the cause and take adequate action"* [50]. This notion underlines the importance of contemporary surveillance studies from different geographical areas and time-periods since such studies may detect emerging trends at an early stage, guide prescription of appropriate empirical antibiotics and inform infection control policy.

As mentioned, hospital-based studies are inappropriate to estimate the occurrence of bacteremia in the general population. However, together with local and nationwide surveillance programs they are useful to monitor the occurrence of bacteremia among hospitalized patients and may provide a measure of prevention and control [28,74,75]. Previous studies have reported on incidences of nosocomial bacteremia and have shown that the occurrence of bacteremia differ greatly by specialty [59,74,76,77]. Studies have also documented that nosocomial bacteremia is associated with increased mortality, length of stay and costs of care [78,79]. Further risk factors for nosocomial bacteremia have been identified such as male sex, recent operative procedures, indwelling intravascular catheters, and nosocomial infections [76,77]. However, to our knowledge no study has addressed the timing of bacteremia among all hospitalized patients and therefore it remains unknown whether patients are at constant risk of bacteremia during hospitalization or if the daily risk (incidence) displays a decreasing or increasing trend with longer admission time. This can only be evaluated by providing denominator data in the form of duration and course of hospitalization for all admitted patients. Knowledge on where and when specific groups of patients are at high risk of bacteremia during hospitalization could help clinicians to identify and prevent bacteremias and hospital hygiene committees to identify problem areas where targeted preventive measures or intensified surveillance are needed [28].

THE PROGNOSIS OF BACTEREMIA

Knowledge on the prognosis of bacteremia is important to patients who wish to know what to expect from their disease, clinicians who wish to identify and modify predictors of death, and healthcare policy makers who wish to investigate whether restructurings of healthcare may improve the prognosis.

Several studies have revealed a dismal short-term prognosis for bacteremia patients with 30-day mortality rates ranging from

12% to 24% [2,3,19,60,68] with even higher rates for patients in intensive care units (41%) [80] or with septic shock (51%) [81]. Fewer studies have assessed long-term mortality after bacteremia with rates ranging from 11% to 63% at one year [18,19,82-89], 49% to 55% at 3 years [18,83] and 63% at 4 years [19]. However, most of these studies were hospital-based [18,19,83], restricted to specific microorganisms [85,88,89], or had limited follow-up time to one year [84].

In recent years, increased attention has been paid to long-term outcomes of severe infections and it has been hypothesized that a bidirectional relationship exist between sepsis and chronic health; poor chronic health predisposes to sepsis and sepsis may in turn worsen chronic health [5,90]. A similar relationship is likely to exist for bacteremia and chronic health. Figure 1 displays a conceptual model for the relationship between acute disease (e.g. bacteremia), chronic disease and death.

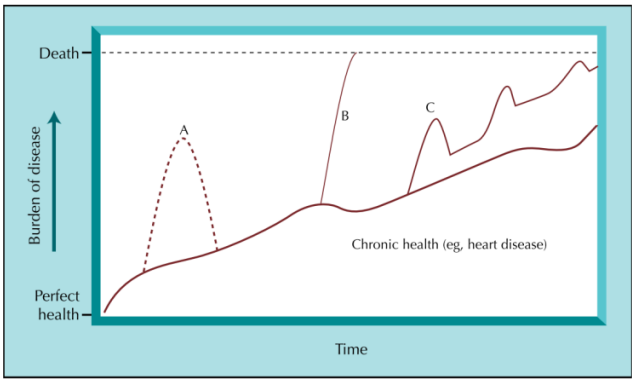

Figure 1
Conceptual model of the relationship of acute disease, chronic disease and death by Yende et al [90] (Reproduced with permission from Springer Science).

Different potential outcomes follow bacteremia: (A) complete recovery; (B) death shortly after bacteremia; or (C) partial recovery and new onset or worsening of existing comorbidity followed by multiple acute events, eventually leading to death. In addition, one could imagine a scenario (D) similar to scenario C but without acute events—such a scenario would also result in curtailed life expectancy. Of note, the conceptual model describes the relationship between bacteremia and outcome on an individual level whereas studies describe the overall effect of bacteremia on outcome in a study population. That is, a study that report excess long-term mortality after bacteremia does not preclude that some patients may fully recover and return to pre-bacteremia health.

We know that scenario B is true as evidenced by the high short-term mortality. The question remains whether long-term survival after bacteremia follows scenario A (full recovery) or scenario C/D (curtailed life expectancy) for those who survive the acute phase of bacteremia. To evaluate this, a comparison of individuals with and without bacteremia is needed. If scenario A is true, we would expect the long-term survival of individuals with and without bacteremia to be comparable (given they differ only with respect to exposure to bacteremia). In contrast, we would expect excess long-term mortality for bacteremia patients if scenario C/D is true.

However, few studies have investigated survival in patients with and without bacteremia and with conflicting results. In hospital-based studies, Leibovici et al. compared the outcome in 1991 bacteremia patients and 1991 non-infected patients matched on sex, age, department, date of hospitalization and a range of comorbidities [19], and found mortality rates of 26% vs. 7% at one month, 48% vs. 27% at one year and 63% vs. 42% at four years. Importantly, the excess mortality was also evident among one-month survivors of bacteremia. Bates et al. compared 142 bacteremia patients with 142 culture-negative controls matched on sex, age, severity of underlying disease and presence of major comorbidity and found that bacteremia patients were in increased risk of death within 30 days (HR 2.3, 95% CI 1.2–4.4) but not hereafter (HR 1.3, 95% CI 0.76–2.1) [18]. Compared with culture-negative patients, Søgaard et al. found no excess mortality for patients with community-acquired gram-negative bacteremia beyond two days of admission and for gram-positive bacteremia beyond seven days of admission [91].

It would be interesting to estimate the combined effect of bacteremia, hospitalization and post-discharge sequelae on survival by comparing long-term mortality in bacteremia patients with that of the general population—especially among bacteremia patients who survived the initial phase of bacteremia. However, there is a striking paucity of such studies. In a hospital-based study, Leibovici et al. observed higher mortality rates among one-month survivors of bacteremia compared with expected age- and sex-standardized mortality rates in the general population (29% vs. 6% at one year and 49% vs. 20% at 4 years) [19]. Skogberg et al. studied the timing of death among 30,523 patients with bloodstream infections in a nationwide Finnish study and found that the hazard rate of death remained increased for only 60 days compared with a sex- and age-adjusted Finnish population [54]. Of note, neither study utilized a matched comparison cohort or accounted for potential confounders when comparing the mortality of bacteremia patients with that of the general population.

Knowledge on causes of death after bacteremia could potentially help determine if increased mortality is a direct consequence of infection (infection as cause of death) or mediated through new onset or worsening of existing comorbidity such as chronic renal failure, diabetes mellitus or cardiovascular disease. In support of the latter, studies have shown that patients with community-acquired bacteremia are at increased risk of myocardial infarction and stroke within 180 days after bacteremia, and venous thromboembolism within 365 days after bacteremia compared with matched population controls [92,93]. Few studies have reported on causes of death and have identified infections, malignancy and cardiovascular diseases as the most common causes of death [19,82,94]. However, none of these studies considered causes of death among long-term survivors of bacteremia and no comparison was made with the general population. Knowledge on causes of death, especially compared with the general population, could eventually help determine if bacteremia survivors can be targeted for specific interventions already at hospital discharge.

Summary

Little is known about the occurrence of bacteremia and trends in the general population and few studies have distinguished between community-acquired, healthcare-associated and nosocomial bacteremia. Despite bacteremia being an important nosocomial infection, we lack knowledge on the daily risk during hospitalization. We know little about long-term mortality and causes of death after bacteremia in particular compared with the general population.

D. Raoult and H. Richet on the question if bacteremia is the most neglected cause of death in Europe [95]:

"It appears that one of the European priorities should be to have an objective evaluation of the incidence and mortality of bacteraemia, in particular of that related to healthcare, eventually by using computer algorithms. This is a prerequisite for any efficient fight against it."

AIMS OF THE THESIS

STUDY I:
To investigate the occurrence of and trends in first-time bacteremia and distribution of microorganisms in the general population; overall and by place of acquisition

STUDY II:
To investigate the overall and daily incidences of bacteremia among hospitalized patients

STUDY III:
To investigate and compare long-term mortality and causes of death after bacteremia with the general population

MATERIALS AND METHODS

SETTING

All three studies were conducted in Funen County, Denmark, which comprises a main island (Fyn) and a number of smaller islands for a total size of 3,099 square km (Figure 2). Funen County consists of mixed rural and urban areas with an 2008 midyear population of 483,123 residents (396,398 were ≥15 years of age). Free tax-funded universal healthcare was provided by general practitioners, one university-affiliated tertiary care center (Odense University hospital) and 7 community-hospitals (Svendborg, Nyborg, Middelfart, Ærø, Langeland, Faaborg and Bogense). The latter three community-hospitals closed during the study period. Additionally, Odense University hospital received patients needing highly specialized treatments from a catchment population outside Funen County of approximately 800,000 residents. All specialities were represented and only patients requiring allogenic bone marrow or solid organ transplantation (except kidney) were referred out of the region for care.

Figure 2
An overview of Denmark with Funen County highlighted in red.

DATA SOURCES

All contacts with the Danish healthcare system are recorded in administrative and research registries at an individual level, and may be used for research purposes conditioned on approval by the relevant authorities. The principles of data linkage and the data sources used in **studies I–III** are described below.

THE DANISH CIVIL REGISTRATION SYSTEM
The Danish Civil Registration System (CRS) is an administrative register established on 2 April, 1968 [96,97]. It holds daily updated information on date of birth, sex, marital status, place of residence, migration and vital status (alive, dead, or disappeared) for all individuals residing in Denmark who 1) were born alive by a mother already registered in the CRS, 2) were baptized and registered in a Danish electronic church register, or 3) have resided legally in Denmark for at least 3 months. Each individual in CRS is assigned a unique non-changeable ten-digit Civil Personal Register (CPR) number that allows unambiguous record linkage between administrative and research registers in Denmark. This principle of data linkage was used in **studies I–III**. Data on place of residence was used in **studies I and III**. Data on marital status was used in **studies I and III**. Data on migration and vital status was used in **study III**.

BACTEREMIA DATABASE
The Danish Observational Registry of Infectious Syndromes (DORIS) is a microbiological research database comprising all bacteremias in Funen County between May 1999 and December 2008. DORIS was established in cooperation between the Department

of Clinical Microbiology and the Department of Infectious Diseases at Odense University Hospital in 2008.

In Funen County, all blood cultures were drawn at hospitals. Protocol dictated that blood cultures were drawn under aseptic conditions by venipuncture and consisted of two aerobic/anaerobic blood culture sets (2x2x10 mL blood). All blood cultures were sent to the Department of Clinical Microbiology at Odense University Hospital and results were recorded in the local Patient Administrative System from May 1999 to December 2005 and the MADS system thereafter [98]. All Blood cultures were incubated and screened for growth of microorganisms for 6 days or until detected positive using the Difco ESP blood culture system (Difco Laboratories, Detroit, USA) in 2000 and the Bactec 9240 system (Becton Dickinson, NJ, USA) thereafter. Routine methods for identification of bacteria are based on conventional characterization [99], the Danish reference program [100], and automated identification using Vitek 2 (bioMérieux, Marcy l'Etoile, France).

Main variables in DORIS include: CPR number, sex, age, date of bacteremia, number of bacteremia episode for each patient, clinical department (at time of venipuncture), clinical specialty (medicine, surgery, intensive care, or pediatrics), place of acquisition (community-acquired, healthcare-associated, or nosocomial), and isolated microorganisms. "Date of bacteremia" was defined as the date of first venipuncture that yielded a positive blood culture. In case this date was missing (12%), we used the never missing date of receipt of blood culture at the Department of Clinical Microbiology, Odense University Hospital. The exact time stamp for blood culture draw was not routinely recorded. Data on bacteremias were used in **studies I–III**.

FUNEN COUNTY PATIENT ADMINISTRATIVE SYSTEM

Funen County Patient Administrative System (FPAS) was established in 1973 and contains data on all admissions to somatic hospitals in Funen County. Data on outpatient and emergency department visits have been recorded since 1989. Data include CPR number, sex, age, dates of admission and discharge at a department level, date of death during hospitalization (if relevant), and discharge diagnoses assigned by the attending physicians according to the ICD-8 until 1993 and the ICD-10 thereafter. We used FPAS to establish the study population and determine the course of hospitalization in **study II**.

THE DANISH NATIONAL REGISTRY OF PATIENTS

The Danish National Registry of Patients (DNRP) was established in 1977 and contains data on all admissions to public somatic hospital in Denmark [101] with outpatient contacts and emergency department visits recorded since 1995. Data completeness is almost 100% [102] and include CPR number, the dates of admission and discharge, as well as surgical procedures and discharge diagnoses assigned by the attending physicians according the ICD-8 until 1993 and the ICD-10 thereafter. The DNRP was used to determine dates of admission and discharge (used to define place of acquisition for bacteremias), and to identify preexisting comorbidities including a history of alcohol dependency in **studies I and III**.

THE DANISH PSYCHIATRIC CENTRAL RESEARCH REGISTER

The Danish Psychiatric Central Research Register contains data on admissions to psychiatric hospitals in Denmark since 1969 [103]. Data include CPR number, the dates of admission and discharge, as well as surgical procedures and discharge diagnoses assigned by the attending physicians according the ICD-8 until 1993 and the ICD-10 thereafter. Alike DNRP, the register was used to determine dates of admission and discharge, and to identify discharge diagnoses associated with a history of alcohol dependency in **studies I and III**.

ODENSE PHARMACOEPIDEMIOLOGICAL DATABASE

Odense pharmacoepidemiological database (OPED) is a regional pharmacy-based prescription register that has captured redeemed prescriptions at pharmacies in Funen County since 1990 with the exception of drugs sold over the counter and drugs not reimbursed by the county authority [104]. We used data on reimbursed Disulfiram (trade name Antabus®) to determine a history of alcohol dependency in **studies I and III**.

THE DANISH CANCER REGISTRY

The Danish Cancer Registry was founded in 1942 and contains data on new cases of cancer in Denmark [105]. Reporting to the cancer registry has been mandatory since 1987. The registry is based on multiple notifications from different data sources and manual quality control routines, which secure a high degree of completeness. We used data on tumor characteristics and date of diagnosis for all new cancers in **study III**.

THE DANISH REGISTER OF CAUSES OF DEATH

The Danish Register of Causes of Death was established in 1875 and contains individual based data on all deaths among residents dying in Denmark [106]. Causes of death are coded according to WHO's rules using the ICD-10 since 1994. For all death certificates it is mandatory to state the underlying cause of death, which is the disease or condition that started the process leading to death. The underlying causes of death were grouped as shown in appendix 1 and used in **study III**.

The principle of data linkage between DORIS, and administrative and healthcare registers is shown below (Figure 3).

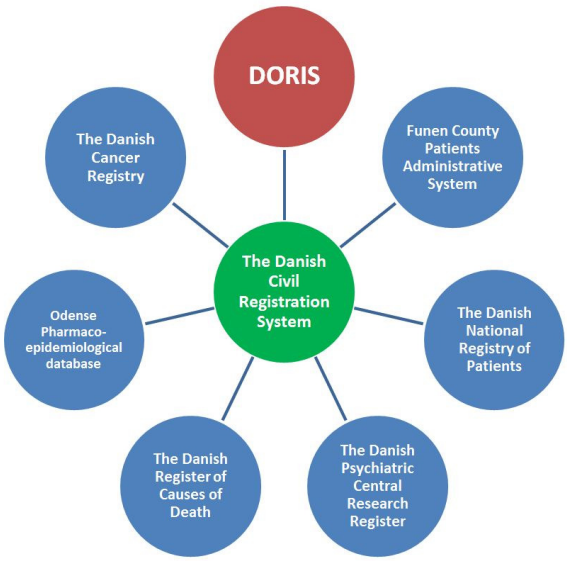

Figure 3
Illustration of the principle of linkage between DORIS, and administrative and research registries using the unique Danish Civil Personal Register number.

DEFINITIONS OF VARIABLES

BACTEREMIA

We used published computer algorithms to derive bacteria episodes in DORIS [30-32]. We defined bacteremia as recognized pathogens detected in ≥ 1 blood culture, or common skin contaminants (coagulase-negative staphylococci, *Bacillus* spp, *Propionibacterium* spp, *Corynebacterium* spp, viridans group streptococci, *Aerococcus* spp, or *Micrococcus* spp) detected in ≥ 2 blood culture sets within 5 days [107,108]. The date of the first positive blood culture set was regarded as the date of bacteremia. Polymicrobial bacteremia was defined as isolation of ≥ 2 different microorganisms, deemed to represent bacteremia, within 2 days [109]. Positive blood cultures with the same microorganisms within 30 days were considered part of the same bacteremia episode. We considered sameness of organisms [107]; that is, *Staphylococcus epidermidis* (species) isolated from one blood culture set and coagulase-negative staphylococci (genus) from another blood culture set within 5 days was reported as bacteremia caused by *Staphylococcus epidermidis*.

Gradel et al. have recently evaluated the performance of a computer algorithm similar to ours against prospective ascertainment of positive blood cultures in a Danish setting [31]. The authors found a high agreement between the clinicians' assessment and the computer algorithm´s definition of positive blood cultures as either "true" bacteremia or contamination (96.6%, Kappa=0.83). A high agreement rate was also seen for monomicrobial vs. polymicrobial bacteremia (95.2%, Kappa=0.76). In line with these results, Leal et al. found an 85% agreement between a similar computer algorithm and manual chart review for classifying positive blood cultures as either "true" bacteremia or contamination [32].

PLACE OF ACQUISITION

We classified bacteremia according to place of acquisition as community-acquired, healthcare-associated or nosocomial.

- Community-acquired bacteremia was defined as draw of the first positive blood culture ≤ 2 days after admission **without** discharge from a hospital or attendance at an outpatient clinic (hematology, nephrology, or oncology) within 30 days prior to the admission.

- Healthcare-associated bacteremia was defined as draw of the first positive blood culture ≤ 2 days after admission **and** discharge from a hospital or attendance at an outpatient clinic (hematology, nephrology, or oncology) within 30 days prior to the admission.

- Nosocomial bacteremia was defined as draw of the first positive blood culture > 2 days after admission.

We denoted the day of admission "Day 1" and thus blood cultures drawn on Day 1, Day 2 and Day 3 defined community-acquired/healthcare-associated bacteremia.

We used a computer algorithm almost similar to that of Gradel et al. to determine place of acquisition [31]. Gradel et al. found an agreement of 83% (kappa 0.57) when comparing the computer algorithm's and physicians' classification of bacteremias as community-onset (community-acquired and healthcare-associated) or nosocomial. The algorithm's ability to distinguish between the presence/absence of healthcare-association yielded a lower agreement of 64% (kappa 0.15). In another study, Leal et al. found an agreement of 85% (kappa 0.78) between a computer algorithm and manual chart review for classification bacteremias according to place of acquisition although their definition of healthcare-associated bacteremia differed modestly from ours [32]. Of note, a given Kappa value does not imply that one method is superior to the other; it merely reflects how often the two methods agree on the classification of bacteremia.

As mentioned previously, the definitions by Friedman et al. are widely used to define place of acquisition [38]. However, we were unable to rigorously comply with these definitions but believe this had only minor impact on our results. First, we were unable to include the use of intravenous therapy at home; however, usage of intravenous therapy was virtually non-existing in Funen County during the study period. Second, we did not consider residence in a nursing home facility; however, the policy in Denmark is to keep even very frail elderly persons in their own homes and only 3278 persons out of approximately 485,000 Funen County residents (0.8%) were registered as nursing home residents in 2008 [110]. Third, in line with many studies, we used healthcare contacts 30 days prior to admission to define healthcare-association as opposed to 90 days as suggested by Friedman et al [2,25,39,46,47]. Nevertheless, this is of minor importance as we have recently shown that using a 90 day window as opposed to a 30 day window to define healthcare-association does not impact 30-day mortality associated with bacteremia [45].

COMORBIDITY

As a measure of patients' comorbidity, we used the Charlson index score [111]. The Charlson index includes 19 major disease categories that are assigned a weighted score according to prognostic severity. We grouped patients in levels of Charlson index scores of 0, 1, 2 and ≥3 points. We calculated the scores based on all previous discharge diagnoses in the Danish National Registry of Patients and the Danish Psychiatric Central Research Register. To ensure equal observation length for all individuals, we included only discharge diagnoses within 6 years prior to the date of bacteremia (or a corresponding index date for population controls in study III). The Charlson index was used to characterize the study population in **study I** and considered a potential confounder in **study III**.

MARITAL STATUS

Marital status (married, never married, divorced¬, or widow[er]) on the date of bacteremia (or a corresponding index date for population controls in study III) was used as a marker of socioeconomic status. A large US cohort study from 2010 of patients hospitalized with sepsis found that single men and women, and divorced men were at greater risk of in-hospital death compared with married men [112]. Marital status was used to describe the study population in **study I** and considered a potential confounder in **study III**.

A HISTORY OF ALCOHOL DEPENDENCY

Alcoholism has been associated with a poor outcome after bacteremia and is a likely confounder [113,114]. We defined a history of alcohol dependency as either: a redeemed prescription for disulfiram; ≥1 discharge diagnosis associated with "chronic alcohol use"; or ≥2 discharge diagnoses associated with "acute alcohol use" within 6 years prior to bacteremia (or a corresponding index date for population controls in study III). ICD-10 codes are

listed in appendix 2. A history of alcohol dependency was used to describe the study population in **study I** and considered a potential confounder in **study III**.

STUDY DESIGN AND STATISTICAL ANALYSES

Table 1 gives an overview of the study designs used in this thesis. Population-based study designs were used for **studies I** and **III**; we included only adult patients (≥ 15 years) who were residents of Funen County on the date of bacteremia. A hospital-based design was used for **study II**; we included all adult patients admitted to hospitals in Funen County, irrespectively of their place of residence.

Table 1. Study settings and periods, designs, and populations for studies I–III.

Study	Setting and period	Study design	Study population
I	Funen County, Denmark, 2000–2008	Population-based observational study	- Residents of Funen County with first-time bacteremia
II	Funen County, Denmark, 2000–2008	Multicenter hospital-based cohort study	- All patients admitted to hospitals in Funen County
III	Funen County, Denmark, 2000–2011	Population-based cohort study	- Residents of Funen County with first-time bacteremia - Population controls

STUDY I

We conducted a population-based observational study to investigate the overall incidence rate and trends in annual incidence rates of first-time bacteremia in Funen County during 2000–2008; overall and by place of acquisition.

In DORIS, we identified all adult residents of Funen County with first-time bacteremia during 2000–2008 in DORIS. From Statistics Denmark we retrieved data on the annual midyear adult population of Funen County, which was used to calculate the person time at risk [110]; per definition each resident contributed with one observation year.

We calculated mean overall and annual incidence rates of bacteremia, overall and by place of acquisition, using the formula [115]:

Incidence rate= (Number of first time bacteremias)/(Total person time at risk)

The overall incidence rate was calculated by dividing all first-time bacteremias during the study period by the cumulative annual midyear populations of Funen County during 2000–2008. The annual incidence rates were calculated by dividing the annual number of first-time bacteremias by the midyear population of Funen County of the corresponding year. We standardized the incidence rates to the sex and age distribution of the 2000 Funen County population using direct standardization to allow for direct comparison of the annual incidence rates. All incidence rates were expressed as bacteremias per 100,000 person years with 95% confidence intervals (CIs) assuming a Poisson distribution.

Trends in annual incidences may be biased by prevalent bacteremias misclassified as first-time bacteremias. Therefore, we imposed an individual 8-month lag period for each individual prior to the date of first-time bacteremia [31]. The decision to use an 8-month lag period was based on the availability of data not included in the study period (May 1999 to December 1999). A patient with bacteremia on 1 January 2000 was assigned a lag period from 1 May 1999 to 31 December 1999 (8 months), whereas a patient with bacteremia on 1 April 2000 was assigned a lag period from 1 August 1999 to 31 March 2000 (8 months). We used this approach to avoid unequal lengths of lag periods. In principle, we also assigned an 8-month lag period to patients with first-time bacteremia later than 1 September 2000; however, had these patients experienced bacteremia within their lag period they would merely have entered the study on an earlier date.

Trends in annual incidence rates were estimated by a Poisson Regression model with calendar time included as a continuous variable rather than a categorical variable as confirmed by the likelihood ratio test. The Poisson Regression model was tested using the Hosmer–Lemeshow goodness-of-fit test and found appropriate. In a sub analysis, we estimated trends in annual incidence rates after excluding common skin contaminants because we observed a high proportion of common skin contaminants in 2000 and 2001 compared with 2002–2008.

Further, we stratified the analyses of incidence rates by sex and age groups to examine if our findings were consistent across subgroups of patients.

Next, we reported the annual number of admissions, used hospital bed days, and performed blood culture sets to investigate if (usage of) healthcare services changed during the study period. We calculated annual incidence rates of bacteremias per 1000 admissions, nosocomial bacteremias per 100,000 bed days, and bacteremias per 100 blood culture set. Trends were estimated using a Poisson regression model.

Finally, we investigated trends in the distribution of microorganisms. We divided the study period into three 3-year periods (2000–2002, 2003–2005 and 2006–2008) and the microorganisms into 16 groups (*Escherichia coli*, *Enterobacter* species, *Klebsiella* species, other *Enterobacteriaceae*, *Pseudomonas aeruginosa*, anaerobic Gram-negative rods, other Gram-negative, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, hemolytic streptococci, *Enterococcus* species, other Gram-positive cocci, Gram-positive rods, fungi and polymicrobial). Trends in proportions and crude incidence rates between the 3-year periods were analyzed using the Chi-squared test for trend and a Poisson regression model, respectively.

STUDY II

We conducted a multicenter hospital-based cohort study among adult patients admitted to somatic hospitals in Funen County to investigate the overall and daily incidences of bacteremia during hospitalization.

From FPAS, we included all patients admitted to somatic hospitals in Funen County during 2000–2008. Outpatients were excluded. Patients were included on the day of admission (Day 1) and followed until their first bacteremia, death, discharge or 31 December 2008, whichever came first. Data on bacteremias between 1 January 2000 and 31 December 2008 were retrieved from DORIS and included the date of bacteremia, isolated microorganisms and department of blood culture draw. Patients were allowed to contribute with multiple bacteremias during the study period but were restricted to one bacteremia per admission.

We calculated the overall incidence of bacteremia per 1000 admissions and per 10,000 bed days with 95% CIs assuming a Poisson distribution. Next, we calculated the number of hospitalized patients and bacteremias for each day of hospitalization (Day 1, 2, 3 … >30) and computed graphs depicting the daily incidence of bacteremia per 10,000 bed days.

To investigate if we could identify groups of patients in a particularly high or low risk of bacteremia, we reiterated the above-mentioned analyses for sex, age groups (15–64, 65–79 and 80+ years), tertiary care center/community hospitals, clinical specialties and microorganisms. The analyses of the daily incidences were restricted to the most prevalent clinical specialties (internal

medicine, abdominal surgery, hematology and oncology) and microorganisms (*Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci and *Streptococcus pneumoniae*) because of few daily events (bacteremias).

For clinical specialties, we considered the department of initial admission due to the complicated nature of patients being transferred one or multiple times between wards and/or clinical specialties. To examine if this decision had any impact on the incidences, we performed a sensitivity analysis in which we excluded patients who were either transferred between hospitals or clinical specialties, or transferred to the ICU. Data on transfer to the ICU were available only from 2004 through 2008.

STUDY III

We conducted a population-based cohort study to investigate and compared long-term mortality and causes of death after bacteremia with the general population.

We included all adult residents of Funen County with a first-time bacteremia in DORIS. For each bacteremia patient we randomly sampled 5 population controls matched on sex, year of birth and place of residency (within Funen County) using the risk set sampling technique. Three bacteremia patients had no controls and were excluded (all were ≥100 year old) and 13 bacteremia patients had less than 5 population controls. The population controls were assigned an index date identical to the date of bacteremia of their corresponding bacteremia patient. Bacteremia patients were eligible as population controls until their first bacteremia; hereafter they contributed with observation time only as cases. The bacteremia patients and population controls were followed from the date of bacteremia (or index date) until death, loss to follow-up or 31 December 2011, whichever came first. The study outcomes were time to death from any cause (all-cause mortality) and time to death from a specific underlying cause of death (cause-specific mortality).

For all-cause mortality, we used the Kaplan-Meier estimator to construct survival curves and calculate cumulative mortality at 30 days, 90 days, 1 year, 5 years and 10 years for both bacteremia patients and population controls. Next, we calculated mortality rates as death per 1000 person years and the risk of death (proportions of patients dying) in predefined follow-up periods after bacteremia (0–30 days, 31–90 days, 91–365 days, 1–5 years and 5 years to end of follow up). To compare mortality in bacteremia patients and population controls, we used Cox regression models stratified on matched sets to calculate unadjusted and adjusted mortality rate ratios (MRRs) for each follow-up period. The Cox regression models were stratified because of the matched cohort design [116]. If a bacteremia patient died during e.g. the 0–30 days follow-up period both that patient and the corresponding population controls were excluded in the subsequent follow-up intervals; hereby, we were able to retain the matching in each follow-up period.

To examine if excess mortality for bacteremia patients was mediated through cancer, we performed a sub-analysis of the MRRs, where we excluded bacteremia patients and population controls who were diagnosed with cancer within -/+ one year of the date of bacteremia/index date. Finally, we stratified the analyses by sex, place of acquisition, clinical department, Charlson Index score, age groups (15–39, 40–64, 65–79, 80+ years), and groups of microorganism(s) in follow-up periods of 0-1 year, 1-5 years and 5+ years.

For cause-specific mortality, we used Cox regression models stratified on matched sets to calculate mortality rates per 1000 person years, unadjusted MRRs and adjusted MRRs in follow-up periods of 0–1 year and 1+ years after the index date [102]. For patients were cancer was the underlying cause of death, we compared the proportions of deaths from specific types of cancers in bacteremia patients and population controls using the chi-squared test.

In the Cox regression models the following factors were a priori considered clinically relevant and adjusted for as potential confounders: comorbidity (Charlson Index score 0, 1, 2, or ≥3), a history of alcohol dependency (yes/no) and marital status (married, divorced, widow[er] or never married). In the Cox regression models were we either excluded cancer patients or stratified by comorbidity, we had to break the matching and instead use a regular Cox regression model adjusted for sex, year of birth, comorbidity, a history of alcohol dependency and marital status.

The proportional hazard assumptions of the Cox regression models for each follow-up period were assessed graphically with log-log plots and found appropriate.

ETHICS

The studies were approved by the Danish Data Protection Agency (2013-41-2579). In accordance with Danish law, observational studies performed in Denmark do not need approval from the Medical Ethics Committee.

MAIN RESULTS

STUDY I

We identified 9408 patients with first-time bacteremia; 7786 were included in the study and 1622 were excluded (1280 patients with residency outside Funen County, 320 patients < 15 years of age and 22 patients with bacteremia during the lag period). The median age of the included patients was 72 years (interquartile range, 60–81) and 54% were males. Of the 7786 included bacteremias, 3565 (46%) were community-acquired, 1806 (23%) were healthcare-associated and 2415 (31%) were nosocomial.

The mean overall incidence rate was 215.7 (95% CI, 210.9–220.5) per 100,000 person years during 2000–2008 including 99.0 (95% CI, 95.8-102.3) for community-acquired, 50.0 (95% CI, 47.7–52.3) for healthcare-associated and 66.7 (95% CI, 64.0–69.4) for nosocomial bacteremia. The incidence rate decreased by 23.3% (95% CI, 17.8%–28.4%) from 254.1 in 2000 to 198.8 in 2008 corresponding to a mean decrease of 3.3% per year (95% CI, 2.4–4.1%) (Figure 4). After excluding common skin contaminants, we still observed a decrease of 2.0% per year (95% CI, 1.1–3.0%). Also, the decreasing trend was observed for both men and women, and across all age groups.

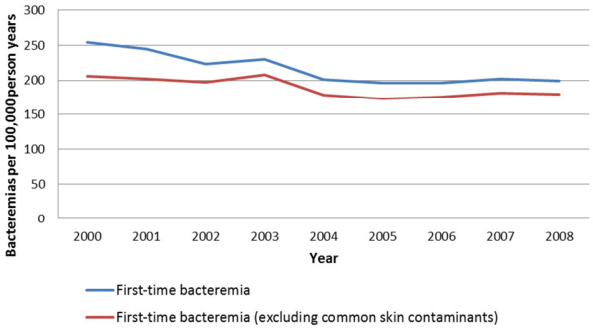

Figure 4
Trends in sex and age standardized incidence rates of first-time bacteremia in Funen County, Denmark, during 2000–2008.

The decreasing trend could not be explained by fewer blood cultures; in fact, the number of performed blood culture sets increased by 28.8% (95% CI, 27.4–30.3%) from 2000 to 2008. In the same period, the number of admissions decreased slightly by 4.9% (95% CI, 4.2%–5.6%) while the number of used hospital bed days decreased markedly by 24.7% (95% CI, 24.5–25.0%).

When stratifying by place of acquisition, we found that the incidence rate of community-acquired bacteremia decreased by 25.6% (95% CI, 17.6–32.8%) from 119.0 per 100,000 person years in 2000 to 93.8 in 2008 corresponding to a mean decrease of 3.7% per year (95% CI, 2.4–4.8%) (Figure 5). The incidence rate of nosocomial bacteremia decreased by 28.9% (95% CI, 19.6–37.2%) from 82.2 per 100,000 person years in 2000 to 56.0 in 2008 corresponding to a mean decrease of 4.2% per year (95% CI, 2.7–5.7%). Finally, the incidence rate of healthcare-associated bacteremia remained more of less stable throughout the study period with a non-significant decrease of 1.3% per year (95% CI, 0.0–3.1%; p=0.17).

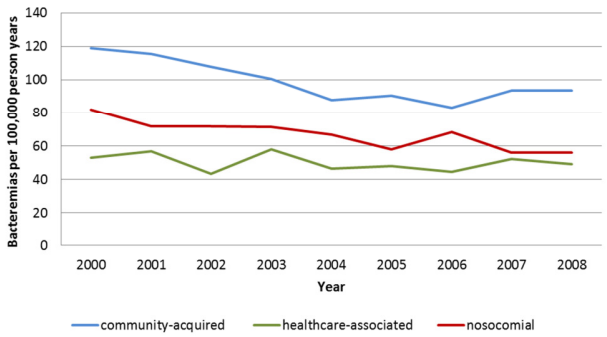

Figure 5
Trends in sex and age standardized incidence rates of first-time bacteremia by place of acquisition in Funen County, Denmark, during 2000–2008.

The most common microorganisms were Escherichia coli (28.3%), Staphylococcus aureus (12.3%), coagulase-negative staphylococci (10.0%) and Streptococcus pneumoniae (9.1%). During the study period, we observed decreasing crude incidence rates for Escherichia coli, Staphylococcus aureus, coagulase-negative staphylococci and Streptococcus pneumoniae, and increasing crude incidence rates for Pseudomonas aeruginosa and enterococci species (p<0.05 for all the mentioned microorganisms).

The figures below display the microorganisms that showed a statistically significant trend (p<0.05) in proportions during the study period for community-acquired (Figure 6), healthcare-associated (Figure 7) and nosocomial bacteremia (Figure 8). Regardless of place of acquisition, the proportion of bacteremias caused by coagulase-negative staphylococci decreased while the proportions caused by Enterococcus species increased.

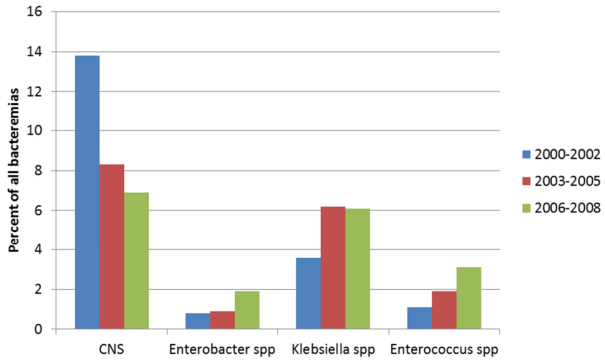

Figure 6
Figure 6. Microorganisms causing community-acquired bacteremias that displayed a statistically significant trend in proportions during the study period. CNS: coagulase-negative staphylococci.

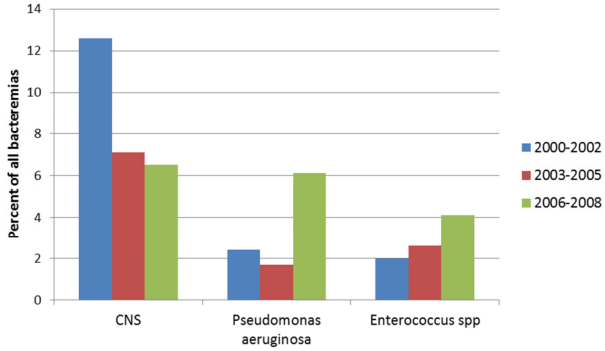

Figure 7
Microorganisms causing healthcare-associated bacteremias that displayed a statistically significant trend in proportions during the study period. CNS: coagulase-negative staphylococci.

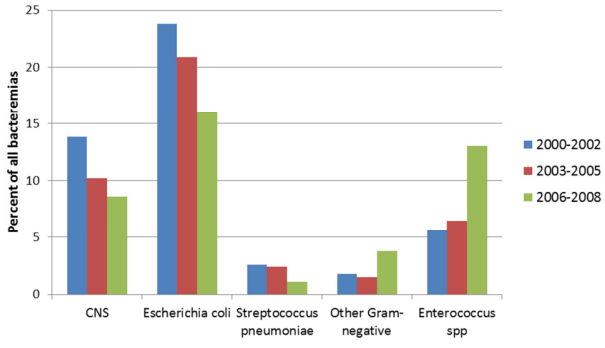

Figure 8
Microorganisms causing nosocomial bacteremias that displayed a statistically significant trend in proportions during the study period. CNS: coagulase-negative staphylococci.

STUDY II

We included 276,586 adult patients with 724,339 admissions to somatic hospitals in Funen County for a total of 4,531,744 bed

days. The median age at admission was 59 years (IQR, 40–73) and 54.2% were females. Most patients were admitted to the tertiary care center (62.9%) and patients were rarely transferred between hospitals (2.8%) or clinical specialties (4.5%), or transferred to the intensive care unit (4.2%). Patients were most often admitted to the Departments of Internal Medicine (29.0%), Abdominal Surgery (14.9%) or Orthopedics (13.2%).

We identified 10,281 first bacteremias per admission in 8818 patients. Compared with non-bacteremia patients, bacteremia patients were more likely to be males (55 % vs. 46%), of older age (median age 69 vs. 59 years), and to have longer length of stay (15 vs. 3 days). Further, bacteremia patients were more often admitted to the tertiary care center (66% vs. 63%), transferred between hospitals (11% vs. 3%) or clinical specialties (21% vs. 5%), transferred to the intensive care unit (20% vs. 4%), and initially admitted to the Departments of Internal Medicine (53% vs. 29%), Hematology (7% vs. 2%), Oncology (7% vs. 5%) or Nephrology (4% vs. 1%).

The overall incidence of bacteremia was 14.2 per 1000 admissions (95% CI, 13.9–14.5) and 23.6 per 10,000 bed days (95% CI, 23.1–24.0). The incidence per 1000 admissions and per 10,000 bed days was highest for males, elderly individuals (>65 years), and patients initially admitted to the Departments of Hematology, Nephrology, Internal Medicine, Urology or Oncology. Among all subgroups of patients, the highest incidences were seen for patients initially admitted to the Department of Hematology with 61.3 bacteremias per 1000 admissions (95% CI, 57.1–65.9) and 123.7 bacteremias per 10,000 bed days (95% CI, 115.1–132.8). Exclusion of patients who were transferred between hospitals or clinical departments, or transferred to the intensive care unit lowered the incidences but did not change the rank order of bacteremias per 1000 admissions or per 10,000 bed days (data not shown).

Almost 20% of the patients were discharged on the day of admission and 75% were discharged within one week of admission. We identified almost half the bacteremias on the day of admission and two-thirds within 3 days of admission while less than 25% of the bacteremias occurred beyond seven days of admission.

The incidence on the day of admission (Day 1) was 68.9 (95% CI, 67.0–70.8) per 10,000 bed days and declined rapidly to approximately 9 per 10,000 bed days on Day 3–7. Hereafter, it increased steadily to around 18 per 10,000 bed days on Day 12 followed by a more or less constant daily incidence (Figure 9).

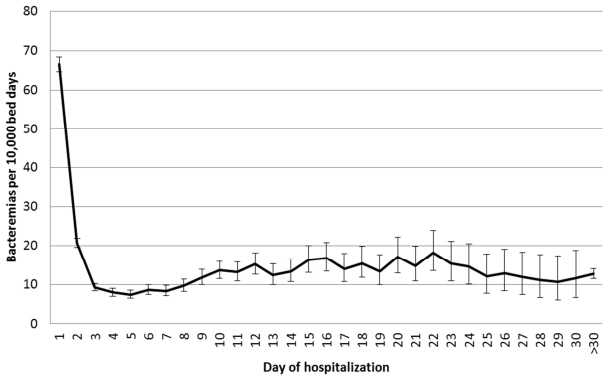

Figure 9
The daily incidence of bacteremia per 10,000 bed days among patients admitted to hospitals in Funen County, Denmark, during 2000–2008.

As displayed in Figure 10–13, we found that the daily incidences varied according to age, admission to the tertiary care center vs. community-hospitals, department of initial admission, and microorganisms. As an example, the incidence was highest for the elderly (80+ years) on the day of admission (Day 1) but lowest beyond 7 days of admission (Figure 10).

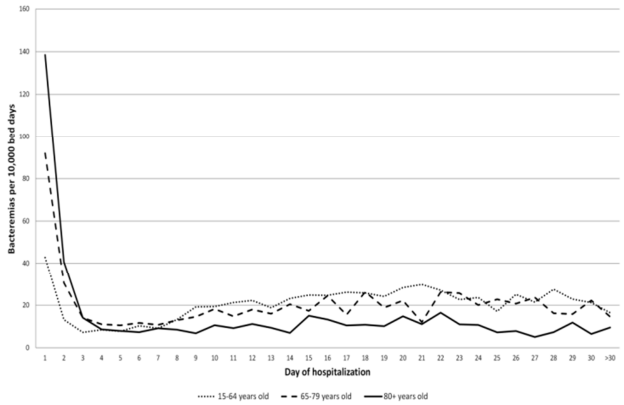

Figure 10
The daily incidence of bacteremia among hospitalized patients by age.

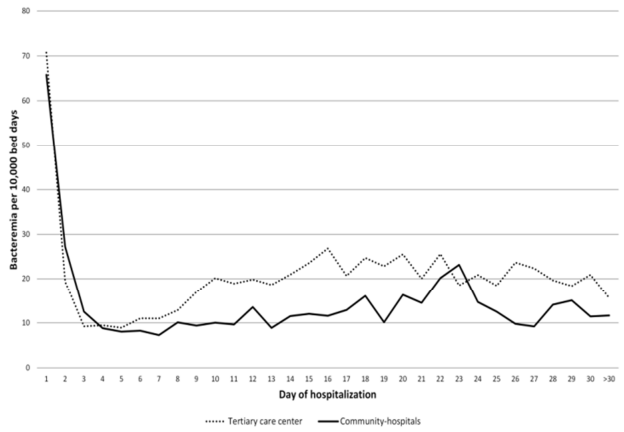

Figure 11
The daily incidence of bacteremia among hospitalized patients by type of hospital.

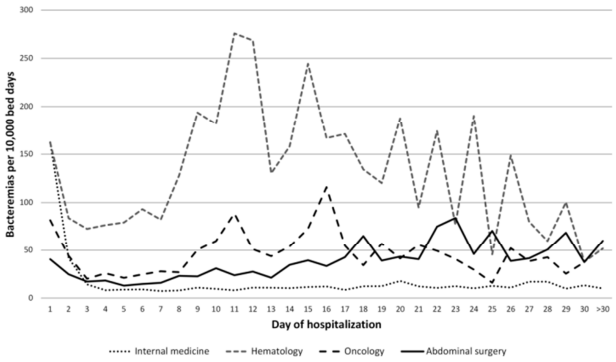

Figure 12
The daily incidence of bacteremia among hospitalized patients by clinical specialties.

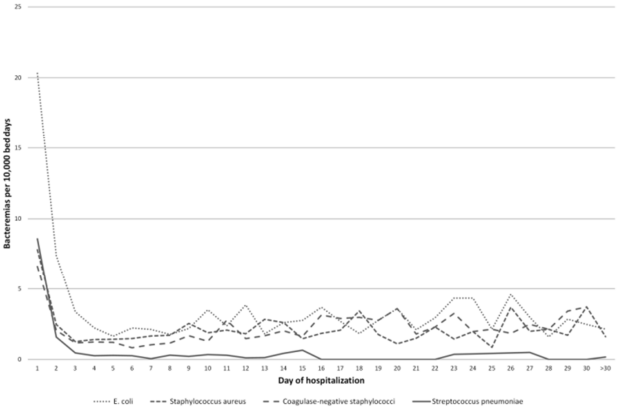

Figure 13
The daily incidence of bacteremia among hospitalized patients by microorganisms.

STUDY III

We included 7783 patients with first-time bacteremia and 38,906 population controls. The median age was 72 years (IQR 59–81) and 54% were male. Compared with population controls, bacteremia patients more often had a history of alcohol dependency (7% vs. 1%) and a Charlson Comorbidity score of 2 (21% vs. 8%) or ≥3 (23% vs. 4%). A total of 118/7783 (1.5%) bacteremia patients were lost to follow-up of whom 115/118 (97%) emigrated.

All-cause mortality

Kaplan-Meier survival curves for bacteremia patients and population controls showed marked differences in long-term survival with a median survival time of 2.2 years for bacteremia patients and more than 12 years for population controls (Figure 14).

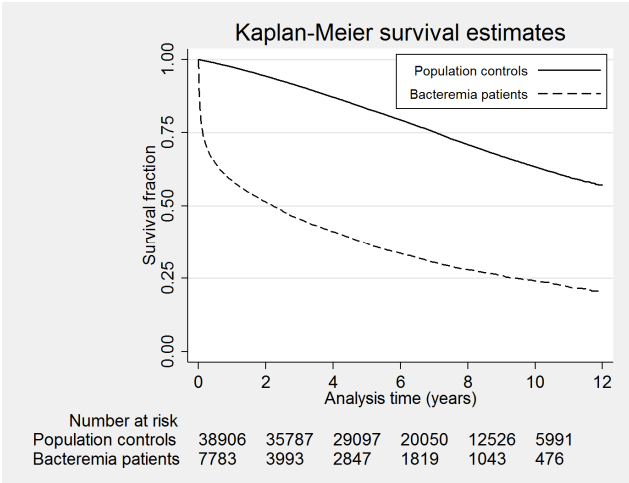

Figure 14
Kaplan-Meier survival curves of bacteremia patients and population controls matched on sex, year of birth, and residency during 12 years of follow-up.

The cumulative mortality for bacteremia patients and population controls was 22.0% vs. 0.2 % (30 days), 30.1% vs. 0.6% (90 days), 41.4% vs. 2.6% (1 year), 63.0% vs. 16.8% (5 years), and 75.8% vs. 36.6% (10 years).

The mortality rates for bacteremia patients were higher in all follow-up periods compared with population controls resulting in excess mortality rates ranging from 3159.0 (95% CI, 3008.8–3309.8) per 1000 person years at risk (PYR) 0–30 days after bacteremia to 35.6 (95% CI, 28.4–42.9) per 1000 PYR from 5 years after bacteremia to end of follow up (Table 2).

Table 2. Risk and relative risk of death among bacteremia patients compared with population controls matched on sex, year of birth, and residency in Funen County, Denmark, during 2000–2008.

	Persons at risk	Deaths (% of persons at risk)	PYR	MR per 1000 PYR (95% CI)	Excess MR per 1000 PYR (95% CI)	Unadjusted MRR (95% CI)	Adjusted MRR[1] (95% CI)
0–30 days							
Bacteremia patients	7783	1714 (22.0)	539	3182.5 (3035.4–3336.8)	3159.0 (3008.8–3309.8)	128.4 (101.9–161.8)	115.3 (88.2–150.9)
Population controls	38,906	75 (0.2)	3191	23.5 (18.7–29.5)	-	1 (Ref.)	1 (Ref.)
31–90 days							
Bacteremia patients	6068	627 (10.3)	935	670.8 (620.3–725.4)	646.2 (593.6–698.9)	30.8 (25.1–37.8)	23.2 (18.5–29.1)
Population controls	38,789	156 (0.4)	6350	24.6 (21.0–28.7)	-	1 (Ref.)	1 (Ref.)
91–365 days							
Bacteremia patients	5440	881 (16.2)	3700	238.1 (222.9–254.4)	211.4 (195.6–227.2)	10.6 (9.5–11.9)	7.4 (6.5–8.4)
Population controls	38,535	764 (2.0)	28,626	26.7 (24.9–28.7)	-	1 (Ref.)	1 (Ref.)
1–5 years							
Bacteremia patients	4556	1608 (36.3)	13,729	117.1 (111.5–123.0)	78.2 (72.4–84.0)	3.7 (3.5–4.0)	2.9 (2.7–3.2)
Population controls	37,423	5055 (13.5)	129,849	38.9 (37.9–40.0)	-	1 (Ref.)	1 (Ref.)
5 years–end of follow up							
Bacteremia patients	2255	604 (26.8)	6783	89.1 (82.2–96.4)	35.6 (28.4–42.9)	2.7 (2.4–2.9)	2.1 (1.8–2.3)
Population controls	24,188	4191 (17.3)	78,473	53.4 (51.8–55.1)	-	1 (Ref.)	1 (Ref.)

PYR: person years at risk; MR: mortality rate; MRR: mortality rate ratio; CI: confidence interval. [1] Computed by stratified Cox regression model (stratified on matched sets) and adjusted for comorbidity (Charlson Index score 0, 1, 2, ≥3), a history of alcohol dependency (yes/no) and marital status (married, divorced, widow[er] or never married).

The adjusted MRR was highest 0–30 days after bacteremia (aMRR 115.3; 95% CI, 88.2–150.9) and decreased in the subsequent follow-up periods; however, the aMMR remained two-fold increased even after 5 years (aMRR 2.1; 95% CI, 1.8–2.3).

Excluding bacteremia patients and population controls diagnosed with cancer within -/+ 1 year of the index date had very little impact on the risk estimates (data not shown).

In the stratified analyses, we were unable to identify any group of bacteremia patients, who had a survival comparable to that of population controls although statistical significance was not reached for all microorganisms among 5 years survivors of bacteremia. Within the first year after bacteremia, factors associated with a high relative risk of death compared with population controls were young age (<65 years old), low comorbidity score, being in the intensive care unit, nosocomial bacteremia and fungemia. Among one-year survivors of bacteremia, young age and fungemia were associated with a particularly unfavorable outcome.

Cause-specific mortality

Cancer and cardiovascular diseases were the most common causes of death (displayed the highest mortality rates) throughout follow-up for both bacteremia patients and population controls (Table 3).

The relative risk of death (risk of death for bacteremia patients compared with population controls) displayed a different pattern. During the first year of follow-up, the relative risk of death was highest for genitourinary diseases (aMRR 100.4; 95% CI, 37.0–272.8), infectious diseases (aMRR 83.6; 95% CI, 38.9–179.7) and blood/immune diseases (aMRR 72.0; 95% CI, 12.9–401.9). Among one-year survivors of bacteremia, the relative risk of death was increased from all specific causes of death with no clear pattern as most 95% confidence intervals overlapped. The highest relative risks was seen for musculoskeletal/skin diseases (aMRR 6.9; 95% CI, 3.2-14.8), in situ/benign neoplasms (aMRR 6.8; 95% CI 3.2-14.1), and infectious diseases (MRR 4.6; 95% CI, 2.8-7.7) but these three causes of death accounted for only 103 deaths among bacteremia patients compared with 599 deaths from cardiovascular diseases and 600 deaths from cancer.

Table 3. Risk, mortality rate, and relative risk of cause-specific death among bacteremia patients compared with population controls in follow-up periods of 0–1 years and 1+ years.

Cause of death	0–1 year			1+ year		
	No. of deaths[1]	Mortality rate per 1000 person years (95% CI)	Adjusted MRR[2] (95% CI)	No. of deaths[1]	Mortality rate per 1000 person years (95% CI)	Adjusted MRR[2] (95% CI)
Cancer						
Bacteremia patients	1200 (37.2)	232.0 (219.2–245.5)	26.3 (21.6-32.0)	600 (27.1)	29.3 (27.0–31.7)	2.8 (2.5-3.2)
Population controls	240 (22.6)	6.3 (5.5–7.1)	1	1065 (22.0)	8.4 (7.9–8.9)	1
Cardiovascular diseases						
Bacteremia patients	657 (20.4)	127.0 (117.7–137.1)	13.0 (11.0-15.3)	599 (27.1)	29.2 (27.0–31.6)	2.2 (1.9-2.4)
Population controls	378 (35.6)	9.9 (9.0–11.0)	1	1676 (34.6)	13.1 (12.5–13.8)	1
Digestive system diseases						
Bacteremia patients	364 (11.3)	70.4 (63.5–78.0)	65.7 (40.8-105.8)	156 (7.1)	7.6 (6.5–8.9)	3.7 (2.8-4.9)
Population controls	42 (4.0)	1.1 (0.8–1.5)	1	188 (3.9)	1.5 (1.3–1.7)	1
Respiratory diseases						
Bacteremia patients	269 (8.3)	52.0 (46.1–58.6)	17.0 (12.7-22.7)	260 (11.8)	12.7 (11.2–14.3)	2.3 (1.9-2.8)
Population controls	115 (10.8)	3.0 (2.5–3.6)	1	615 (12.7)	4.8 (4.5–5.2)	1
Infectious diseases						
Bacteremia patients	158 (4.9)	30.5 (26.1–35.7)	83.6 (38.9-179.7)	47 (2.1)	2.3 (1.7–3.1)	4.6 (2.8-7.7)
Population controls	14 (1.3)	0.4 (0.2–0.6)	1	64 (1.3)	0.5 (0.4–0.6)	1
Genitourinary diseases						
Bacteremia patients	111 (3.4)	21.5 (17.8–25.8)	100.4 (37.0-272.8)	84 (3.8)	4.1 (3.3–5.1)	5.4 (3.6-8.1)
Population controls	13 (1.2)	0.3 (0.2–0.6)	1	99 (2.0)	0.8 (0.6–1.0)	1
Endocrine diseases						
Bacteremia patients	98 (3.0)	18.9 (15.5–23.1)	15.3 (8.6-27.3)	110 (5.0)	5.4 (4.5–6.5)	3.7 (2.6-5.2)
Population controls	44 (4.1)	1.2 (0.9–1.6)	1	150 (3.1)	1.2 (1.0–1.4)	1
Injury/poisoning						
Bacteremia patients	73 (2.3)	14.1 (11.2–17.8)	14.1 (8.3-23.8)	44 (2.0)	2.2 (1.6–2.9)	2.1 (1.4-3.2)
Population controls	39 (3.7)	1.0 (0.8–1.4)	1	126 (2.6)	1.0 (0.8–1.2)	1
Ill–defined causes						
Bacteremia patients	70 (2.2)	13.5 (10.7–17.1)	10.4 (6.4-16.7)	87 (3.9)	4.2 (3.4–5.2)	2.3 (1.7-3.2)
Population controls	58 (5.5)	1.5 (1.2–2.0)	1	232 (4.8)	1.8 (1.6–2.1)	1
Nervous system diseases						
Bacteremia patients	54 (1.7)	10.4 (8.0–13.6)	14.1 (8.1-24.9)	65 (2.9)	3.2 (2.5–4.0)	2.4 (1.7-3.3)
Population controls	30 (2.8)	0.8 (0.6–1.1)	1	187 (3.9)	1.5 (1.3–1.7)	1
Mental disorders/drug abuse						
Bacteremia patients	50 (1.6)	9.7 (7.3–12.8)	5.6 (3.5-9.0)	91 (4.1)	4.4 (3.6–5.5)	1.8 (1.4-2.4)
Population controls	57 (5.4)	1.5 (1.2–2.0)	1	321 (6.6)	2.5 (2.3–2.8)	1
Musculoskeletal/skin diseases						
Bacteremia patients	47 (1.5)	9.1 (6.8–12.1)	47.1 (15.4-144.3)	31 (1.4)	1.5 (1.1–2.2)	6.9 (3.2-14.8)
Population controls	11 (1.0)	0.3 (0.2–0.5)	1	38 (0.8)	0.3 (0.2–0.4)	1
In situ/benign neoplasms						
Bacteremia patients	43 (1.3)	8.3 (6.2–11.2)	42.9 (14.6-126.0)	25 (1.1)	1.2 (0.8–1.8)	6.8 (3.2-14.1)
Population controls	7 (0.7)	0.2 (0.1–0.4)	1	38 (0.8)	0.3 (0.2–0.4)	1
Blood/immune diseases						
Bacteremia patients	21 (0.7)	4.1 (2.7–6.2)	72.0 (12.9-401.9)	9 (0.4)	0.4 (0.2–0.8)	1.6 (0.6-4.4)
Population controls	9 (0.8)	0.2 (0.1–0.5)	1	27 (0.6)	0.2 (0.2–0.3)	1
No cause of death reported						
Bacteremia patients	7 (0.2)	1.4 (0.7–2.8)	5.6 (1.3-23.8)	4 (0.2)	0.20 (0.07–0.52)	0.9 (0.2-4.5)
Population controls	5 (0.5)	0.1 (0.1–0.3)	1	16 (0.3)	0.13 (0.08–0.20)	1
Total no. of deaths (Bacteremia patients/population controls)	3222/1062			2212/4842		

[1] Number of cause-specific deaths and percent of all deaths for bacteremia patients and population controls, respectively. [2] Computed by stratified Cox regression model (stratified on matched sets) and adjusted for comorbidity (Charlson index score 0, 1, 2, ≥3), a history of alcohol dependency (yes/no) and marital status (married, divorced, widow[er] or never married). MRR: mortality rate ratio; CI: confidence interval.

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Accuracy is key when interpreting study findings and implies that the estimates of interest are measured with little random error (high precision) and little systematic error (high validity). Systematic error comprises selection bias, information bias and confounding, which together with precision have to be critically appraised before making inferences about study results. In the following we will explore the presence of selection bias, information bias and confounding, and together with precision discuss the potential impact on the studies in this thesis. Rothman et al. [115] state:

"*The objective of an epidemiologic study is to obtain a valid and precise estimate of the frequency of a disease or of the effect of an exposure on the occurrence of a disease in the source population of the study*."

Selection bias

Selection bias may occur if individuals theoretically eligible for study are omitted from the study. As a consequence the association between exposure and outcome may differ between those included and those not included in the study [115]. Selection bias may also occur if individuals lost to follow-up differ from those who remain in the study with respect to exposure or outcome (informative censoring).

Information bias

Information bias can result from measurement errors in the needed information and can be divided into differential and nondifferential misclassification. Nondifferential misclassification occurs when the measurement error of exposure or outcome is equally distributed among exposed and unexposed subjects; it always biases the estimates towards the null value given exposure/outcome is binary. Differential misclassification occurs when the measurement error is unequally distributed among exposed and unexposed subjects; it can bias the estimates both towards and away from the null value.

Confounding

Confounding can be considered confusion of effects. An apparent association of exposure and outcome may in part or fully be explained by an unequal distribution of a factor (the confounder) between exposed and unexposed subjects. A factor that is on the causal pathway from exposure to outcome is not considered a confounder. Confounding can lead to an underestimation or overestimation of effect [115].

Precision

We used 95% confidence intervals to express the statistical precision of our estimates. Statistical precision implies little variance and can be improved by increasing the study size if the number of outcomes also increases. The 95% confidence intervals can be interpreted as a 95% certainty that our estimates contain the true value of the measurement of interest in the target population [117].

STUDY I

Information bias

All inhabitants of Funen County were under constant surveillance for bacteremia due to the population-based design and ascertainment of all blood cultures drawn within the region. Therefore, we consider any undetected bacteremia patients misclassified as "bacteremia-free" due to lack of information. This could happen in several ways and the consequence would be an *underestimation* of the true incidence rate of first-time bacteremia.

First, we cannot rule out that few individuals with bacteremia did not seek medical attention; some may have had very mild symptoms of infection while others had rapidly fatal infections. Second, some patients were likely successfully treated by their general practitioner without the need for hospitalization. In support of this, we have recently shown that patients with community-acquired bacteremia in the Emergency Department may not necessarily appear severely ill; one-third presented without fever and one-third presented without sepsis [118]. Third, some bacteremias remained undetected if blood cultures were not performed. The decision to draw blood cultures was at the attending clinicians' discretion as no uniform protocol existed. Fourth, some admitted critically ill bacteremia patients probably died prior to blood culture draw. Fifth, blood cultures may have yielded false negative results if appropriate antibiotics were administered prior to blood culture draw at either hospitals or by general practitioners. Sixth, we were unable to include residents of Funen County with bacteremia who were either admitted to or transferred to hospitals outside Funen County. Seventh, our computer algorithm has probably to some degree misclassified contaminations as true bacteremias and vice versa, which could lead to either an *underestimation* or an *overestimation* of the true incidence rate of bacteremia.

These potential information biases could also have affected our trend estimates if the impact of the biases changed during the study period. However, this is not easily evaluated because it is virtually impossible to assess trends in bacteremias outside hospitals among non-blood cultured individuals. Further, we lacked data on the timing of blood cultures and administered antibiotics as well as blood cultures drawn outside Funen County. However, we did observe that more blood cultures were drawn towards the end of the study period, which probably reduced the number of undetected bacteremias. Also, initiation of the Surviving Sepsis Campaign in 2003 may have prompted protocoled management of severely ill patients including draw of blood cultures prior to administration of antibiotics [119].

We aimed to estimate the incidence rate of first-time (ever) bacteremia. Therefore, patients who had experienced bacteremia prior to the study period should be excluded from the study. Failure to do so leads to an *overestimation* of the incidence rate of first-time bacteremia. Our trend estimates would also be influenced since prevalent bacteremias are likely to be clustered at the beginning of the study period as recurrent bacteremias often occur within months [120]. Consequently, inclusion of prevalent bacteremias would result in an *underestimation* of an increasing trend and an *overestimation* of a decreasing trend. We sought to reduce this potential bias by imposing the before mentioned lag period. To examine if the length of the lag period was appropriate, we performed additional analyses where we extended the lag period by 12 months and 24 months, respectively, without any impact on our trend findings (results not shown).

When calculating incidence rates, Funen County can be considered an open dynamic cohort were individuals may enter or exit the cohort one or several times. Individuals entered the cohort if they moved into Funen County or reached the age of 15 years (in our studies) whereas they exited the cohort if they left Funen County, died or experienced the event of interest. An unbiased estimate of the total person time at risk for the cohort could be calculated as the sum of the individual person times at risk. We used a simpler and widely accepted approach: we defined the total person time at risk (denominator) as the cumulative annual mid-year population of Funen County without censoring observation time for individuals who experienced first-time bacteremia [115]. As long as the population growth occurred steadily and the event of interest was rare, we expect only a slight *underestimation* of the incidence rates of bacteremia.

In conclusion, we have likely *underestimated* the overall incidence rate of bacteremia whereas we may have either underestimated or *overestimated* our trend estimates dependent on the direction and magnitude of the potential biases.

Place of acquisition may have been misclassified for some patients although no gold standard exists to compare our results against. As discussed earlier, there was a high agreement between a computer algorithm similar to ours, and both chart review and the physicians' clinical judgment. For bacteremias with missing dates of venipuncture (12%), we used the never missing date of receipt at the Department of Clinical Microbiology. This may pose a problem as the dates of venipuncture and receipt sometimes differed. We examined bacteremias were *both* the date of venipuncture and receipt were available and found that the date of receipt was equal to the date of venipuncture in around half the cases, delayed by one day in around half the cases, and delayed for more than one day in only 3% of the cases. Consequently, few bacteremias with missing dates of draw may have been misclassified as nosocomial rather than community-acquired/healthcare-associated. The number of blood cultures with a missing date of draw varied from year to year but showed no clear trend and are therefore unlikely to have influenced our trends estimates by place of acquisition.

Precision

We were able to report our overall incidence rates and incidence rates by place of acquisition with high statistical precision (narrow 95% confidence intervals). Trend estimates were expressed with modestly wider 95% confidence intervals but the overall decreasing trends and the decreasing trend for community-acquired and

nosocomial bacteremia were still highly statistically significant ($p<0.001$).

STUDY II

Selection bias

Patients entered the study on the day of admission to a hospital in Funen County. However, patients transferred from hospitals outside Funen County are likely to have stayed in hospitals for days (or months), which may give rise to referral bias. Because transferred patients had hospital stay before inclusion in our cohort and because the incidence decreased with increasing admission time, we may have *underestimated* the true incidence of bacteremia during the first days of admission at hospitals in Funen County. Conversely, we may have *overestimated* the incidence during the first days of admission as transferred patients may be at a particularly high risk of bacteremia because of the indication for transfer (e.g. meningitis, spondylodiscitis, or the need for surgery or intensive care). Also, the incidences in the stratified analyses may have been affected as transferred patients are often younger and admitted to highly specialized wards (neurosurgery, thoracic surgery and the intensive care unit) compared with non-transferred patients (data not shown). Detection bias may have occurred if physicians were more likely to look for bacteremias among patients *a priori* known to be at high risk of bacteremia such as the elderly or patients admitted to the Department of Hematology or the intensive care unit. Conversely, the elderly or immunocompromised may have had fewer blood cultures drawn as they often present with no or vague symptoms of infection [121].

Information bias

As described under information bias in **study I**, we probably did not ascertain all eligible bacteremia patients and consequently *underestimated* the true overall incidences. However, our estimates were not biased by bacteremia patients who did not seek medical care as the hospital-based design of this study prompted that the study population consisted of hospitalized patients only.

Precision

The extremely large cohort of admitted patients and more than 10,000 bacteremias resulted in high statistical precision and allowed for stratified analysis of the overall incidences with narrow 95% confidence intervals. The daily incidences were also estimated with high statistical precision; however, the stratified analyses by department of initial admission or microorganisms yielded much wide 95% confidence intervals as few bacteremias occurred per day of admission. For the same reason we were unable to calculate daily incidences for most microorganisms and initial departments of admission.

STUDY III

Selection bias

In cohort studies, informative censoring due to loss to follow-up may pose a problem. However, this is unlikely to have occurred in our study as we used prospective population-based registries (the Danish Civil Registration System) with complete follow-up. Only few patients emigrated and emigration is normally considered non-informative and very few individuals emigrated in our study.

Selection bias may have occurred if the association between exposure (bacteremia) and outcome (mortality) differed between those included and those not included in the study. Selection into the cohort of bacteremia patients depended on blood culture draw, and as mentioned earlier patient characteristics or vague symptoms of infection may have influenced the indication for blood culture draw. This could have biased the relative mortality estimates in subgroup analyses of e.g. age or comorbidity. Also, we have probably *overestimated* the impact of bacteremia on mortality because we considered only patients with bacteremia who were hospitalized and had blood cultures drawn. Studies have shown that most patients included in bacteremia databases are severe cases of systemic infection [16,17], whereas individuals with bacteremia treated by general practitioners without the need for hospitalization are probably less severely ill and have a better prognosis. Conversely, we have possibly *underestimated* the impact of bacteremia on mortality if bacteremia remained undetected among patients who died prior to blood culture draw. Further, we have possibly *underestimated* the relative risk of death for bacteremia patients compared with population controls if we have inappropriately sampled population controls who had experienced undetected bacteremia during the study period.

Information bias

Bacteremia patients presenting with severe symptoms are probably more likely to have antibiotics administered prior to blood culture draw compared with less ill patients, which could result in false negative blood culture results. This would lead to differential misclassification and an *underestimation* of the mortality associated with bacteremia and conservative relative mortality estimates.

All-cause mortality is unlikely to have been misclassified whereas causes of death may have been both differentially and non-differentially misclassified. The quality of the cause of death registration on death certificates is known to vary [106]. Studies have shown that autopsies, which are rarely performed in Denmark, often result in revision of the presumed cause of death [122,123]. We reported causes of death in follow-up periods of 0–1 years and >1 year after the index date. Differential misclassification may have occurred during the first year of follow-up as bacteremia patients are more likely than population controls to have their death attributed to certain categories of causes of death as a diagnosis of bacteremia may direct the clinicians' attention towards "infectious diseases" although the true underlying cause of disease was cancer (cause of death recorded as pneumonia rather than pulmonary cancer). This may have resulted in *overestimation* or *underestimation* of the relative risk for specific causes of death. Deaths that occurred later than one year after bacteremia were much less likely to be affected by a diagnosis of bacteremia unless the patients experienced recurrent bacteremias or had an unresolved long-term infection. Also, the retrospective design of our study meant that causes of death were recorded independent of any study hypothesis. Consequently, any misclassification of the underlying causes of death among long-term survivors of bacteremia is likely to be nondifferential and thus bias the relative risk estimates towards the null value (conservative estimates).

We retrieved information on chronic diseases from the Danish National Registry of Patients and since data were prospectively recorded independently of our research hypothesis this effectively rules out differential misclassification. However, the attending physicians may have misclassified the discharge diagno-

ses. The positive predictive values of Charlson conditions recorded in the Danish National Registry of Patients have been shown to be high [124] whereas the sensitivity of Charlson conditions compared with chart review is lower [125]. Also, comorbidities of bacteremia patients are likely more comprehensively recorded due to previous hospitalizations compared with population-based controls. As a result, we may have *underestimated* our relative risk estimates because we have not fully adjusted for the "true" burden of comorbidity among population controls.

Confounding

We controlled for confounding by adjusting for comorbidity, marital status and a history of alcohol dependency. Sex and year of birth were not adjusted for because we accounted for these factors in the matched design and the statistical models. The observed excess long-term mortality for bacteremia patients may in part be explained by residual confounding or unmeasured confounding. Residual confounding results from improper categorization or misclassification of the confounder variables. Unmeasured confounders may include smoking, obesity, low educational level and low income which have all been identified as predictors of short-term mortality among bacteremia patients [126,127]. Other likely confounders include level of physical activity, functional status and nutritional status as well as reimbursed medication although the latter may partly have been accounted for in the Charlson comorbidity index.

Precision

In general, we were able to express our estimates with relative high precision as many deaths occurred during follow-up. However, some of the relative risk estimates displayed wide 95% confidence intervals because of few events, e.g. for all-cause mortality among 5-years survivors (especially for microorganisms) and for cause-specific mortality among one-year survivors. In these subgroups of patients, the low number of events may cause models to be over-fitted and may have resulted in type 2 errors.

DISCUSSION OF THE RESULTS IN RELATION TO THE EXISTING LITERATURE

STUDY I

Incidence rates of bacteremia

The overall incidence rate in our study (215.7 per 100,000 person years) was comparable to or higher than previously reported (95 to 215 per 100,000 person years) [2,3,50-52,54,60,64-68]. Several possible explanations for this discrepancy exist.

First, some studies did not ascertain all eligible individuals as healthcare was either not provided for all residents within the catchment area [53,60,68], or the included laboratories did not handle all blood cultures drawn within the catchment area [65,66]. Second, all studies except one [60] included children who constitute a low incidence population (except those <1 year) [51,52]. Third, some studies excluded all bacteremia caused by common skin contaminants [53,64]. Fourth, incidence rates depend on blood culture rates as studies have found a positive correlation between the number of drawn blood cultures and the incidence rate of bacteremia [2,51]. A Finnish nationwide surveillance study found an average blood culture rate of 3209/100,000 population, which is 30% lower than observed in our study (4220/100,000 population) [51]. In the same Finnish study, blood culture rates varied between health districts by a factor of two, which may easily explain modest variations in bacteremia incidences within or between countries. Fifth, incidence rates depend on the underlying sex and age structure of the study population as males and the elderly are at the highest risk of bacteremia [68].

Conversely, we may have underestimated the incidence rate since we included only first-time bacteremias as opposed to all bacteremias in most other studies. A Danish study by Jensen et al. found that 12% of bacteremia patients experienced recurrence within one year [120]. Thus, we would have observed modestly higher incidence rates if we had included all bacteremias.

To put the occurrence of bacteremia in perspective, the incidence rate of bacteremia was comparable to that of stroke in Denmark during 2003–2012 (200 per 100,000 person years for women and 260 per 100,000 person years for men) [128] and acute myocardial infarction (AMI) for women (156 per 100,000 person years) in 2008 [129].

Few studies have reported incidence rates of bacteremia by place of acquisition. The incidence rate of nosocomial bacteremia in our study was in line with previous studies [2,60] whereas the incidence rates of community-acquired and healthcare-associated bacteremia were higher [2,53,60,64,71]. Most likely this can be attributed to the abovementioned reasons. Further, incidence rates of healthcare-associated bacteremia are also influenced by criteria used to define healthcare-association and differences in healthcare structures between institutions.

Trends in incidence rates of bacteremia

We found that the overall incidence rate decreased by 23.3% during 2000–2008 (3.3% per year), which is in conflict with previous studies that have reported either no trend [50] or an increasing trend [2,3,51,54,65,67,71]. This decreasing trend is not easily explained especially since the number of blood cultures increased by more than one-fourth during the study period. As such, it seems that a threshold has been reached where more blood cultures do not result in the detection of more bacteremias. In favor of a general trend, we found that the incidence decreased for both males and females, and all age groups. In 2001, we implemented a new blood culture system but changes in blood culture systems are normally associated with increased detection rates of microorganisms in part due to larger blood volumes [69]. We were unable to find any study that compared the performance of the two blood culture systems (Difco ESP and Bactec 9240 blood culture system) used during our study period.

Søgaard et al. observed that the overall incidence rate of bacteremia peaked in Northern Jutland, Denmark in 2004 and decreased by as much as 7% during 2005–2006 (data retrieved from the authors) [2]. It would be interesting to see if contemporary data from Northern Jutland could confirm that the incidence rate of bacteremia has peaked in Denmark.

Few studies have reported trends in incidence rates by place of acquisition. We can only speculate why the incidence rate of community-acquired bacteremias decreased. The threshold for seeking healthcare upon early signs of infections may have decreased or antibiotics may increasingly be prescribed to patients presenting with signs of infection. In support of this, aggregated data on the amount of antibiotics sold in Denmark showed a 35% increase from 2000 to 2008 with a 70% increase in broad spectrum antibiotics [130]. In Denmark, by far the most antibiotics is prescribed in primary health care [131]. The decreasing incidence rate of nosocomial bacteremia may be explained by a shift in healthcare from in-hospital care towards outpatient care as evi-

denced by fewer admission and hospitals bed days in our study. This may also explain why the incidence rate of healthcare-associated bacteremia did not decrease as more patients were at risk of healthcare-associated bacteremia. For nosocomial bacteremia early removal of bladder/intravascular catheters, increased focus on hospital hygiene, and timely adequate antibiotic therapy for patients suspected of infections may also be of importance.

Søgaard et al. reported increasing incidence rates regardless of place of acquisition during 1992–2006. However, they also noted that the incidence rate of nosocomial bacteremia decreased considerably from 2002 to 2006 (77 to 57 per 100,000 person years) concomitantly with fewer used bed days [2], which agree with our findings. Laupland et al. confirmed the increasing trend for healthcare-associated bacteremia but found no trend for community-acquired and nosocomial bacteremia in Calgary, Canada during 2000–2008 [68]. Finally, Laupland et al. reported unchanged incidence rates of community-onset (community-acquired and healthcare-associated) bacteremia in two studies from Victoria, Canada during 1998–2005 [70] and in Calgary, Canada during 2000–2004 [53].

Microorganisms

In accordance with previous studies, we found the most common microorganisms to be *Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci and *Streptococcus pneumoniae* [50-54]. We observed a decreasing trend for *Streptococcus pneumoniae* with lower incidences in 2006–2008 compared with 2000–2005. This may conceivably be explained by a possible outbreak of invasive pneumococcal disease in Denmark during 2002–2004 [132] whereas increased vaccine coverage is unlikely to have affected our estimates. The estimated vaccine coverage of the 23-valent pneumococcal vaccine was less than 3% for people aged ≥65 years in Denmark between 1998 and 2007 and declining [132]. The 7-valent pneumococcal conjugate vaccine has previously been shown to introduce herd immunity resulting in a significant decline in invasive pneumococci disease in adults [133] but was not launched for routine use in Children in Denmark until October 2007. The decreasing incidence rates of *Staphylococcus aureus* bacteremia in our study disagrees with a multinational population-based surveillance study that found no overall trend during 2000–2008. However, in that study a decreasing trend was in fact seen for included centers in North Denmark and Copenhagen City [134]. Of concern, the incidences rates of enterococcus species and *Pseudomonas aeruginosa* increased in our study. A recent Danish study by Pinholt et al. [135] found that the incidence rate of enterococcus species increased during 2006–2009 and in line with our findings this was mainly caused by *Enterococcus faecium* (described in the published article equivalent to study I in this thesis). Parkins et al. reported that the incidence rate of Pseudomonas aeruginosa increased in Canada during 2000–2006 [136] and similar to our findings this was related to an increase in healthcare-associated bacteremias. In contrast, Al-Hasan et al. reported no trend in *Pseudomonas aeruginosa* in a different Canadian region during 1997–2006 [85].

STUDY II

The overall incidence of 14.2 bacteremias per 1000 admissions in our study falls within the wide range of incidences reported in previous studies (2.3 to 26.9 per 1000 admissions) [58,60,137,138] and is in line with studies from Spain (14.7 per 1000 admissions) [60] and Israel (14.2 per 1000 admissions in 2004) [138]. Surveillance studies of nosocomial bacteremia have reported lower rates; the nationwide SCOPE Project from the USA reported 6 bacteremias per 1000 admissions [59], and a study comprising 4 hospitals in Finland reported 2.7 bacteremias per 1000 admissions [74]. Comparing incidences reported from hospital-based studies can be problematic for many of the same reasons as discussed for population-based studies (study I). In addition, hospital-based studies often differ considerably with factors not limited to patient characteristics, blood culturing rates and services provided by the included hospitals [40,51]. Also, some studies rely on discharge diagnoses to identify bacteremia rather than microbiological databases [76].

We observed the highest incidences for males, elderly individuals (>65 years) and patients initially admitted to the Departments of Hematology, Nephrology, Internal Medicine, Urology or Oncology. These findings agree with studies that have identified male sex, higher age, hemodialysis, chemotherapy, and diseases of the blood as risk factors for nosocomial bacteremia 76,139. In agreement with our results, previous studies of nosocomial bacteremia have found that patients admitted to the Departments of Internal Medicine or Oncology are at high risk of bacteremia while patients admitted to the Departments of Gynecology and Obstetrics, or Orthopedics are at low risk [74,139].

To our knowledge, our study is the first to report on the daily incidence of bacteremia during hospitalization. The incidence was highest on the day of admission and in line with previous studies we identified two-thirds of all bacteremia during the first three days of admission (community-onset) [2,68,140]. Although these bacteremias are non-preventable at hospitals, focus should remain on early identification as appropriate antibiotic therapy reduces mortality [73].

Models that predict the risk of nosocomial bacteremia have been developed [139,141] and although useful for identifying patients in high risk of bacteremia, these models make no inference about the timing of bacteremia during the course of admission. Our study provides new knowledge on this topic. The incidence was highest on the day of admission likely because most blood cultures are drawn upon admission resulting in detection of community-onset bacteremias. The daily incidence of bacteremia increased from Day 3 to Day 12, which likely reflects that patients staying in hospitals for a prolonged period of time are at increased risk of bacteremia due to nosocomial infections, complications to surgical procedures, the presence of intravascular or urinary catheters, stay in the intensive care unit, mechanical ventilation and malnutrition [76].

The daily incidence remained constant beyond Day 12; however, this translates into a linear increase in the cumulative incidence with increasing time spend in hospitals. Therefore, a potential reduction in nosocomial bacteremias could be achieved by reducing the number of hospital bed days. Nonetheless, patients with nosocomial bacteremia may belong to a group of severely ill patients that cannot be discharged earlier from hospitals. In support of this, studies have reported that patients often had central venous catheter (61–77 %), stayed in the intensive care unit (55%), received mechanical ventilation (40%), or received total parenteral nutrition (24%) at the time of nosocomial bacteremia [58,74]. We were unable to retrieve such detailed and validated clinical data from registries for use in this thesis.

Further, we lacked data on the number and timing of all blood cultures drawn in Funen County, which would otherwise have allowed us to calculate the daily incidence of bacteremia per blood culture. This would have been of interest since the clinicians' decision to draw blood cultures presumable serves as a marker of patients in high risk of bacteremia. Data on blood cul-

tures would also allow us to evaluate if a low overall incidence for a specific clinical specialty was simply a consequence of few drawn blood cultures.

STUDY III

This population-based cohort study confirms the poor short-term prognosis associated with bacteremia and contributes with new knowledge on long-term mortality and excess mortality compared with the general population.

We found a high 30-day mortality of 22%, which is in line with previous population-based studies (13% to 24%) [2,3,54,68]. A nationwide surveillance study in Finland during 2004–2007 reported a 30-day mortality of only 13% [54], which may in part be explained by inclusion of children with low mortality rates. Inclusion of recurrent bacteremias may also have introduced survival bias as bacteremia survivors may be more "robust" and less likely to die from subsequent episodes.

To our knowledge, our study is the first population-based study to include and report mortality beyond one year among all bacteremia patients. Our 1-year mortality of 41% is modestly higher compared with a Danish study of monomicrobial bacteremias (35%) [84] and a Canadian study of community-onset bacteremia (25%) [82]. This discrepancy can in part be explained by inclusion of polymicrobial and nosocomial bacteremias in our study, which are associated with a poor prognosis. Mortality in studies generally differs according to the prevalence of known predictors of death such as male sex, older age, underlying comorbidity and specific microorganisms [82,83]. Disease severity at the time of bacteremia is also important but is rarely available. We have previously shown that 30-day mortality is higher for bacteremia patients with Streptococcus pneumoniae with severe sepsis or septic shock compared with no or mild sepsis (18% vs 6%) [17].

We are aware of only two studies that have compared the survival of bacteremia patients with the general population. Leibovici et al. conducted a hospital-based study among 1991 bacteremia patients ≥18 years of age and found that the risk of death was increased compared with the expected survival in the Jewish population of Israel after standardization for the age and sex structure of the study population [19]. Among one-month survivors of bacteremia, the mortality was 29% (1 year) and 49% (4 years) as compared with an expected mortality of 6% (1 year) and 20% (4 years). In a Finnish nationwide population-based cohort study, Skogberg et al. found that bacteremia patients were in increased risk of death for only 60 days compared with the general Finnish population after adjusting for the sex and age distribution of the study population [54]. Neither study adjusted for potential confounders as mortality for bacteremia patients was compared with national mortality estimates rather than sampled individuals from the general population. The importance of considering preexisting comorbidity is apparent in the study by Leibovici et al. as 25% of the bacteremia patients had malignancy and 24% had atherosclerotic heart diseases [19]. The prevalence of these conditions is expected to be much lower in the general population.

We found that the relative risk of death for bacteremia patients was highest within months but remained increased even among 1 and 5-year survivors of bacteremia. In support, studies on community-onset sepsis have found an increased risk of death for up to 5 years compared with non-septic hospital controls [142] and non-septic population controls [143,144] after adjusting for preexisting comorbidities. As discussed earlier, unmeasured confounding may have accounted for part of the observed excess mortality. However, adjusting for comorbidity, a history of alcohol dependency, and marital status only lowered the relative risk from 2.7 (95% CI, 2.4–2.9) to 2.1 (95% CI, 1.8–2.3). Therefore, it would probably take one or more very strong confounders with a high prevalence among bacteremia patients to lower the relative risk estimates enough to include 1 in the 95% confidence interval.

We found malignancy and cardiovascular disease to be the most common causes of death. In agreement, Laupland et al. observed that patients with community-onset bacteremia died from malignancy (39%), cardiovascular diseases (24%) and infections (7%) [82] during 5 years of follow-up. During the first year of follow-up, we found that bacteremia patients often died from genitourinary diseases, infectious diseases, blood/immune diseases and digestive system diseases compared with population controls. These categories of diseases probably reflect the underlying cause of bacteremia and may help clinicians identify the primary focus of infection especially in the absence of an apparent focus. To our knowledge, only Leibovici et al. have compared causes of death after bacteremia with a control group. As expected, infections were a common cause of death within months¬ after bacteremia compared with a matched control group without infection [19]. Pedersen et al. reported infections as the primary cause of death in 62% (61/99) of bacteremia patients who died prior to notification of positive blood culture [145] but no comparison group was available.

We found that one-year survivors of bacteremia were in increased risk of death from all specific causes of death compared with population controls with no distinct differences for the most common causes of death as most 95% confidence intervals overlapped. However, this does not preclude that bacteremia may be predictive of specific post-bacteremia comorbidities in subgroups of patients, which may in turn lead to death. As an example, community-acquired bacteremia patients have recently been shown to be at increased risk of myocardial infarction or stroke within 180 days of bacteremia compared with matched population controls and within 30 days compared with admitted non-bacteremic controls [93]. Also, survivors of severe sepsis have been shown to be at increased risk of cardiovascular events within one year compared with unmatched population controls, matched population controls, and matched hospital controls [146].

CONCLUSIONS AND PERSPECTIVE

In this thesis, we have described the occurrence and prognosis of bacteremia in terms of incidence and trends in the general population, incidence among hospitalized patients, and mortality and causes of death after bacteremia compared with the general population.

We found that incidence rates of bacteremia in Funen County was higher compared with other studies from Western countries, but also that incidence rates are not easily compared between studies. To facilitate comparison, studies should report blood culture rates as well as sex and age specific incidences, which would allow for standardization to a common standard population such as the 1960 Segi population [147] and the newer 2000 WHO standard population [148] alike the principle used in cancer research. Surprisingly, we observed that the incidence rate decreased by almost one-fourth during the study period despite more blood cultures being drawn. The decreasing trend pertained to the first half of the study period but was evident for both community-acquired and nosocomial bacteremia as well as for

males and females, and all age groups. This speaks in favor of a general trend. The exact reasons for the observed trend are difficult to pin point but we speculate that fewer nosocomial bacteremias were associated with the continuous decrease in admissions and hospital bed days. Fewer community-acquired bacteremias were possibly related to an increased use of antibiotics; however, we did not have data on used antibiotics on an individual level to support this claim. Studies on the usage of primary health care could potentially elucidate if improved diagnostics or a lowered threshold for treating less severe infections with antibiotics has contributed to the prevention of bacteremias. We observed a change towards microorganisms with a high level of intrinsic antibiotic resistance (*Enterococcus* species and *Pseudomonas aeruginosa*) and this finding highlights the need for continued surveillance of distribution of microorganisms and revision of empirical antibiotics regimens.

We found that the risk of bacteremia for hospitalized patients differed markedly with patient characteristics and clinical specialties, and varied with time spent in hospitals. These findings can potentially help save resources by focusing surveillance on patients in high risk of bacteremia such as patients initially admitted to the Departments of Hematology, Nephrology and Oncology. Conversely, less attention can be paid to patients initially admitted to the Departments of Gynecology/Obstetrics or Orthopedics as these departments accounted for only 4% of all bacteremias but 25% of all admissions and hospital bed days. Differences in healthcare structures between countries may hamper the generalizability of these results; however, our findings highlight that important differences exist in incidences of bacteremia between e.g. clinical specialties, which can be used to direct clinicians' attention towards patients in high risk of bacteremia and to plan targeted preventive measures. To characterize the population at risk of nosocomial bacteremia, future studies should aim to provide data on the number and timing of blood cultures, clinical data at the time of blood culture draw and administered antibiotics during hospitalization. Implementation of electronic medical records will hopefully facilitate this.

We have shown that bacteremia is associated with high short-term and long-term mortality as well as continued excess mortality compared with the general population. Bacteremia patients had a median survival of only two years and were in two-fold increased risk of death even 5 years after bacteremia compared with population controls. Likely, unmeasured confounding accounted for some of this excess mortality and it would be interesting to confirm our findings in large cohort studies in other settings that include detailed information on factors not limited to comorbidities, redeemed prescriptions, prior admissions, personal income, educational level, usage of primary health care and other potential confounders. Given the large number of bacteremia-survivors discharged from hospitals every year, we believe that more attention should be paid to the long-term consequences of bacteremia. In terms of prevention, future studies should focus on identifying (modifiable) predictors of readmission, recurrent bacteremia and death among bacteremia survivors. It should also be recognized that the clinical course and outcomes of bacteremia probably differ with different foci of infection and disease severity, and these factors should be taken into account if possible. Other outcomes such as quality of life, cognitive impairment and functional disability are also of great importance to bacteremia survivors and deserve attention. Finally, even though deaths among one-year survivors of bacteremia did not pertain to one or more specific causes of death, bacteremia could still be a predictor of new onset of worsening of existing comorbidity in subgroups of patients. Studies on the interplay between bacteremia and chronic disease could help identify patients at risk of post discharge complications and could potentially help target bacteremia survivors for specific interventions already at hospital discharge.

SUMMARY

Bacteremia is associated with increased morbidity and mortality, and ranks among the top seven causes of death in Europe and North America. The occurrence of bacteremia has increased for decades while short-term prognosis has remained unchanged or improved only slightly. Consequently, we are facing an increased number of bacteremia survivors for whom we know little about long-term survival and causes of death. Contemporary knowledge on the epidemiology and outcome of bacteremia is important to assess its impact on public health and is a prerequisite for any effective prevention and improvement of prognosis.

This thesis is based on data from a bacteremia database (The Danish Observational Registry of Infectious Syndromes) comprising all bacteremias in Funen County, Denmark, between May 1999 and December 2008. Data on bacteremias were cross-linked with various administrative and research healthcare registries and we conducted 3 studies on adult bacteremia patients with **the aims**:

To investigate the occurrence of and trends in first-time bacteremia and distribution of microorganisms in the general population; overall and by place of acquisition **(study I)**

To investigate the overall and daily incidences of bacteremia among hospitalized patients **(study II)**

To investigate and compare long-term mortality and causes of death after bacteremia with the general population **(study III)**

Study I: In a population-based observational study, we identified 7786 residents of Funen County with first-time bacteremia for an overall incidence rate of 215.7 per 100,000 person years including 99.0 for community-acquired, 50.0 for healthcare-associated and 66.7 for nosocomial bacteremia. The overall incidence rate decreased by 23.3% (95% CI, 17.8%–28.4%) from year 2000 to 2008 (3.3% per year, p<.001) due to decreasing rates of community-acquired bacteremia (3.7% per year, p<0.001) and nosocomial bacteremia (4.2% per year, p<0.001). The incidence rate of healthcare-associated bacteremia remained more of less stable throughout the study period (p=0.17). The crude incidence rates decreased for *Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci and *Streptococcus pneumoniae*, and increased for *Pseudomonas aeruginosa* and *enterococci* species (p<0.05 for all the mentioned microorganisms). Regardless of place of acquisition, the proportion of bacteremias caused by coagulase-negative staphylococci decreased while the proportion caused by Enterococcus species increased.

Study II: In a multicenter hospital-based cohort study, we included 276,586 adult patients with 724,339 admissions to hospitals in Funen County for a total of 4,531,744 bed days. Among the hospitalized patients, we identified 10,281 first-time bacteremias per admission for an overall incidence of 14.2 per 1000 admissions and 23.6 per 10,000 bed days; highest for males, elderly individuals (>65 years), and patients initially admitted to the Departments of Hematology, Nephrology, Internal Medicine, Urology or Oncology. The daily incidence was highest on the day of admission and declined rapidly to a low level on Day 3–7. Hereafter it increased steadily until Day 12 followed by more or less constant daily incidences. The daily incidences varied considerably with patient and clinical characteristics.

Study III: In a population-based cohort study, we included 7783 patients with first-time bacteremia and 38,906 population controls matched on sex, year of birth and residency. We found that the cumulative mortality in bacteremia patients and population controls was 22.0% vs. 0.2% (30 days), 41.4% vs. 2.6% (1 year), and 75.8% vs. 36.6% (10 years). Bacteremia patients were consistently at increased risk of death compared with population controls throughout 12 years of follow-up and the risk of death remained twofold increased even among 5-year survivors of bacteremia (adjusted MRR 2.1; 95% CI, 1.8–2.3). The most common causes of death after bacteremia were cancer and cardiovascular diseases. Compared with population controls, bacteremia patients were at the highest risk of death from genitourinary diseases and infectious diseases within one year of bacteremia. Among one-year survivors of bacteremia, the risk of death was increased for all major causes of death compared with population controls.

We **conclude** that the occurrence of bacteremia is decreasing in the general population. However, bacteremia is associated with a very poor short and long-term prognosis and the risk of death remains increased for years compared with the general population. The most common causes of death after bacteremia are cancer and cardiovascular diseases. Among hospitalized patients, the incidence of bacteremia is highest within days of admission and varies with patient and clinical characteristics.

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APPENDIX

APPENDIX 1

The 15 categories of underlying causes of death classified according to The International Classification of Diseases, 10th Revision:

For infectious diseases, the codes were A00–B99; malignant neoplasms C00–C96; in situ/benign neoplasms D00–D49; blood/immune diseases D50–D89; endocrine diseases E00–E90; mental disorders /drug abuse F00–F99; nervous system diseases G00–G99; cardiovascular diseases I00–I99; respiratory diseases J00–J99; digestive system diseases K00–K93; musculoskeletal/skin diseases L00–L99 and M00–M99; genitourinary diseases N00–N99; injury/poisoning S00–T98,V,W,X and Y; ill-defined causes H00–H95, O00–O99, P00–P96, Q00–Q99 and R00–R99; No cause of death reported.

APPENDIX 2

The International Classification of Diseases, 10th Revision codes used to define “chronic alcohol use” were E244, E529A, F10[2–9], G312, G621, G721, I426, K292, K70*, K852, K860, L278A, O354, P043, T500A, X65[1-2], Z502, Z714 and Z721, and codes used to define “acute alcohol use” were F10[0–1] and T51*.