Community-acquired *Staphylococcus aureus* bacteremia: Studies of risk and prognosis with special attention to diabetes mellitus and chronic heart failure

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THE FOUR ORIGINAL PAPERS ARE

I. Smit J, Søgaard M, Schønheyder HC, Nielsen H, Thomsen RW. Classification of healthcare-associated *Staphylococcus aureus* bacteremia: Influence of different definitions on prevalence, patient characteristics, and outcome. Infection Control & Hospital Epidemiology 2016;37:208-211.

II. Smit J, Søgaard M, Schønheyder HC, Nielsen H, Frøslev T, Thomsen RW. Diabetes and risk of community-acquired *Staphylococcus aureus* bacteremia: A population-based case-control study. European Journal of Endocrinology 2016;174:631-639.

III. Smit J, Thomsen RW, Schønheyder HC, Nielsen H, Frøslev T, Søgaard M. Outcome of community-acquired *Staphylococcus aureus* bacteraemia in patients with diabetes: A historical population-based cohort study. PLoS One 2016;11:e0153766.

IV. Smit J, Adelborg K, Thomsen RW, Søgaard M, Schønheyder HC. Chronic heart failure and mortality in patients with community-acquired *Staphylococcus aureus* bacteremia: A population-based cohort study. BMC Infectious Diseases 2016;16:227.

THESIS OUTLINE

Staphylococcus aureus bacteremia (SAB) is a serious clinical syndrome associated with considerable morbidity and a 30-day mortality of 20-40% in developed countries [1-3]. High age and presence of chronic diseases are recognized as some of the most important risk and prognostic factors for SAB [1-2, 4-5]. Due to population aging and lifestyle-related factors, the prevalences of diabetes mellitus and chronic heart failure (CHF) are rapidly increasing worldwide and in western countries in particular [6-10]. Nevertheless, there is a paucity of data specifically elucidating the influence of diabetes and CHF on SAB risk and prognosis. Such information is important to extend our knowledge about the clinical course of patients with SAB and contributes to improvement of preventive measures and clinical care for patients suffering from these chronic diseases. Therefore, we used population-based registries and medical databases to investigate whether diabetes is associated with an increased risk of community-acquired SAB (CA-SAB) and whether presence of diabetes and CHF influence prognosis. SAB acquired during admission to the hospital is strongly associated with concurrent diseases and surgical procedures [11-12], which may distort the association between diabetes, CHF, and the risk and prognosis of SAB considerably. Therefore, aiming to elucidate the association between these chronic conditions and SAB in the general population, we chose to focus on CA-SAB in this thesis.

The thesis is based on four papers referred to in the text by Roman numerals (I-IV). The first paper is a methodological study portraying some of the challenges associated with the classification of SAB. Study II investigates diabetes as a risk factor for CA-SAB and the third paper ascertains the prognostic impact of diabetes in patients with CA-SAB. Finally, in the fourth study, the association between underlying CHF and CA-SAB outcome is assessed.

The background outlines the three central conditions SAB, diabetes, and CHF, including a review of the existing literature in relation to the aims of the thesis. The subsequent chapters include a summary of the methods used and results obtained in studies I-IV, discussion of the main results in relation to the existing literature, methodological considerations, and finally conclusion and perspective

BACKGROUND

S. aureus bacteremia

S. aureus is both a commensal bacterium and a major human pathogen with the propensity to cause a broad spectrum of clinical disease across all age groups [1,13]. *S. aureus* colonizes asymptomatically the skin and mucosa of approximately 30% of healthy persons [14-19]. In addition to its frequent carriage as a commensal, *S. aureus* is a leading cause of skin and soft tissue infections (~90% of staphylococcal infections), bone and joint infections, wound infection, infective endocarditis, and infections related to medical devices [13, 20-22]. In most cases, *S. aureus* infections remain localized to the affected organ, however the body´s protective mechanisms cannot always restrict the infection and staphylococci may subsequently gain entry to the bloodstream causing *S. aureus* bacteremia (the suffix ´-emia´ relates to the blood) [23].

SAB is defined as ´the isolation of *S. aureus* bacteria from one or more peripheral venous blood culture samples collected from a patient with associated relevant symptoms and signs of systemic infection´ [24]. *S. aureus* is a rare contaminant as shown in prospective studies with a total of 1,809 SAB episodes of which only 27 (1.5%) were considered to represent contamination [24]. Considering the serious clinical consequences associated with SAB, it is recommended that the isolation of *S. aureus* from blood cultures should always be regarded as clinically significant [24]. The precondition of acquisition in the community implies that the origin of the *S. aureus* infection is rarely observed. To avoid speculative distinctions between primary and secondary foci, it is prudent to prioritize the site of infection that is the most probably source of the bloodstream infection when the first positive blood culture was drawn, based on symptoms and clinical signs, additional microbiological findings, and imaging results.

In Denmark, there is a long tradition of research on SAB. Since 1957, SAB has been surveyed on a national basis by collection of blood culture isolates. The *Staphylococcus* Laboratory at Statens Serum Institut has undertaken strain characterization and retrieval of clinical and epidemiological information on the patient level [25]. Since the inception of this cohort, numerous studies have provided valuable insight into different aspects of SAB epidemiology including antibiotic resistance [26-27], clinical characteristics [28-30], incidence [31-32], and outcome [31-32]. Although bacteremia with methicillin-resistant *S. aureus* (MRSA) constitutes a major challenge in many countries, bacteremia with methicillin-susceptible *S. aureus* (MSSA) represent the most common type of SAB in most parts of the world [3]. In Denmark, the prevalence of MRSA bacteremia has remained uniquely low (~ 2%) during the past three decades [25, 33], though a slight increase in prevalence has been observed in recent years (2.9% in 2014) [25].

The population incidence of SAB ranges from 10 to 35 per 100,000 person years in the industrialized world [31-32, 34-36]. In Denmark, the incidence of SAB increased from 18.2 per 100,000 person years to 30.5 per 100,000 person years between 1981 and 2000. Of note, annual rates increased by 6.4% for CA-SAB compared with only 2.2% for hospital-acquired SAB (HA-SAB) [32]. Since 2000, the incidence of SAB in Denmark has continued to rise reaching an incidence rate of 34.9 per 100,000 person years in 2014 [25]. During the past 50 years, the rates of hospital admissions, outpatient contacts, and complex invasive medical interventions have increased exponentially. Thus, increased exposure to the healthcare system may explain part of the observed increase in SAB incidence. On the other hand, the increasing incidence of SAB may also reflect demographic changes, e.g., an aging population and the increasing longevity of patients with chronic diseases due to medical progress [1]. In addition, the indications for obtaining blood cultures may have widened during the period and improvements in blood culture technology may further have influenced the incidence [37].

Once established, SAB is associated with substantial morbidity and mortality [2-3, 38-40]. In the pre-antibiotic era, all-cause mortality in patients with SAB ranged between 75% and 83% [41]. Although the introduction of effective antibiotics in the 1940s and 1950s radically improved SAB management, studies from different settings around the world have demonstrated that the 30-day all-cause mortality associated with SAB have plateaued at 20-35% [3, 39, 42-43]. These results are corroborated by the aforementioned surveillance reports from Statens Serum Institut demonstrating an almost constant 30-day mortality of approximately 25% during the years 1998-2014 [25]. SAB may also have important non-lethal outcomes including discomfort, pain, decreased functional status, long-term financial costs, and SAB recurrence (2-10% of patients) [44-46].

Clinical manifestations and management of *S. aureus* bacteremia

The presentation of SAB varies greatly and the clinical course is difficult to predict [1, 47-48]. Non-specific findings of fever, hypotension, tachycardia, and leukocytosis are common, nevertheless no anamnestic features or clinical signs are considered pathognomonic of SAB [1, 47]. More than 30% of patients with SAB develop more than one focus of infection [48-51], thus the full extent of *S. aureus* infection may not be obvious at presentation and the clinical picture may change several times during the course of infection. Adding to the complexity, the symptoms and findings may originate from the organ that was initially infected (e.g., a skin infection), from hematogenous or contiguous spread to another organ (e.g., infective endocarditis), or potentially from a combination of local and systemic infection [1,47].

SAB is closely associated with the clinical syndrome of sepsis which compromises physiologic, pathologic, and biochemical abnormalities elicited by the infectious process [52]. During the past two decades, sepsis has been almost synonymous with the systemic inflammatory response syndrome (SIRS) caused by confirmed or suspected infection. Sepsis with organ dysfunction or hypoperfusion was further classified as severe sepsis, which could eventually progress to septic shock [53-54]. However, due to inadequate specificity and sensitivity of the SIRS criteria, updated definitions of sepsis were proposed in 2016 [55]. According to the Third International Consensus Definitions for Sepsis and Septic Shock

(Sepsis-3), sepsis should be defined as ´life-threatening organ dysfunction caused by a dysregulated host response to infection´. Sepsis may intensify to septic shock, defined as a subset of sepsis in which ´particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone´. Of note, since patients with evidence of organ dysfunction or hypoperfusion are encompassed by the 2016 definitions of sepsis and septic shock, the use of the term severe sepsis is no longer recommended [55]. Septic shock, although definitions vary slightly between previous studies, has been demonstrated to occur in approximately 10-40% of patients with SAB [2].

Although clinical guidelines for the management of SAB are available [24, 56-58], the evidence guiding optimal treatment unfortunately remains poor. As demonstrated by a recent comprehensive review [59] assessing the clinical management of SAB, only a single study fulfilled the GRADE (grading of recommendation, assessment, development, and evaluation) criteria [60] for high-quality evidence. Despite the need for additional evidence based on well-designed studies, early identification and control of the infective focus (or foci) and appropriate antibiotic therapy are widely accepted as the two mainstays of SAB management [1, 24, 59]. Although estimates vary between different clinical settings, SAB is complicated by infective endocarditis (IE) in approximately 25-38% of cases, which is often clinically indistinguishable from SAB without the presence of IE [47-48, 59]. The risk of IE is highest among patients with congenital heart disease, prosthetic heart valves, intracardiac devices, and previous episodes of IE, although ~ 50% of cases of IE develop in SAB patients with no previous history of heart valve disease [22, 47, 59]. Because the presence of IE is decisive for clinical monitoring and treatment, echocardiography of all patients with SAB is recommended by most recent guidelines [59]. Effective antimicrobial therapy for SAB requires careful selection of a proven agent administered with optimal frequency and sufficient dosage [24, 47, 59]. The optimal duration of antibiotic therapy remains controversial, however, and continues to rest mainly on clinical traditions. Still, receipt of antibiotic therapy for less than two weeks has been associated with increased risk of relapse in patients with SAB [61-62], thus a minimum of two weeks of intravenous antibiotic treatment is recommended by the majority of current SAB guidelines [56, 58-59, 63].

Classification of *S. aureus* bacteremia

SAB can be classified in several ways, e.g., as MSSA or MRSA [1, 47] or as monomicrobial or polymicrobial (64-65). Central to this thesis, SAB is classified according to whether the infection has arisen in the community (CA-SAB) or during hospitalization (HA-SAB) [66]. In 1975, McGowan et al. [67] defined community-acquired bacteremia as presence of positive blood cultures on admission or within the two first days in the hospital and hospital-acquired bacteremia as occurring on or after the third day in the hospital, and this approach was adapted in a subsequent study on bacteremia by Brenner et al. [68]. Later, in 1988, Garner et al. [69] published definitions of acquisition on behalf of the Centers for Disease Control and Prevention (CDC) stating that classification of infections should be based on individual assessment using all available clinical data and not rely solely on prespecified time windows. Nevertheless, a pragmatic 48-hour cut-off between infection diagnosis and the time of hospital admission to distinguish between community and hospital acquisition has been used in most previous studies of SAB [3, 35, 39, 70-73].

Since the initial introduction of the CA and HA categories, the health care system has experienced major organizational changes and increasingly complex medical services are now being provided in the patients´ homes or in outpatient hospital clinics. Thus, it might not always be adequate to label infections simply as CA, and in 2002 a separate healthcare-associated (HCA) group was proposed by Siegman-Igra et al. [74] and by Friedman et al. [75], respectively, to extend the definition of CA bacteremia (detailed criteria are provided in Table 1). SAB is particularly often seen in patients with frequent contact to the healthcare system [1, 11-12], hence correct classification on admission is pivotal. Nevertheless, there is no international consensus on the definition of HCA bacteremia (including HCA-SAB) [76] which may influence negatively the validity of the estimates and render comparison of SAB studies difficult. Indeed, as evident from a review of the existing literature (Table 2), rather different definitions of HCA-SAB have been employed in previous studies.

Table 1. Initial definitions of healthcare-associated (HCA) bacteremia.

Study, year of publication	**HCA bacteremia criteria** *Blood culture performed within 2 days of admission and the following:*
Siegman-Igra Y, et al., 2002 [74]	1. Discharge from hospital 2 to 30 days previously *or* 2. Admission from nursing home *or* 3. Patients with long-term intravenous devices, for hemodialysis, chemotherapy or parenteral nutrition *or* 4. Chronic hemodialysis *or* 5. Invasive procedure previously or at hospital admission

Friedman D, et al., 2002 [75]	1. Received intravenous therapy at home, wound care or specialized nursing care through a healthcare agency, family or friends; or had self-administered intravenous medical therapy in the 30 days before the infection *or* 2. Attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the previous 30 days *or* 3. Were hospitalized in an acute care hospital for 2 or more days in the previous 90 days *or* 4. Resided in a nursing home or long-term care facility

Table 2. Previously used definitions of healthcare-associated (HCA) infection in studies of *S. aureus* bacteremia.

Study, year of publication	**HCA-bacteremia criteria**
Jacobsson G, et al., 2007 [77]	*Blood culture performed within 2 days of admission and:* 1. Nursing home residence *or* 2. Reception of healthcare at home
Asgeirsson H, et al., 2010 [35]	*Blood culture performed within 2 days of admission and:* 1. Hospital admission for >2 days within 90 days of the current hospitalization
Paulsen J, et al., 2015 [39]	*Blood culture performed within 2 days of admission and:* 1. Received intravenous therapy at home, wound care or specialized nursing care through a healthcare agency, family or friends, or had self-administered intravenous medical therapy in the 30 days before the infection *or* 2. Attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the previous 30 days *or* 3. Were hospitalized in an acute care hospital for ≥ 2 days in the previous 30 days *or* 4. Resided in a nursing home or long-term care facility
Yahav D, et al., 2016 [4]	*Blood culture performed within 2 days of admission and:* 1. Previous hospitalization of ≥2 days during previous 90 days *or* 2. Clinic visit during previous 30 days *or* 3. Home IV therapy or chemotherapy or wound treatment during the previous 30 days *or* 4. Patients arriving from long-term care facilities
Forsblom E, et al., 2016 [78]	1. Blood culture performed ≥48 hours after hospital *or* 2. Admission from long-term care facility *or* 3. Hemodialysis within the preceding two months

Established risk and prognostic factors for *S. aureus* bacteremia

Several factors are associated with increased risk of SAB. First of all, age is one the strongest risk factors for SAB [34-36, 79], for example the incidence of SAB is >100 per 100,000 person-years among patients aged more than 70 years [34] compared with only 4.7 per 100,000 person-years in healthier U.S. military personnel of younger age [80]. Further, male gender constitutes one of the most consistent risk factors for SAB with male-to-female ratios of approximately 1.5 [35, 79-81]. However, the excess risk of SAB observed among elderly and male persons may partly be explained by more frequent contacts to the healthcare system and presence of comorbid conditions. Indeed, comorbidity is associated with markedly increased risk of SAB [1, 31, 47]. As an example, a recent Danish cohort study demonstrated that patients with end-stage renal disease experienced an almost 30 times increased risk of SAB compared with population controls [82]. The risk was most pronounced among patients receiving dialysis, which is corroborated by surveillance reports from the US demonstrating that the incidence of SAB is more than 100 times higher among dialysis patients compared with the healthy US population [83]. According to a Danish cohort study, the risk of SAB in patients living with HIV is 24 times that of persons without HIV [84]. Part of the overall increased risk among patients with HIV may, however, have been driven by a higher prevalence of injection drug abuse, which has been associated with increased risk of SAB [85-87]. Finally, the presence of medical devices in general and venous catheters in particular is associated with considerable increased SAB risk [1, 88].

Several of the abovementioned risk factors for SAB also constitute important prognostic factors for SAB. Consistent across a multitude of studies, age remains the single most important prognostic factor of all-cause 30-day mortality in patients with SAB [1-3, 89]. Female gender has been associated with increased mortality in previous studies [31, 90-91], yet the mechanisms underlying this association remain unclear. The place of acquisition (HA, CA, HCA) has also been investigated as a potential prognostic factor. Although a recent Norwegian cohort study [39] observed an improved outcome associated with CA-SAB, the majority of previous studies have not been able to demonstrate notable differences in 30-day mortality between patients with CA-SAB and HA-SAB, respectively [3, 31-32, 42-43, 73]. Notwithstanding, some studies on bacteremia (including SAB) have suggested that patients with HCA infection are at increased risk of death as compared to patients with CA infection [92-94]. Furthermore, the prognosis of SAB varies considerably by infective focus, *viz* respiratory focus and IE are associated with high mortality, whereas osteoarticular focus and SAB related to use of intravascular access devices are associated with a better outcome [2-3, 95]. Moreover, failure to identify the infective focus [89, 96] and presence of multiple foci in particular impart a poor prognosis [48, 50, 97-98]. In addition to being a risk factor of SAB, presence of accumulated comorbidity also represents an important prognostic factor [31-32, 99]. Although there is a paucity of in-depth data assessing the prognostic influence of specific comorbid conditions, chronic kidney disease requiring dialysis [40, 89, 100], liver cirrhosis [65, 101], cancer [2, 102], and alcohol-related conditions [31, 40] have all been suggested to be associated with poor outcome in patients with SAB. The presence of septic shock is strongly associated with poor outcome, with 30-day mortalities ranging between 38-86% [2]. Still, the wide variation in outcome observed in these previous studies may partly be explained by differences in sepsis definitions and study populations [2]. Finally, as touched upon in relation to the clinical management of SAB, early identification and control of the infective focus and appropriate antibiotic treatment are of importance for SAB outcome [24, 59, 103].

Diabetes

Diabetes is a major cause of morbidity and mortality on a global scale. According to reports from the International Diabetes Federation, 1 in 11 of the world´s population currently suffers from diabetes and every 6 seconds a person dies from this disease [104]. Diabetes is a chronic multisystem metabolic disease resulting from insufficient insulin secretion, insulin action, or a combination of both [105-107]. Due to the complex clinical presentation of diabetes and the potential presence of a mixture of phenotypes, classification of the disease is not always straightforward. Still, the American Diabetes Association recommends that diabetes is classified into four major categories: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types of diabetes [105]. Type 1 diabetes is most commonly seen in patients aged less than 40 years and stems from autoimmune destruction of pancreatic beta cells leading to insulin deficiency. Type 2 diabetes is most frequently diagnosed in patients older than 30-40 years, but may develop at any age. It is characterized by variable degrees of insulin secretion, insulin resistance, and increased hepatic glucose production. Type 2 diabetes accounts for the vast majority (>90%) of those with diabetes [105, 108].

Owing to population ageing, increasing obesity, and inactive lifestyle, the prevalence of type 2 diabetes is on the increase globally [6-8, 104]. Still, increased diagnostic activity and longer survival of patients with diabetes due to earlier diagnosis or improved anti diabetes therapy may underlie part of the observed increase in prevalence. Approximately 415 million people are afflicted by diabetes worldwide, and this is expected to increase to as many as 642 million people by 2040 [104]. In line with this, approximately 320,000 Danish residents are currently living with diabetes, and the prevalence is estimated to rise by more than 20,000 patients each year [109-110]. Diabetes has a negative effect on patients´ quality of life and is strongly associated with reduced life expectancy [111-112]. In addition, patients with diabetes with poor glycemic control and patients with a long history of diabetes are at increased risk of a microvascular and macrovascular diabetes complications [111,113]. These complications may affect multiple organ systems, thus diabetes is strongly associated with risk of ischemic heart disease [114], chronic heart failure [115], cerebrovascular disease [114], and peripheral neuropathy and peripheral arterial disease which may lead to diabetic foot ulcers [116]. Moreover, diabetes is a leading cause of chronic kidney disease and blindness in the industrialized world [117]. Finally, patients with diabetes are often characterized by advanced age and concurrent chronic conditions (e.g., chronic obstructive pulmonary disease and cancer) adding further to the disease burden [111-112].

Chronic heart failure

CHF constitutes a staggering health problem affecting more than 23 million adults worldwide [9-10]. In Denmark, an estimated 60,000 persons suffer from CHF leading to more than 11,000 hospital admissions annually [118]. The American College of Cardiology guidelines describes heart failure as ´a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill or eject blood´ [119]. It is important to recognize that CHF is not a single disease but a clinical syndrome with a multitude of clinical presentations rendering its diagnosis a considerable challenge. CHF can arise from a variety of causes that may co-exist and interact with each other in an individual patient, still ischemic heart disease, hypertension, and valvular heart disease remain among the most frequent underlying causes [9-10, 119-121]. In addition, there is evidence that obesity and diabetes are associated with risk of CHF independently of clinical coronary disease and hypertension [115, 122]. The presence and severity of CHF is usually classified according to the New York Heart Association (NYHA) functional classification (stage I-IV) or by the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) classification system (stage A-D) [119-120]. The former is based solely on exercise capacity and the symptomatic status of the diseases, whereas the latter takes into account both risk factors for

CHF and documented presence of structural heart disease. Both classification schemes have, however, been demonstrated to be valuable tools for predicting prognosis in patients with CHF [119-120]. Adding to the burden of CHF, the disease is often preceded and/or complicated by other cardiovascular conditions including cardiomyopathy, valvular heart disease, and atrial fibrillation [119-120]. Although the mortality from CHF appears to have declined in recent decades [9-10], the one-year all-cause mortality following diagnosis remains at 20% in Denmark [118]. Additionally, CHF is associated with high rates of readmissions imposing a heavy burden on patients´ quality of life and healthcare systems [10, 123].

Diabetes, chronic heart failure, and *S. aureus* bacteremia

Diabetes may influence the risk and prognosis of CA-SAB for a number of reasons. Of chief importance, diabetes and CA-SAB share several important risk and prognostic factors counting advanced age and presence of concurrent chronic conditions. Furthermore, it may be that patients with diabetes complications are at particularly increased risk of CA-SAB. For instance, diabetic foot ulcers degrade normal skin barriers [116] which may allow staphylococci to enter the abutting tissues or ultimately the bloodstream. Moreover, diabetes is strongly associated with development of chronic kidney disease requiring dialysis [113, 117], both of which have been suggested to be associated with increased risk and poor prognosis in patients with CA-SAB [82-83].
Diabetes affects several aspects of the cellular and humoral immunity. Neutrophil leukocytes represent the most important cellular defense against *S.aureus* infections [124-125]; however, chemotaxis, adhesion and intracellular killing are impaired in patients with diabetes [126-127]. Furthermore, there is strong evidence indicating that diabetes is associated with chronic low-grade inflammation. Hence, increased levels of C-reactive protein and interleukin 6 have been demonstrated to precede the development of type 2 diabetes in healthy persons and increased levels of proinflammatory cytokines (including interleukin 6) are associated with manifest diabetes [128-129]. In contrast, cytokine responses to an acute infectious challenge have been suggested to be blunted in patients with diabetes [130-131], thus, the impact of diabetes on cytokine responses may be envisaged to affect both the risk and outcome of CA-SAB. In line, there is evidence suggesting that patients with diabetes with serious systemic infection may be protected from severe complications such as respiratory failure through a less active inflammatory cascade [132]. On the other hand, hyperglycemia is associated with increased coagulation and subsequent risk of thrombotic events which may have a negative effect on outcome [133].
Finally, colonization with *S. aureus* may be associated with increased risk of infection including SAB [14, 134]. Some previous studies have suggested that patients with diabetes are more frequently colonized with *S. aureus* than patients without [135], whereas other studies have observed no differences in prevalence of colonization associated with diabetes [136-137]. Thus, the potential role of *S. aureus* colonization for the risk and prognosis of SAB among patients with diabetes is not well understood.

In line with diabetes, CHF may also be speculated to influence the prognosis of patients with CA-SAB. As mentioned above, SAB is strongly associated with sepsis and the latter has been demonstrated to affect myocardial function negatively through various mechanisms counting maldistribution of coronary blood flow, cytokine-induced neutrophil activation and myocardial injury, and complement-triggered myocyte contractile failure. Thus, patients with sepsis may be challenged by ventricular dilatation, reduced ejection fraction, and decreased ability to mount a sufficient cardiovascular output despite the presence of increased catecholamine levels [52, 138-140]. As patients with CHF are characterized by insufficient cardiac pump function at baseline, it might be speculated that these patients are particularly at risk of circulatory collapse and subsequent death when challenged by SAB. Additionally, CHF is strongly associated with advanced age and multiple morbidities which, as described previously, represent some of the most important prognostic factors for CA-SAB [2-3, 31, 89, 99].

Literature review

Searching the Medline and Embase databases from the earliest available date until September 2016, we conducted a literature review to identify and summarize existing knowledge on 1) the influence of different definitions of HCA infection on HCA-SAB prevalence, clinical characteristics, and outcome, 2) the influence of diabetes on CA-SAB risk and prognosis, and 3) the influence of CHF on outcome from CA-SAB.
No restrictions concerning language were applied and conference abstracts were also included. The entire literature review was supervised by an experienced medical librarian and we customized the search for each database using both controlled thesaurus terms and natural language terms for synonyms. We assessed the title and abstract of each paper and selected all relevant studies fulfilling the PICO criteria [142], i.e. information was available on the study population, the exposure, the comparison group, and the outcome. The reference lists of all selected papers were then reviewed for additional works of relevance and we further ascertained papers indicated as relevant by Medline and Embase for each selected paper. Finally, if we through our previous work were aware of additional relevant studies not identified by the search, these were also included (n=3).

Study I

A few previous studies have touched upon whether different definitions of HCA infection influence the prevalence, clinical characteristics, and outcome of patients with *S. aureus* infection. In 2005, Folden et al. [143] observed an almost doubling of the HCA-MRSA infection prevalence with use of two different classification schemes. Two later studies [144-145] compared epidemiological criteria with criteria based on antimicrobial susceptibility patterns for classifying HCA-MRSA infection and obtained discrepant results. Moreover, Leung et al. [146] and Gradel et al. [66], respectively, demonstrated that the use of different time windows to define HCA infection did not notably influence the prevalence of HCA-MRSA infection [146], nor the results of prognostic models in patients with bacteremia [66]. Nevertheless, all the previous studies had different primary objectives and none assessed

specifically the influence of different HCA infection definitions in patients with SAB. In addition, the majority of these previous studies were limited by small and selected sample sizes [143-144, 146], which may have biased the results.

Study II

A limited number of previous studies have included diabetes among a number of other potential risk factors for SAB. In an American cohort study, Bryan et al. [147] reported the incidence of SAB being three times higher among patients with diabetes as compared to patients without. These first results were later corroborated by two Canadian cohort studies in which Laupland et al. [148-149] found diabetes to be associated with an increased risk of invasive *S. aureus* infection and SAB in particular. In line with this, a Swedish cohort study investigating several risk factors for invasive *S. aureus* infection (including SAB) identified diabetes as one of the most important risk factors (unadjusted OR=8.2 (95% CI, 6-12)) [77]. Results from an Italian case-control study [72] investigating risk factors for SAB demonstrated an increased risk of diabetes associated with CA-SAB, and in an American cohort study comprising emergency department patients suspected of infection [150], patients with diabetes experienced a two-fold risk of MRSA bacteremia compared to patients without diabetes. Finally, in a Spanish cohort study on SAB, Hernandez et al. [151] found that diabetes was associated with SAB of unknown origin.

However, none of these studies investigated diabetes as a risk factor for SAB as the primary aim and lacked detailed information on diabetes exposure (e.g., duration of diabetes or presence of diabetes complications). Although their findings appear fairly consistent, the limitations of the individual studies are considerable, e.g., selected study populations [72, 147, 150-151], inclusion of non-incident SAB cases [147, 77], and limited numbers of patients with diabetes (n<60) [77, 147-150].

Study III

Four cohort studies were among the first to touch upon the influence of diabetes on SAB outcome [147, 152-154]. In a cohort study on SAB, Cluff et al. [152] observed an in-hospital mortality of 17% among patients with no comorbidity compared with as high as 69% among patients with diabetes. In contrast, Cooper et al. [153] observed no difference in in-hospital mortality among patients with diabetes and without diabetes in a cohort study on SAB, and this finding was corroborated by Bryan et al. [147] who demonstrated comparable in-hospital mortality among patients with and without diabetes in a later SAB cohort study. Yet, increased in-hospital mortality was found among patients with diabetes in a later study by Maradona et al. [154]. More recent studies continue to be characterized by inconsistent results. An American SAB cohort study by Mylotte et al. [42] found a 2.5-fold increased risk of 30-day mortality, which was supported by a cohort study from New Zealand [71]. Moreover, in an American RCT subgroup analysis on patients with SAB and concurrent endocarditis, Kanafani et al. [155] reported an all-cause mortality at 6 weeks of 22.1% in patients with diabetes vs. 11.4% in patients without. On the other hand, in-hospital mortality did not differ in a Canadian cohort study on invasive *S. aureus* infection [148] or in a Swiss cohort study on SAB [40]. These findings were corroborated by results from a cohort study by Kaasch et al. [3] who found no association between diabetes and increased 30-day mortality in patients with SAB.

Nevertheless, a number of important limitations should be taken into account in the interpretation of these prior results. The majority of the studies were conducted in tertiary care centers [40, 42, 152-155], which increases the risk of selection bias [158-159] and hampers the generalizability of the results [160-161]. In addition, limited numbers of patients with SAB [40, 42, 152-155] and diabetes [40, 42, 71, 147-148, 152-155], respectively, and restriction of the follow-up to the in-hospital period [42, 147-148, 154] may have influenced the findings.

Study IV

A few previous studies have included CHF among a variety of variables in their prognostic models [39-40, 156-157]. In a Swiss single-center SAB cohort study, Kaech et al. [40] reported a 2.5-fold increased risk of death within 90 days associated with CHF. In a later Columbian cohort study specifically investigating cancer patients with SAB, Cuervo et al. [156] observed an adjusted HR as high as 10.6 (95% CI, 1.8-63.7) for 90-day SAB-related death among patients with CHF compared to patients without. Lin et al. [157] conducted a cohort study in Taiwan on patients with persistent MRSA bacteremia suggesting that CHF was associated with increased 30-day mortality and, finally, a Norwegian cohort study assessing SAB outcome [39] demonstrated that patients with CHF were more than two times likely to die during 30 days of follow-up, compared with patients without CHF. However, CHF was only included among a variety of variables in these previous studies and none of them assessed the prognostic influence of SAB as the primary objective. Moreover, the prior results may in part be explained by small [39-40, 156-157] and selected study populations [39-40, 156-157] including few patients with CHF (n<70), and insufficient adjustment for concomitant comorbid conditions [40] may also have influenced the results

Limitations of the existing literature

In summary, little is known about whether differences in the definition of HCA infection influences the prevalence of HCA infection, patient characteristics, and outcome. The few previous studies on this subject had other primary objectives and none assessed specifically the impact of different definitions of HCA infection in patients with SAB. Although a number of previous studies have included diabetes among a variety of variables in their statistical models, data elucidating the association between diabetes and SAB remain sparse. Moreover, the prior studies yielded inconsistent results and the majority were restricted by selected and small sample sizes (including few patients with diabetes), insufficient confounder control, and incomplete follow-up, which may further have limited their results. Analogous with diabetes, there is a scarcity of in-depth data elucidating the influence of CHF on SAB prognosis and previous results may be influenced by selection bias rendering comparison to other settings difficult. Thus, considerable gaps in the available

knowledge exist and evidence derived from population-based studies is needed.

Aims of the thesis

I. To investigate whether different definitions of healthcare-associated infection affect the proportion of patients classified as HCA-SAB, and whether the prevalence of patient characteristics and mortality reported in the HCA-SAB group vary by disparate definitions.
II. To investigate the risk of CA-SAB comparing patients with and without diabetes overall and according to characteristics of diabetes (e.g., diabetes type, duration of diabetes, and presence of diabetes complications).
III. To investigate the influence of diabetes on 30-day all-cause mortality in patients with CA-SAB overall, among patients with and without recent healthcare contacts, and according to characteristics of diabetes (in particular diabetes type, duration, and presence of diabetes complications).
IV. To investigate 90-day all-cause mortality in patients with CA-SAB comparing patients with and without CHF overall and according to presence of CHF-related conditions (e.g., cardiomyopathy and valvular heart disease), CHF severity, and duration of CHF.

METHODS

Setting

The four studies were conducted during January 1, 2000 and December 31, 2011 in the Northern and Central Regions of Denmark, within a population of approximately 1.8 million residents. During the study period, a reform of local government merged four counties into two health regions: Central Denmark Region and North Denmark Region, collectively referred to as Northern Denmark. The study setting is served by two university hospitals and a decreasing number of regional hospitals (22 regional hospitals in 2000 versus 7 regional hospitals in 2011). Tax-supported, unfettered healthcare is available for the entire Danish population and all patients hospitalized with acute conditions are treated free of charge in these public hospitals.

Data sources

We conducted all four studies using routinely recorded data from population-based medical registries and databases. All Danish residents are given a unique 10-digit identification number (the Civil Registration Number) upon birth or immigration, which facilitates unambiguous linkage of records between the data sources [162-163] (Figure 1).

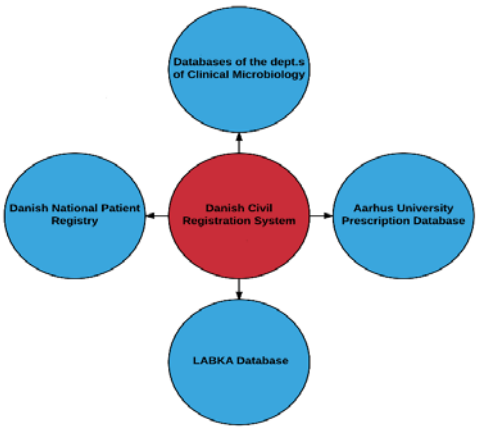


Figure 1. Data sources in studies I-IV.

Databases of the departments of clinical microbiology (studies I-IV)

Data on SAB were retrieved from the laboratory information systems (hereafter referred to as databases) of the departments of clinical microbiology which provided diagnostic bacteriology for the entire catchment area. During the study period, Central Denmark Region was served by three departments of clinical microbiology located in Aarhus (Aarhus University Hospital), Viborg (Regional Hospital of Viborg), and Herning (Regional Hospital West Jutland), while North Denmark Region was served by one department of clinical microbiology in Aalborg (Aalborg University Hospital). Data were obtained as part of everyday clinical practice and included the date and hour of the blood draw, number of bacterial isolates, and susceptibility to a range of antibiotics. For a small subset of blood cultures the date of receipt in the laboratory was substituted due to missing information. Blood cultures were requested by the attending physician and blood samples were obtained by biotechnicians. Throughout the study period, the BacT/Alert blood culture system (bioMérieux, Marcy l'Etoil, France) was utilized at all hospital sites. In North Denmark Region, a standard blood culture for adults included one set with three bottles (two aerobic and one anaerobic bottle), whereas the standard for adults included two sets with two bottles each (one aerobic and one anaerobic bottle) in Central Denmark Region.
S. aureus was identified by horse plasma tube coagulase test or an equivalent commercial latex agglutination test and susceptibility testing was conducted locally by disk diffusion. All blood culture isolates were subsequently submitted to the Staphylococcal Reference Laboratory at Statens Serum Institut (Copenhagen) for national surveillance [25], definitive identification, and serotyping. Screening for methicillin resistance differed between hospital sites during 2000-2002, however from 2003 onwards, the cefoxitin disk diffusion test was used both locally and at Statens Serum Institut [164-165]. Detection of the *mecA* gene cassette was conducted by in-house polymerase chain reaction (PCR) or the EVIGENE™ hybridization test.

The Danish Civil Registration System (studies I-IV)
The Danish Civil Registration System (DCRS) was established in 1968 [162-163]. This registry keeps track of demographic data (including gender, age, and marital status) and vital statistics including date of birth, changes in address, dates of immigrations and emigrations, and exact date of death. The DCRS is electronically updated daily, which ensures virtually complete patient follow-up.

The Danish National Patient Registry (studies I-IV)
The Danish National Patient Registry (DNPR) tracks information on all citizens admitted to Danish non-psychiatric hospitals since January 1, 1977 [166-167]. From 1995 onwards, the register was expanded to include data on emergency department visits and outpatient clinics as well. Each record includes the dates of admission and discharge, data on surgical procedures, one physician-assigned primary diagnosis and one or more optional secondary diagnoses, classified according to the International Classification of Diseases, 8th revision until the end of 1993 and the 10th revision thereafter (the 9th revision was never applied in Denmark). Since 1996, surgical procedures have been recorded with the Nordic Medico Statistical Committee Classification of Surgical Procedures codes [168]. Of note, reporting to the DNPR is mandatory.

The LABKA database (studies II-IV)
The clinical laboratory information system (LABKA) research database is maintained by the Department of Clinical Epidemiology, Aarhus University Hospital [169]. This database keeps laboratory test results using NPU codes (Nomenclature, Properties, Units) and local analysis codes for blood samples obtained during visits to general physicians and hospitals in Northern Denmark, since 1997 and 2000, respectively. In addition, the exact time of blood sample collection is recorded.

The Aarhus University Prescription Database (studies I-IV)
The Aarhus University Prescription Database (AUPD), also maintained by the Department of Clinical Epidemiology at Aarhus University Hospital, holds individual-level data on all reimbursable prescriptions dispensed at community pharmacies in Northern Denmark since 1998 [170]. Each record logs data on the prescription redemption date and the type and quantity of medication dispensed according to the Anatomical Therapeutic Chemical (ATC) classification system.

Study designs
Using the data sources described above, we conducted a cross-sectional study (study I), a case-control study (study II), and two cohort studies (studies III and IV). The study period, 1 January 2000 and 31 December 2011, was the same for all studies. Table 4 provides an overview of the design of the four studies. According to Danish legislation, individual informed consent is not required for studies based entirely on registry data. All studies were approved by the Danish Data Protection Agency (ref. no. 2012-41-0942).

Study populations
In all four studies the population of interest was patients with SAB. Detailed information on SAB was available in the databases of the departments of clinical microbiology and we defined eligible cases as patients aged ≥15 years with one or more positive blood cultures with *S. aureus* as the only isolate. Because SAB recurrence is associated with risk and prognosis [45-46], we restricted the study population to patients with incident SAB, defined as no previous SAB diagnosis within at least five years of the current SAB episode. SAB was defined as community-acquired if the first positive blood culture had been drawn within two days of admission and hospital-acquired (HA-SAB) if the first positive blood culture had been obtained >2 days after admission. In studies II-IV, patients with CA-SAB and healthcare contacts within 30 days of the current admission were further sub-classified as healthcare-associated SAB (HCA-SAB) if one or more of the following criteria were met: hospital admission, visit to hospital outpatient surgical clinics, visit to hospital hematology, oncology, or nephrology clinics. SAB patients admitted from nursing homes or long-term care facilities were classified as CA-SAB if they did not fulfill the HCA-SAB criteria.
In study I, a descriptive cross-sectional study, we included all patients with SAB. However, as mentioned in relation to the thesis outline, HA-SAB is associated with several factors including concurrent disease and invasive procedures, which might introduce a risk of confounding the association between diabetes, CHF and the risk and prognosis of SAB. Therefore, to reduce the risk of bias, we restricted our study population to patients with CA-SAB in studies II-IV. In study III, a case-control-study, we randomly selected 10 population controls from the DCRS on the date the first positive blood culture was drawn, matched to each CA-SAB case by age, gender, and residence. The risk set sampling technique was applied [171], requiring that the population controls had to be alive and at risk of a first CA-SAB at the time the corresponding case was diagnosed. Population controls were assigned an index date identical to that of the corresponding case.

Exposures
HCA infection definitions (study I)
In order to classify patients as CA-SAB, HA-SAB or HCA-SAB, we collected a complete history of all patients´ hospital contacts and preadmission medication use via the DNPR and AUPD. Patients with SAB were first classified as either CA-SAB or HA-SAB. Based on our review of the literature, we then suggested five different definitions of HCA infection (the criteria are provided in Table 3) and patients were classified as HCA-SAB or ´true´ CA-SAB according to each definition. To allow comparisons among groups, we ranked the definitions in a decreasing order concerning stringency of criteria.

Table 3. Five definitions of healthcare-associated (HCA) *S. aureus* bacteremia.

	Definition	Criteria *Blood culture performed within 2 days of admission and the following:*
Highest level of stringency	**1.**	▪ Any hospital inpatient admission within the previous 30 days
↓	**2.**	▪ Any hospital inpatient admission within the previous 30 days *or* ▪ Hospital outpatient clinic visit including surgery or visits to clinics of oncology, hematology or nephrology within the previous 30 days
↓	**3.**	▪ Any hospital inpatient admission within the previous 30 days *or* ▪ Any type of hospital outpatient clinic visit within the previous 30 days
↓	**4.**	▪ Any hospital inpatient admission within the past 90 days *or* ▪ Any type of hospital outpatient clinic visit within the previous 30 days
Lowest level of stringency	**5.**	▪ Any hospital inpatient admission within the past 90 days *or* ▪ Any type of hospital outpatient clinic visit within the previous 30 days *or* ▪ Antibiotic or immunosuppressive treatment 30 days prior to admission

Diabetes (studies II and III)

In studies II and III, patients with diabetes were identified using a previously validated method [131] incorporating two databases: the DNPR [166-167], and the AUPD [170]. First, the DNPR provided information on all patients with a discharge or outpatient diagnosis of diabetes registered at any time prior to the index date. Second, the AUPD allowed for identification of patients with at least one recorded prescription for any anti diabetes drug at any time predating the index date. To further optimize the identification of patients with diabetes, we employed the LABKA database [169] to identify patients with a glycosylated hemoglobin A1c (HbA1c) level confirming diabetes (≥ 6.5% (48 mmol/mol)) measured at any time before the index date. We classified patients as type 1 diabetes if they were aged up to 30 years at diagnosis and were treated with insulin as monotherapy and had no history of oral anti diabetes medication, or as type 2 diabetes (all other patients with diabetes).

We calculated the duration of diabetes as the time passed between the first record of diabetes (in any of the three registers) and the date the first positive blood culture was drawn. Data on all Hba1c measurements from the LABKA database within 12 months of the index date were obtained, which allowed us to assess the level of preadmission glycemic control (only the most recent Hba1c measurement before the index date was used in our analyses). In study 3, we further retrieved data on blood glucose levels on admission among patients with diabetes.

Using the DNPR, we collated data on the presence of macrovascular-, and microvascular complications. During the study period, no consistent or specific diagnostic codes were used for diabetic foot ulcers. Therefore, in study 2, we constructed two proxies of diabetic foot ulcers by identifying 1) patients with diabetes with conditions associated with diabetic foot ulcers (i.e., neuropathy and/or peripheral atherosclerosis or vascular disease) and 2) diabetes patients with previous lower-extremity ulcer diagnoses or ulcer-related procedures as described elsewhere [172]. Finally, we assessed the preadmission renal function of the study participants utilizing the most recent creatinine measurement from an outpatient hospital clinic or general practitioner one year to seven days prior to the index date and subsequently computed glomerular filtration rates (eGFR) using the four-variable version of the Modification of Diet in Renal Disease equation [173].

Chronic heart failure (study IV)

In study IV, we utilized the DNPR to identify patients diagnosed with CHF at any time before the current admission. CHF was defined as any previous hospital discharge diagnosis or outpatient diagnosis of congestive heart failure, pulmonary edema with mention of heart failure, left ventricular failure, unspecified heart failure, cardiomyopathy, or hypertensive heart disease with congestive heart failure (with or without hypertensive renal disease or renal failure). We further disaggregated patients with CHF into five subcategories of CHF-related conditions: 1) cardiomyopathy (with or without any of the following diagnoses), 2) heart valve disease (with or without any of the other diagnoses except cardiomyopathy), 3) previous myocardial infarction (with or without atrial fibrillation), 4) atrial fibrillation only, and 5) none of the above diagnoses.

The DNPR [166-167] does not include information on the severity of CHF. Therefore, as a surrogate measure of increasing CHF severity, we categorized patients according to daily dosage of filled prescriptions of loop-diuretics: non-users (no loop-diuretics), low dose (≤40 mg/day), medium dose (41-80 mg/day), high dose (81-159 mg/day), and very high dose (≥160 mg/day). We also calculated mean loop-diuretic dosages by dividing the number of dispensed tablets by a dispensing time interval of 180 days, as described previously [174-175]. All data on preadmission loop-diuretic use were collated from the AUPD. Finally, duration of CHF was computed as the time passed between the first diagnosis of CHF and the date the first positive blood culture was drawn.

Outcomes

HCA-SAB prevalence proportions (study I)

In study I, the prevalence proportion of patients classified as HCA-SAB according to each of five HCA-definitions represented the primary outcome. Secondary outcomes were the prevalence of patient characteristics (e.g., age, gender, comorbidity) and 30-day all-cause mortality by each HCA-SAB definition.

CA-SAB (Study II)

In study II, the main outcome of interest was incident CA-SAB. A detailed case definition of CA-SAB is given in the section describing the study populations.

All-cause mortality (studies I, III-IV)

Information on vital status was obtained from the DCRS. In study I, 30-day mortality was assessed as a secondary outcome, whereas 30-day mortality constituted the primary outcome in study III. In study IV, the main outcome was 90-day mortality. Some previous studies have observed considerable additional mortality after 90 days and suggested that long-term survival should be taken into account in prognostic studies involving patients with SAB [176-178]. Nevertheless, due to the acute and fulminant course of SAB, we consider it likely that the majority of deaths within up to 90 days after SAB are causally related to the infection and that the majority of additional deaths beyond 90 days are determined predominantly by the presence of coexisting morbidity. This is corroborated by results from a German cohort study on SAB (n=200) specifically ascertaining this problem. The investigators found that mortality after SAB plateaued after 90 days among patients with little comorbidity, whereas an additional 13% of patients with severe comorbidity died after 90 days [179].

Distinguishing between death directly attributable to infection (i.e., CA-SAB) and death related to presence of preexisting morbidity is difficult and may potentially introduce bias, especially when historical data are used [178]. Therefore, in studies III-IV, we decided to assess all-cause mortality only, which we consider a robust and clinically meaningful outcome.

Covariates

In all studies, we obtained information on a wide range of covariates. Demographic data were used to characterize the study populations, while other variables were included for confounder adjustment or to examine different effects across subgroups of patients.

Demographic data (studies I-IV)

Using the DCRS, we collected data on age, gender, and marital status on the date the first positive blood was drawn (or on the corresponding index date for controls). Unfortunately, we did not have detailed data on educational level or socioeconomic status, therefore marital status (married, divorced or widowed, never married) was utilized as a proxy and included as a factor in the stratification (study III) and in the adjustments (studies II-IV) [180].

Comorbidity (studies I-IV)

To assess the burden of comorbidity for each study participant and to evaluate the potential influence of preexisting disease on SAB risk and prognosis, we identified comorbid conditions included in the Charlson Comorbidity Index (CCI) [181] from all inpatient and outpatient discharge diagnoses recorded in the DNPR. We applied a look-back period of ten years prior to (but excluding) the admission date or corresponding index date for the population controls in study II. The CCI assigns between 1 to 6 points to 19 major disease categories and has previously been validated for use with hospital discharge registry data in medical databases for the prediction of mortality [182]. We computed aggregate Charlson Comorbidity Index (CCI) scores for each study participant, and defined three levels of comorbidity: low (CCI-score=0), intermediate (CCI-score=1-2), and high (CCI-score=>2). In studies II and III, diabetes represented the exposure variables, therefore we separated this condition from the CCI and the index was designated as a modified CCI (m-CCI). In line, a m-CCI excluding congestive heart failure was applied in study IV.

Using the same look-back period (10 years), we also obtained data on a number of conditions not included in the CCI, counting hypertension, osteoporosis, dialysis within 30 days of the current admission/index date, and conditions related to drug or alcohol abuse.

Laboratory test results

In addition to the laboratory test results related to diabetes, we obtained data on plasma C-reactive protein measurements (study III) and white blood cell counts (study IV) from the LABKA database on the date the first positive blood culture was drawn. These data were used to explore potential differences in inflammatory responses to infection among exposed and unexposed patients.

Preadmission medication use (studies I-IV)

To characterize the study populations, and because some types of medications might influence the risk and prognosis of CA-SAB [183-185], we retrieved data on prescriptions redeemed prior to the current admission or the index date from the AUPD. In studies II-IV, we obtained information on any systemic antibiotic therapy and antineoplastic and immunomodulating agents within 30 days of the current admission or index date. In studies II-IV, additional data were collated on any previous use of angiotensin-converting-enzyme inhibitors, beta blockers, low-dose acetylsalicylic acid, and statins.

Statistical analysis

Contingency tables with demographic data and clinical characteristics were constructed for each study, and all odds ratios (ORs) and mortality rate ratios (MRRs) were obtained with corresponding 95% confidence intervals (CIs). The potential confounding factors included in the multivariate adjustments were carefully selected a priori based on the existing knowledge on risk and prognostic factors for CA-SAB, which we consider preferable to data-driven selection processes (e.g., stepwise selection or change-in-estimate) [186].

To assess potential differences in effect in subgroups of patients (effect measure modification), we conducted stratified analyses when relevant. Moreover, because the risk and prognosis of CA-SAB may differ among patients with and without recent preadmission healthcare exposure [92-94], we reran all analyses in studies II-IV restricting the study cohort alternately to patients with CA-SAB and HCA-SAB, respectively. We conducted all statistical analyses using STATA 11.2 for Windows (STATA, College Station, TX, USA).

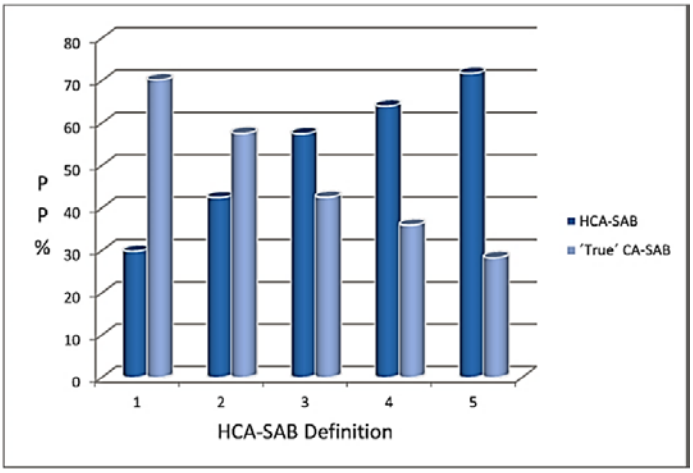
Prevalence (study I)
First, we computed prevalence proportions (PPs) of patients classified as HCA-SAB by each HCA definition and presented the results graphically for comparison. Next, PPs for patient characteristics and outcomes according to each of the five HCA definitions were estimated. Finally, we compared the five HCA groups with each other and to the group including all CA-SAB patients (i.e. ´true´ CA-SAB and HCA-SAB). Thirty-day all-cause mortality was estimated using the Kaplan-Meier method.

Risk (study II)
Due to the matched design of study 2, we used conditional logistic regression to calculate crude and adjusted ORs of CA-SAB for persons with diabetes compared to persons without diabetes. When risk set sampling is applied, the odds ratios represent unbiased estimates of corresponding rate ratios in a similar cohort study [158]. We further categorized diabetes exposure by diabetes type, duration of diabetes, the quality of the glycemic control, diabetes complications including diabetes foot ulcers, and preadmission renal function. All analyses were adjusted for marital status, m-CCI score, alcohol-related conditions, any statin use before the index date, and antibiotic treatment within 30 days of the index date. Using conventional logistic regression with additional adjustment for the matching factors, stratification was performed according to gender, age group, and m-CCI level.

Mortality (studies III and IV)
Time-to-event data were applied to investigate the influence of diabetes (study III) and chronic heart failure (study IV) on CA-SAB outcome, respectively. Follow-up began on the date the first positive blood culture was obtained, and all patients were followed until death, migration, or end of follow-up, whichever came first. The Kaplan-Meier method (1 – survival function) was used to compute and graphically display 30-day mortality in study III and 90-day mortality in study IV.
In study III, we used Cox proportional hazards regression to compare 30-day mortality rates for CA-SAB patients with and without diabetes as a measure of MRRs. Furthermore, we conducted stratified analyses according to gender, age category, marital status, and m-CCI level, and in a subgroup analysis restricted to patients with diabetes, we elucidated 30-day mortality by diabetes duration, the quality of glycemic control, diabetes complications, level of glucose on admission, and baseline preadmission renal function. The analyses were adjusted for age, gender, m-CCI score, hypertension, alcohol-related conditions, marital status, and use of statins and antibiotics before admission. In the analyses assessing the influence of diabetes complications on mortality, the complication in question was excluded from the m-CCI prior to adjustment.
In study IV, a Cox proportional hazards regression model was applied to compute MRRs comparing 90-day mortality among CA-SAB patients with versus without CHF. Ninety-day mortality was further analyzed in subgroups of patients according to a number of CHF related conditions (e.g., concomitant valvular heart disease or atrial fibrillation), CHF severity (as measured by daily loop-diuretic dosage), and CHF duration. In studies III and IV, the assumption of proportional hazards in all Cox models was assessed graphically with log-minus-log plots and found appropriate.

RESULTS
Study I
Study I included 4,385 patients hospitalized with incident SAB. Patients were most frequently male (60%), median age was 69 years (interquartile range (IQR), 57-79), and 70% had one or more conditions registered in the CCI. As little as 0.6% had MRSA bacteremia. A total of 2,638 (60.2%) were CA-SAB and 1,747 (39.8%) HA-SAB. Figure 2 presents the proportional distribution of HCA-SAB according to each of the five definitions. The proportion of patients classified as HCA-SAB increased considerably from 29.8% of all CA-SAB episodes when the most stringent definition was applied (Def. 1) to 71.7% when using the least stringent definition (Def. 5). Correspondingly, the proportion of patients classified as ´true´ CA-SAB decreased from 70.2% with the most stringent definition (Def. 1) to 28.3% with the least stringent definition (Def 5.).

Figure 2. Prevalence proportions (PP) of patients classified as healthcare-associated (HCA) *S. aureus* bacteremia (SAB) and ´true´ community-acquired (CA) SAB by definition 1-5.



As shown in Table 4, the distribution of age, gender, and CCI score in patients with HCA-SAB varied little across the different definitions.

Table 4. Prevalence proportions of patient characteristics and 30-day mortality by definition 1-5 of healthcare-associated (HCA) *S.aureus* bacteremia (SAB).

	Definition 1	Definition 2	Definition 3	Definition 4	Definition 5
		Decreasing stringency of HCA-SAB definitions →			
n (% of all CA-SAB)	787 (29.8)	1115 (42.3)	1517 (57.5)	1688 (64.0)	1892 (71.7)
Age >75 years	238 (30.2)	307 (27.5)	464 (30.6)	561 (33.2)	649 (34.3)
Male gender	454 (57.7)	663 (59.5)	914 (60.3)	1019 (60.4)	1147 (60.6)
MRSA-SAB	5 (0.6)	6 (0.5)	11 (0.7)	12 (0.7)	12 (0.6)
CCI score					
Low (0)	119 (15.1)	136 (12.2)	229 (15.1)	262 (15.5)	355 (18.8)
Intermediate (1-2)	286 (36.3)	371 (33.3)	540 (35.6)	612 (36.3)	690 (36.5)
High (≥3)	382 (48.5)	608 (54.5)	748 (49.3)	814 (48.2)	847 (44.8)
30-day mortality	195 (24.8)	252 (22.6)	344 (22.7)	406 (24.1)	468 (24.7)

CA-SAB: community-acquired SAB. MRSA-SAB: methicillin-resistant SAB. CCI: Charlson Comorbidity Index.

Contrasting patients classified initially as CA-SAB (i.e. ´true´ CA-SAB and HCA-SAB) with patients in the Def.1 group, patients with CA-SAB patients were more frequently older than 75 years (35.9% vs 30.2%), more likely to be male (61.3% vs. 57.7%), and more frequently characterized by a low CCI score (27.5% vs. 15.1%).

Study II

For study II, we included 2,638 patients with incident CA-SAB and 26,379 population controls. The median age of the study participants was 69 years (IQR, 56-79) and the majority was male (61%). Forty-two percent of all CA-SAB patients had recently been in contact with the healthcare system (HCA-SAB), and a considerably higher proportion of cases than controls (69.3% vs. 27.8%) had one or more hospital-diagnosed comorbidities.

As outlined in Table 5, diabetes was strongly associated with increased risk of CA-SAB. We observed no notable differences in risk estimates for cases with and without recent healthcare contacts, respectively.

Table 5. Unadjusted and adjusted odds ratios (ORs) for community-acquired *S. aureus* bacteremia according to presence of diabetes.

	Cases	Controls	Unadjusted OR (95% CI)	Adjusted[1] OR (95% CI)
Diabetes				
Absent	1,925 (73.0)	23,884 (90.5)	1.0 (ref.)	1.0 (ref.)
Present	713 (27.0)	2,495 (9.5)	3.7 (3.4-4.1)	2.8 (2.5-3.1)

[1]Adjusted for: conditions included in the modified Charlson Comorbidity Index, marital status, alcohol-related conditions, any statin use predating the index date, and antibiotic therapy within 30 days of the index date.

In analyses stratified according to characteristics of patients with diabetes, the increased risk of CA-SAB remained robust across all strata. Nevertheless, compared to patients without diabetes the risk of CA-SAB was most pronounced among patients with type 1 diabetes (aOR=7.2 (95% CI, 3.9-13.0)), patients with ≥10 years of diabetes history (aOR=3.8 (95% CI, 3.2-4.6)), patients with a Hba1c ≥9% (aOR=5.7 (95% CI, 4.2-7.7)), and patients with diabetes complications, in particular microvascular disease (aOR=5.5 (95% CI, 4.2-7.2)).

The risk of CA-SAB appeared slightly higher among female patients compared to males (adjusted ORs 3.2 (95% CI, 2.6-3.8) vs. 2.5 (95% CI, 2.2-2.9). Furthermore, the relative impact of diabetes was most pronounced in younger patients and in patients without coexisting morbidities.

Study III

In study III, we included 2,638 patients with CA-SAB, including 713 (27.0%) with diabetes. The median age of patients with and without diabetes was comparable (71 vs. 68 years), and there were slightly more men among patients with diabetes (63.4% vs. 60.5%). Among patients with diabetes, 44% were classified as HCA-SAB compared to 42% among patients without diabetes. Patients with diabetes had considerably more comorbidity registered in the m-CCI, including CHF (23.0% vs. 9.6%), cerebrovascular disease (16.3% vs. 10.3%), and peripheral vascular disease (22.9% vs. 8.6%), as compared to patients without diabetes.

The overall 30-day cumulative mortality in patients with diabetes was 25.8% and 24.3% in patients without DM, yielding an aMRR of 1.01 (95% CI, 0.94-1.20). The corresponding estimates according to type of SAB are given in Table 6.

Table 6. Unadjusted and adjusted 30-day mortality in incident *S. aureus* bacteremia (SAB) patients with versus without diabetes.

	n	30-day mortality (95% CI)	Unadjusted MRR (95% CI)	Adjusted[2] MRR (95% CI)
All SAB				
No diabetes	1925	24.3 (22.5–26.3)	1.00 (ref.)	1.00 (ref.)
Diabetes	713	25.8 (22.8–29.2)	1.07 (0.90–1.27)	1.01 (0.84–1.20)
Type 1 diabetes	40	5.0 (1.3–18.6)	0.19 (0.47–0.75)	0.59 (0.14–2.39)
Type 2 diabetes	673	27.0 (23.9–30.6)	1.13 (0.95–1.34)	1.01 (0.85–1.21)
CA-SAB				
No diabetes	1125	24.9 (22.5–27.5)	1.00 (ref.)	1.00 (ref.)
Diabetes	398	30.4 (26.1–35.2)	1.26 (1.02–1.56)	1.13 (0.91–1.41)
HCA-SAB				
No diabetes	800	23.5 (20.7–26.6)	1.00 (ref.)	1.00 (ref.)
Diabetes	315	20.0 (15.9–24.9)	0.84 (0.63–1.11)	0.84 (0.62–1.14)

MRR: unadjusted mortality rate ratio. CA-SAB: community-acquired SAB. HCA-SAB: healthcare-associated SAB. Adjusted for: age, gender, marital status, conditions included in the modified Charlson Comorbidity Index, hypertension, alcohol-related conditions, any previous statin use prior to admission, and antibiotic therapy 30 days prior to admission.

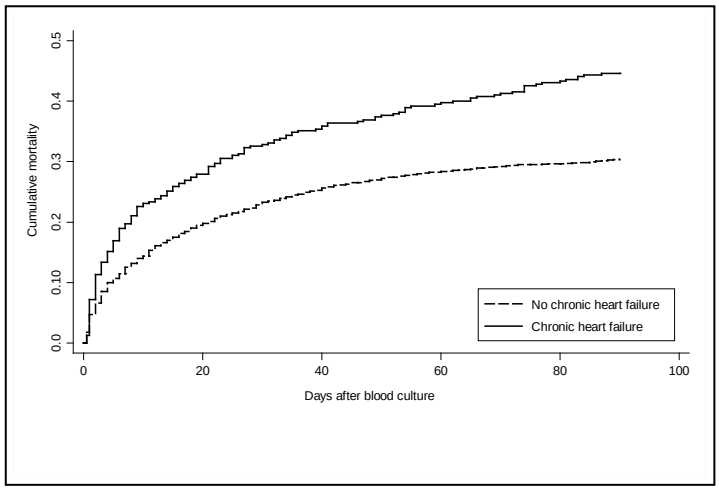
We observed no notable differences in 30-day mortality according to gender, age group, marital status, or m-CCI level in patients with and without diabetes. Duration of diabetes did not notably influence 30-day mortality. Thus, compared with 0-3 years of diabetes duration, the aMRRs were 0.72 (95% CI, 0.47-1.12) for 3-5 years of diabetes history and 0.87 (95% CI, 0.59-1.27) for > 10 years. In line, the estimates of 30-day mortality did not differ notably according to other characteristics of patients with diabetes, including the level of glycemic control, glucose level on admission, or the presence of micro- or macrovascular complications.

Study IV

Study IV included 2,638 patients with incident CA-SAB, of whom 390 (14.8%) had CHF. The majority of patients with CHF were males (64.9% vs 60.6%) and the median age was 77 (IQR, 70-82) and 67 (IQR, 54-78) years for patients with vs. without CHF, respectively. The proportion with HCA-SAB was comparable among patients with and without CHF (48% vs. 41%). Patients with CHF had a higher prevalence of hospital-diagnosed comorbidity than patients without CHF, including diabetes (31.0% vs. 12.8%), hypertension (49.5% vs. 20.4%), and renal disease (33.3% vs. 13.6%).

Figure 3 presents Kaplan-Meier curves for 90 days of follow-up. The cumulative mortality in patients with CHF compared with patients without CHF was 44.6% *cf.* 30.4% after 90 days, corresponding to an MRR of 1.60 (95% CI, 1.36-89) and an aMRR of 1.24 (95% CI, 1.04-1.48). Restricting the study cohort alternately to patients with and without recent healthcare contacts did not influence the estimates notably.

Figure 3. Cumulative 90-day mortality in patients with incident community-acquired *S. aureus* bacteremia with versus without chronic heart failure.



Compared with patients without CHF, the excess 90-day mortality was most prominent among CHF patients with concomitant valvular disease (aMRR=1.73 (95% CI, 1.26-2.38)) and CHF patients with a daily loop-diuretic dosage greater than 160 mg/day (aMRR=1.62 (95% CI, 1.21-2.18)).

In addition, CHF duration of less than three years was associated with increased mortality (aMRR=1.43 (95% CI, 1.14-1.78)), whereas longer duration of CHF was not found to be associated with a poor outcome, as compared to patients without CHF. The estimates of 90-day mortality did not differ notably across gender, age categories, or m-CCI level.

DISCUSSION

Comparison with the existing literature

The following section provides a discussion of our results in relation to the existing literature and possible mechanisms underlying our findings are briefly touched upon.

Study I

To our knowledge, this is the first study to specifically investigate whether different definitions of HCA infection influences the prevalence, patient characteristics and outcome in patients with SAB. An American cross-sectional study investigating MRSA infection prevalence, reported that the prevalence of HCA-MRSA infection was 51% with use of CDC nosocomial infection criteria compared with 95% according to use of healthcare risk factor exposure criteria (e.g., recent hospitalization or residence in a long-term care facility) [143]. However, the study was restricted to a limited number of patients (n=100) from a single tertiary-care center, which may have biased the findings. Two American cross-sectional studies of different size (n=352 and n=2,151, respectively) examined the prevalence of HCA- vs. CA-MRSA infections dependent on epidemiological classification criteria and antimicrobial co-resistance [144-145]. Using these different classification schemes, McCarthy et al. [144] reported comparable prevalences of HCA-MRSA infections (54% vs. 44%), whereas the prevalences of HCA-MRSA differed dependent on the definition (37% vs. 54%) according to Sievert et al. [145]. Still, in the study by McCarthy et al. [144], the definition of HCA-MRSA was not explicitly described, and the study by Sievert et al. [145] was limited by missing data. In a Canadian cross-sectional study of 100 patients with MRSA infection, Leung et al. [146] found no notable difference in HCA-MRSA prevalence with use of either a 4-week or 12-month look-back window (reclassification error rate=2%). A Danish cohort study of 56,606 patients with bacteremia examined whether use of different time windows to distinguish between CA-, HCA-, and HA bacteremia influenced the results of prognostic models [66]. In accordance with our results, no difference in 30-day mortality was observed for HCA patients in relation to a 30- or 90-day time window. However, the applied definitions of HCA differed only with regard to time windows and in-depth comparisons of patient characteristics according to different HCA definitions were beyond the scope of the study. In contrast to our study, infections were classified as either HCA or CA in all but one of the previous studies [66], i.e., HA infection was not considered a distinct entity and these patients were included by the HCA infection definition. In addition, the majority of the previous studies included other types of *S. aureus* infection than SAB. Thus, a direct comparison with our results may not be straightforward.

Based on our observations, we may speculate that the substantial differences in HCA-prevalence observed in previous studies of SAB is more dependent on differences in definitions of HCA infection and less so on actual discrepancies in local settings and the populations being studied. Further, comparing patients with CA-SAB and HCA-SAB, we observed no differences in 30-day mortality. This is in contrast to results from some previous studies of bacteremia (including SAB) reporting an increased risk of death associated with HCA infection [92-94], which may be driven partly by increased colonization and infection with MRSA [187-188]. Our study was conducted in a setting with very low MRSA prevalence among SAB isolates, and this might partly explain our observations.

Study II

To the best of our knowledge, we present the first report assessing diabetes as a risk factor for SAB as the primary aim of the study. An American cohort study [147] including 397 patients with SAB reported that the number of SAB episodes was 3.0 per 1000 patient-years in patients with diabetes compared with 1.2 per 1000 patient-years in patients without. Nevertheless, only 46 patients with diabetes were enrolled in the study and the inclusion of non-incident cases may have inflated the results. In two previous cohort studies from Canada and Sweden [77, 148], respectively, the investigators elucidated whether diabetes was associated with increased risk of invasive *S. aureus* infection, defined as the identification of *S. aureus* from blood, cerebrospinal fluid, pleural or synovial fluid, or aseptically obtained surgical-tissue samples or deep-tissue aspirates. The Canadian study reported an unadjusted RR of 7.7 (95% CI, 5.0-9.7) for invasive *S. aureus* infection associated with diabetes [148], which was in line with the Swedish results (unadjusted OR=8.2 (95% CI, 6-12)) [77]. Yet, in the Canadian study the number of patients with diabetes was determined solely on survey estimates and the use of a composite end point (invasive *S. aureus* infection) may render interpretation and direct comparison with our results difficult. An Italian combined case-control and cohort study including 165 patients with SAB reported an increased risk of diabetes associated with CA-SAB (aOR=6.21 (95% CI, 1.62-23.77)) [72] whereas, interestingly, no increased risk was observed for HA-SAB or HCA-SAB. Still, the investigators used controls sampled from hospital wards, which may not be an optimal comparison group when assessing specifically the risk of CA-SAB [189]. Furthermore, an American cohort study of 5,630 emergency department patients suspected of infection identified diabetes as a risk factor for MRSA bacteremia (aOR=2.02 (95% CI, 1.13-3.61)) [150], and Hernandez et al. [151] observed an association between diabetes and SAB of unknown origin (aOR=1.72 (95% CI, 1.01-2.91)) in a Spanish cohort study including 78 patients with SAB. However, the number of patients with diabetes was not provided in the American study and only 24 patients with diabetes were included in the Spanish study, therefore the validity of these findings is difficult to assess. Finally, it should be noted that unlike our work, no previous study has provided detailed estimates of SAB risk stratified according to characteristics of patients with diabetes or according to age, gender, and comorbidity level.

Several mechanisms may underlie our findings. When we adjusted for comorbid conditions the association between diabetes and CA-SAB attenuated, which may indicate that the observed risk associated with diabetes is at least partly driven by general frailty secondary to the presence of multiple comorbidities. Also, specific diabetes complications may increase the risk of CA-SAB, which is supported by our findings of particularly high CA-SAB risk among diabetes patients with foot ulcers and patients with poor kidney function. Moreover, as described in the background section of the thesis, diabetes influences immune responses through different pathways [125-133], which can lead to generally decreased immunity and subsequent increased risk of systemic infection, yet the design of our study did not allow for investigation of these potential mechanisms. Summarizing the results from our study and previous studies, there is substantial evidence of diabetes being associated with increased risk of CA-SAB.

Study III

To the best of our knowledge, no previous study has investigated the prognostic influence of diabetes in patients with SAB as the primary aim. However, a number of previous studies on SAB have assessed diabetes among a variety of potential prognostic factors with highly conflicting results. In an American cohort study of 185 patients with SAB, Cluff et al. [152] observed an in-hospital mortality of 17% among patients with no comorbidity compared with 69% among patients with diabetes, which was supported by results from a Spanish cohort (n=274) [154] reporting an increased risk of SAB-related in-hospital death associated with diabetes (p=0.054). Yet, only a limited number of patients with diabetes were included in both studies (n=26 and n=44, respectively) and post-discharge follow-up was missing. In a more recent American cohort study on 293 patients with SAB, Mylotte et al. [42] observed an aOR for 30-day mortality of 2.4 (95% CI, 1.2-4.7) associated with diabetes, which was supported by results from a SAB cohort study from New Zealand (n=424) reporting a corresponding a 30-day mortality RR of 1.5 (95% CI, 1.0-2.4) [71]. However, both studies were conducted at tertiary care centers which increases the risk of selection bias [159]. Moreover, the American study was not restricted to incident cases which may falsely inflate the outcome measures [160]. Kanafani et al. [155] reported an all-cause mortality at 6 weeks of 22.1% in patients with diabetes vs. 11.4% in patients without in an American RCT subgroup analysis on 235 patients with SAB. However, the study population was restricted to patients with SAB and concomitant infective endocarditis which may hinder a direct comparison with our results.

In contrast, other previous studies have observed no association between diabetes and increased mortality in patients with SAB. In an American cohort of 397 patients with SAB, Cooper et al. [153] observed almost similar in-hospital mortality among patients with diabetes (n=27) and without diabetes (n=34), and an American cohort study including 397 patients with SAB reported an in-hospital mortality of 15.8% among patients with diabetes vs. 24.8% in patients without [147]. Still, as both of these studies were limited by restriction to in-hospital mortality and insufficient control for concurrent comorbid conditions these results should be interpreted cautiously. In concordance, no difference in in-hospital mortality was reported in a Canadian cohort study on invasive *S. aureus* infection (n=264) [148] and in a Swiss cohort study of 308 patients with SAB [40]. Yet, the exact estimates on mortality associated with diabetes were not given in these papers, and again, follow-up after discharge was not available. Finally, these findings were corroborated by results from one of the hitherto largest cohort studies on SAB (n=3,395) conducted in a multi-national setting where Kaasch et al. [3] reported an adjusted HR for 30-day all-cause mortality of 1.12 (95% CI, 0.95-1.33) associated with diabetes. In contrast to our study, none of the previous studies ascertained the impact of diabetes duration, the level of glycemic control, or presence of diabetes complications.

The mechanisms underlying our null results are not entirely clear. Comparing patients with and without diabetes, we observed no differences in the inflammatory response as measured by the plasma C-reactive protein level on admission and the distribution of important prognostic markers (including age, gender, and m-CCI level) were roughly the same. Furthermore, the majority of patients in our study were > 65 years old and suffered from multiple chronic diseases, which may suggest that the high mortality associated with CA-SAB is conveyed primarily by the accumulated burden of comorbidity, age, and gender and less so by individual comorbidities such as diabetes. In consideration of the inconsistency and limitations of previous studies, our work provides clarification and firm evidence that diabetes is not associated with increased mortality in patients with CA-SAB.

Study IV

Our study is, to the best of our knowledge, the first to specifically investigate CHF as a prognostic factor in patients with SAB, and our results are corroborated by the existing quite limited literature. Kaech et al. [40] reported an unadjusted OR for 90-day mortality of 2.4 (95% CI, 1.0-5.6) associated with CHF in in a study of 308 patients with SAB. Yet, the results may be inflated due to insufficient adjustment for comorbid conditions, and unfortunately, follow-up was restricted to in-hospital mortality. A Columbian cohort study by Cuervo et al. [156] observed an adjusted HR of 10.6 (95% CI, 1.8-63.7) for 90-day SAB-related mortality associated with CHF, which is markedly higher than the corresponding estimate in our study (aMRR=1.24 (95% CI, 1.04-1.48)). However, the Columbian study included only 9 patients with CHF and, as indicated by the wide CI, cautious interpretation is indeed warranted. Moreover, the study was restricted to patients with cancer and misclassification of SAB-related death may also have influenced the results. In a Taiwanese cohort study including 227 patients with persistent MRSA bacteremia, an increased 30-day mortality associated with CHF (aOR=2.85 (95% CI, 1.44-5.65)) was observed [157]. Yet, the study employed a highly selected study population hampering the external generalizeability and, again, only a limited number of patients with CHF were available for analysis (n=63). Finally, a Norwegian cohort study (n=374 patients with SAB) [39] reported an aOR for 30-day mortality of 2.4 (95% CI, 1.21-4.80) associated with CHF. In contrast to our

study, the influence of CHF on SAB prognosis was not assessed according to CHF related conditions, severity of CHF, or CHF duration in this or any of the other previous studies. The explanation for the observed increased mortality in patients with CHF in our study is most likely multifactorial. Compared to patients without CHF, patients with CHF were characterized by advanced age, a higher m-CCI score, and a higher prevalence of CHF-related conditions (e.g., valvular heart disease) which are factors that influence outcome substantially in patients with SAB [1-2, 9-10, 119]. Furthermore, patients with concomitant valvular heart disease experienced the highest mortality in our study and it may be speculated that valvular heart disease can lead to pulmonary edema and circulatory collapse secondary to severe systemic infection. Nevertheless, valvular heart disease also constitutes one of the most important risk factors for infective endocarditis [22], which may have contributed to the dismal prognosis of this particular subset of patients with CHF. Yet, due to lack of access to clinical and echocardiographic data, we were unfortunately not able to investigate this potential mechanism further. In summary, our data combined with previous results provide strong evidence that CHF constitutes an important prognostic factor in patients with CA-SAB.

Methodological considerations

Systematic and random error (chance) represent important threats to the internal validity of all observational studies and must therefore be carefully considered before inferring causal associations [159]. Systematic error entails selection bias, information bias, and confounding bias whereas random error refers to the statistical precision of the estimates [159]. In the following, the potential influence of bias and random error will be evaluated for each study.

Selection bias

Selection bias is defined as a systematic error arising from the procedures to select study participants and/or from factors that influence study participation [159]. The bias comes about when the association between exposure and outcome differs for study participants and non-participants. The association between exposure and outcome among non-participants is rarely known, hence selection bias must usually be inferred as opposed to being observed [159].

In a cross-sectional design (study I), selection bias may occur if the study population is not representative of the background population. However, because we used routinely collected data from population-based databases within the Danish unfettered and tax-supported healthcare system, we were able to capture all patients with incident SAB in Northern Denmark, thereby considerably reducing the risk of selection bias [190]. Thirty-day mortality was assessed using the daily updated and virtually complete DCRS, thus we consider loss to follow-up highly improbable.

The results of our case-control study (study II) could have been influenced by selection bias if the inclusion of cases and controls into the study was dependent on exposure status, i.e., diabetes. We cannot entirely rule out that contact to the healthcare system is more frequent among patients with diabetes and physicians may be more attentive to early signs of infection in patients with versus without diabetes. Consequently, a higher proportion of CA-SAB cases could have been hospitalized among patients with diabetes and time to blood culture draw and initiation of appropriate antibiotic treatment may thus have been shorter in patients with diabetes. Such surveillance bias would inflate the risk CA-SAB associated with diabetes. Nevertheless, due to the acute and fulminant clinical presentation of CA-SAB [1, 47-48], we consider it less likely that presence or absence of diabetes should have substantially influenced the triage and clinical care of patients. In addition, previous studies from our setting on pneumococcal bacteremia and pneumonia, respectively, demonstrated no differences in microbiological results, levels of inflammatory markers on admission, antibiotic treatment, and proportion of patients with at least one blood culture taken when comparing patients with and without diabetes [131, 191].

In studies III and IV, selection bias would be of concern if the association between the exposure (diabetes and CHF, respectively) and the outcome (mortality) differed between study participants and non-participants, or if loss to follow-up occurred. As in study I, we ascertained vital status via the DCRS, which is virtually complete [162-163], therefore we do not consider loss to follow-up an issue.

The study populations in studies III and IV included all residents in Northern Denmark who were hospitalized with a first time episode of CA-SAB. Nevertheless, detection of CA-SAB may be influenced by admission patterns and timing of blood culture draw. Thus, we cannot preclude that a small proportion of patients with CA-SAB were not captured if some patients were hospitalized at a hospital outside of the study setting, if they had been treated with antibiotics prior to admission, or if they died before a blood culture had been obtained. If either of these factors were particularly related to patients with diabetes or patients with CHF selection bias may have arisen and mortality would subsequently be underestimated in these patient groups. Moreover, we cannot preclude that physicians may have a lower threshold for admitting patients with diabetes and patients with CHF on suspicion on infection, which also would lead to an underestimation of mortality. Yet, in studies III and IV, the proportion of patients classified as HCA-SAB did not differ between exposed and non-exposed patients, and we saw no notable differences in levels of inflammatory markers, or the proportion of patients who had received preadmission antibiotic treatment. Although this argues against, we cannot entirely dismiss the presence of some selection bias in studies III and IV, which may have led us to underestimate mortality among CA-SAB patients with diabetes and CHF.

Information bias

Information bias refers to misclassification of exposure, outcome, or data on potential confounders. Non-differential misclassification arises when the probability of misclassification is the same across compared groups, and differential misclassification is introduced when the probability of being

misclassified differs between the comparison groups. Non-differential misclassification of dichotomous variables will usually bias the estimate towards unity, whereas the effect of non-differential misclassification is difficult to predict [159].
We cannot entirely rule out misclassification of patients with HCA-SAB (the exposure) in study I. Patients with previous inpatient admissions and hospital outpatient clinic contact were identified in the DNPR which includes highly valid data on admission data [166-167]. As we did not have direct access to data on chemotherapy and dialysis, contacts to outpatient hospital clinics of oncology, hematology and nephrology were utilized as proxies and this may have introduced misclassification of some patients in previous or current treatment courses. Nevertheless, such misclassification would most likely be non-differential and thereby lead to more conservative estimates.
Further, we did not have data on nursing home residence or specialized home care, which is frequently included in definitions of HCA infection. Still, the majority of elderly people in Denmark does not live in specially adapted homes but in common housing where personal help and medical services are provided in the home by the local municipality [192]. Therefore, we consider it unlikely that the addition of this factor would have considerably influenced our results. Nevertheless, we acknowledge the relevance of these exposures and consider them important for any definition of HCA infection.

In study II, misclassification of the exposure or confounder data could possibly have influenced the results. To identify patients with diabetes, we applied a previously validated method [131] incorporating data that were retrieved prospectively and independently of the study purpose. Moreover, information on diabetes and characteristics of patients with diabetes (e.g., diabetes duration and diabetes complications) was retrieved in the same way for cases and controls, which virtually eliminates the risk of non-response bias and recall bias. Still, we may have missed some patients with diabetes, especially if they were treated with diet and lifestyle changes alone. Nevertheless, we expect such misclassification to be evenly distributed among cases and controls (i.e., non-differential), thereby biasing the estimates towards the null.

During the study period, diabetic foot ulcers were not coded consistently with unique diagnostic codes. Thus, we constructed two separate proxy variables using data on 1) conditions related to foot ulcers and 2) previous lower-extremity ulcer diagnoses, which may have led to some misclassification. Still, both variables suggested a high risk of CA-SAB associated with diabetic foot ulcers, and we consider it unlikely that misclassification alone could explain such high risk estimates. Furthermore, clinical data would most likely be preferable to registry data for determining the duration of diabetes and for distinguishing between type 1 and type 2 diabetes. Yet, we find it most likely that any misclassification of these factors would be non-differential, which would lead to more conservative estimates.

Data on comorbidity were obtained prior to the index date, thus information on this potential confounder was not influenced by case status. However, we obtained information on comorbidity including alcohol-related conditions using discharge diagnoses recorded in the DNPR, and misclassification of these factors due to incorrect data entry or lack of data entry of available information could potentially have biased our results. Although the PPV of the discharge diagnoses used in our study have been demonstrated to be high in the DNPR [182], the existence of some misclassification of the diagnoses in this database cannot be entirely precluded. Yet, any misclassification of comorbidity would most likely be non-differential and thus diminish the contribution of this factor to the association between diabetes and risk of CA-SAB. On the other hand, if the diagnostic coding of patients with diabetes was more complete due to surveillance bias or higher rates of hospitalizations, the misclassification of comorbidity may have been non-differential thereby overestimating or, potentially, underestimating the risk of CA-SAB associated with diabetes.
Prescription data for confounder adjustment were collated from the AUPD. Although it may vary by drug type, an estimated completeness of this database is 96% based on cross-tabulation of insulin prescriptions with hospitalization records of diabetes mellitus [170]. Unfortunately, we lacked information on drug adherence, yet patient copayment is required and misclassification due to nonadherence is probably negligible.

In cohort studies (studies III and IV**)**, information bias may arise from collection of erroneous information on exposure status, outcome status, or potential confounding variables [159]. The primary outcome was all-cause mortality in both studies. Information on vital status was collected from the DCRS which is updated on a daily basis and practically complete [162-163], therefore misclassification of mortality seems highly unlikely.

In study III, diabetes constituted the exposure of interest. As discussed in relation to study II, information on diabetes was obtained using a validated method [131] and collected prospectively and independently of our study hypothesis. Thus, we consider the introduction of differential misclassification of the exposure variable unlikely. In study IV, we identified patients with CHF using a range of hospital or hospital outpatient discharge diagnoses registered in the DNPR. This method for capturing patients with CHF has not been validated, thus some misclassification of CHF exposure cannot be entirely precluded. However, two recent Danish validation studies demonstrated positive predictive values for CHF in the DNPR of 81% [193] and 100 % [182], respectively. Further, any misclassification would most likely be non-differential and thus lead to underestimation of our results. CHF severity is optimally evaluated using data on the ejection fraction and by the American College of Cardiology Foundation/American Heart Association classification system or New York Heart Association Functional Class [119-120], and the use of loop-diuretic dosages may have introduced misclassification of CHF severity in our study. Hence, if some patients used loop-diuretics on other indications than CHF

(e.g., concomitant chronic kidney failure), this may have diluted any differences between severe and less severe CHF. However, such misclassification may most likely be non-differential and may not explain our overall results.

In studies III and IV, misclassification of comorbidity and preadmission medication could also have influenced our results. Data on comorbidity was retrieved from the DNPR, and as mentioned previously some misclassification of the diagnoses in this database cannot be entirely precluded. Still, such misclassification would most likely be non-differential and thereby attenuate the influence of comorbidity on the association between the exposure (diabetes and CHF, respectively) and the outcome (mortality). On the other hand, the coding of comorbidity might be more complete among patients with diabetes or CHF due to more frequent contact to the healthcare system or surveillance bias. Such differential misclassification could potentially lead to both overestimation and underestimation of the contribution of comorbidity to the association between diabetes or CHF and mortality. As described in relation to study II, the validity of the data in the AUPD has been demonstrated to be high [170], hence we do not consider it likely that misclassification of preadmission medication has influenced the results of studies III and IV notably.

Confounding

Confounding is defined as a bias occurring when a measure of association between exposure and outcome is confused with or distorted by the effect of third (confounding) factor [159]. By definition, a confounder is associated with the exposure and the outcome and does not constitute an intermediate link in the chain of causation between exposure and outcome [159]. In contrast to selection bias and information bias, the risk of confounding can be reduced in the design phase of a study (e.g., by restriction, and matching) and in the analysis phase (e.g., by stratification and multivariate adjustment) [160]. Although these methods were applied in studies I-IV, our results may still be affected by residual and unmeasured confounding. Residual confounding may stem from misclassification of the potential confounding factors or use of too crude categories of confounders, which may lead to loss of information. Unmeasured confounding may have been introduced by confounding from known factors, which we were not able to adjust for [159].

Study I described the influence of different definitions of HCA-SAB on the outcomes HCA-SAB prevalence, patient characteristics, and mortality. As the study was a strictly descriptive study, with no statistical comparisons or examination of exposure-outcome associations, we do not consider it likely that confounding should have influenced the results of study I.

In study II, we restricted the study population to patients with CA-SAB to reduce the risk of confounding associated with HA-SAB. Further, cases and controls were matched by age, gender, and residence to prevent confounding fromthese factors. Finally, at the analysis level, multivariate adjustment and stratification by potential confounders were conducted.

We used the Charlson Comorbidity Index (CCI) to adjust for comorbidity. The CCI is the most extensively studied and validated comorbidity index for predicting mortality, and this also pertains to patients with SAB [31, 89, 99, 182, 194-195]. Still, the index has not been validated for predicting the occurrence of subsequent diseases and may therefore not be considered optimal for adjustment of comorbidities in studies assessing risk. Yet, accumulated comorbidity constitutes one of the most important risk factors for SAB [1, 31, 47], and the CCI includes most of the single chronic diseases suggested to be associated with SAB (e.g., chronic renal disease and cancer). Residual confounding may have arisen from misclassification of the conditions included in the m-CCI due to erroneous coding or from differences in coding related to diabetes status. However, as described in relation to information bias, the discharge diagnoses included in the CCI have previously been shown to have high positive predictive values [182]. Furthermore, we consider it unlikely that coding differences due to surveillance bias alone could explain risk estimates of the magnitude observed in study II. Moreover, we chose to adjust for m-CCI level (low, intermediate, high) in lieu of individual disease categories, which might have introduced residual confounding due to improper categorization. However, rerunning the analyses while adjusting for individual disease categories left the estimates virtually unchanged.

We also adjusted for preadmission use of statins and antibiotics, however the use of other types of medications may also be associated with diabetes and risk of CA-SAB. Immunosuppressive therapy, for instance, could potentially confound the association between diabetes and CA-SAB risk [1], yet very few study participants had received this type of treatment prior to admission (0.3%). Thus, we ultimately chose to exclude this factor from the adjustment, which did not change the estimates. Furthermore, we consider medication use as a direct consequence of diabetes (e.g., insulin or metformin) to constitute a part of the exposure´s (diabetes) effect and, therefore, this factor was not considered a potential confounder.

Unfortunately, we did not have data on smoking, body mass index, and functional or nutritional status, which could potentially confound the association between diabetes and risk of CA-SAB [196]. Nevertheless, we were able to adjust for several lifestyle-related comorbidities in our analyses (e.g., cardiovascular disease and chronic pulmonary disease), thereby partly accounting for these potential confounders.

As in study II, we only included patients with CA-SAB in studies III and IV thereby reducing the risk of confounding associated with HA-SAB. In addition, we performed stratification and multivariate adjustment for potential confounders at the analytical level.

In studies III and IV, we utilized a modified CCI (m-CCI) and we cannot entirely preclude that this might influence the index´ ability to predict mortality. Moreover, diseases not included in the m-CCI may represent a risk of confounding in studies III and IV. Yet, as previously mentioned, the CCI includes the majority of chronic diseases associated with SAB

and we included alcohol-related conditions and hypertension in the adjustment. In some previous studies of SAB outcome [185, 197], the investigators adjusted for severity of SAB-related disease as measured by the Acute Physiology And Chronic Health Evaluation score, the Pitt bacteremia score, or other comparable scores [198-200, 201]. However, we consider severity of disease to constitute a part of the causal pathway leading from diabetes and CHF, respectively, to mortality in patients with SAB. Therefore, this factor does not meet the definition of a potential confounder and should not be adjusted for in studies assessing SAB prognosis.

As previously mentioned, the infective focus is associated with SAB outcome [2-3, 95], however due to the historical design of our studies, data on the infective focus were not available. If the infective foci were differently distributed among patients with our without diabetes (study III) or among patients with or without CHF (study IV), this could potentially have confounded our mortality rate estimates. Moreover, we did not have data on in-hospital clinical care including antibiotic therapy, ICU admission, and surgical procedures and differences related to diabetes or CHF, respectively, could potentially have influenced our assessment of mortality. We may also speculate that if the post discharge follow-up differed among patients with or without diabetes or patients with or without CHF, this too may have played a role for our results.

As for study II, data on potential confounders such as smoking, body mass index, and functional status were not available. Furthermore, socioeconomic status is associated with SAB outcome [2, 202], but unfortunately, data on educational level and personal income were lacking. However, we adjusted for marital status although this admittedly represents a somewhat crude proxy [180]. Still, as healthcare is unrestricted and free in Denmark, we do not consider it likely that differences in socioeconomic status could explain our observation in studies III and IV.

Precision

No amount of statistical treatment can correct for systematic error arising from the study research design, yet by increasing the sample size it is possible to improve the statistical precision of a given study [158, 160]. We employed 95% CIs to evaluate the precision of the associations in studies II-IV. Rather than using significance testing (with associated p-values), we preferred to consistently report effect sizes together with uncertainty metrics (i.e., 95% CIs). Unfortunately, CIs are often used simply to judge whether it contains the null value or not, thereby converting it to a significance test. However, we believe that confidence intervals should rather be interpreted as quantitative measures indicating the magnitude of effect and degree of precision, with less attention paid to the precise location of the boundaries of the confidence interval [161, 203]. Due to the considerable number of cases and outcomes in our studies, the main analyses in studies II-IV yielded statistically precise estimates, as indicated by narrow CIs. Furthermore, the estimates remained robust in most subgroup analyses in studies. We were, nevertheless, limited by sparse data on patients aged less than 40 years (studies I-IV), patients with type 1 diabetes (studies II-III), and characteristics of patients with diabetes (studies II-III).

External validity

External validity is the degree to which the results of a study are applicable in other settings [161]. Our studies were conducted in an area with low prevalence of MRSA (0.5%). Although this facilitated a clean focus on MSSA, it might have impeded the applicability of our results to other settings with significant MRSA prevalence. Nevertheless, assuming a low risk of systematic error and taking into account the high precision of our estimates, we consider it likely that our results are generalizable to other settings and countries with similar lifestyle and free, unrestricted access to healthcare and prescription drugs including anti diabetes therapy and medications for CHF.

CONCLUSIONS

Based on our results and the subsequent evaluation of the methodology applied in the four studies, the following main conclusions were drawn:

Study I

We demonstrated that the prevalence of patients classified as HCA-SAB varied considerably with use of different definitions of HCA infection. Of note, using the least stringent definition of HCA-SAB more than doubled the prevalence of patients classified as HCA-SAB compared with the most stringent definition. In addition, use of different definitions of HCA-SAB influenced the distribution of patient characteristics, whereas the estimates of 30-day all-cause mortality remained comparable.

Study II

We found that diabetes was a strong risk factor for CA-SAB. Compared with persons without diabetes, the influence of diabetes on CA-SAB risk was most apparent among patients with type 1 diabetes, patients with a long diabetes history, patients with inadequate glycemic control, and patients with diabetes complications in general and microvascular disease in particular. Moreover, the impact of diabetes on relative CA-SAB risk was particularly pronounced among patients aged less than 60 years and among patients with no other comorbidities.

Study III

The study provided firm evidence against an association between diabetes and 30-day all-cause mortality in patients with CA-SAB. The prognosis remained comparable among patients with and without recent preadmission healthcare contacts, respectively, and no notable differences in mortality were demonstrated according to age, gender, marital status, or comorbidity level. Furthermore, characteristics of patients with diabetes (e.g., diabetes duration, quality of glycemic control, and diabetes complications) did not influence the 30-day mortality.

Study IV

Compared with patients without CHF, patients with CHF experienced a 24% increase in 90-day all-cause mortality. The excess risk of death was particularly pronounced in patients with CHF with concomitant valvular heart disease, patients with a short history of CHF, and patients using high daily dosages of loop-diuretics. Ninety-day mortality did not differ notably across strata of gender, age groups, and comorbidity levels.

CLINICAL IMPLICATIONS AND PERSPECTIVES

This thesis highlights some of the challenges associated with the classification of SAB and extends our existing knowledge of CA-SAB with special attention to underlying diabetes and CHF. We found that the prevalence of patients classified as HCA-SAB varied substantially when different definitions of HCA infection were used. In addition to underlining the necessity for caution when designing, interpreting, and comparing studies on SAB, these results emphasize the need for an evidence-based consensus definition of HCA infection. Ideally, this should distinguish between different infectious disease syndromes and take local epidemiological and microbiological characteristics into account.

Our results further provide evidence that diabetes constitutes a considerable risk factor for CA-SAB, although this condition is not a prognostic factor. This underlines the importance of improved preventive care for patients with diabetes and particularly good infection surveillance among patients with a long history of diabetes and patients with diabetes complications. Moreover, our observations of a gradually increased risk of SAB with successive increases in HbA1c levels may help to further motivate patients and physicians to maintain an optimal Hba1c level at all times. Still, some questions remain unanswered. The exact biological mechanisms behind the increased risk of SAB continue to be unclear and should be further elucidated. In particular, as our results indicate that presence of diabetic foot ulcers is associated with very high risk of SAB, we would like to investigate this potential mechanism further using accurate clinical and microbiological data on this important diabetes complication. In addition, bacterial vaccines have proven effective in the prevention of invasive infection from *Haemophilus influenzae* type b (Hib) [204] and *Streptococcus pneumoniae* [205], yet an effective staphylococcal vaccine is still not available [206]. Nevertheless, recent vaccine studies have shown promising results [207-208] and vaccination for staphylococci might be considered as part of the preventive measures for high-risk patients with diabetes in the future.

The high mortality observed among SAB patients with CHF implies that this subset of patients may benefit from increased clinical attention. As described in relation to the background section, the association between sepsis and myocardial function is highly complex and further research is needed to investigate which specific pathophysiological mechanisms underlie the association between CHF and SAB outcome. Moreover, the potential role of heart valve disease and infective endocarditis merits further investigation, preferably in prospective clinical studies involving clinical microbiologists, cardiologists, and infectious diseases specialists.

In our studies, we observed an overall 30-day all-cause mortality of ~25% associated with CA-SAB. This is of considerable clinical and public health concern and there is a major incentive to prevent and optimize the clinical management of this clinical syndrome. In recent years, systematic infectious disease specialist consultation (IDC) has been investigated as a strategy to optimize the quality of care for patients with SAB [209-215]. According to a recent systematic review and meta-analysis of 18 studies (patients with SAB=5,337), IDC was associated with improved control of the infective focus and antibiotic therapy as well as reduced risk of 30-day, 90-day mortality, and SAB relapse [216]. Thus, ICD can be a promising step toward standardizing and enhancing the management of SAB and in turn facilitate improved patient outcomes. Nevertheless, further well-designed studies are warranted to validate the results and refine the specific elements of the intervention.

SUMMARY

Community-acquired *Staphylococcus aureus* bacteremia (CA-SAB) is a serious infection with detrimental clinical effects. Chronic diseases constitute some of the most important risk and prognostic factors for CA-SAB. The prevalence of diabetes and chronic heart failure (CHF) is rapidly increasing on a global scale, nevertheless, there are few data available specifically elucidating the influence of these chronic conditions on CA-SAB risk and outcome.

Therefore, to extend the current knowledge, we aimed to I) elucidate the impact of different definitions of healthcare-associated (HCA) infection on the prevalence of HCA-SAB, patient characteristics, and mortality, II) to investigate whether diabetes is a risk factor for CA-SAB, III) to ascertain the prognostic influence of diabetes on CA-SAB outcome, and IV) to investigate the influence of CHF on mortality in patients with CA-SAB.

The thesis is based on a cross-sectional study, a case-control study, and two cohort studies, all conducted in Northern Denmark, 2000-2011. Utilizing the unique civil registration number assigned to all Danish residents, we linked data from the local departments of clinical microbiology, the Danish Civil Registration System, the Danish National Patient Registry, the LABKA database, and the Aarhus University Prescription Database.

In study I, we included 4,385 patients with SAB. The proportion of patients classified as HCA-SAB ranged between 29.8% and 71.7% across five different definitions of HCA infection. Use of different definition of HCA infection also influenced the distribution of patient characteristics, whereas estimates of 30-day mortality remained unchanged (~ 24%). Study II included 2,638 patients with CA-SAB and 26,379 population controls matched by age, gender, and residence. We found diabetes to be strongly associated with an increased risk of CA-SAB (adjusted odds ratio=2.8 (95% CI, 2.5-3.1)). Compared with persons without diabetes, the increased CA-SAB risk was most apparent among patients with type 1 diabetes, patients with a long diabetes history, patients with poor glycemic control, and patients with diabetes complications. In study III, we included 2,638 patients with CA-SAB, of whom 713 (27.0%) had diabetes. After adjustment for potential confounders, the mortality rate ratio for patients with diabetes was 1.01 (95% CI, 0.84-1.20) after 30 days of follow-up.

No notable differences in 30-day mortality were observed among patients with and without recent healthcare contacts, and the finding remained robust according to gender, age, comorbidity level, and characteristics of patients with diabetes (e.g. diabetes type and duration of diabetes). In study IV, CHF was associated with a 24% increase in 90-day mortality in patients with CA-SAB. The excess risk of death associated with CHF was most pronounced among patients with concomitant valvular disease and patients using very high doses of loop diuretics, as compared to patient without CHF.

In conclusion, we observed considerable variation in the proportion of patients classified as HCA-SAB when different definitions of HCA infection were applied. Diabetes was associated with a substantially increased risk of CA-SAB, whereas CA-SAB outcome was virtually unaffected by diabetes. In contrast, patients with CHF experienced increased 90-day mortality compared with patients without CHF.

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