Diagnosis and prognosis of patients with community-acquired bacteremia

PhD Thesis

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Preface

This PhD thesis is based on studies carried out during my employment at the Department of Clinical Microbiology.

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Mette Søgaard, November 2009

This PhD thesis is based on the following studies:

I. Søgaard M, Nørgaard M, Schønheyder HC. First notification of positive blood cultures and the high accuracy of the gram stain report. J Clin Microbiol 2007; 45:1113-1117.

II. Søgaard M, Nørgaard M, Pedersen L, Sørensen HT, Schønheyder HC. Bacteremia and mortality in medical ward patients with blood cultures taken within two days of admission. A Danish cohort study. [manuscript in preparation]

III. Søgaard M, Schønheyder HC, Riis A, Sørensen HT, Nørgaard M. Short-term mortality in relation to age and comorbidity in older adults with community-acquired bacteremia: a population-based cohort study. J Am Geriatr Soc 2008; 56:1593-1600.

Corrections to study I:
Table 2: Gram-positive cocci, chains/diplococci; 807/818 (not 707/818) correct Gram stain evaluations/total. Yeasts; 90/90 (not 90/92) correct Gram stain evaluations/total.
Table 4: There were 23 (not 22) *Citrobacter* spp. of which 9 were peritrichous.

Contents

Abbreviations

BC	Blood culture
BSI	Bloodstream infection
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CoNS/CNS	Coagulase-negative staphylococci
EARSS	European Antimicrobial Resistance Surveillance System
ED	Emergency department
ESBL	Extended spectrum beta-lactamase
GPCC	Gram-positive cocci in clusters
HDR	Hospital discharge registry
ICD	International classification of diseases
ICU	Intensive care unit
IQR	Interquartile range
KPC	*Klebsiella pneumoniae* carbapenemase
MRR	Mortality rate ratio
NPV	Negative predictive value
NS	Not stated
OR	Odds ratio
PPV	Positive predictive value
RCT	Randomized controlled trial
Se	Sensitivity
STARD	The Standards for Reporting of Diagnostic Accuracy
SIRS	Systemic inflammatory response syndrome
Sp	Specificity
UTI	Urinary tract infection

1 Introduction

Bacteremia is associated with high morbidity and mortality. Despite the availability of effective antibiotics and improved treatment of circulatory failure and organ dysfunctions, the 30-day mortality from bacteremia still averages 20%. Mortality may be even higher in older patients with coexisting chronic diseases. Prompt detection and treatment is therefore an important goal for improving patient prognosis[1].

Improving prevention and treatment of community-acquired bacteremia requires a better understanding of the disease and its prognosis. Randomized controlled trials (RCTs) do not provide all necessary information, since they usually involve small sample groups in which vulnerable patient groups such as the elderly and those with coexisting chronic diseases are often underrepresented. In order to study the entire spectrum of bacteremia patients, we need large studies with valid sources of information, prospective data collection, and complete follow-up.

In the present thesis we used population-based registries to examine 1) the accuracy of the first notification of bacteremia based on Gram stain and wet-mount microscopy; 2) the prognostic impact of community-acquired bacteremia in medical patients with blood cultures taken within the first two days of hospital admission; and 3) the impact of age and comorbidity on mortality from community-acquired bacteremia.

1.1 Introduction to bacteremia

What is bacteremia?

Bacteremia is a clinical entity associated with the presence of viable bacteria in the bloodstream, as evidenced by blood cultures in which contamination has been effectively ruled out[2-4]. Candidemia is included in the collective term bacteremia, and in the daily clinical setting the diagnosis of bacteremia is based on bacterial or fungal growth in blood cultures that has etiological significance as determined by joint clinical and microbiological assessment[3].

An historical perspective of bacteremia

Bacteria in the blood was described for the first time in 1850 by the French physician Casimir-Joseph Davaine (1812-1882), who observed bacteria in blood from animals with anthrax and designated the bacteria *Bactéridie charbonneuse*. The term **bacteremia** (bactériémie) was coined in 1872 by Edmé Vulpian (1826-1887) to emphasize the pathogenic role of the now accepted phrase 'bacteria in the blood'. Other contemporary terms were **pyemia** and **septicemia** (for a review, see Bulloch[5]).

Blood cultures were originally considered a research tool. However, in the beginning of the twentieth century, blood cultures became valued by clinicians as a diagnostic tool. At this time, the use of blood cultures was closely tied to the study of endocarditis, and one of the earliest comprehensive studies of bacteremia was published by Emmanuel Libman[6]. Many of the early studies centered on a single bacterium or bacterial group, including pneumococci[7;8], *Staphylococcus aureus*[9;10], and Gram-negative rods[11;12]. However, the introduction of antimicrobial chemotherapy generated an interest in studies that were not restricted to a single group of pathogens[13]. In 1979, McGowan et al published bacteremia surveillance data from Boston City Hospital; these data were collected from a total of 12 years during the time period 1935 to 1957[14]. Similar studies conducted at other locations followed this report [15] and created greater interest in nosocomial bacteremia. Two of the most influential papers on bacteremia were published by Weinstein et al in 1983[2;16]. These twin papers reported data from a hospital-based cohort study and included a detailed set of definitions that formed a foundation for many later studies, including this thesis. To our knowledge, the first population-based studies of bacteremia were conducted in North Carolina in the seventies and early eighties[17;18]. The first prospective bacteremia registries were developed at St. Thomas's Hospital, London[19], and at Beilinson Hospital in Petah Tiqva, Israel[20]. The North Jutland County Bacteremia Research Database (now North Denmark Region Bacteremia Research Database) was established in 1992.

Since the early 1980s, the term bloodstream infection (BSI) has been used as an alternative to bacteremia, especially in relation to infection control activities. 'BSI' primarily denotes cases without a definite focus of infection; the term is sometimes preferred because it encompasses both bacteremia and fungemia. Nonetheless, bacteremia, as an historic term, include both bacteremia and fungemia, because bacteria were originally classified as a subdivision of fungi (Schizomycetes)[21].

The origin of bacteremia

It is important to determine whether the infection is acquired inside or outside the hospital setting: The place of acquisition is closely related to risk factors for bacteremia, the focus of the infection, microbial agent (Figure 1), antibiotic resistance, and prognosis[16]. The Centers for Disease Control and Prevention (CDC) surveillance definitions of bacteremia include only nosocomial infections[22], and many studies have used a hospital stay greater than two days as a cut-off criterion for nosocomial infection[23-28]. However, Leibovici et al[29] have shown that such a distinctive threshold for hospital-type pathogens probably does not exist, emphasizing that the distinction between community-acquired and nosocomial infections should be based on all available clinical information[22;29]. Infections likely to be present or incubating at hospital admission are considered community-acquired. An increasing number of patients, however, have frequent contact with hospitals, e.g. for hemodialysis or chemotherapy, and it may not

always be appropriate to categorize their infections as community-acquired. In our studies, we therefore considered patients with a hospital stay within 30 days prior to admission or who have regular hospital visits as belonging to a separate health care-related group[30;31]. This 30-day period is in agreement with Siegman-Igra et al[32]; however, 30 days may be too short as a cut-off period, since previous hospitalization has been shown to affect antimicrobial susceptibility to subsequent bacteremia for up to 360 days after hospital discharge[33]. Accordingly, others have proposed that 90 days up to one or even more years after hospital stay be considered when categorizing bacteremia origin, especially with regard to persistent colonization with methicillin-resistant *S. aureus*[31;34;35].

Causative microorganisms

The distribution of causative microorganisms depends on the focus[36;37] and origin of bacteremia (Figure 1). In the laboratory, most microorganisms are identified to the species level and characterized according to their pattern of antibiotic susceptibility. This helps guide antibiotic therapy and can direct the examination towards the focus of infection. If a microorganism is not readily identified, it may be recognized as belonging to a certain genus or to a provisional group such as coagulase-negative staphylococci (CoNS).

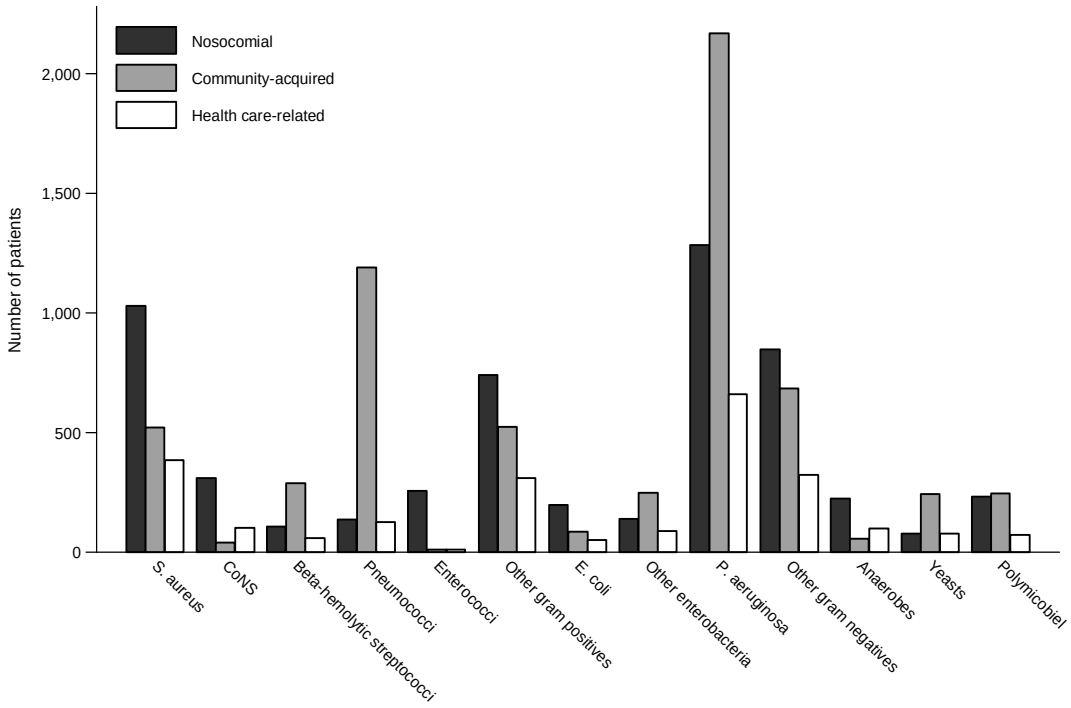


Figure 1. Distribution of causative microorganisms according to the origin of bacteremia in North Jutland County, Denmark, 1992-2006.

Bacteremias can be grouped together based on the similarity of the isolated microorganisms (e.g. Gram-negative rods). In this thesis, we refer to these groups as types of bacteremia. Studies conducted in the 1980s reported a shift from Gram-negative bacteremia towards Gram-positive bacteremia[38;39]. Possible reasons for this shift include empirical antibiotic regimens

designed primarily against Gram-negative pathogens that have selected out resistant Gram-positive pathogens, increased use of long-term intravascular catheters and surgically implanted foreign material, as well as the spread of antibiotic resistance among Gram-positive organisms[40]. However, the studies were not confined to incident bacteremias, and often there was no distinction between community-acquired bacteremia and nosocomial bacteremia; this may also have influenced the findings of these studies.
Among community-acquired bacteremias in North Jutland County, Denmark, *Escherichia coli* has remained the most commonly isolated pathogen, followed by *Streptoccus pneumoniae* and *S. aureus*[41]. This rank order is similar to that reported by recent studies in England and Wales[42], Olmsted County, MN, USA[43], and Canada[44]. *E. coli* bacteremia most often arises from focal infections of the urinary and gastrointestinal tracts; most infections originate in the community, and the highest incidence rates are observed among elderly patients[27;45].

Portal of entry and focus of bacteremia

Bacteremia implies a failure of the protective mechanisms of the body that serve to restrict an infection to its primary site[46-48]. The bacteria may be transiently introduced into the blood, e.g. through breaks in skin and mucosal barriers, which may or may not lead to symptoms (Figure 2, route 1). Transient bacteremias are probably not uncommon, and under normal circumstances may have no impact on health, as circulating bacteria are promptly inactivated and filtered out by the liver and spleen[49]. However, in some individuals, transient bacteremia may enable microorganisms to establish an infection elsewhere in the body. Prior to removal by the body's normal clearance mechanisms, the circulating microorganisms may find haven in a damaged tissue or organ, a locus minoris resistentiae. Well-know examples are endocarditis and hematogenous osteomyelitis. Other portals of entry include drainage from the primary or secondary focus of infection via the lymphatic system to the bloodstream and direct entry from contaminated intravascular devices such as catheters or graft materials[46-48] (Figure 2, route 2). It can be difficult to distinguish between primary and secondary foci, since the pathophysiological events are often putative and unobserved. Certain bacteria, such as Salmonella and *Yersinia enterocolitica*, may silently reach the blood stream through Peyer's patches[50;51].

The distribution of foci varies according to the origin of infection and causative microorganisms, but the most frequent foci in patients with community-acquired bacteremia are the urinary, respiratory, gastrointestinal, and hepatobiliary tracts[41]. Despite the best efforts of physicians, the focus of infection remains unknown in about 20% of patients with community-acquired bacteremias which have been associated with increased mortality[41].

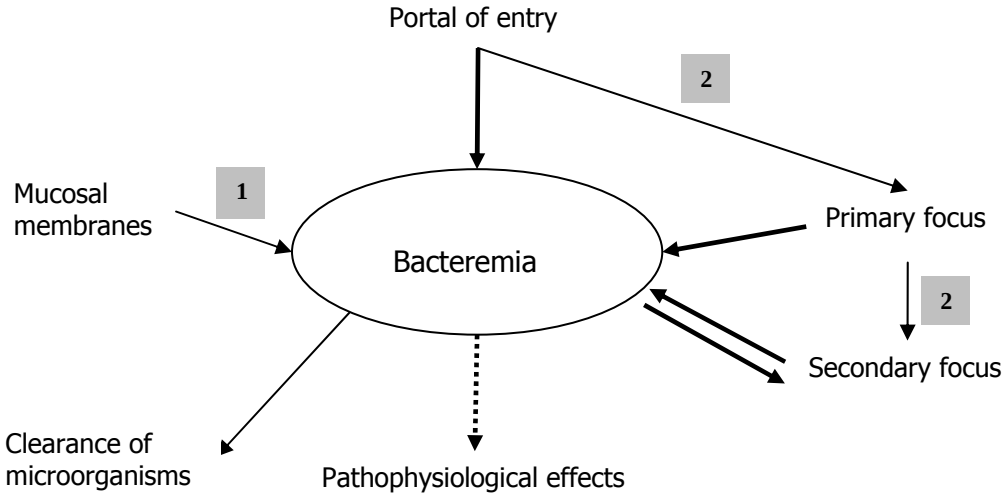


Figure 2. Relationship between portals of entry, focus of infection, and bacteremia. [1] Denotes transient bacteremia accompanying, e.g., tooth brushing, dental, and medical procedures; [2] denotes the spread from focal infections that are not observed clinically. The portal of entry and the primary focus may be the same.

Interrelationship between bacteremia and sepsis

Understanding bacteremia epidemiology has long been complicated by a rather indiscriminate use of the terms bacteremia, sepsis, and septicemia in many studies. In 1991, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) convened a Consensus Conference at which a set of definitions for sepsis and its sequelae were proposed; these definitions were revised in 2001[52]. At this conference, the term systemic inflammatory response syndrome (SIRS) was developed to indicate a clinical response arising from a nonspecific insult characterized by two or more of the following: tachycardia, fever or hypothermia, tachypnea, leukocytosis, or leukopenia. SIRS can be triggered by a variety of insults, including localized or general infection, trauma, burns, or sterile inflammatory processes (Figure 3). When SIRS is the result of a confirmed infectious process, it is termed sepsis. Sepsis may intensify over time to severe sepsis, i.e. sepsis with organ dysfunction or hypoperfusion, and eventually to septic shock[53-55]. Bacteremia has been documented in no more than 50% of patients with sepsis, severe sepsis, or septic shock[55-58]. Conversely, almost all patients with bacteremia (using our definition) fulfil the criteria for sepsis[3;4;59;60], and between 7% and 24% of bacteremia patients are reported to have septic shock[56;61;62].

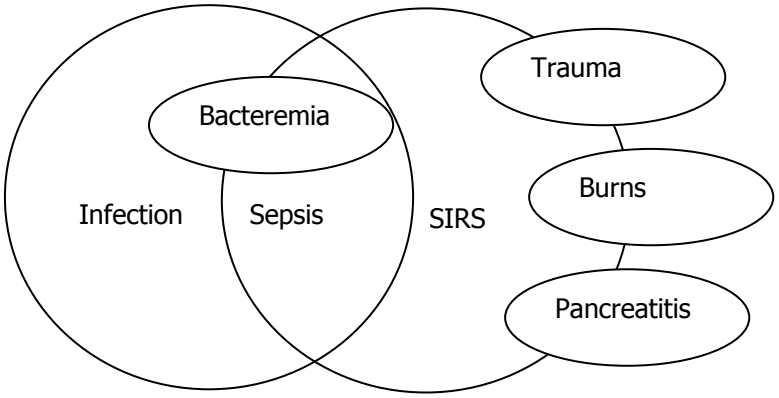


Figure 3. The interrelationship between systemic inflammatoty response (SIRS), sepsis, and infection. Modified from Bone[57].

The burden of bacteremia

The first reported population-based incidence rates of bacteremia are from the 1970s and 1980s[17;18], and the incidence rates have increased by almost 150% over the last 25 years . Recent population-based studies of bacteremia have estimated incidence rates of bacteremia to be between 125 per 100,000 person-years in Finland[63] to 156 per 100,000 person-years in females and 237 per 100,000 person-years in males in Olmsted County, MN[43]. However, only a few studies have distinguished between nosocomial, health-care related, and community-acquired infections[18;44], and the distributions and temporal trends for these categories of bacteremia are unknown. Close to half of all episodes are categorized as community-acquired bacteremia[16;62;64], and the 1:1.5 ratio of community-acquired bacteremia to nosocomial bacteremia has been relatively constant in Denmark over the last few decades, with increasing occurrence of both types. In Calgary, Canada, Laupland et al[44] recently reported that the overall annual incidence rate of community-acquired bacteremia was 81.6 per 100,000 person-years during the period 2000 to 2004.

Figure 4 shows the incidence of bacteremia in North Jutland County, 1992-2006. The incidence increases markedly with patient age, and worldwide, bacteremia may become an even more common clinical problem as the average life expectancy increases in most countries. A previous population-based Danish cohort study reported an increase in the incidence of bacteremia in North Jutland County from 76 per 100,000 person-years in 1981 to 153 per 100,000 person-years in 1994[65]. Between 1992 and 2006, we found that the incidence of bacteremia in North Jutland County increased from 119 to 166 per 100,000 person-years, corresponding to an age- and sex-standardized incidence rate ratio of 1.40 (95% CI 1.20-1.62). We observed this increase for all age groups, but it was most pronounced in the oldest age group, ≥ 80 years; the elderly (> 64 years), especially males, had substantially higher incidence rates that corresponded well with previous findings[43;44;63].

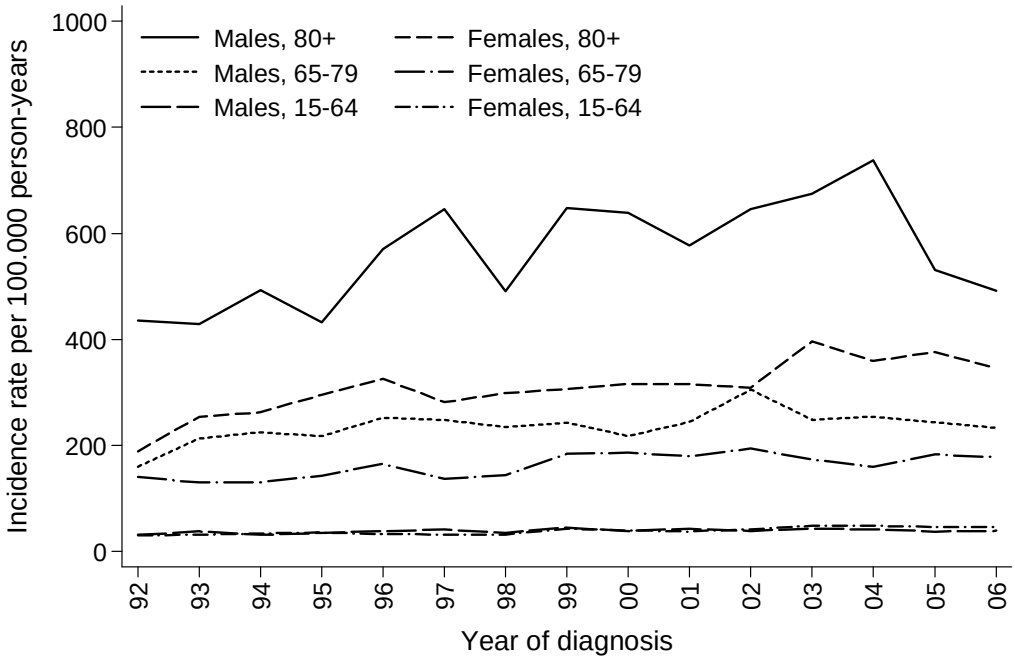


Figure 4. Standardized incidence rates of first-time episodes of bacteremia in North Jutland County, Denmark, 1992-2006, according to age and gender.

There are several plausible explanations for the rising incidence of bacteremia. The increase may be associated with demographic changes, e.g. an aging population plus increasing longevity of patients with chronic diseases. Alternatively, it may also be be due to the increased use of invasive procedures and immunosuppressive drugs, chemotherapy, transplantations, and increasing antimicrobial resistance[66;67]. Since we observed an increasing incidence within each age group, factors other than an aging population must be involved. A potential increase in the ascertainment of bacteremia must also be taken into account[68]. For example, physicians may have lowered their threshold for ordering blood cultures, and blood culture technology has definitely improved (see below).

1.2 Diagnosis of bacteremia

Blood cultures

After more than 100 years, blood cultures are still considered the gold standard for diagnosing and managing patients with bacteremia. A blood culture denotes a sample of blood drawn by venipuncture and inoculated into one or more blood culture bottles with attention to aseptic technique; if more than one bottle is inoculated with each venipuncture, this is frequently referred to as a blood culture set[69;70]. A positive blood culture deemed clinically relevant either establishes or confirms a diagnosis of infection. Moreover, it provides one or more microorganisms for susceptibility testing which, in turn, allows targeted antibiotic treatment[70;71]. From a prognostic standpoint, a positive blood culture provides evidence that the host defenses have failed to contain the infection at its primary location and/or that the physician has failed to

remove, drain, or otherwise eradicate the focus of infection[70;71]. The validity of blood culture as a diagnostic test is dependent on physician behavior (that is, the choice of the number and timing of culture sets) and clinical judgment (that is, the estimation of the pre-test probability of bacteremia and interpretation of the results)[72].

Blood culture technology

The most important technical advance in blood cultures in the past 30 years has been the development of automated, continuously monitored blood culture systems that provide growth readings every 10 to 20 minutes throughout the day to detect positive cultures as quickly as possible[73]. However, the single most important factor in determining the sensitivity of the blood culture is still the volume of blood drawn for culture[69;74-76]. Other important factors include the timing of the culture, the number of cultures taken, the ratio of blood to broth, the incubation length, the incubation atmosphere, and additives to the blood culture media[2;69;71;72;77;78].

Utilization of blood cultures

There are few data regarding the utilization of blood cultures. In 1990, an international collaborative group collected data from 67 medical centers in the USA (n=12), Europe (n=44), and Asia (n=11). Assuming a bed occupancy of 90%, a median blood culture rate of 20 per 1000 hospital days (range 6-84) could be calculated for 24 hospitals in 6 European countries[79]. In Finland, the national annual rate of blood cultures has increased from 2766 per 100,000 person-years in 1995 to 3685 per 100,000 person-years in 2002[63]. Among ambulatory outpatients in the Calgary Health Region, Canada, Laupland et al reported an annual blood culture rate of 89.4 per 100,000 person-years[80].

The European Antimicrobial Resistance Surveillance System (EARSS) collects annual data on the number of blood cultures performed at participating hospitals in 29 countries on a voluntary basis. Data for the entire country of Denmark were available for 2006, and the number of blood culture sets was 300,000, equivalent to 52 per 1000 hospital days[81]. In 2007 the median culturing frequency for all participating countries was 36 per 1000 hospital days, ranging from 4 in Lithuania to 114 in Israel. In the UK and France, rates were 36 and 56, respectively, and for the Scandinavian countries, rates clustered around 50[82]. For comparison, the blood culture rate in North Jutland County was 20 per 1000 hospital days in 1995 and 35 per 1000 hospital days in 2006. This rate was below the national average, likely due in part to the practice of sampling one blood culture set with 3 bottles rather than two sets with two bottles each (see page 36).

Indications

Despite the essential role that detection plays in the prognosis of patients with bacteremia, there are no clear guidelines concerning indications for obtaining blood cultures. The 9th edition of Harrison's Principles of Internal Medicine, published in 1980[83], states that blood cultures

should be obtained from all febrile patients who have rigors, who are seriously ill, who are thought to have endocarditis or intravascular infection, or who are immunosuppressed. Other indications include fever or hypothermia, hypotension, leukocytosis, and changes in mental status[84-86]; these are also the criteria for SIRS. However, it is noteworthy that these criteria have been criticized for being too sensitive and nonspecific[87;88].

Prior studies of hospitalized patients have developed criteria for rational ordering of blood cultures, but these guidelines are frequently ignored in clinical practice[89-92]. Nonetheless, physicians have been shown to inaccurately predict bacteremia risk, often overestimating the patient's likelihood of bacteremia[93]. As a result, the proportion of positive blood cultures remains at 5-10%[37;63;94]. In a retrospective study of 432 blood culture episodes in patients admitted to medical wards, Justesen et al[94] found that 46.1% of all patients who had blood cultures taken had a rectal temperature below 38.5°C, as did 28% of patients who had positive blood cultures. The febrile response may be blunted or absent in some patient populations, such as immunocompromised patients, patients with end-stage renal disease, infants, and older patients, who often present with more vague symptoms than younger patients[95;96]. Accordingly, local guidelines in North Jutland County emphasize that the decision to order blood cultures should be preceded by a clinical assessment and caution against standard orders specifying a set level of pyrexia. Nonetheless, some blood cultures may be ordered as part of a "routine fever work-up", more to rule out bacteremia than in response to clinical suspicion of bacteremia. On the other hand, it is likely that some patients with a likelihood of bacteremia do not have blood cultures taken, and patients' underlying disorders may also influence the indications for performing blood cultures.

Clinical impact of positive blood cultures

Prompt and appropriate empirical treatment is associated with improved survival in patients with bacteremia[97-101]. As a consequence, empirical antibiotic treatment is recommended for patients with suspected bacteremia while culture results are pending. Nonetheless, up to 40% of all patients with bacteremia receive inadequate antibiotic treatment prior to the first notification of a positive blood culture[97;102-104]. Although broad-spectrum empirical antibiotics may appear to be an attractive choice, their use can result in increased costs and adverse events as well as in increased selective pressure for antibiotic resistance. Therefore, an important task for the microbiological laboratory is to provide timely reports on positive blood cultures that can be used to guide antibiotic therapy.

Most laboratories report the results of blood cultures in three stages (Figure 5). The first notification is typically a preliminary report based on the result of a Gram stain of positive cultures. At the time of the preliminary report, 12% to 20% of the patients may not have started antibiotic treatment; for 30% to 45% of the patients, the Gram stain result is followed

by a change in empirical treatment[41;103-107]. A second notification is normally provided within 12-24 hours of the preliminary report; this one includes a provisional or definitive identification of the isolated microorganism(s) accompanied by the antimicrobial susceptibility pattern. Further changes in treatment may be made on the basis of these results.

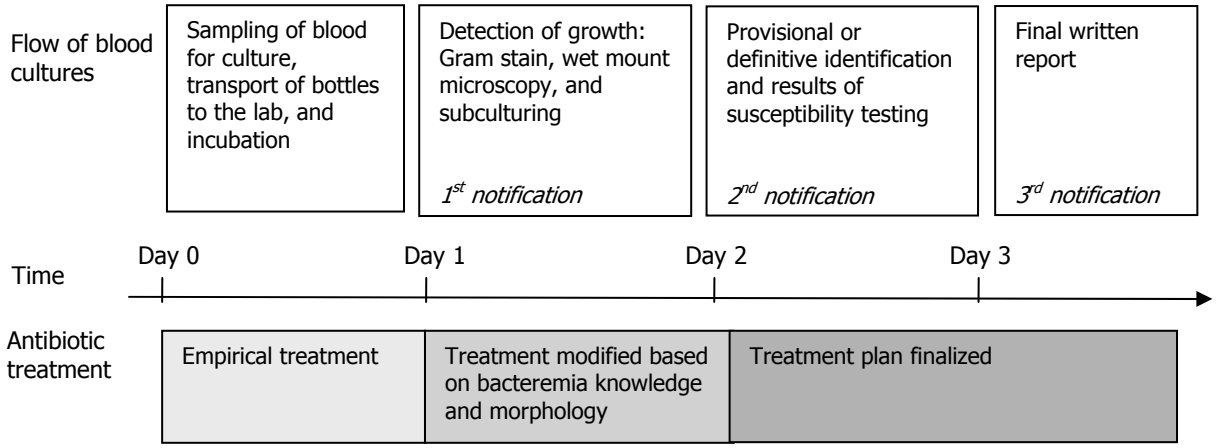


Figure 5. Flowchart of blood cultures yielding bacterial growth; the attending physicians are notified by the physicians in the Department of Clinical Microbiology when blood cultures yield significant growth. The times indicated in the figure are approximate.

Adjustments in treatment results in targeted treatment, limited use of broad-spectrum antibiotics, and cost savings[102;108-111]. Recent studies have shown that Gram stain results combined with information on whether the infection was community-acquired or nosocomial may further improve the appropriateness of the antibiotic treatment[112;113]. In addition to having direct implications for antibiotic treatment, the Gram stain result may also prompt further diagnostic and therapeutic interventions, including a search for the focus of infection in patients without an apparent one. However, the sooner blood culture results become available, the better the compliance with the recommendations of the microbiologist[105;108;109]. Thus, the Gram stain report has greater impact on antimicrobial treatment than provision of cultural identification and antimicrobial susceptibility test results[103;107;114]. It is likely that physicians are reluctant to change treatment after two or three days in patients whose status is improving or to reduce the spectrum of antibiotic treatment in very ill patients in whom it is uncertain that the blood isolate(s) is the only pathogen with a role in infection.

The Gram stain as a diagnostic test

The Gram stain was developed by the Danish physician Hans Christian Joachim Gram in 1884[21]. It has been said to be "perhaps the most useful rapid test for blood cultures (…) because the results allow physicians to initiate or modify empirical antimicrobial therapy"[115]. The Gram stain is a differential stain that divides bacteria into two groups: Gram-positive organisms, which retain the primary crystal violet dye and appear deep blue or purple, and Gram-negative organisms, which are decolorized due to loss of the primary stain and subsequently take up the counterstain, which is usually safranin or carbol-fuschin[116;117]. Wet-mounts, long a tradition in

Danish clinical microbiology for use in conjunction with Gram staining of positive blood cultures[118], help detect and determine the morphology of microorganisms. In addition, wet-mounts are used to further classify bacteria according to their motility pattern as nonmotile, peritrichous, or polar[119;120]. Thus, Gram stain, in conjunction with bacterial morphology (cocci vs. rods), the arrangement of the bacteria (clusters vs. chains), and the bacterial motility pattern, can be used to make a presumptive identification of bacteria or yeast to guide empirical antibiotic treatment (Figure 6). In particular, polar motility of Gram-negative rods may indicate *Pseudomonas aeruginosa* and other aerobic bacteria that require extended antibiotic coverage. Motility is rarely observed with other bacterial groups, but among Gram-positive rods it may be an important clue to *Listeria monocytogenes*.

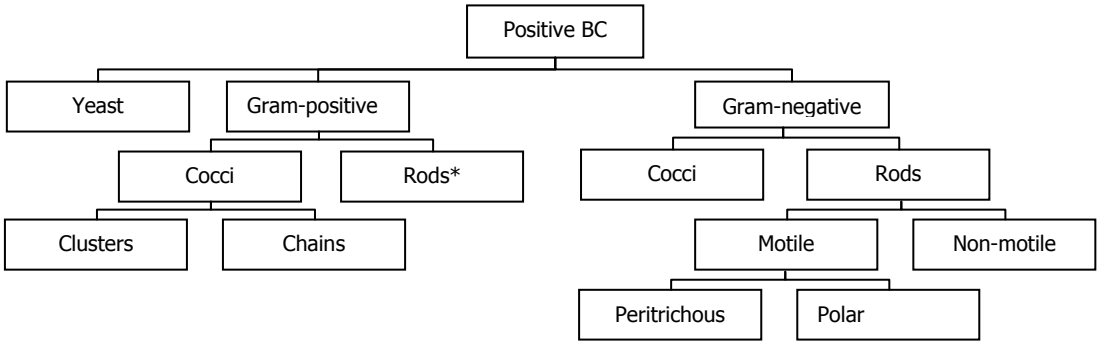


Figure 6. Presumptive identification of bacteria and yeast in positive blood cultures (BC) on the basis of Gram stain and wet mount microscopy. *Motility may also be observed with Gram-positive rods (e.g. *L. monocytogenes*, *Bacillus*, and *Clostridium*).

Berg[121] described the movement of peritrichous bacteria as gently curved 'runs', driven by the counterclockwise rotation of flagella, that are terminated by chaotic events called tumbles that arise when the flagella rotate clockwise. Conversely, aerobic bacteria, mostly mono- or lophotrichous bacteria, exhibit linear or curvilinear runs that lack tumbling and cease rapidly in the absence of air.

Accuracy of the Gram stain

Despite the acknowledged importance of the first notification of positive blood cultures, the accuracy of the Gram stain result has not been studied by many groups. We searched the MEDLINE database using the terms and limiting the search to English language articles:

- (gram[All Fields] AND ("staining and labeling"[MeSH Terms] OR ("staining"[All Fields] AND "labeling"[All Fields]) OR "stain"[All Fields])) AND ("bacteraemia"[All Fields] OR "bacteremia"[MeSH Terms] OR "bacteremia"[All Fields]) AND (("blood"[All Fields] OR "blood"[MeSH Terms]) AND "culture"[All Fields] OR "culture"[MeSH Terms])) [yielded 62 articles].

Additional studies were identified by searching the reference lists of selected publications. We found only 7 studies that reported estimates of the accuracy of Gram stain reports (Table 1).

Here we use the term accuracy to mean the amount of agreement between the information from the test under evaluation and the reference standard[122]. According to Guyatt et al[123], a valid diagnostic study does the following:

1. assembles an appropriate spectrum of patients
2. applies both the diagnostic test and the reference standard to all patients
3. interprets each test blind to the other
4. repeats itself in a second independent ("test") set of patients

The Standards for Reporting of Diagnostic Accuracy (STARD) Initiative recently published guidelines to improve the quality of reporting of studies of diagnostic accuracy[122]. However, only two of the studies on the accuracy of the Gram stain were published after these guidelines were issued, and all of the studies lack some information concerning design, conduct, and analysis needed to assess the potential for bias and to evaluate whether the results can be generalized. Moreover, most studies were hampered by small sample sizes[106;124-126], and only 4 provided estimates of sensitivity and specificity[125-127].

Rand et al[128] found that the Gram stain was misread in 57 (0.7%) of 8,253 patients with positive blood cultures. However, only major errors were reported, defined by the authors as errors in which the original Gram stain reported a single organism with a Gram stain that was the opposite of the Gram stain in the final determination (Gram-positive rather than Gram-negative, or vice versa). As a consequence, confusing Gram-positive cocci in clusters (presumed *Staphylococcus* sp.) with Gram-positive cocci in chains (presumed streptococci or *Enterococcus* sp.) was not regarded as a major error, even though the clinical implications are quite different. In comparison with the error rate of 0.7% reported by Rand et al[128], Cunney et al[106] reported an error rate of 5%. Other studies regarding the accuracy of Gram staining focused on differentiation of staphylococci and streptococci[125-127], *Candida albicans* from *Candida* non-*albicans*[28], or detection of *S. pneumoniae* in Gram stains[124].

Table 1. Studies on the accuracy of the Gram stain report of positive blood cultures.

Authors, country, year	Study period	Aim	Data collection	Study population	Reference standard	Results
Rand et al[128], USA, 2006	2002-2003	To examine the occurrence of errors in Gram stain reports	Retrospective	8.253 positive BCs, yeast excluded	Cultural identification	In 57 (0.7%) BCs there was "major" non-concordance between Gram stain and cultural identification. - 22 Gram-positive cocci - 10 Gram-positive rods - 25 Gram-negative rods 28 were polymicrobial.
Cunney et al[106], USA, 1997	NS	To assess the impact of BC results on antibiotic treatment	Prospective	132 bacterial isolates in 123 patients, contaminants excluded	Cultural identification	7/132 (5%) were misread by Gram stain: 4 isolates were not found by Gram stain, 2 *Haemophilus* sp. were seen as Gram-negative and Gram-positive cocci, respectively, and 1 *Acinetobacter* sp. were misread as Gram-positive diplococci.
Murdoch et al[125], New Zealand, 2005	NS	To differentiate *S. aureus* from CoNS in Gram stains	Prospective	150 positive BacT/Alert BCs and 100 positive BACTEC BCs with GPCC	Cultural identification	BacT/Alert: Se=89%, Sp=98%, PPV=97%, NPV=92% BACTEC: *S. aureus* and CoNS could not be differentiated.
Agger et al[127], USA, 1977	1973-1976 and 1976-1977	To differentiate staphylococci from streptococci in Gram stains	Retrospective and prospective	569 positive BCs with Gram-positive cocci	Cultural identification	Preponderance of clusters: 97.4% Se and 95.3% Sp for staphylococci. Preponderance of chains: 95.3% Se and 99.2% Sp for streptococci. 9/18 cultures with *S. pneumoniae* revealed diplococci. *S. aureus* and CoNS could not be differentiated.
E. Wald[126], USA, 1982	1979-1980	To differentiate staphylococci from streptococci in Gram stains	Prospective	107 positive BCs with Gram-positive cocci	Cultural identification	Preponderance of clusters: 98.8% Se and 96.3% Sp for staphylococci Preponderance of chains: 96.3% Se and 98.8% Sp for streptococci
Harrington et al[28], USA, 2007	NS	To differentiate *Candida albicans* from non-*albicans*	Prospective	60 patients with fungemia	Cultural identification	Presence of clustered pseudohyphae on Gram stain: Se=85%, Sp=97%, PPV=96%, NPV=89% for *C. albicans*.
Merlino et al [124], Australia, 2000	NS	To evaluate identification of *S. pneumoniae* in Gram stains	Retrospective	27 positive BCs with *S. pneumoniae*	Cultural identification	12 Gram stains revealed diplococci. 15 Gram stains revealed cocci in chains.

BC, Blood culture; NS, not stated; GPCC, Gram-positive cocci in clusters; CoNS, coagulase-negative staphylococci; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

1.3 Prognosis of patients with bacteremia

Prognosis (from the Greek *pro-gnosis*, meaning 'foreknowledge') means foreseeing, predicting, or estimating the risk of future outcomes[129]. In medicine, prognosis commonly relates to the risk of an individual developing a particular state of health (an outcome) over a specified time[130;131]. Outcomes are often events, such as death, but may also be conditions like disease progression, discomfort, disability, or dissatisfaction[130;132;133].

Purpose of prognostic studies

Physicians study prognosis to predict, understand, and change the outcomes of disease[132]. However, prognostic studies are important not only to clinicians but also to patients, who wish to know what to expect from their disease and whether their condition can be improved. Additionally, healthcare policy makers need information on whether prognoses can be changed by changing the organization of healthcare[129;130;134;135]. Box 1 summarizes some of the reasons for conducting prognostic studies[135;136].

- To guide clinical decision-making, including treatment selection and patient counselling
- To improve understanding of the disease process
- To improve the design and analysis of clinical trials (for example, risk stratification)
- To assist in comparing outcome between treatment groups in non-randomized studies by allowing adjustment for case-mix
- To define risk groups based on prognosis
- To predict disease outcomes more accurately or at a lower cost

Box 1. Purpose of prognostic studies[135;136].

Prognostic factors for bacteremia

Prognostic factors for bacteremia can be defined as inborn patient characteristics or exposures that are causally associated with an adverse outcome of bacteremia. A number of prognostic factors overlap with risk factors for bacteremia, foremost old age and comorbidity. Age in itself may not be a prognostic factor, but rather a proxy for other related conditions that directly or indirectly influence outcome[131;137] (Figure 7). These factors include intrinsic aspects of aging such as immunosenescence[138] and increasing frailty, as well as diagnostic difficulties and potential differences in treatment and clinical quality associated with old age. Older adults are more likely to present with attenuated or atypical manifestations of infections than younger adults[95;96] and may be more likely to develop infections caused by antibiotic-resistant pathogens[25]. Both of these can delay diagnosis and treatment. Other factors are functional and nutritional status, which have been associated with increased mortality in the elderly population[24;62].

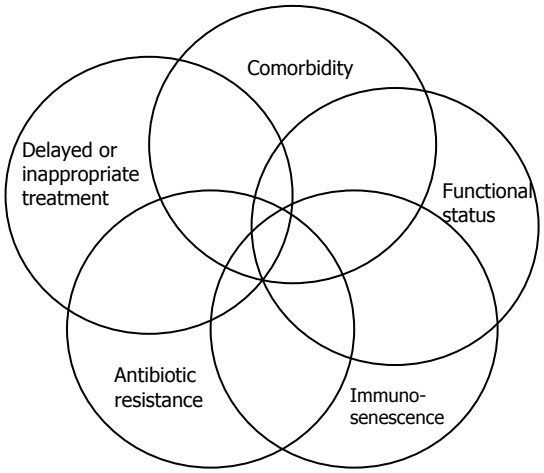


Figure 7. Conceptual model of the aspects of aging that may influence outcomes in patients with bacteremia. The model makes no assumptions about the relative influence of each of these, but does indicate that they are all, to some extent, related to one another (adapted from Crnich and Zimmerman[137])

Age-related increases in the frequency and severity of comorbidity have also been suggested to contribute to the discrepancy in outcomes between older and younger adults[139-142]. Comorbidity has been defined as "any distinct additional clinical entity that has existed or that may occur during the clinical course of a patient with an index disease under study"[143;144]. In bacteremia, comorbidity may cause delays in diagnosis and antibiotic treatment, influence prognosis, and confound associations in studies. There is a variety of methods to measure comorbidity, ranging from a simple count of existing diseases to the use of severity-weighted indices[145]. In 1962, the McCabe classification was developed to control for comorbidity in patients with Gram-negative bacteremia[146;147]; this classification system is used frequently in bacteremia studies[56;58;148]. According to this classification, comorbid diseases are classified into three categories: rapidly fatal, ultimately fatal, and nonfatal. The classification thus depends on the investigator's knowledge and judgment of the prognosis of the underlying illness, and, as a consequence, is only valid in prospective studies. In retrospective studies, misclassification may occur if the classification is influenced by knowledge of the outcome. The most widely used comorbidity index is the Charlson Comorbidity Index[145;149]. This index was developed by Mary Charlson[149] at the Cornell Medical Center in New York. During a one-month period in 1984, all patients admitted to medical services (n=559) were evaluated at admission, and all comorbid diseases were recorded. A one-year follow-up was obtained for these patients, and the prognostic impact of individual comorbid diseases was determined in a proportional hazards model. The diseases were categorized into 19 distinct medical conditions and each was assigned a weight based on the relative mortality rate. Thus, a weighted index was created that accounted for the number and the seriousness of comorbid diseases[149]. The Charlson comorbidity index was subsequently validated in a cohort of 685 breast cancer patients. Over the last two decades, the index has been adapted for hospital discharge data in ICD-based databases and has been used to control for comorbidity for a variety of diseases, including bacteremia[41;141;142;150;151].

A number of bacteremia-related factors have also been associated with a poor prognosis. These include an unknown focus of infection[41;152], certain microbial agents, such as yeast [153;154], *P.*

aeruginosa [155], and polymicrobial bacteremia[156], and, though debated, bacterial antibiotic resistance[157]. Figure 8 shows examples of prognostic factors that are likely to play a role in the prognosis of bacteremia patients. There are a number of physiologic derangements that have been associated with bacteremia prognosis[58], some of which are included in intensive care scoring systems such as APACHE II[158] and SAPS[159]. Whether to include these measures of disease severity in an epidemiological study depends on the type of study and on the time at which they are measured in relation to the bacteremia episode. There are two main types of epidemiological outcome studies, explanatory studies and prediction studies[131]. In prediction studies, the purpose is to predict the outcome for individual patients as accurately as possible by including all independent prognostic factors that might improve prediction in the model[130;160;161]. In explanatory studies of bacteremia, the aim is to understand mechanisms of action, including pathobiology and causes of mortality, by isolating the effects of specific variables[143]. Thus, statistical models are used to evaluate the causal role of one or more prognostic factors while simultaneously adjusting for the confounding effect of other factors[131]. The factors that are included as potential confounders should fulfil specified criteria (page 30). For disease severity to be considered a confounder, it should be measured before the onset of bacteremia, otherwise it may constitute a variable in the causal pathway leading from bacteremia to death rather than a variable controlling for the patient's baseline state and risk of mortality[162]. Conversely, in prediction studies, measures of severity may improve prediction and may thus be included in the model[131]. Measurement of disease severity poses a particular challenge in patients with community-acquired infections because it is difficult to assess disease severity at the optimal time point i.e. immediately before the actual onset of bacteremia[157;163;164]. This time point may be impossible to determine in the community setting and will rarely coincide with the time that a blood specimen is obtained for culture.

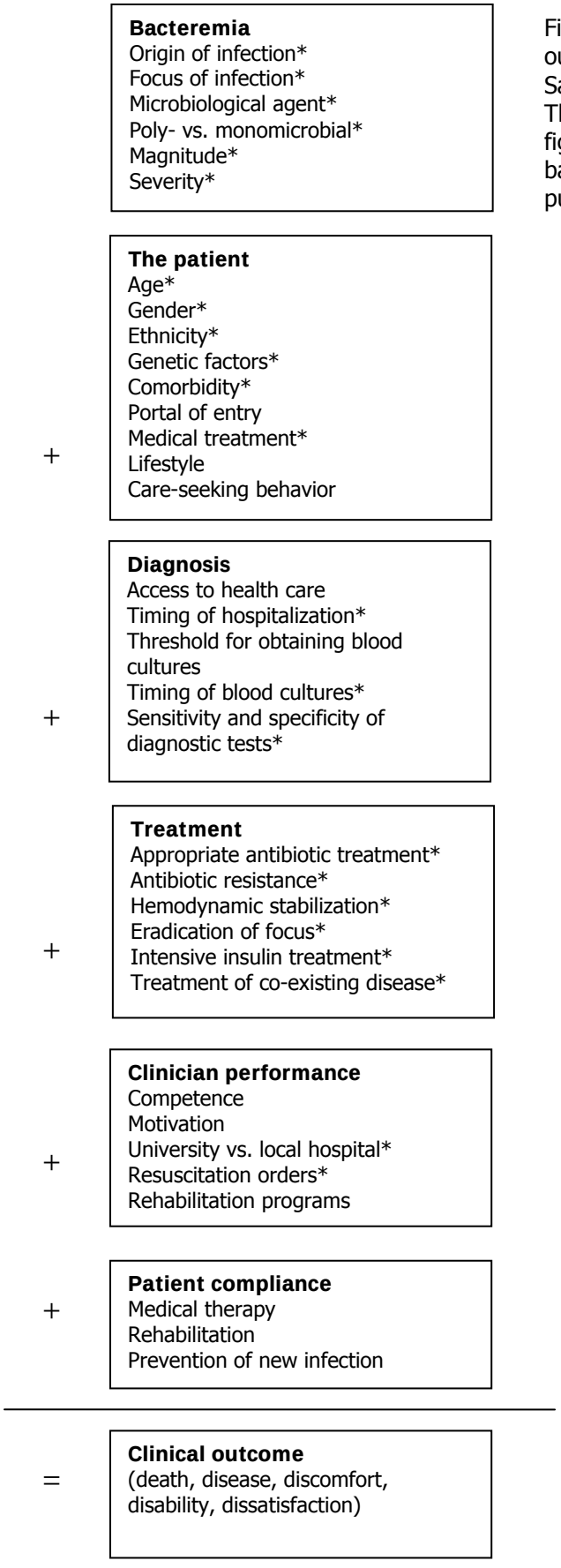


Figure 8. Factors that influence bacteremia outcome. This figure is modified from Sackett's figure in "Clinical Epidemiology"[133]. The "patient" box was added to the original figure, and the text has been adapted for bacteremia. Factors substantiated by published data are marked with an asterisk.

Prognosis studies

Studies comparing the prognosis of bacteremia patients and patients without bacteremia

There are several studies of the prognosis of bacteremia, but only a few have compared mortality in patients with and without bacteremia. We conducted a Medline search with the following query:

- "Prognosis"[Mesh] AND ("Bacteremia"[Mesh] OR bloodstream infection) AND ("Matched-Pair Analysis"[Mesh] OR "Comparative Study"[Publication Type]) [yielded 228 articles].

We limited the search to include only English language studies in humans. Additional studies were found by searching the references from identified references. However, many of the studies compared the prognosis of different antibiotic treatments (n=31), infections with susceptible vs. resistant pathogens (n=9), or nosocomial vs. community-acquired infections (n=4).

In a study at two Vancouver hospitals, Roberts et al[165] included 1972 positive blood culture episodes categorized as either clinically significant bacteremia, transient bacteremia, bacteremia of indeterminate significance, or contaminated blood cultures. Mortality in patients with positive blood cultures was compared with mortality in an age- and gender-matched control group of 1244 patients with negative blood cultures, with the finding that mortality was higher in the patients with positive blood cultures, regardless of category, than in controls. Bates et al[56] examined the prognostic impact of bacteremia in a sample of adult patients who had blood cultures performed[56]. That study reported an adjusted relative 30-day mortality of 2.3 (95% CI 1.2-4.4) and an adjusted relative one-year mortality of 1.3 (95% CI 0.76-2.1) in 142 bacteremia patients compared to 142 patients with negative blood cultures matched by age, gender, severity of underlying disease, and major comorbidity[56]. In comparison, in a hospital-based cohort study from Israel, Leibovici et al[62] found increased mortality among bacteremia patients up to 4 years after the infection compared to a control group comprising 1991 inpatients without any infectious diseases, matched for age, sex, department, date of admission, and underlying disorders (Table 2). Two other cohort studies identified bacteremia as a predictor of in-hospital mortality in ICU patients with sepsis, severe sepsis, or septic shock with relative risks around 1.6[58;166]. However, none of these studies specifically addressed patients with suspected community-acquired bacteremia, and all had some limitations, including study populations restricted to selected groups of sepsis patients[58;166], lack of long-term follow-up[58;166], small sample size[56], and failure to adjust for coexisting chronic diseases[166].

Studies on the impact of age and comorbidity on prognosis

We searched Medline to identify articles on the association between bacteremia prognosis, age, and/or comorbidity using the following terms:

- ("Bacteremia"[Mesh] OR bloodstream infection) AND (mortality OR "Prognosis"[Mesh]) and age [yielded 986 articles].
- ("Bacteremia"[Mesh] OR bloodstream infection) AND (mortality OR "Prognosis"[Mesh]) and comorbidity [yielded 116 articles].

We limited the search to studies in humans that were published in English. In addition, reference lists of selected publications were searched for other relevant articles. Several studies were identified in this manner, most of which were purely predictive studies examining a wide range of prognostic factors. Few studies had age as the primary exposure [167-170]. Table 3 summarizes the selected studies that examine age and/or comorbidity as prognostic factors for bacteremia; reviews are not shown.

Age is a prognostic factor of mortality in patients with bacteremia, but the association between age and mortality is not clear. As is evident in Table 3, comparisons of previous studies are complicated by the selected study populations, such as ICU patients[139;167], cancer patients[170], patients in geriatric hospitals[26], different age categories, or differences in outcome, as well as adjustment for different sets of covariates. Other limitations include uncontrolled confounding by diseases other than bacteremia[25;169;171] and a lack of control groups[26;172]. An increased burden of comorbidity, which is closely related to advanced age, may also explain in part the higher mortality among older patients. However, we are not aware of studies examining the association between age and mortality of bacteremia patients with increasing levels of comorbidity. Moreover, previous studies on the impact of comorbidity were mostly hospital-based and restricted to selected patient groups, such as critically ill patients[139] and patients with *S. aureus*[141] or enterococcal bacteremia[173].

Table 2. Studies comparing the prognosis of patients with and without bacteremia.

Authors, country, year	Study period	Setting	Study design	Patients	Adjustment	Risk estimates	Results
Roberts et al[165], 1991, Canada	1984-1987	Vancouver General Hospital and the British Columbia Cancer Agency	Matched cohort study	1972 positive BCs (1244 clinically significant, 144 transient, 519 contaminated, and 65 of indeterminate significance) 1244 patients with negative BCs, matched for age and gender	No	Mortality rates	In-hospital mortality Significant bacteremia: / Controls: Day 0 1.8% / 0.4% Day 2 7.8% / 1.3% Day 5 12.6% / 2.4% Day 10 18.0% / 4.5% Day 20 22.8% / 6.5% Day 30 27.3% / 7.3%
Bates et al[56], 1995, USA	November 1988-February 1989, May-June 1989, August 1990	Brigham and Women's Hospital, Boston, MA	Matched cohort study	142 bacteremia patients 142 matched patients with negative BCs 155 patients with contaminated BCs	Yes	MRR	30 day mortality: Bacteremia: 16%; negative BCs: 8% Adj. MRR = 2.3 (1.2-4.4) 1-yr mortality: Bacteremia: 30%; negative BCs: 23% Adj. MRR = 1.3 (0.76-2.1)
Leibovici et al[62], 1995, Israel	March 1988-October 1992	Beilinson Medical Center, Petah Tiqva	Matched cohort study	1991 bacteremia patients 1991 patients without an infection, matched for underlying disorders and other covariates	No	Mortality rates and median survival	Bacteremia patients: / Controls Mortality rates: / Mortality rates: 1 month: 26% / 1 month: 26% 6 month: 43% / 6 month: 43% 1 year: 48% / 1 year: 48% 4 year: 63% / 4 year: 63% Median survival: / Median survival: 16, 2 months / > 75 months
Brun-Buisson et al[58], 1995, France	January-February 1993	Multicenter study in 170 ICUs	Cohort study	742 with documented severe sepsis 310 with culture-negative sepsis	Yes	MRR	Bacteremia within 3 days after severe sepsis: MRR = 1.7 (1.1-2.8) Bacteremia was not associated with 28-day mortality (estimates not given).
Laupland et al[166], 2004, Canada	1999-2000	Three multidisci-plinary ICUs in the Calgary Health Region	Population-based cohort study	1,981 patients admitted to ICU - 100 with bacteremia and SIRS - 1,504 with SIRS without bacteremia	Yes	OR	In-hospital mortality: Crude OR, bacteremia = 1.6 (1.1-2.2) Adj. OR, bacteremia = 1.1 (0.7-1.8)

BC, blood culture; MRR, mortality rate ratio; OR, odds ratio.

Table 3. Studies of the impact of age and/or comorbidity on the prognosis of patients with bacteremia.

Authors, country, year	Study period	Setting	Study design	Type of infection	Number	Age groups	Adjustment	Risk estimates	Results
Blot et al[167], Belgium, 2009	1992-2006	The ICU of Gent University Hospital	Cohort	Nosocomial bacteremia	1228 episodes 984 patients	45-64 65-74 ≥75	Yes	MRR	In-hospital mortality: 45-64: (ref) 65-74: 1.3 (1.0-1.5) ≥75: 1.7 (1.3-2.2)
Lee et al[168], Taiwan, 2007	2001-2002	The ED, National Taiwan University Hospital	Cohort	Community-acquired bacteremia	890 patients	18-64 65-84 ≥85	Yes	MRR	90-day mortality, adj. MRR = 1.01 (1.0-1.02) per one year increase in age
Tal et al[26], Israel, 2005	1998-2000	Harzfeld Geriatric Hospital, Gedera	Cohort	Bacteremic UTI	191 patients	75-84 85-94 ≥95	Yes	OR	Age not associated with in-hospital mortality (estimates not given) Adj. OR per comorbid disease= 1.3 (1.0-1.6)
Nørgaard et al[170], Denmark, 2005	1992-2002	North Jutland County	Population-based cohort	Bacteremia	358 with hematological malignancies	15-59 60-79 ≥80	Yes	MRR	7-day adj. MRR: 15-59: (ref) 60-79: 1.6 (0.8-3.1) ≥80: 1.8 (0.7-4.4) 30-day adj. MRR: 15-59: (ref) 60-79: 1.7 (1.1-2.7) ≥80: 2.3 (1.2-4.3) A linear relationship between age and mortality was indicated
Greenberg et al[172], USA, 2005	1996-1998	Ben Taub General Hospital, Houston, TX	Cohort	Bacteremia	238 episodes 234 patients	65-74 ≥75	Yes	OR	No significant association between age and in-hospital mortality (estimates not given)
Pedersen et al[41], Denmark, 2003	1992-1997	North Jutland County	Population-based cohort	Community-acquired bacteremia	1844 patients	15-64 65-74 75-84 ≥85	Yes	OR	30-day mortality: 15-64: (ref) 65-74: adj. OR 1.1 (0.8-1.6) 75-84: adj. OR 1.5 (1.0-2.1) ≥85: adj. OR 1.9 (1.3-2.9) Comorbidity: Index 0: (ref) Index 1-2: adj. OR 1.6 (1.2-2.3) Index ≥ 3: adj. OR 3.0 (2.1-4.3)
Lesens et al[141], France, 2003	2001-2002	Pooled data from two university-affiliated hospitals	Cohort	*S. aureus* bacteremia	166 patients	<70 ≥70	Yes	MRR	Mortality 3 months after completion of antibiotic treatment: <70: (ref) ≥70: 1.05 (1.03-1.07) Comorbidity: Charlson score <3: (ref) Charlson score ≥3: OR = 3.0 (1.3-5.5)

Authors, country, year	Study period	Setting	Study design	Type of infection	Number	Age groups	Adjustment	Risk estimates	Results
Gavazzi et al[25], France, 2002	1998	Pooled data from 46 hospitals	Cohort	Bacteremia	1740 episodes	65-74 75-84 ≥85	Yes, but not comorbidity	OR	Overall: no differences in 7-day mortality among age groups Community-acquired bacteremia: 65-74: 10.1% 7-day mortality ≥85: 14.2% 7-day mortality
McClelland et al[169], USA, 1999	1994-1998	Duke University Medical Center, NC	Cohort	*S. aureus* bacteremia	438 patients	18-60 ≥65	Yes	OR	12-week mortality: 18-60: (ref) ≥65: 2.21 (1.32-3.70)
Deulofeu et al[24], Spain, 1998	1991-1993	Hospital General de Granollers	Cohort	Bacteremia	242 patients	<65 ≥65	Yes	OR	In-hospital mortality: ≥65: 1.9 (0.4-8.5) >1 underlying disease: 1.6 (0.4-5.8) Low functional status: 11.7 (3.2-43)
Stroud et al[173], USA, 1996	1989-1993	Pooled data from 4 hospitals within a geographical region	Cohort	Enterococcal bacteremia	145 patients	<55 ≥55	Yes	OR	In-hospital mortality: <55: (ref) ≥55 = 4.1 (1.7- 10.0) Comorbidity: Index <3: (ref) Index ≥3: 6.6 (2.6-16.5)
Pittet et al[139], Switzerland, 1993	1984-1988	The surgical ICU, University Hospital of Geneva	Cohort	Bacteremia and sepsis	225 episodes 176 patients	<65 ≥65	Yes	OR	In-hospital mortality: <65: (ref) ≥65: 6.3 (2.8-14.5) Number of comorbidities: Adj. OR = 1.16 (1.04-1.23) per pre-existing comorbidity (linearly correlated with in-hospital mortality, $r^2 = 0.92$)
Leibovici et al[171], Israel, 1993	1988-1990	Beilinson Medical Center, Petah Tiqva	Cohort	Bacteremia	995 patients	60-79 ≥80	Yes	OR	In-hospital mortality: Median survival: 60-79: 30% 60-79: 42 days ≥ 80: 35% ≥ 80: 29 days Adj. OR 1.1 (1.0-1.2) for 60-79 yrs, NS for ≥ 80 (age = continuous)

MRR, mortality rate ratio; UTI, urinary tract infection; OR, odds ratio.

Prognosis of bacteremia: methodological considerations

The overall goal of an epidemiologic study is to obtain valid and precise estimates of the frequency of disease or the effect of an exposure on a given outcome in the source population of the study[174]. The first step to achieve this is to establish the research question and state the aims clearly and quantitatively so that the parameter to be measured is certain[175].

Source population

The source population gives rise to the subjects for the study and is defined by the selection methods of the study. A good choice of study subjects ensures that the parameter estimated in the study is an accurate estimate of that parameter in the population of interest[175]. Ideally, study subjects should represent a random sample of a defined geographical population. However, most of the previous studies of the prognosis of patients with bacteremia have been hospital-based and conducted within different settings and selected patient groups. Conversely, there are few population-based studies conducted within a precisely defined and identified population[41;58;139;166;170]. Moreover, in many studies the authors have not distinguished between first episodes and later episodes, and often the bacteremias are grouped together regardless of the place of acquisition.

Exposures

Exposure means proximity and/or contact with the source of a disease agent in such a manner that transmission of the agent or the harmful effect of the agent can occur[143]. In epidemiological studies, exposure is often used in a broader sense to mean all traits that are capable of affecting the outcome; including, for example, genetic factors[176]. The timing of the record of exposure and the occurrence of the outcome is key: A study may be described as prospective if the exposure measurement could not be influenced by the study outcome[177]. The choice of what is considered 'exposure' depends solely on the study hypothesis.

Outcome

Ideally, descriptions of prognosis should include the full range of disease manifestations that would be considered important to patients In a study of patients with suspected Gram-negative sepsis, Perl et al[148] found that survivors reported more physical dysfunction and perceived their general health as poor more often than individuals in the general US population. Very few other bacteremia studies have evaluated health outcomes other than death[132]. Death, however, can be measured several ways, and definitions used for assessing mortality associated with bacteremia have been variable in previous studies: Some evaluated all-cause mortality[41;167;168;170], while others evaluated only mortality directly attributable to bacteremia[56]. Weinstein et al[16] distinguished between deaths directly related to sepsis, indirectly related to sepsis, or unrelated to sepsis. We find, however, that any sharp distinction between "infection-

attributable" and "nonattributable" mortality may be problematic, as it is difficult to distinguish between the contribution of the septic process and that of underlying disorders[62;165]. In addition, it is difficult to obtain valid information through our registries about cause-specific death[178]. In this thesis, we have used overall mortality as the outcome measure in the prognostic studies because we find this to be a robust and relevant outcome in bacteremic patients.

Choice of follow-up

Patients should be followed long enough for the outcome to occur or be prevented[132]. Therefore, the length of follow-up should correspond to the study hypothesis and aim. Bacteremia has a time-dependent progression that reflects the dynamic interplay of the infectious agent, the host's innate and specific immune responses, and therapeutic interventions, including antibiotic treatment. Therefore different follow-up times may be necessary to describe the prognosis of bacteremia. In a classical study of pneumococcal bacteremia, Austrian & Gold[8] analyzed survival in three historical cohorts, one of which was untreated, one treated with antiserum, and one treated with penicillin G. Despite major improvements in prognosis with advancement of treatment, mortality was virtually the same in all three groups within the first 5 days of follow-up. The fact that early mortality was unaffected by antimicrobial therapy emphasizes the role of the systemic inflammatory response. Roberts et al[165] studied the time pattern of mortality and observed that mortality continued for at least 20 days after the first positive blood culture for most patients, regardless of the type of bacteremia and foci of infection.

Many earlier bacteremia studies may be limited due to censoring of follow-up for mortality after discharge[24;139;167;171;173]. Use of in-hospital mortality may provide a false sense of improved outcome over time if there has been a shift towards earlier hospital discharge; accordingly, an international group of sepsis experts has recommended that follow-up should extend for at least 90 days after diagnosis[88]. However, there are only a few long-term prognostic studies of bacteremia[56;62;179], probably due to the difficulties of individual follow-up in many countries during the post-discharge period.

Occasionally, a cohort's definition will require that everyone meeting this definition must have survived for a specified period[180]. If a study uses a time window from entry into the cohort to define exposure, e.g. community-acquired bacteremia, then classification of patients with community-acquired bacteremia requires that they survive for a certain length of time. Thus, patients who die shortly after the start of follow-up may not be classified as having community-acquired bacteremia[181-183]. In this way, patients with community-acquired bacteremia are given an artificial survival advantage that is termed the immortal time bias (Figure 9).

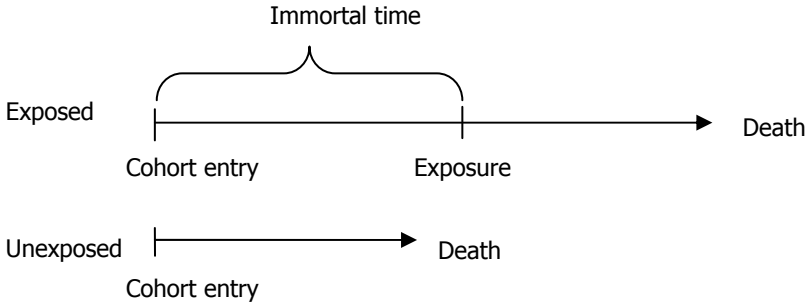


Figure 9. Illustration of immortal time bias. If exposure is defined after cohort entry, the time between cohort entry and exposure, for those who become exposed, is 'immortal'.

The bias arises when the analysis fails to account for this period of immortality; the magnitude of the bias depends on the length of the time window and the risk of the outcome in this period[184]. One approach to avoiding this bias is to classify the immortal person-time as unexposed, until the exposure actually happens, and as exposed thereafter[181;182]. Alternatively, time zero can be redefined as the day after the selected exposure time, thereby excluding the follow-up period used to define exposure[181-183]. This has important implications for the design of **study II.**

Random and systematic errors

Errors in estimation are traditionally classified as either random or systematic. Random error is due to chance, and its estimated magnitude is presented as confidence intervals and p-values in the statistical analysis. The opposite of random error is precision. Systematic errors in estimates are commonly referred to as biases. Biases can be classified into three general categories: selection bias, misclassification bias, and confounding. Selection biases are distortions that result from the procedures used to select subjects and from factors that influence study participation[174]. Misclassification bias is caused by measurement error of exposure or outcome. This misclassification can be either differential (the exposure status is misclassified differentially of the outcome status or vice versa) or non-differential (the exposure status is misclassified independently of the outcome status, or vice versa)[174]. In the last term, confounding literally means 'mixing together', and in explanatory bacteremia studies, this implies that the effect of the exposure under study (e.g. age) is mixed with or masked by the effect of another factor (e.g. comorbidity) on the outcome of bacteremia. To act as a confounder in a study of mortality in patients with bacteremia, a factor must 1) in itself be a risk factor for mortality, 2) be unevenly distributed between the comparison groups, and 3) not be a consequence of the infection[174]. As shown in Table 4, a number of tools are available to deal with bias at the design and analytical stages.

Table 4. Tools to minimize types of bias[174;185;186]

Type of bias	Tools
Selection bias (at the design stage only)	Selecting only incident cases Restricting identification of incident cases to a given geographical area to reduce referral bias Minimizing the number of patients lost to follow-up Implementing a procedure to track those who drop out
Information bias (at the design stage only)	Standardizing the measurement process Using objective, previously defined criteria for defining exposure and disease
Confounding bias (at the design or analytical stage)	Randomization Matching Exclusion Restriction Standardization Stratification Multivariate analysis and modeling

Conclusion

There is little data regarding the accuracy of the first notification of a positive blood culture given to the attending physician, even though it is standard practice to use this notification to guide treatment. A few studies have compared mortality in bacteremic patients with mortality in patients without bacteremia, but none have distinguished between community-acquired bacteremia and nosocomial bacteremia. Among patients with community-acquired bacteremia, several aspects of the association between age, comorbidity, and outcome of bacteremia are poorly understood; in particular, it has not been clear whether age-related levels of comorbidity can explain the higher mortality in older patients. Use of medical databases, which offer the opportunity for large-scale, population-based research using archived data, may help overcome some of the limitations of the published studies[187]. In this thesis, we focused on medical patients with community-acquired bacteremia because we wanted a more homogeneous study population; we also wanted to restrict confounding from underlying disorders and interventions[131].

2 Aims of the thesis

1. To evaluate the accuracy of preliminary blood culture reports based on Gram stain and wet-mount microscopy (**Study I**).

2. To examine the prognostic impact of positive blood cultures on early mortality (within 3-7 days), short-term mortality (days 8-30), and long-term mortality (days 31-180) in medical patients with blood cultures taken within the first two days of admission (**Study II**).

3. To examine (i) the association between increasing age and mortality in patients with community-acquired bacteremia; (ii) whether the level of comorbidity has an impact on mortality adjusted for age; and (iii) the association between age and mortality in bacteremia patients with increasing levels of comorbidity (**Study III**).

3 Materials and Methods

3.1 Study design

Study I

To evaluate the accuracy of preliminary blood culture reports, we conducted a cross-sectional study using blood culture data from the years 1996, 2000, 2001, and 2003. We restricted the study to blood cultures with one morphological type; for patients with bacteremia, only the first positive blood culture was included. By excluding repeated positive blood cultures in patients with bacteremia and Gram stain reports with more than one morphological type, our study focused on those Gram stain reports most likely to influence clinical decision making.

Studies II and III

Studies II and **III** were conducted as cohort studies within an open population. The study outcome was all-cause mortality, whose associations with study exposures were estimated by mortality rate ratios (MRR) obtained by comparing mortality in exposed and unexposed patients. In **study II**, the exposed group consisted of patients with community-acquired bacteremia, while the unexposed (reference) group consisted of patients with negative blood cultures. In **study III**, the exposed groups consisted of patients older than 64 years of age (subdivided into age groups of 65-79 years and ≥80 years) or with medium or high levels of comorbidity. The unexposed groups consisted of patients in the youngest age group (15-64) and/or patients with a low level of comorbidity. For further details, see Table 5.

3.2 Setting

All three studies were conducted in the region formerly known as North Jutland County, Denmark, which had approximately 500,000 inhabitants during the study periods (mean population 1995: 488,303; 2006: 495,090). The population is primarily Caucasian, and there is a mix of rural and urban areas. As in all of Denmark, the entire population in the county was provided with free, tax-supported health care by the National Health Service, allowing free access to the county's seven public hospitals. All patients hospitalized with acute conditions are treated in these public hospitals, one of which (Aalborg Hospital) serves as both a district hospital for the greater Aalborg area (~200,000 inhabitants) and as a referral hospital.

3.3 Data sources

Below is a detailed description of the data sources used in this thesis.

The Civil Registration System

Since 1968, all residents in Denmark have been registered in the Civil Registration System and given a unique number which is used in all national registries to identify that person[188;189]. This allows accurate linkage among Danish registries (Figure 10). For **studies II** and **III**, we also obtained information from the Civil Registration System regarding marital status (married, never married, divorced or widowed), vital status (dead or alive), date of death, and residence of the study population members.

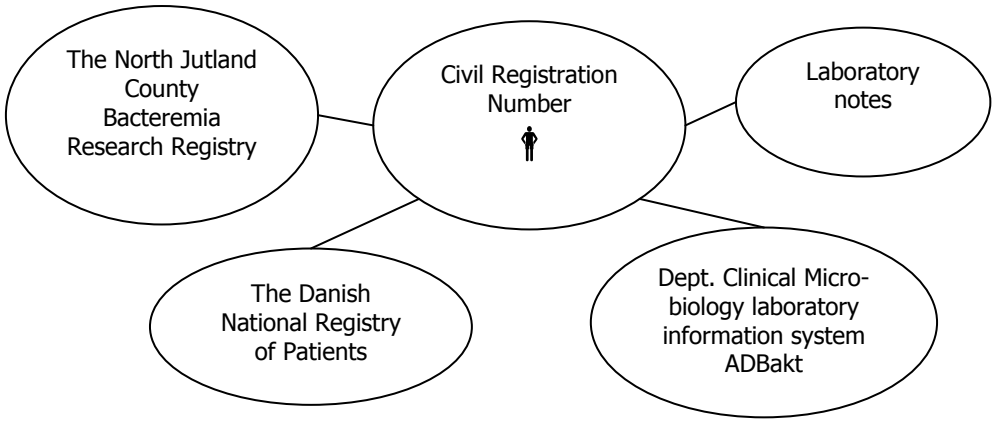


Figure 10. Data sources for **studies I-III**

The North Jutland County Bacteremia Research Registry

Since 1981, all episodes of bacteremia in North Jutland County have been registered in the microbiologic County Bacteremia Research Registry[37;104;189]. This registry is maintained by the Department of Clinical Microbiology at the Aalborg Hospital, which provides bacteriological services, including blood cultures, for the entire county. When a blood culture is positive, the physician on call at the Department of Clinical Microbiology notifies the attending physician by telephone, and both physicians jointly assess the relevance to the patient based on the general condition, underlying comorbidity, portals of entry, the likely focus of infection, and appropriateness of ongoing antibiotic treatment (if any). Since 1992, the clinical information and advice given has been routinely registered on a paper form concurrently with the clinical episode as part of standard working practice in the department. On this paper form, follow-up contacts are also recorded. These forms are the basis for registration of bacteremia in the registry. The registry contains the following data: date of venipuncture, bacterial isolates and susceptibility patterns, patient age, gender, date of admission, presumed focus of infection, empirical antibiotic treatment, and civil registration number.

The Danish National Registry of Patients

The Danish National Registry of Patients is an administrative public registry. It includes data for over 99.5% of the non-psychiatric hospitalizations in Denmark since January 1, 1977[189;190]. Since 1995, outpatient data have been included in the registry as well. The recorded information includes the patient's civil registration number, the dates of admission and discharge, the surgical procedure(s) performed, and up to 20 physician-given discharge diagnoses, classified according to the Danish version of the International Classification of Diseases (ICD-8 before 1994 and ICD-10 after 1994; ICD-9 was never used in Denmark).

The microbiological laboratory information system

We obtained data on contaminated (**study I**) and negative (**study II**) blood cultures from the electronic laboratory information system (ADBakt, Autonik, Ramsta, Sköldinge, Sweden) at the Department of Clinical Microbiology, Aalborg Hospital. Information included the patient's civil registration number, the date that the cultures were taken, the length of incubation, and the species and results of antibiotic susceptibility testing. For blood culture isolates submitted to the national reference laboratory (Statens Serum Institut) for national surveillance schemes, the database was updated with typing results.

Review of laboratory notes

In **study I**, we abstracted the Gram stain result, bacterial motility, and species diagnosis from the technician's notes. Independent of the primary investigator, the data were tabulated by one of the co-authors (HCS) and by a technician in the Department of Clinical Microbiology. In cases with inconclusive reports, missing data, or disagreement with the results of cultural identification, the primary investigator (MS) reviewed the laboratory notes.

3.4 Definition of study population, exposure, and outcomes in studies II and III

Table 5 gives an overview of the design of **studies II** and **III**. The classifications of the variables are described in detail below. The study populations included all adult (≥15 years old) medical patients who had one or more blood cultures taken within the first two days of hospital admission and who had no previous blood cultures (**study II**), or who were diagnosed with their first episode of community-acquired bacteremia during the study period (**study III**).

The term medical patients refers to patients admitted to the Departments of Internal Medicine and to the allied specialty departments. Aalborg Hospital has 7 departments of internal medicine with the following specialties: endocrinology, gastroenterology, geriatrics, hematology, infectious diseases, nephrology, and pulmonary diseases. The 4 allied specialty departments include the Departments of Cardiology, Medical Oncology, Neurology, and Rheumatology. Patients with acute cardiac or cerebrovascular disease are admitted directly to the appropriate

departments. Other acute patients are normally admitted to an acute care medical unit; transfer to an intensive care unit (ICU) may take place subsequently. Each district hospital has a Department of Medicine, and there is access to an ICU at two of the hospitals.

Table 5. Design of the cohort studies, studies II and III.

Study	Period	Source population	Exposure	Outcome(s)
II	1995-2006	Medical patients ≥ 15 years who had blood cultures during the first 2 days of admission	Positive blood culture	3-7, 8-30, and 31-180 day mortality
III	1995-2004	All medical patients ≥ 15 years with a first episode of community-acquired bacteremia	Age and comorbidity	7-day and 30-day mortality

Blood cultures

Two different blood culture systems were used during the study periods. In 1995, the Colorbact system (Statens Serum Institut, Copenhagen, Denmark) was used. In this system, blood is sampled directly into two aerobic and one anaerobic blood culture bottles containing culture broth[191]. Starting in 1996, the BacT/Alert system (bioMerieux, Marcy l'Etoile, France) was used; in this system, blood is also sampled directly into two standard aerobic (SA) bottles and one standard anaerobic (SN) bottle. In 1999, one of the SA bottles was replaced by a bottle with the BacT/Alert FAN blood culture medium in order to enhance recovery of fastidious organisms[192]. The presence of activated charcoal in the FAN bottle may hamper microscopy examination, especially for Gram stain, and the use of two kinds of media was considered advantageous. For adult patients, the nominal volume per blood culture was 20-22 ml for the Colorbact system and 28-32 ml for the BacT/Alert system. Volume information was obtained by periodic weighing of blood culture bottles upon receipt (H.C. Schønheyder, personal communication). At least 30 ml of sample volume is generally recommended for blood culture[69;70;86]; the current practice in our Department, i.e. use of three broth culture bottles per set, is intended to achieve this volume for all adult patients.[193]. Compared to the standard practice of obtaining two blood culture sets, this obviates the need for two venipunctures and thus facilitates patient management. With two bottles per set, the sample volume may be critically low if the second set is not drawn due to logistic or technical difficulties or because instituting antibiotic treatment is given priority[3]. The drawback is that the significance of some opportunistic pathogens cannot be confirmed by an independent blood sample.

Blood culture bottles with a positive growth index were unloaded at fixed times between 8 a.m. and 8:30 p.m. and examined immediately by a technician. Technicians with less than two years of experience were supervised by more experienced colleagues. The compound microscopes were equipped with 100x achromatic oil objectives, which are also suited for phase-contrast microscopy, and Koehler illumination was checked daily. Wet-mount preparations were immediately examined by phase-contrast microscopy, and smears for Gram staining were fixed

by flame fixation and stained using acetone for decolorization and safranin as a counterstain. The motility (wet-mount), Gram stain reaction, morphology, and bacterial arrangement were recorded in a laboratory note. Positive blood cultures were subcultured onto plate media selected according to the Gram stain result, and isolates were routinely identified by a combination of conventional and commercial methods according to the most recent versions of Danish reference documents[194;195] and the Manual of Clinical Microbiology[117]. On the basis of the microscopy results, a first notification was made by telephone to the attending physicians, and the antibiotic treatment was adjusted if it was inappropriate. As soon as a tentative or, sometimes, definitive species diagnosis and antibiotic susceptibility pattern were obtained, a second notification was made either to confirm or adjust antibiotic treatment. No written preliminary report was issued, and the definitive report was normally issued when the growth status for all three bottles was obtained.
Negative blood cultures were incubated for a total of 6.7 days, after which a written report was sent to the attending physicians.

Bacteremia

We identified all adult county residents who had their first episode of bacteremia recorded in the North Jutland County Bacteremia Research Registry from January 1, 1995 to December 31, 2006 (or 2004 in **study III**). Coagulase-negative staphylococci, *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* were regarded as contaminants unless they were isolated from two or more separate blood culture sets[2]. Exceptions to this rule were made if an identical isolate was obtained from another relevant specimen, e.g. from an intravenous catheter[196;197]. We classified the bacteremias as community-acquired, nosocomial, and care-related as described on page 6-7. In **study I**, we excluded patients with polymicrobial bacteremia, defined as blood cultures with more than one pathogen isolated within 24 hours[2].

The bacteremia episodes were further classified according to the isolated pathogen. We categorized the bacteremias as follows: Gram-positive, Gram-negative, and polymicrobial or fungemia (studies II and III). In study I, we further subdivided the bacterial pathogens into 6 groups according to Gram stain characteristics and morphology: Gram-positive cocci in clusters, Gram-positive cocci in chains or diplococci, Gram-positive rods, Gram-negative cocci, Gram-negative rods, and yeasts. Bacterial motility was classified as peritrichous, polar, or non-motile.

The focus of infection was defined as the organ or tissue infected at the time when the bacteremia became clinically apparent and a blood culture was drawn[198]. Determining the focus of infection was based on all information obtained during admission from microbiological samples, clinical findings, and imaging. In studies II and III, we categorized the focus as urinary, respiratory, abdominal or hepatobiliary, miscellaneous (central nervous system, muscles, skin, joint and bones, genital system, and intravascular devices), or unknown.

Exposures

As noted earlier, in **study II** exposure was defined as a positive blood culture within the first two days of hospital admission. We used this two-day time window to identify the source population at risk of community-acquired bacteremia. In **study III**, exposures were age and level of comorbidity (see below).

Comorbidity

In **study III**, comorbidity was the exposure under study, and in **study II**, comorbidity was a potential confounder of the main association under study (i.e. blood culture result and mortality). We classified comorbidity according to the Charlson Comorbidity Index[145;149]. We calculated the score based on all previous discharge diagnoses recorded in the Hospital Discharge Registry before the date of admission. We defined three levels of comorbidity on the basis of the Charlson index scores: 0 ("low"), corresponding to patients with no recorded underlying diseases according to the Charlson index; 1-2 ("medium"); and >2 ("high").

Outcome

The outcome in prognostic **studies II** and **III** were mortality rate and cumulative all-cause mortality after 7, 30, or 180 days of follow-up. Follow-up started on the third day of admission in study II and on the date of the first positive blood culture in study III. We did not attempt to determine the cause of death (e.g. the fraction of deaths attributable to bacteremia). However, because we used patients with negative blood cultures as a reference population in study II, we surmise that the MRRs in that study reflect the impact of bacteremia on mortality.

3.5 Definition of other variables

Empirical antibiotic treatment

In **study III**, we adjusted the analyses for appropriateness of empirical antibiotic therapy administered at first notification of the positive blood culture in the Bacteremia Registry. Therapy was regarded as appropriate if it was given intravenously (with the exception of fluoroquinolones and metronidazole) and if the blood isolate(s) were susceptible to one or more of the antibiotic drugs. If isolates were found to be resistant, or if the doses or the form of administration were insufficient, the empirical treatment was considered inappropriate[41]. In some cases the patient had already died at the time of first notification, or the treatment was completed or had ceased because the patient was terminally ill and a decision had been made to withhold therapy.

Marital status

Through the civil registration number we obtained information about marital status (married, never married, divorced or widowed) on the date of the first positive blood culture. In studies II and III, we used marital status to control for differences in social status. More elaborate

measures of socioeconomic status have been used in other studies. However, studies have shown that the risk of mortality for individuals who are widowed, divorced, or single is 1.2- to 2.5-fold higher than for those who are married[199-201]; for infections, Martin et al[202] reported that the odds of hospitalization with *Salmonella typhimurium* were 8.1 (95% CI 2.1-33) times higher for persons living alone. The beneficial effect of marriage is probably explained by the higher socioeconomic status and healthier lifestyle it confers.

3.6 Statistical analyses

In **study I**, we evaluated the accuracy of Gram-staining and wet-mount microscopy using the cultural identification results as a reference standard. According to Gram stain characteristics and morphology, we defined 6 morphologic groups: Gram-positive cocci in clusters, Gram-positive cocci in chains or diplococci, Gram-positive rods, Gram-negative cocci, Gram-negative rods, and yeasts. Bacterial motility was classified as peritrichous, polar, or non-motile. For each group we estimated the performance characteristic of Gram staining (sensitivity, specificity, positive predictive values (PPV), and negative (NPV) predictive values)[132] (Table 6).

Table 6. A two by two table illustrating evaluation of the Gram staining performance characteristics using Gram-negative rods as an example.

		Cultural identification: Gram-negative rods	
		Yes	No
Gram stain result: Gram-negative rods	Yes	True positive	False positive
	No	False negative	True negative

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{true positives + false negatives}}$$

$$\text{Specificity} = \frac{\text{true negatives}}{\text{true negatives + false positives}}$$

$$\text{PPV} = \frac{\text{true positives}}{\text{true positives + false positives}}$$

$$\text{NPV} = \frac{\text{true negatives}}{\text{true negatives + false negatives}}$$

To quantify the maximum impact of a potential selection bias due to missing data, we repeated the analyses with the assumption that all missing data was incorrect. We further evaluated the Gram stain and wet-mount results for the predominant pathogens at the species level. Bacterial motility was assessed for the most frequent motile Gram-negative species.
We estimated 95% CIs as exact binomial confidence intervals (the Clopper-Pearson interval).

In **study II**, the exposure definition of patients with community-acquired bacteremia, was made over the first two days of hospital admission; thus, follow-up started on the third day of admission and extended for 180 days, until death or migration, whichever came first[181]. To compare mortality in patients with positive and negative blood cultures over different time periods, we categorized the follow-up time into three intervals: early mortality (within the first 3 to 7 days), short-term mortality (days 8 to 30), and long-term mortality (31 to 180 days). Kaplan-Meier curves were calculated for these outcomes. To compare the risk of death between patients with at least one positive blood culture obtained within the first two days of admission and patients with negative cultures, we then used Cox regression analysis to compute crude and adjusted MRRs for each time period with 95% CIs. Study period (1995-1998, 1999-2002, 2003-2006), age at the date of blood culture (15-39, 40-59, 60-79, 80 years and older), gender, marital status (married, never married, divorced or widowed), and level of comorbidity were considered as potential confounders, and all models included adjustments for these variables. We were concerned that the indications for blood culture might vary among patients and that some cultures might be taken to rule out bacteremia, so we conducted an analysis that was restricted to patients with a primary or secondary discharge diagnosis of infectious disease (ICD-10 codes A00-B99, G00-G02, I32, I33, I41, J00-J06, J10-J18, J20-J22, J36-37, J85-J86, K65, L00-L03, L080, L088-L080, M00-M01, N10, N12, N30, N39.0). We also conducted a Cox regression analysis with categorization of bacteremia into different types (Gram-positive, Gram-negative, and polymicrobial or fungemia). We further stratified the analysis by comorbidity level.

In **study III**, follow-up began on the date that the patient's first positive blood culture was drawn, and the patients were followed until death, migration or for 30 days, whichever came first. We calculated Kaplan-Meier survival curves and product limit estimates of 7- and 30-day mortality according to the main study variables: age group (15-64 years, 65-79 years, and ≥80 years), gender, marital status, level of comorbidity (according to Charlson score categories), type and focus of bacteremia, and whether the initial antibiotic treatment was appropriate. Spline regression is an alternative to using categorized variables[203], and we used quadratic splines to smooth the crude 7- and 30-day mortality curves with linear restrictions imposed on both tails in order to assess graphically the relationships between age or comorbidity and bacteremia mortality. To compare the risk of death in the different age groups and levels of comorbidity, we used Cox proportional hazards analysis to compute the MMRs for 7- and 30-day mortality with 95% CIs, controlling for gender, marital status, type of bacteremia, focus of infection, and whether or not the initial antibiotic treatment was appropriate. Comorbidity and age were included in the Cox model as continuous variables when examining the impact of age and comorbidity, respectively. To assess the impact of age at different levels of comorbidity, we chose bacteremia patients in the youngest age group with low comorbidity as our reference group, against which we compared the mortality rates. For each of the remaining combinations

of age and comorbidity, we created a binary variable that indicated age group and level of comorbidity which we entered into the Cox regression model[204].

We assessed the assumption of proportional hazards in the Cox regression model using log(-log(survival)) plots as well as by goodness-of-fit testing on the basis of Schoenfeld residuals. All estimates were obtained with corresponding 95% CIs. Analyses were performed using Stata Statistical Software v. 9.0 (Stata Corp., College Station, TX, USA). The studies were approved by the Danish Data Protection Agency (Record no. 2006-41-7413).

4 Results

The main results of the three studies are summarized below.

4.1 Study I

Among 6461 positive blood cultures obtained during the four selected study years, we excluded 438 (7%) polymicrobial cultures and 130 (2%) records lacking information on either bacterial morphology or the Gram stain reaction. Thus, our study sample constituted 5893 blood cultures, of which 1985 (34%) were contaminated. Sensitivity, specificity, and PPV and NPV of the Gram stain are shown in Table 7. Assuming that all 130 excluded records were false negatives and following the overall distribution would change the sensitivities as follows: Gram-positive cocci in clusters 95.5% (95% CI 96.8-98.2), Gram-positive cocci in chains/diplococci 94.8% (95% CI 93.1-96.2), Gram-negative cocci 89.7% (95% CI 75.8-97.1), Gram-positive rods 89.3% (95% CI 86.6-91.6), Gram-negative rods 96.6% (95% CI 95.7-97.3), and yeasts 95.7% (95% CI 89.5-98.8).

Table 7. Performance characteristics of the Gram stain with culture-based identification as a reference

	Gram-positive cocci, clusters	Gram-positive cocci, chains/ diplococci	Gram-negative cocci	Gram-positive rods	Gram-negative rods	Yeasts
Total	2107	833	38	620	2203	92
No. correct / No. observed	2101/2129	807/818	35/37	566/584	2175/2217	90/90
Sensitivity	99.7 (99.4-99.9)	96.8 (95.4-97.8)	92.1 (78.6-98.3)	91.3 (88.8-93.4)	98.7 (98.2-99.2)	97.8 (92.4-99.7)
Specificity	99.3 (98.9-99.5)	99.8 (99.6-99.9)	100 (99.9-100)	99.7 (99.5-99.8)	98.9 (98.5-99.2)	100 (99.9-100)
PPV	98.7 (98.1-99.2)	98.7 (97.6-99.3)	94.6 (81.8-99.3)	96.9 (95.2-98.2)	98.1 (97.5-98.6)	100 (96.0-100)
NPV	99.8 (99.7-99.9)	99.5 (99.3-99.7)	100 (99.9-100)	99.0 (98.7-99.2)	99.2 (98.9-99.5)	100 (99.9-100)

The comparatively low sensitivity for Gram-positive rods (91.3%, 95% CI 88.8-93.4) was mainly due to *Bacillus* spp. and *Clostridium* spp. Nearly half of the *Bacillus* isolates (45.3%) were recorded as Gram-negative rods, corresponding to a sensitivity of 48.4% (95% CI 35.8-61.3). Likewise, 7 of 35 *Clostridium* spp. were reported as Gram-negative rods (n=6) or as a mixture of Gram-negative and Gram-positive rods (n=1). The sensitivity of the preliminary diagnosis for *Clostridium* spp. was 80% (95% CI 63.1-91.6). The sensitivity was close to 100% for all

predominant bacterial pathogens with non-hemolytic streptococci being the only distinctive exception (sensitivity 91.3%, 95% CI 86.2-94.9).
Overall, the sensitivity of the wet-mount report varied from 30% to 70% for bacterial species with peritrichous motility (Table 8). A total of 100 bacteria displayed polar motility, of which one quarter were enterobacteria (with the major serovar being the *Salmonella* serovar).

Table 8. The motility patterns of the predominant motile Gram-negative bacteria as assessed by wet-mount microscopy.

		Motility			
Species	n	Not-motile (%)	Peritrichous (%)	Polar (%)	Not stated (%)
Enterobacteria					
E. coli	1263	651 (50.8)	602 (47.0)	6 (0.5)	23 (1.7)
Citrobacter spp.	23	11 (47.8)	9 (39.1)	0	3 (13.1)
Enterobacter spp.	111	25 (22.5)	69 (62.2)	3 (2.7)	14 (12.6)
Morganella morganii	20	3 (15.0)	14 (70.0)	0	3 (15.0)
Proteus spp.	72	19 (26.4)	37 (51.4)	4 (5.6)	12 (16.6)
Serratia marcescens	24	10 (41.7)	10 (41.7)	0	4 (16.6)
Salmonella serovar	42	5 (11.9)	23 (54.8)	11 (26.2)	3 (7.3)
Other enterobacteria[a]	7	4 (57.1)	2 (28.6)	0	1 (14.3)
Pseudomonas aeruginosa	118	36 (30.5)	10 (8.5)	62 (52.5)	10 (8.6)

4.2 Study II

During the period from January 1, 1995 to December 31, 2006, we identified 179,917 patients admitted to medical departments of whom 35,673 had at least one blood culture taken within the first two days of admission. After exclusion of patients hospitalized within the preceding 30 days (n=6084), patients with non-community-acquired bacteremia (n=316), and patients dying before the third day of admission (n=663), our study population included 28,610 medical patients. Of these patients, 2,520 (8.8%) had positive blood cultures.

Patients with positive blood cultures were older (median age 72 years, IQR 59-81 years) than patients with negative blood cultures (median age 68 years, IQR 50-79 years), and 53.0% had medium or high comorbidity index scores compared with 49.8% among patients with negative cultures. In the 2,520 patients with positive blood cultures, 1,091 (43.3%) had Gram-positive bacteremia, 1,281 (50.8%) Gram-negative, and 148 (5.9%) polymicrobial bacteremia or fungemia (there were only 3 cultures with fungemias).

Figure 11 shows mortality curves displaying 180 days of follow-up from the third day of admission, stratified according to blood culture result and type of bacteremia. The 3-7 day mortality was 3.7% in patients with negative cultures and 5.1% in patients with community-acquired bacteremia, resulting in an adjusted MRR of 1.2 (95% CI 1.0-1.4) (Table 9). The

highest mortality rates were observed among patients with polymicrobial bacteremia or fungemia (3- to 7-day mortality = 10.1%), followed by patients with Gram-positive bacteremia (3- to 7-day mortality = 5.8%). Compared to patients with negative cultures, the adjusted MRR was 2.0 (95% CI 1.2-3.4) for patients with polymicrobial bacteremia or fungemia, 1.5 (95% CI 1.2-1.9) for patients with Gram-positive bacteremia, and 0.8 (95% CI 0.6-1.1) for patients with Gram-negative bacteremia (Table 9).

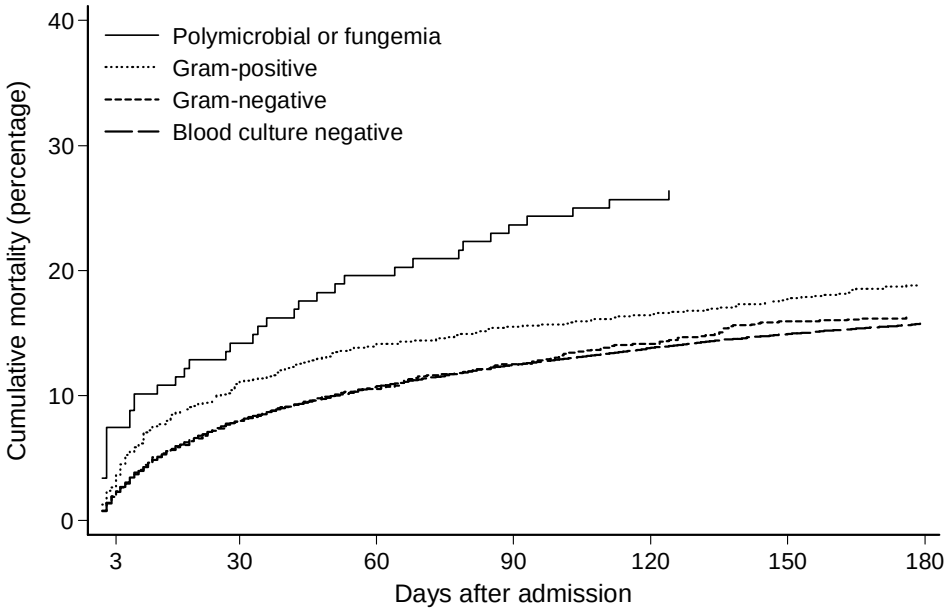


Figure 11. Mortality curves stratified according to blood culture result and type of bacteremia.

Death within 8-30 days after admission occurred in 4.5% of patients with negative cultures vs. 4.9% of patients with community-acquired bacteremia. The adjusted MRR was 0.9 (95% CI 0.8-1.1) for patients with bacteremia compared with patients with negative blood cultures. In patients with Gram-positive bacteremia, mortality was 20% higher than in patients with negative cultures (adj. MRR = 1.2, 95% CI 0.9-1.6), whereas mortality in patients with Gram-negative bacteremia and patients with polymicrobial bacteremia or fungemia was slightly lower than in patients with negative cultures (Table 9).

We extended follow-up to day 180 after admission for patients who were alive on day 30. During days 31-180, 9.3% of patients with community-acquired bacteremia died compared with 8.4% of the patients with negative blood cultures (Table 9). Adjusted MRR for days 31-180 days for patients with community-acquired bacteremia was 0.9 (95% CI 0.8-1.1) compared with culture-negative patients. Only polymicrobial bacteremia or fungemia was associated with increased long-term mortality (adj. MRR 1.4, 95% CI 0.9-2.2). Differences in comorbidity did not have a major impact on the MRR estimates (not shown).

Table 9. Crude and adjusted risk of death within 3-7, 8-30, and 31-180 days of admission among medical patients with one or more blood cultures taken within the first two days of hospital admission.

Blood culture result	3-7 days after admission			8-30 days after admission **			31-180 days after admission ***		
	7-day mortality (95% CI)	Crude MRR (95% CI)	Adj.* MRR (95% CI)	30-day mortality (95% CI)	Crude MRR (95% CI)	Adj. MRR (95% CI)	180-day mortality (95% CI)	Crude MRR (95% CI)	Adj. MRR (95% CI)
Negative	3.7 (3.5-3.9)	1.0 (ref)	1.0 (ref)	4.5 (4.3-4.8)	1.0 (ref)	1.0 (ref)	8.4 (8.1-8.8)	1.0 (ref)	1.0 (ref)
Positive	5.1 (4.3-6.0)	1.4 (1.1-1.6)	1.2 (1.0-1.4)	4.9 (4.1-5.8)	1.1 (0.9-1.3)	0.9 (0.8-1.1)	9.3 (8.2-10.5)	1.1 (1.0-1.3)	0.9 (0.8-1.1)
Gram-positive	5.8 (4.6-7.4)	1.6 (1.2-2.0)	1.5 (1.2-1.9)	5.6 (4.3-7.1)	1.2 (0.9-1.6)	1.2 (0.9-1.5)	8.9 (7.2-10.8)	1.1 (0.9-1.3)	1.0 (0.8-1.3)
Gram-negative	3.8 (2.9-5.0)	1.0 (0.8-1.3)	0.8 (0.6-1.1)	4.3 (3.3-5.6)	1.0 (0.7-1.3)	0.8 (0.6-1.0)	8.2 (6.7-10.1)	1.1 (0.9-1.3)	0.9 (0.7-1.0)
Polymicrobial or fungemia	10.1 (6.2-16.3)	2.7 (1.6-4.4)	2.0 (1.2-3.4)	4.5 (2.1-9.8)	1.0 (0.5-2.3)	0.8 (0.3-1.7)	14.2 (9.2-21.6)	1.8 (1.1-2.9)	1.4 (0.9-2.2)

* Adjusted for age, gender, level of comorbidity, marital status, and calendar period. ** For patients alive at day 8. *** For patients alive at day 31.

4.3 Study III

We identified 2,851 patients with community-acquired bacteremia (median age 74 years, IQR 61-82 years). The prevalence of patients with a medium or high level of comorbidity was similar in the two oldest age groups (69% and 65%, respectively) but considerably higher than in the reference group of patients aged 15-64 years (44%).

Seven-day mortality increased with age (from 8% among patients younger than 65 years to 14% for patients 80 years and older) and with level of comorbidity (from 7% among patients with a low level to 15% among patients with a high level of comorbidity) (Table 10). The corresponding MRRs are shown in Table 10.

Table 10. Crude and adjusted risk of death within 7 or 30 days in patients with a first-time admission for community-acquired bacteremia according to age and level of comorbidity.

				Mortality rate ratio (MRR)	
Prognostic factor	n	Dead, n	Mortality (95% CI)	Crude (95% CI)	Adjusted* (95% CI)
Age					
7-day					
15-64	851	65	8% (6-10)	1.0 (reference)	1.0 (reference)
65-79	1091	112	10% (9-12)	1.4 (1.0-1.8)	1.4 (1.0-2.0)
≥80	909	125	14% (12-16)	1.8 (1.4-2.5)	1.6 (1.1-2.2)
30-day					
15-64	851	95	11% (9-13)	1.0 (reference)	1.0 (reference)
65-79	1091	179	16% (14-19)	1.5 (1.2-1.9)	1.5 (1.2-2.0)
≥80	909	192	21% (19-24)	2.0 (1.6-2.5)	1.8 (1.4-2.3)
Level of comorbidity					
7-day					
Low (0)	1134	82	7% (6-9)	1.0 (reference)	1.0 (reference)
Medium (1-2)	1122	129	12% (10-14)	1.6 (1.2-2.1)	1.4 (1.0-1.8)
High (>2)	595	91	15% (13-18)	2.2 (1.6-2.9)	1.5 (1.1-2.0)
30-day					
Low (0)	1134	127	11% (10-13)	1.0 (reference)	1.0 (reference)
Medium (1-2)	1122	200	18% (16-20)	1.6 (1.3-2.1)	1.5 (1.2-1.8)
High (>2)	595	139	23% (20-27)	2.2 (1.7-2.8)	1.7 (1.4-2.2)

*Adjusted for gender, marital status, type of bacteremia, focus of infection, and appropriateness of empirical antibiotic treatment.

After 30-days of follow-up, mortality increased from 11% among patients younger than 65 years to 21% for patients 80 years and older and from 11% in patients with low comorbidity to 23% in patients with high comorbidity (Table 10). The corresponding 30-day MRR estimates are shown in Table 10. The smoothed age-mortality curve indicated that both 7- and 30-day mortality increased linearly except for a plateau between 50 and 65 years of age (Figure 12). We also found an almost linear increase in 7- and 30-day mortality with increasing levels of comorbidity.

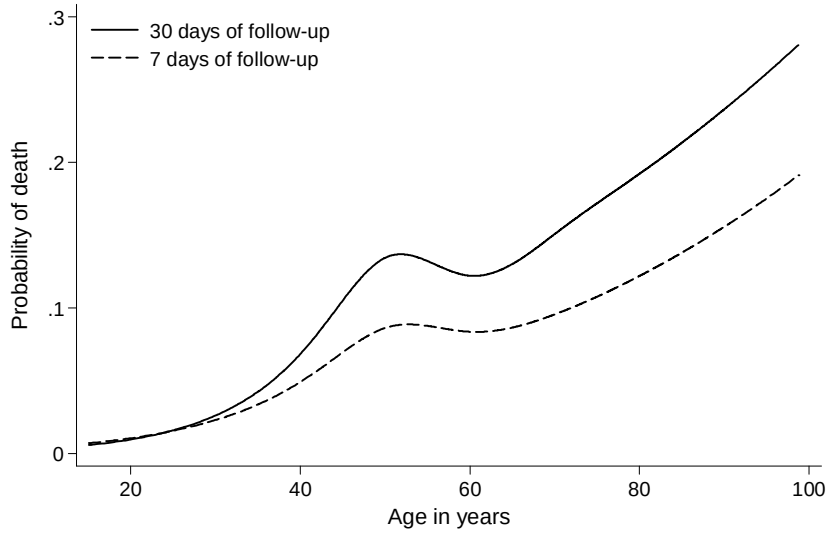


Figure 12. Estimated 7- and 30-day mortality from community-acquired bacteremia related to age.

The combined effects of age and comorbidity are shown in Table 11, with patients in the youngest age group and with a low level of comorbidity serving as the reference. Judging from these data, there is no synergistic effect between age and comorbidity, i.e. the joint effects of age and comorbidity does not exceed the sum of their individual effects on mortality[131].

Table 11. Crude and adjusted risk of death within 30 days for patients with a first-time admission for community-acquired bacteremia according to age group and level of comorbidity.

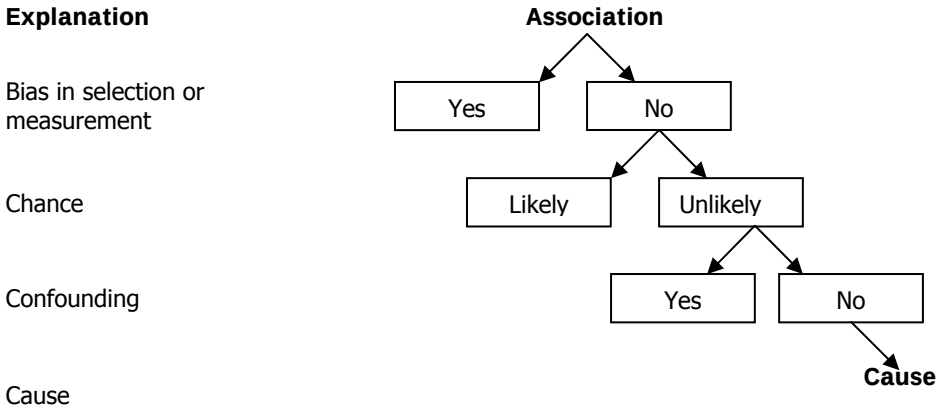
	Charlson Comorbidity Index		
Age group	Low (0)	Medium (1-2)	High (>2)
15-64			
n	477	259	115
Dead, n	35	39	21
Mortality, %	7.3	15.1	18.3
MRR (95% CI)			
Crude	1.0 (reference)	2.1 (1.4-3.4)	2.6 (1.5-4.5)
Adjusted*	1.0 (reference)	2.0 (1.2-3.2)	2.2 (1.3-3.9)
65-79 years			
n	335	482	274
Dead, n	39	78	62
Mortality, %	11.6	16.2	22.6
MRR (95% CI)			
Crude	1.6 (1.0-2.6)	2.3 (1.5-3.4)	3.3 (2.2-5.0)
Adjusted*	1.9 (1.2-3.1)	2.4 (1.6-3.6)	3.8 (2.4-5.8)
≥80			
n	322	381	206
Dead, n	53	83	56
Mortality, %	16.5	21.8	27.2
MRR (95% CI)			
Crude	2.3 (1.5-3.6)	3.2 (2.1-4.7)	4.1 (2.7-6.3)
Adjusted*	2.3 (1.4-3.6)	3.3 (2.2-5.1)	3.3 (2.1-5.2)

Note: reference group = patients in the youngest age group and with low comorbidity.
*Adjusted for gender, marital status, type of bacteremia, focus of infection, and appropriateness of empirical antibiotic treatment.

5 Methodological considerations

As in all observational studies, systematic errors due to the lack of randomization may affect the validity of our findings. We must therefore critically evaluate alternatives to causal interpretation before interpreting the findings as evidence of causality[132]. Specifically, we need to consider how problems in selection and information, confounding factors, and statistical imprecision may have influenced our estimates (Figure 13)[132].

Figure 13. Association and cause [132].



5.1 Study I

We assessed the accuracy of preliminary blood culture reports based on Gram stain and wet mount microscopy findings using cultural identification as the reference standard. Our study was conducted in a clinical setting using a sample of positive blood cultures covering the spectrum of bacterial and fungal morphotypes likely to be encountered when using the Gram stain. Some factors may have affected the validity of our findings.

First, when evaluating the culture-based identification, the technicians were not blinded to the Gram stain and wet-mount microscopy results, which may have led us to overestimate the performance characteristics of Gram stain and wet-mount microscopy[122;123;205]. However, as a new Gram stain is performed when there are discrepancies between cultural identification and the initial Gram stain findings, we consider this a rather theoretical source of error. Second, 2% of the blood culture records were excluded because we lacked information on either Gram stain or morphology results. This may have been due to problems with the interpretation of the smears, and could also result in an overestimation of the accuracy of the Gram stain results. However, even if all the excluded blood cultures were classified incorrectly by Gram stain (as a worst case scenario), the sensitivity would still be around or above 90%. Third, we chose to include more than one contaminated blood culture per patient because subsequent cultures are evaluated *de novo* with no prejudice from previous findings. This led, however, to a preponderance of staphylococci, which had the best performance characteristics. Finally,

because of the retrospective nature of the study, there was no way to systematically determine whether the few observed discrepancies were due mainly to interpretive or technical errors.

5.2 Studies II and III

Selection bias

Selection bias can arise in cohort studies from the method of selecting study participants or from factors affecting study participation. As a result, the association between exposure and disease may differ between participants and non-participants in the study[131]. In our **studies II** and **III**, we used the Civil Registration System to obtain data on vital status and as this registry is almost complete, our loss to follow-up was negligible[188].
Since we used different source populations in the studies, we will discuss selection of each study population separately.

The study population of **study II** consisted of adult patients (≥ 15 years) with one or more blood cultures taken during the first two days of admission to a medical department and no previous blood cultures. Selection into this cohort thus depended on the indications for having a blood culture taken. Thus, in a clinical setting with a low threshold for taking blood cultures, a relatively higher proportion of the patients would have negative blood cultures compared with a clinical setting with more rigorous guidelines. This may have influenced the relative mortality estimates.

Study III was a cohort of all medical patients who were hospitalized for the first time with community-acquired bacteremia in North Jutland County, 1995-2004. Detection of community-acquired bacteremia depends on admission patterns and the timing of blood cultures. Selection bias could arise if the indications for taking a blood culture differed according to age and level of comorbidity. Patients with comorbidity are probably seeking health care more often than patients without comorbidity. If their physicians are more alert to early signs of infection, then milder cases of bacteremia may be diagnosed among patients with comorbidities. This would lead to an underestimation of the relative mortality among patients with and without comorbidities. Conversely, we may have missed some cases of bacteremia if the patients died before blood cultures were taken. If this occurred to a greater extent to older patients or to patients with a higher level of comorbidity, mortality may have been underestimated in these groups. This would lead to more conservative relative mortality estimates. Because this study focused on community-acquired bacteremia, mortality may also have been underestimated in the elderly if fewer blood cultures were taken in the older patients or if they were postponed because of symptoms that were vague.

Information bias

Information bias can arise if the information collected about study subjects is erroneous[131]. These errors may result in misclassification of the exposure or the outcome. The misclassification can be either differential or non-differential, depending on its distribution among comparison groups. In both studies, our outcome was all-cause mortality, which is unlikely to be misclassified.

In **study II**, we may have misclassified some patients with community-acquired bacteremia as having negative blood cultures if their bacteremia was detected after the first two days of admission (which we used as the time window for defining community-acquired bacteremia). This window is arbitrary, although widely accepted. Moreover, some patients with negative cultures may have had bacteremia that was undetected, e.g. if they received antimicrobial therapy prior to cultures being obtained[206]; this may have led to more conservative relative mortality estimates. Unfortunately, we cannot determine the extent to which this is a problem, as there is no gold standard test for bacteremia and false-negatives cannot currently be identified.

Age and comorbidity were the prognostic factors in **study III.** Because we used routine hospital discharge data to identify patients with comorbidity, some coding errors may have occurred. The validity of discharge diagnoses registered in the National Patient Registry is variable, but is generally high for most prevalent diseases, including diabetes, myocardial infarction, chronic obstructive pulmonary disease, and cancer[190;207]. The Charlson index[149] is one of the most extensively validated comorbidity indices for predicting mortality[145], including mortality in patients with bacteremia[141;142;170;208]. The index has been shown to have a high specificity, but a more variable sensitivity, and thus cannot control for confounding from comorbidity as effectively as clinical data[209]. Any misclassification of the comorbidity level among our study participants would most likely bias the relative mortality towards the null.

Confounding

We controlled for confounding in both study design and analysis. At the design stage, we restricted the studies to medical patients with blood cultures taken during the first two days of admission (**study II**) or community-acquired bacteremia (**study III**). In the analysis, we adjusted for age, gender, comorbidity, marital status, calendar period (**study II**), type and focus of infection (**study III**), and appropriateness of the empirical antibiotic treatment (**study III**). We obtained information on these potential confounders from existing registries, and any lack of specificity in these routinely recorded data may have reduced our ability to completely control confounding. Thus, our estimates could still be affected by residual, unmeasured, or unknown confounding[131]. Residual confounding results from improper categorization and misclassification of one or more confounding variables, such as age and comorbidity. In **study**

III, we categorized age into three age groups (15-64, 65-79, ≥ 80) and used quadratic splines to depict the relationship between age, or comorbidity, and bacteremia mortality[203].

As mentioned previously, the Charlson index may have variable sensitivity. The accuracy of discharge diagnoses from previous admissions used in the Charlson index is, however, unlikely to be affected by the results of blood cultures obtained during the first two days of the current admission. Thus, in **study II**, any misclassification should bias the observed mortality estimates toward unity. However, it is possible that comorbidity is recorded more accurately in younger patients than in elderly patients. In **study III**, we therefore cannot preclude that residual confounding due to misclassification may have influenced our findings, although the stratified analysis found that restriction to patients without recorded comorbidity did not change the association between age and mortality. This argues against the idea that residual confounding from comorbidity, which was not captured by the Charlson index, could explain the association between age and mortality. We categorized the Charlson comorbidity index score into 3 levels. While this may result in loss of information and efficiency, it is useful for descriptive purposes and for familiarizing the investigator (and the reader) with the data[174]. Furthermore, this categorization has been used in several previous studies[170;208;210] and Lesens et al[141], for example, dichotomized the index (<3 and >=3) in their study of *S. aureus* bacteremia. Moreover, earlier studies reported almost no differences between comorbidity scores modeled as a continuous variable or in several categories[211].

In **study III**, we found that patients aged 80 years or older were more likely than younger patients to receive inappropriate empirical antibiotic treatment, and we were able to take this into account in the analysis. It is likely that suboptimal treatment of the elderly is not limited to antibiotic treatment[212]. Suboptimal supportive treatment, for example, could worsen the prognosis of bacteremia. The timing of appropriate empirical antibiotic treatment is also an important concern[213]. Unfortunately, we did not have access to data that was accurate enough to determine either the delay in seeking medical attention or the delay in initiating antibiotic therapy.

One concern in our studies is the lack of information in health registries on important variables which may hinder our ability to control for confounding[214;215], such as lifestyle-related factors[216]. We adjusted for marital status, an important aspect of social support, but unfortunately we did not have data on other socio-economic factors such as income, education, or occupation. We also lacked information on nutritional and functional status, as well as clinical details such as disease severity. However, as mentioned earlier (page 21) disease severity may represent a link in the causal chain between age and mortality following bacteremia. We acknowledge that clinical data might have provided better insight into the biological mechanisms underlying the observed associations in **studies II** and **III**.

Precision

We have used 95% confidence intervals throughout this thesis to report the precision of the estimates. The width of the confidence intervals indicates the amount of random error in our estimates; even in our large cohorts, some of the mortality estimates in subgroup analysis had wide intervals, such as the mortality estimates in patients with polymicrobial bacteremia or fungemia.

6 Discussion in relation to the existing literature

6.1 Study I

First notification of positive blood cultures and the high accuracy of the Gram stain report

Few studies have reported on the accuracy of the Gram stain for positive blood cultures (Table 1), and to the best of our knowledge, no previous study has assessed the information on motility from wet mounts. Because the preliminary blood culture reports in our study were based on a combined evaluation of Gram stain findings and wet mount microscopy, we were not able to separate the contribution of these two tests.

Cunney et al[106] reported a discrepancy between Gram stain results and cultural identification in 7 of 132 isolates, resulting in an error rate of 5%. This result is similar to our findings, as we observed nonconcordance between the initial Gram stain findings and the subsequent culture in 119 of the 5893 blood cultures (2%). Our error rate is slightly higher than that reported by Rand and Tillan[128] (57 of 8253 positive blood cultures, 0.7%). In contrast to our study, Rand and Tillan included polymicrobial bacteremias, which accounted for 28 of the reported errors. However, their study included only major errors rather than all discrepancies between Gram stain and cultural identification. In the study by Rand and Tillan[128], an *Acinetobacter* sp. was isolated in 5 of 13 cultures in which the Gram stain was initially read as Gram-positive cocci or rods. *Acinetobacter* spp. have been reported to stain Gram-positive quite easily, despite proper Gram stain technique[217], however, the 24 included *Acinetobacter* spp. in our study were all reported as Gram-negative rods. Our findings also agree with the limited available data on the accuracy of differentiating staphylococci and streptococci morphologically on the Gram-stained smear[126;127].

Technicians' assessment of bacterial motility was less accurate than the Gram stain results. Classification of motility is based on a subjective evaluation. The motility patterns displayed by aerobic bacteria may, however, be equivocal, and the motility ceases rapidly in the absence of air. Motility can be judged more reliably by inoculation and incubation of serum broth for a few hours, but this is incompatible with expedient notification[218].

6.2 Study II

Bacteremia and mortality in medical ward patients with blood cultures taken during the first two days of admission. A Danish cohort study

We are aware of only two studies that compare the prognosis of patients with positive and negative blood cultures[56;165]. In agreement with our findings, Roberts et al[165] found that patients with bacteremia had a considerably higher in-hospital mortality in the first 30 days

after a positive blood culture than did patients with negative blood cultures. Within this time window, the difference in mortality was higher than in our study, possibly due to the fact that only in-hospital mortality was measured and that the patients with negative cultures were matched only by age and gender and not by comorbidity. Similar to us, Bates et al[56] found that short-term mortality was higher among bacteremia patients (adjusted 30-day MRR = 2.3, 95% CI 1.2-4.4), whereas 1-year mortality was less affected (adjusted 1-year MRR = 1.3, 95% CI 0.8-2.1) when compared with patients with negative blood cultures. In comparison, Leibovici et al[62] found that bacteremia was associated with severely curtailed long-term survival when compared patients had the same underlying conditions but were not suspected to have an infection; mortality within 6 months of follow-up was 43% in the bacteremia patients and 19% among controls[62]. In patients with severe sepsis and septic shock, Brun-Buisson et al[58] showed that bacteremia was associated with mortality within 3 days of ICU admission (OR = 1.7, 95% CI 1.1-2.8) but not 28 days after admission. More recently, Laupland et al[166] found that bacteremia was associated with a 60% increase in in-hospital mortality (crude OR = 1.6, 95% CI 1.1-2.2) among Canadian ICU patients with systemic inflammatory response syndrome. When they adjusted for variables that reflected the acute response to infection, the adjusted OR was 1.1 (95% CI 0.7-1.8). This suggests that the effect of bacteremia is mediated by the severity of the systemic inflammatory response.

Brun-Buisson et al[58] found that mortality within 3 days of ICU admission was associated with an increasing number of failing organs and variables reflecting the acute response to infection. Mortality within up to 28 days of admission was additionally associated with the severity of the underlying disease and with pre-existing organ insufficiency[58]. Underlying disorders and comorbidities may have influenced the physician's decision to obtain blood cultures. However, the association between blood culture status and early, short-term, and long-term mortality remained robust in analyses restricted to patients with a primary or secondary infectious disease discharge diagnosis or analyses stratified according to the level of comorbidity. As mentioned previously (page 28-29), it is difficult to distinguish the contribution of the septic process from that of underlying disorders[62]. Nevertheless, because we used patients with negative blood cultures as reference, we surmise that the MRRs reflect the impact of the infection per se on mortality.

6.3 Study III

Short-term mortality in relation to age and comorbidity in older adults with community-acquired bacteremia: A population-based cohort study

Several studies have addressed the impact of age on bacteremia mortality with conflicting results[24-26;41;139;141;167-173]. However, the majority of studies that found that age had no impact on mortality included only patients older than 65 and often made no distinction between old (65-79) and old-old (80 years and older); these studies not only failed to demonstrate

differences between younger and older people, but also found no differences within the older population[26;172]. Other studies dichotomized patients into two groups: young and old[24;139;141;169;173]. However, identifying older people using a cut point that is around age 65, which generally corresponds to retirement age, is probably unsatisfactory. Thus, our data extends the previous studies. Other than a plateau between the ages of 50 and 65, we showed an almost linear association, between age and mortality that corroborates findings in subpopulations of patients with hematological malignancies[170] and *S. aureus* bacteremia[141].

Comorbidity, functional status, and nutritional status, rather than age itself, have been suggested as risk factors for mortality[24;25]. However, in many previous studies, differences in comorbidity were not taken into account in the statistical analysis[25;171]. Different levels of comorbidity between the age groups may therefore have confounded the observed association between age and mortality. In agreement with previous studies[24;26;41;139;141;173], we found that comorbidity is a predictor of mortality and that 7- and 30-day mortality increased linearly with the Charlson index score. The relative impact of age on mortality was higher among patients with no comorbidity. Still, we found a 49% higher 30-day mortality in patients aged 80 years and older with high comorbidity compared with patients aged 15-64 years with high comorbidity. Thus, even though comorbidities are highly relevant for predicting the outcome, it is unlikely, according to our findings, that they fully account for the differences in mortality observed between age groups. In a recent study of 984 critically ill patients with nosocomial bacteremia, Blot et al[167] found that in patients aged 65 and older, the observed in-hospital mortality exceeded the 95% CIs of the APACHE II predicted mortality. In middle-aged patients (45-64 years), the observed mortality did not deviate from the predicted mortality, suggesting that the negative impact of infection is higher in the elderly.

Other possible explanations for the increased mortality with advancing age are progressive deterioration of the immune system, diagnostic difficulties, and potential differences in treatment and clinical quality associated with old age. The febrile response may be blunted or absent, and older patients with infections often present with symptoms that are more vague than in younger patients[95;96] This can delay diagnosis and treatment. We found that the prevalence of bacteremias with an unknown focus increased with age and with increasing levels of comorbidity. Failure to determine the focus has been associated with increased mortality in patients with bacteremia[41;152] and may reflect both nonspecific symptoms and the higher likelihood that older patients die before further diagnostic investigations are undertaken.

7 Main conclusions

Based on the results and an examination of potential bias, confounding factors, and chance in the three studies, we drew the following conclusions:

7.1 Study I

We found that Gram staining performed by experienced technicians in a routine setting was highly accurate with performance characteristics close to 100% for all main morphological groups. In contrast, the information on bacterial motility gained from the wet-mounts was less accurate.

7.2 Study II

We found an approximately 20% increased short-term mortality among patients admitted to medical wards with community-acquired bacteremia compared to patients with negative blood cultures. For patients with polymicrobial bacteremia or fungemia, the mortality increase persisted for at least 180 days. In patients with Gram-negative bacteremia, mortality throughout follow-up was very similar to patients with negative blood cultures. The level of comorbidity did not seem to influence the association between blood culture status and mortality.

7.3 Study III

We found that aging was a strong prognostic factor for mortality in patients with community-acquired bacteremia admitted to a medical ward. Comorbidity was also associated with a fatal outcome, but increasing levels of comorbidity with increasing age did not entirely explain the effect of age on bacteremia mortality.

8 Perspectives

The high burden and costs of community-acquired bacteremia are estimated to increase with an aging population[219]. While the incidence of bacteremia has increased over the last several decades[63;65], the associated mortality rates have remained constant or decreased slightly[39;65].

Early appropriate antibiotic treatment is a mainstay in bacteremia treatment[157]. In this thesis, we found that the first notification of a positive blood culture was highly accurate, which provides the attending physicians with a presumptive identification that can be used to guide empirical antibiotic treatment. Very recently, multiplex real-time PCR technologies and microarrays have been developed that allow direct identification of bacteria and fungi in blood as well as identification of resistance genes such as *mecA*, ESBL, and KPC[220;221]. The expense, as well as the requirement for both technical equipment and trained personnel, have so far precluded general applicability of these technologies for routine purposes[222]. Still, there are other promising tools that can discriminate between patients at high and low risk of bacteremia, including biomarkers such as procalcitonin[223] and medical decision support systems[224].

The Danish national healthcare system provides an optimal setting for conducting large population-based studies of bacteremia. The civil registration numbers makes it possible to unambiguously link medical databases and administrative registries and thereby build large cohorts with detailed longitudinal data that include complete hospital history, comorbidity data, and complete long-term follow-up data. Our studies have, however, also exposed some of the weaknesses in the Danish health care databases. The main weakness is the lack of clinical data. We could not grade the level of sepsis based on the fact that a blood culture was obtained, nor did we know the indications for obtaining the cultures. In a future study, we would like to compare the pathophysiological response in patients with positive and negative blood cultures. The ideal study, however, would be a prospective study in which all patients are screened with blood cultures at admission, and the clinical details are recorded. We hope that implementation of electronic medical records will allow more detailed characterization of patients with bacteremia and their treatments for future studies. Another weakness is that bacteremia may have important health outcomes other than death[148], but such outcomes are not generally recorded. These include chronic disability due to sequelae, pain and discomfort, emotional distress, and long-term financial costs both for the individual patient and for society.

Our finding that more than 1 in 5 patients who is either aged ≥80 years or has a high level of comorbidity will not survive 30 days after the date of blood culture is of great concern, both from a clinical and a public health standpoint, especially since bacteremia may be preventable. Urinary tract infections, which were the likely source of infection in more than one third of the elderly patients with bacteremia, should be recognized early and treated promptly. Other

important precautions are hydration and limited use of urinary catheters. The 23-valent pneumococcal vaccine has been associated with an approximately 50% reduction of the incidence of invasive pneumococcal disease among adults and the immunocompetent elderly[225]. However, in contrast to the USA, where up to 80% of individuals over age 65 have received the pneumococcal vaccination[226], the estimated vaccine coverage in 1998 to 2007 among the Danish elderly is at most 17/1000 individuals (1.7%)[227]. Moreover, it was not until 2007 that a 7-valent pneumococcal conjugate vaccine was introduced into the Danish childhood immunization program. Currently, a vaccine for group B streptococcal infection is under development[228] and, in the future, sequencing of complete bacterial genomes is expected to provide an important contribution to vaccine development[229].

Another important concern is that elderly patients and patients with high comorbidity received inappropriate empirical antibiotic treatment more often than other patients. These findings emphasize the importance of up-to-date clinical guidelines for optimal treatment of bacteremia and sepsis, such as the Surviving Sepsis Campaign[1] and the national patient safety campaign "Operation Life"[230]. In future studies, we would like to examine age-related disparities in antibiotic treatment and outcome of community-acquired bacteremia and also determine the extent to which comorbidity influences the choice of empirical antibiotic treatment.

Our knowledge about preventable or modifiable risk factors and prognostic factors for community-acquired bacteremia in a population-based setting remains limited. In particular, the mechanisms by which age affects bacteremia mortality is far from understood. Despite the increasing prevalence of chronic diseases with age, our knowledge about how these diseases co-occur in the same individual is insufficient. It is hoped that in the future, joint efforts by researchers in disciplines including clinical-, molecular-, and pharmacoepidemiology, clinical microbiology, infectious diseases, and clinical medicine will provide new insights into the etiology, course, and prevention of community-acquired bacteremia. Future work may also uncover potential sources of infection and reservoirs in humans, animals, and nature[231]. The continuous development of the North Jutland County Bacteremia Research Registry is a priority and includes full integration with departmental and hospital information systems. The registry has inspired interregional cooperation on bacteremia research, and key information on bacteremia cases is currently recorded in the same format by three Danish departments of clinical microbiology on a routine basis. This holds promise of a much wider population base for future bacteremia studies[232]. In 1969, Martin[233] made a plea for an American national bacteremia registry. Although this goal has not yet been accomplished in the USA, it is feasible in a country like Denmark.

9 Summary

Bacteremia and fungemia are associated with high morbidity and mortality. Prompt and accurate detection is therefore important for improving patient care. Despite the availability of effective antibiotics and improved supportive care, the 30-day mortality still average 20% and is even higher in patients of advanced age who often have coexisting chronic diseases. One way to improve our knowledge of the course and outcome of bacteremia is to conduct observational studies based on existing data in large healthcare databases.

This thesis is based on one cross-sectional study and two cohort studies conducted in the former North Jutland County, Denmark, and are based on data from the Civil Registration System, the North Jutland County Bacteremia Research Database, the Danish National Registry of Patients, the microbiological information system in North Jutland, and laboratory notes. The aims were to examine 1) the accuracy of the first notification of bacteremia based on Gram stain and wet-mount microscopy, 2) the prognostic impact of bacteremia in medical patients with blood cultures taken within the first two days of admission, and 3) the impact of age and comorbidity on bacteremia mortality.

In **study I**, Gram stains of 5,893 positive blood cultures performed by experienced technicians were highly accurate, with sensitivities in the range 91.3-99.7%, specificity 98.9-100%, PPV 94.6-100%, and NPV 99.0-100%. The sensitivity for the most frequent species was in the range 91.3-100%, with non-hemolytic streptococci having the lowest value (sensitivity 91.3%; 95% CI 86.2-94.9). The information on bacterial motility obtained from wet mounts was less accurate.

In **study II**, we included 28,610 medical patients with blood cultures taken during the first two days of hospital admission. Follow-up started on the third day of admission. We found that short-term mortality was increased slightly in patients with community-acquired bacteremia compared to patients with negative blood cultures. The mortality increase conferred by bacteremia was highest in the first week of follow-up (adjusted MRR 1.2, 95% CI 1.0-1.4) but persisted for at least 180 days among patients with polymicrobial bacteremia and fungemia (180-day adjusted MRR 1.4, 95% CI 0.9-2.2). Gram-positive bacteremia, polymicrobial bacteremia, and fungemia were associated with the highest mortality, whereas mortality in patients with Gram-negative bacteremia was similar to mortality in patients with negative blood cultures.

In **study III**, we found that mortality from community-acquired bacteremia increased linearly with age and level of comorbidity. A greater burden of comorbidity in elderly people did not, however, fully explain the association between age and mortality. Thus, at each level of comorbidity, increasing age adversely affected the outcome.

We conclude that the first notification of positive blood cultures given to the attending physicians is highly accurate and can be used to make a presumptive identification that can guide empirical antibiotic treatment. Although this is currently standard practice, the accuracy has not previously been confirmed in a study of this scale. All patients with blood cultures had high mortality that was only slightly higher among patients with bacteremia. In the bacteremia patients, mortality increased with age and level of comorbidity. This is a public concern since average life expectancy is increasing in most countries.

10 Danish summary

Bakteriæmi og fungæmi er alvorlige infektioner med en 30-dages dødelighed omkring 20%; for ældre patienter, som ofte har kroniske sygdomme, er dødeligheden endnu højere. Hurtig og præcis diagnostik er afgørende for at fremme optimal behandling. Observationelle studier baseret på eksisterende registre kan bidrage med viden om faktorer forbundet med en dårlig prognose.

Denne afhandling er baseret på et tværsnitsstudie og to kohortestudier udført i det tidligere Nordjyllands Amt. Studierne bygger på data fra CPR-registret, Bakteriæmidatabasen for Nordjyllands Amt, Landspatientregistret, den regionale kliniske mikrobiologiske afdelings prøveregister samt bioanalytikernes arbejdssedler. Formålene med studierne var at undersøge 1) nøjagtigheden af den første udmelding (notifikation) om en positiv bloddyrkning, som er baseret på Gram farvning og mikroskopi af et fugtigt præparat, 2) den prognostiske betydning af bakteriæmi hos medicinske patienter, der har fået foretaget bloddyrkning inden for de første to indlæggelsesdøgn, og 3) betydningen af alder og komorbiditet for overlevelsen ved bakteriæmi.

I **studie I**, omfattende 5893 positive bloddyrkninger, fandt vi at Gram farvning udført af erfarne bioanalytikere var meget nøjagtig med en sensitivitet på 91,3-99,7%, specificitet 98,9-100%, PPV 94,6-100%, og NPV 99,0-100%. For de hyppigste bakteriearter var sensitiviteten 91,3-100% med den laveste værdi observeret for de non-hæmolytiske streptokokker (sensitivitet 91,3%; 95% CI 86,2-94,9%). Derimod var vurderingen af bevægelighed mindre nøjagtig og ofte misvisende.

I **studie II** inkluderede vi 28.610 medicinske patienter, som var bloddyrkede inden for de først to døgn af indlæggelsen. Follow-up startede på 3. indlæggelsesdag. Vi fandt, at patienter med bakteriæmi havde en let forøget dødelighed i forhold til patienter med negativ bloddyrkning. Denne overdødelighed var højest i den første uge efter indlæggelsen (justeret MRR 1.2, 95% CI 1.0-1.4), dog havde patienter med polymikrobiel bakteriæmi og fungæmi en forøget dødelighed gennem hele den 180 dage lange opfølgningsperiode (justeret MRR 1.4, 95% CI 0.9-2.2). Dødeligheden var højest blandt patienter med Gram-positiv bakteriæmi, polymikrobiel bakteriæmi og fungæmi, mens patienter med Gram-negativ bakteriæmi havde stort set den samme dødelighed som patienter med negativ bloddyrkning.

I **studie III** fandt vi, at dødeligheden for samfunds-erhvervet bakteriæmi tiltog lineært med stigende alder og niveau af komorbiditet. Uanset niveauet af komorbiditet var stigende alder associeret med en dårligere prognose, og et højere niveau af komorbiditet blandt ældre synes derfor ikke at kunne forklare sammenhængen mellem alder og dårlig prognose.

Sammenfattende viser de tre studier, at den første information om en positiv bloddyrkning, som gives til den behandlende læge, er meget pålidelig og derfor kan danne grundlag for valg af den empiriske behandling. Dødeligheden er høj blandt alle bloddyrkede patienter, og i forhold til

patienter med negativ bloddyrkning har patienter med bakteriæmi kun en lettere forøget dødelighed. Både alder og komorbiditet er forbundet med dårlig prognose, hvilket bør vække bekymring, da gennemsnitslevealderen er stigende.

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12 Appendix: studies I-III

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First Notification of Positive Blood Cultures and the High Accuracy of the Gram Stain Report[▽]

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When blood cultures turn positive, the attending physicians are usually notified immediately about Gram stain findings. However, information on the accuracy of Gram staining is very limited. We examined the accuracy of preliminary blood culture reports provided by a regional laboratory in an observational study including the years 1996, 2000 to 2001, and 2003. We used data from computer files and technicians' laboratory notes. The study was restricted to cultures with one morphological type. Using cultural identification as a reference, we estimated the sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) for the following defined morphological groups: gram-positive cocci in clusters, gram-positive cocci in chains or diplococci, gram-positive rods, gram-negative cocci, gram-negative rods, and yeasts. We further evaluated the Gram stain and wet mount findings for the most frequent bacterial species/groups. We obtained 5,893 positive blood cultures and the following results for the defined groups: sensitivity, range of 91.3 to 99.7%; specificity, 98.9 to 100%; PPV, 94.6 to 100%; and NPV, 99.0 to 100%. The sensitivity for the most frequent species was in the range 91.3 to 100%, with nonhemolytic streptococci having the lowest value (sensitivity, 91.3%; 95% confidence interval, 86.2 to 94.9%). Wet mount reports were less accurate (sensitivity of 30 to 70% for species with peritrichous motility), and *Enterobacteriaceae* (notably *Salmonella* spp.) accounted for 25% of the reports stating polar motility. In conclusion, we demonstrated a high accuracy of Gram stain reports, whereas wet mount microscopy was generally less accurate.

Bacteremia is a serious condition with an overall in-hospital mortality above 20% (15, 19). Early administration of appropriate empirical antibiotic treatment has repeatedly been associated with improved survival in patients with bacteremia (5, 15, 27), yet up to 40% of all patients with bacteremia receive inadequate antibiotic treatment until the first notification of a positive blood culture (5, 6, 22, 24). Therefore, an important task for the microbiological laboratory is to provide expedient reports on positive blood cultures that may guide antibiotic therapy.

The first notification of a positive blood culture is typically based on the Gram stain result. At this time, 12 to 20% of the patients may not have started antibiotic treatment, and in another 30 to 45% of patients, the Gram stain result is followed by a change in the empirical treatment (2, 7, 19, 22, 24). The Gram stain report has been shown to have a much greater impact on antimicrobial treatment than provision of cultural identification and antimicrobial susceptibility test results (17, 22), and recently Hautala et al. (12) reported that combining Gram stain results with information on whether the infection was hospital or community acquired could further improve the appropriateness of the antibiotic treatment. Besides the direct implications for antibiotic treatment, the Gram stain result may also prompt further diagnostic and therapeutic interventions.

Despite the acknowledged importance of the first notification, the accuracy of the Gram stain result has only been addressed sporadically, and the studies have mainly focused on distinction of either contaminants from true bacteremia (3, 13) or staphylococci from streptococci (1, 28). Therefore, we conducted this study to evaluate the accuracy of the preliminary blood culture reports based on Gram stain and wet mount microscopy.

MATERIALS AND METHODS

Setting. We conducted this observational study in North Jutland County, Denmark (population of approximately 500,000), using blood culture data from the years 1996, 2000, 2001, and 2003. We restricted the study to blood cultures with one morphological type: for patients with bacteremia, only the first positive blood culture was included. Patients were admitted to one of seven public hospitals, of which one (Aalborg University Hospital) served as both the district and referral hospital. The Department of Clinical Microbiology, Aalborg Hospital, provided bacteriological services, including blood cultures, for the entire county.

Blood cultures. The BacT/Alert blood culture system (bioMerieux, Marcy l'Etoile, France) was used throughout the study period. Blood cultures were obtained due to a physician's suspicion of an infection, and in adult patients three blood culture bottles were routinely inoculated at bedside using one needle. In 1996, a blood culture included two standard aerobic (SA) bottles and one standard anaerobic (SN) bottle; during the other 3 years, one SA bottle was substituted for by an aerobic FAN bottle. The nominal volume of blood per set was 28 to 32 ml for adults. For infants and preschool children, one pediatric aerobic FAN bottle was used.

Positive bottles were unloaded at 8:00 a.m., 11:00 a.m., 14:00 p.m., and 8:30 p.m. and immediately examined by a technician. Technicians with less than 2 years of experience were supervised by more experienced colleagues. The compound microscopes were equipped with ×100 achromatic oil objectives also suited for phase-contrast microscopy, and Koehler illumination was checked daily. Wet mount preparations were immediately examined by phase-contrast microscopy, and smears for Gram staining were fixed by flame fixation and stained using acetone for decolorization and safranin as counterstain. The motility (wet mount), Gram stain reaction, morphology, and bacterial arrangement

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TABLE 1. Distribution by calendar year of the 5,893 positive blood cultures with one morphological type on Gram stain grouped according to Gram stain characteristics, arrangement, and morphology

Yr	No. (%)[a]:						
	Cocci			Rods		Yeasts	Total
	Gram positive, clusters	Gram positive, chains/diplococci	Gram negative	Gram positive	Gram negative		
1996	428 (35.0)	241 (17.5)	8 (0.7)	104 (8.5)	467 (38.2)	3 (0.3)	1,224 (100)
2000	595 (37.5)	198 (12.5)	8 (0.5)	246 (15.5)	511 (32.2)	28 (1.8)	1,586 (100)
2001	603 (38.6)	210 (13.4)	11 (0.7)	156 (10.0)	563 (36.0)	20 (1.3)	1,563 (100)
2003	481 (31.6)	211 (13.9)	11 (0.7)	114 (7.5)	662 (43.6)	41 (2.7)	1,520 (100)
Total	2,107 (35.8)	833 (14.1)	38 (0.6)	620 (10.5)	2,203 (37.4)	92 (1.6)	5,893 (100)

[a] For patients with bacteremia, only the first positive blood culture was included.

were recorded on a laboratory note. The positive blood cultures were subcultured onto plate media selected in accordance with the Gram stain result, and isolates were routinely identified by a combination of conventional and commercial methods (18).

The laboratory's proficiency was assured by participation in the UK External Quality Assessment Scheme as well as national quality control programs. All isolates of streptococci, pneumococci, meningococci, and yeasts were referred to Statens Serum Institut (Copenhagen, Denmark) as part of a national surveillance program. All microbiological information was recorded in a laboratory information system (ADBakt, Autonik, Ramstra, Sköldinge, Sweden).

Data on positive blood cultures. We defined bacteremia as bacterial or fungal growth in blood culture, where a combined clinical and microbiological assessment effectively ruled out contamination (30). Coagulase-negative staphylococci, *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* were regarded as contaminants unless they were isolated from two or more separate blood cultures or special risk factors were known to be present. All episodes of bacteremia (and fungemia) in the county since 1981 have been registered in the North Jutland County Bacteremia Registry (23, 24), which we used to identify the first positive blood culture for all episodes of bacteremia occurring during the 4 years studied.

Information on contaminated blood cultures was retrieved from the laboratory information system, and for all cultures, we abstracted information on Gram stain result, bacterial motility, and species diagnosis from the technician's notes; these data were tabulated independently of the main investigator. We defined six main groups according to Gram stain characteristics and morphology, namely: gram-positive cocci in clusters, gram-positive cocci in chains or diplococci, gram-positive rods, gram-negative cocci, gram-negative rods, and yeasts. Bacteria were classified by motility as peritrichous, polar, or nonmotile.

Data analysis. We evaluated the accuracy of Gram staining and wet mount microscopy using the results obtained by cultural identification as a reference standard. For each of the six defined groups, we estimated the performance characteristic of Gram staining (sensitivity, specificity, and positive and negative predictive values [PPV and NPV, respectively]) (9). Using gram-negative rods as an example, sensitivity refers to the proportion of gram-negative rods identified by culture that were determined as such by Gram stain. The specificity describes the ability of the initial Gram stain to rule out a certain combination of Gram staining and morphology. For gram-negative rods, the specificity refers to the proportion of blood cultures with isolates other than gram-negative rods that were classified accordingly in the initial Gram stain examination (i.e., the numerator was the number of blood cultures not identified as being gram-negative rods in the initial Gram staining and the denominator was the number of all blood cultures that were not classified as gram-negative rods by cultural identification). The PPV is the probability that gram-negative rods seen on Gram stain were identified as such by culture. The NPV is the probability that a morphotype different from that of gram-negative rods is not identified as gram-negative rods by culture.

To quantify the maximum impact of a potential selection bias caused by missing data, we repeated the analyses assuming that all missing data had been incorrect. We further evaluated the Gram stain and wet mount results for predominant pathogens at the species level. Bacterial motility was assessed for the most frequent motile gram-negative species.

Estimates are presented with 95% confidence intervals (CI). Statistical analyses were performed using Stata Statistical Software v.9.0 (Stata Corp., College Station, TX).

RESULTS

Among 6,461 positive blood cultures obtained during the study period, 438 (7%) were polymicrobial with more than one morphological type and 130 (2%) records lacked information on either bacterial morphology or Gram stain reaction. Thus, our study sample included 5,893 blood cultures, of which 1,985 (34%) were contaminants. The distribution of recovered isolates grouped according to Gram stain characteristics is shown in Table 1. Sensitivity, specificity, PPV, and NPV of the Gram stain are given in Table 2. These estimates remained stable

TABLE 2. Performance characteristics of the Gram stain with culture-based identification as reference

Pathogen[a]	No. of correct Gram stain evaluations/ total	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
Cocci					
Gram-positive, clusters	2,101/2,129	99.7 (99.4–99.9)	99.3 (98.9–99.5)	98.7 (98.1–99.2)	99.8 (99.7–99.9)
Gram-positive, chains/ diplococci	707/818	96.8 (95.4–97.8)	99.8 (99.6–99.9)	98.7 (97.6–99.3)	99.5 (99.3–99.7)
Gram negative	35/37	92.1 (78.6–98.3)	100 (99.9–100)	94.6 (81.8–99.3)	100 (99.9–100)
Rods					
Gram positive	566/584	91.3 (88.8–93.4)	99.7 (99.5–99.8)	96.9 (95.2–98.2)	99.0 (98.7–99.2)
Gram negative	2,175/2,217	98.7 (98.2–99.2)	98.9 (98.5–99.2)	98.1 (97.5–98.6)	99.2 (98.9–99.5)
Yeasts	90/92	97.8 (92.4–99.7)	100 (99.9–100)	100 (96.0–100)	100 (99.9–100)

[a] Pathogens are grouped according to their Gram stain characteristics, morphology, and arrangement.

TABLE 3. Evaluation of Gram stain results for predominant bacterial pathogens or groups

Pathogen	No. of correct Gram stain evaluations/ total	% Sensitivity (95% CI)
S. aureus	592/592	100 (99.4–100)
Streptococci		
Hemolytic	151/152	99.3 (96.4–100)
Nonhemolytic	167/183	91.3 (86.2–94.9)
Enterococci	103/106	97.2 (96.7–99.4)
Pneumococci	386/392	98.5 (96.7–99.4)
Meningococci	33/34	97.1 (84.7–99.9)
Enterobacteria	1841/1859	99.0 (98.5–99.4)
E. coli	1275/1282	99.5 (98.9–99.8)
Citrobacter spp.	23/23	100 (85.2–100)
Enterobacter spp.	110/111	99.1 (95.1–100)
Klebsiella spp.	296/305	97.1 (94.5–98.6)
Morganella morganii	19/20	95.0 (75.1–99.9)
Proteus spp.	72/72	100 (95.0–100)
Serratia marcescens	24/24	100 (85.8–100)
Salmonella serovar	42/42	100 (91.6–100)
Other enterobacteria[a]	7/7	100 (59–100)
Pseudomonas aeruginosa	117/118	99.2 (95.4–100)

[a] Includes two *Hafnia alvei* isolates, one *Pantoea agglomerans* isolate, two *Yersinia enterocolitica* isolates, and one unidentified enterobacterium.

across the 4 years studied (data not shown). Assuming that all of the 130 excluded records were false negative and distributed as in Table 1, this led to the following sensitivities: gram-positive cocci in clusters, 95.5% (95% CI, 96.8 to 98.2%); gram-positive cocci in chains/diplococci, 94.8% (95% CI, 93.1 to 96.2%); gram-negative cocci, 89.7% (95% CI, 75.8 to 97.1%); gram-positive rods, 89.3% (95% CI, 86.6 to 91.6%); gram-negative rods, 96.6% (95% CI, 95.7 to 97.3%); and yeasts, 95.7% (95% CI, 89.5 to 98.8%).

The comparatively low sensitivity for gram-positive rods (91.3%; 95% CI, 88.8 to 93.4%) was mainly caused by *Bacillus* spp. and *Clostridium* spp. Nearly half of the *Bacillus* isolates (45.3%) were recorded as gram-negative rods corresponding to a sensitivity of 48.4% (95% CI, 35.8 to 61.3%). Likewise, 7 of 35 *Clostridium* spp. were reported as gram-negative rods ($n = 6$) or as a mixture of gram-negative and gram-positive rods ($n = 1$). The sensitivity of the preliminary diagnosis for *Clostridium* spp. was 80% (95% CI, 63.1 to 91.6%). Leaving out *Bacillus* spp. and *Clostridium* spp. from the analysis, the remaining gram-positive rods had a sensitivity of 97.3% (95% CI, 95.5 to 98.5%), specificity of 99.7% (95% CI, 99.5 to 99.8%), PPV of 96.6% (95% CI, 94.6 to 98.0%), and NPV of 99.7 (95% CI, 99.6 to 99.9%).

Table 3 shows sensitivity at the species level for the predominant bacterial pathogens. The sensitivity was close to 100% for all listed pathogens, with nonhemolytic streptococci being the only distinctive exception. Sixteen Gram-stained smears with nonhemolytic streptococci were misread and initially reported as gram-negative rods ($n = 2$), gram-positive cocci in clusters ($n = 9$), or gram-positive rods ($n = 5$). Furthermore, all *Acinetobacter* spp. included in the study were reported as gram-negative rods on Gram stain, corresponding to a sensitivity of 100% (95% CI, 85.8 to 100.0%).

Overall, the sensitivity of the wet mount report varied from 30% to 70% for bacterial species with peritrichous motility (Table 4). A total of 100 bacteria were recorded as displaying a polar pattern of motility; one-quarter of these were enterobacteria, of which a *Salmonella* serovar accounted for the major part.

DISCUSSION

In this study of more than 5,800 positive blood cultures, we found that the Gram stain reports were highly accurate and remained so over the years studied. The performance characteristics for the main morphological groups were close to 100% and only slightly lower for gram-positive rods, in accordance with the propensity of both *Bacillus* spp. and *Clostridium* spp. to appear gram negative (4).

The use of wet mounts in association with Gram staining for positive blood cultures to determine the morphology of organisms, gross structure, and motility has a long tradition in Danish clinical microbiology. We cannot determine to what extent the information gained from the wet mounts (beside the information on motility) may have contributed to the technicians' accurate assessment of the Gram stain. It is our impression

TABLE 4. Motility patterns of predominant motile gram-negative bacteria assessed by wet mount microscopy

Species (*n*)	No. (%) of isolates with motility type:			
	Nonmotile	Peritrichous	Polar	Not stated
Enterobacteria				
E. coli (1,263)	651 (50.8)	602 (47.0)	6 (0.5)	23 (1.7)
Citrobacter spp. (22)	11 (47.8)	8 (39.1)	0	3 (13.1)
Enterobacter spp. (111)	25 (22.5)	69 (62.2)	3 (2.7)	14 (12.6)
Morganella morganii (20)	3 (15.0)	14 (70.0)	0	3 (15.0)
Proteus spp. (72)	19 (26.4)	37 (51.4)	4 (5.6)	12 (16.6)
Serratia marcescens (24)	10 (41.7)	10 (41.7)	0	4 (16.6)
Salmonella serovar (42)	5 (11.9)	23 (54.8)	11 (26.2)	3 (7.3)
Other enterobacteria (7)[a]	4 (57.1)	2 (28.6)	0	1 (14.3)
Pseudomonas aeruginosa (118)	36 (30.5)	10 (8.5)	62 (52.5)	10 (8.6)

[a] Includes two *Hafnia alvei* isolates, one *Pantoea agglomerans* isolate, two *Yersinia enterocolitica* isolates, and two unidentified enterobacteria.

that the use of wet mounts aids in the interpretation of Gram stains (mostly with respect to the arrangement of gram-positive cocci and weakly stained gram-negative organisms, which may appear more distinct in wet mounts). In this study, the information on bacterial motility gained from the wet mounts was less accurate and in some instances misleading. Polar motility of gram-negative rods is given particular attention because it may indicate *Pseudomonas aeruginosa* and other aerobic bacteria, which require extended antibiotic coverage. However, considering the variation in motility displayed by *P. aeruginosa*, we find that the absence of polar motility in wet mount microscopy should not be used to rule out *P. aeruginosa*.

The strengths of our study are its large size, coverage of the service for an entire county, and collection of data on blood cultures, bacteremia, and microbiological findings independently of the study, making investigators' bias unlikely. Thus, the study in itself did not influence the diagnostic process, and bias due to differential diagnostic effort was prevented. By excluding repetitious positive blood cultures in patients with bacteremia and Gram stain reports with more than one morphological type, our study focused on those Gram stain reports most likely to influence the clinical decision making. We included all contaminated blood cultures since this is basically a post hoc classification based on multiple criteria including the diagnosis obtained by culture and a clinical assessment (30). Still, other factors could affect the validity of our findings. First, when evaluating the culture-based identification, the technicians were not blinded to the results of Gram stain and wet mount microscopy. This may have led us to overestimate the performance characteristics of Gram stain and wet mount microscopy (16). Second, 2% of the blood culture records were excluded because we lacked information on either the Gram stain result or morphology. This may have been due to problems with the interpretation of the smears and could also cause an overestimation of the accuracy of the Gram stain results. However, even if all of the excluded blood cultures were classified incorrectly by Gram stain (worst case scenario), the sensitivity would still be around or above 90%. Third, because of the retrospective nature of the study, there was no way to systematically determine whether the few observed discrepancies between Gram stain and culture-based identification were mainly due to interpretative or technical errors. Gram stains from the FAN medium may be more difficult to interpret because of the presence of charcoal particles (29), but the accuracy was not negatively affected by the introduction of the FAN medium in 1999. Decolorizing is the most critical part of the Gram staining procedure, and we believe that the use of 100% acetone instead of a 50:50 mixture of acetone and 95% ethyl alcohol, as recommended in the *Manual of Clinical Microbiology* (18), may explain part of the observed decolorization of, especially, *Bacillus* spp. and *Clostridium* spp.

The performance of the Gram stain is dependent on the interpreter, and even though this study was conducted in a routine setting and reflects everyday practice, an important premise is that most technicians undertaking the direct microscopy are highly skilled. Our results may therefore not apply to other settings.

Reports on the accuracy of the Gram stain for blood cultures are very sparse. A study by Cunney et al. (7) reported a discrepancy between Gram stain results and cultural identification in 7 of 132 isolates (5%). These results corroborate our results as we observed nonconcordance between the initial Gram stain and the subsequent culture in 119 of the 5,893 blood cultures (2%). This proportion was somewhat lower (57 of 8,253 positive blood cultures) in the study by Rand and Tillan (21), but their study focused only on those errors that had the greatest potential for patient harm. Our findings also agree with the limited data available on the accuracy of differentiating staphylococci and streptococci morphologically on the Gram-stained smear (1, 28). However, only the study by Cunney et al. (7) specified whether contaminants were included in the evaluation. *Acinetobacter* spp. have been reported to stain gram positive despite proper Gram stain technique (11), and in the study by Rand and Tillan (21), an *Acinetobacter* sp. was isolated in 5 of 13 cultures where the Gram stain initially was read as gram-positive cocci or rods. Our data set included 24 *Acinetobacter* spp. which were all reported as gram-negative rods.

Several studies have demonstrated that reporting of blood culture results considerably increases the proportion of bacteremic patients who receive appropriate antibiotic treatment (5, 15, 27). Bouza et al. (5) found that the odds of death increased 1.2-fold for each day until definitive identification was available (odds ratio = 1.2; 95% CI, 1.05 to 1.4%). This and the improvement in antibiotic treatment on the basis of microbiological data underlie the potential benefit of applying rapid microbiological detection and testing methods as previously shown (8, 26). A range of other promising direct tests for rapid identification (including direct inoculation in automated systems, hybridization, and PCR) has been described in recent years (10, 14, 20, 25). Still, our study emphasized that Gram staining performed and interpreted by experienced technicians is inexpensive, fast, and highly accurate.

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Bacteremia and mortality in medical ward patients with blood cultures taken within two days of admission: A Danish cohort study

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Abstract

Objectives: To compare mortality in patients with community-acquired bacteremia with mortality in patients with negative blood cultures and to determine the effects of the type of bacteremia and level of comorbidity on mortality.

Design: A cohort study, using medical databases in Denmark.

Setting: North Jutland County, Denmark, 1995-2006.

Participants: Adults with blood cultures taken within two days of admission to a medical ward (n = 28,610).

Main outcome measures: Observed mortality and mortality rate ratios (MRRs) within 3-7, 8-30, and 31-180 days after admission in bacteremia patients and patients with negative blood cultures, adjusted for potential confounders.

Results: Mortality in the 2,520 (8.8%) bacteremic patients versus 26,090 patients with negative blood cultures was as follows: 5.1% vs. 3.7% (3-7 days), 4.9% vs. 4.5% (8-30 days), and 9.3% vs. 8.4% (31-180 days) after admission, corresponding to adjusted MRRs of 1.2 (95% confidence interval (CI): 1.0-1.4), 0.9 (95% CI: 0.8-1.1), and 0.9 (95% CI: 0.8-1.1), respectively. Compared with patients with negative cultures, mortality increases after 3-7 days were highest among patients with Gram-positive bacteremia (adjusted MRR=1.5, 95% CI: 1.2-1.9) and polymicrobial bacteremia or fungemia (adj. MRR=2.0, 95% CI: 1.2-3.4) whereas patients with Gram-negative bacteremia had the same risk of dying as patients with negative cultures (adj. MRR=0.8, 95% CI: 0.6-1.1). Only polymicrobial bacteremia and fungemia were associated with increased mortality within 31-180 days (adj. MRR=1.4, 95% CI: 0.9-2.2) compared to blood culture-negative patients. Stratification by level of comorbidity did not affect the estimates.

Conclusion: Short-term mortality was slightly higher in bacteremia patients than in patients with negative blood cultures, and the increased mortality was associated with Gram-positive bacteremia and polymicrobial bacteremia or fungemia. The increased 180-

day mortality in patients with polymicrobial bacteremia or fungemia may be due to confounding by underlying disorders.

Introduction

Community-acquired bacteremia is a common and serious condition with a hospitalization rate around 80 per 100,000 population-years (1) and a 30-day mortality greater than 15% (2). Mortality results from a dynamic interplay of factors that include the infectious agent, the host's immune response, underlying disorders, and therapeutic interventions; these factors, and their effects, can be difficult to distinguish from each other (3;4).

A comparison of mortality in patients with positive and negative blood cultures is likely to reflect the contribution of bacteremia to mortality. Two previous cohort studies suggested that compared to blood culture-negative patients, bacteremia was associated with a two to three-fold higher 30-day mortality (4;5) but had a smaller effect on 1-year mortality (MRR 1.3, 95% CI: 0.76-2.1) (5). In comparison, an Israeli cohort study found that mortality was two-fold higher 6 months after admission in patients with bacteremia and remained increased for up to four years, when compared with patients who had the same underlying conditions but were not suspected of having an infection (3). Two other cohort studies identified bacteremia as a predictor for in-hospital mortality in ICU patients with sepsis, severe sepsis, or septic shock with relative risks around 1.6 (6;7). None of these previous studies distinguished between community-acquired bacteremia and nosocomial bacteremia; other limitations included lack of long-term follow-up (4;6;7), small sample size (5), and no adjustment for coexisting chronic diseases (4;7).

We therefore conducted a cohort study in medical ward patients who had one or more blood cultures taken within two days of admission, in order to compare mortality in patients with bacteremia and patients with negative blood cultures while adjusting for potential confounders. Our aims were to examine (i) the association between blood culture status, defined as positive (i.e. bacteremia) or negative blood cultures, and early mortality (within

3-7 days of hospital admission), short-term mortality (within days 8-30), and long-term mortality (within 31-180 days): (ii) the association between blood culture status and mortality according to the type of bacteremia: and (iii) the association between blood culture status and mortality according to levels of comorbidity.

Material and methods

Study setting and population

We conducted this study in North Jutland County, Denmark (population ~500,000) between January 1, 1995 and December 31, 2006. The study included all adult patients (≥15 years) with one or more blood cultures taken within two days of admission to one of 17 medical departments in the county; the medical departments represented general internal medicine and seven medical specialties, as well as cardiology, medical oncology, neurology, and rheumatology departments. Included patients had no previous blood cultures and no hospital contact during the preceding 30 days (Figure 1). All county residents have access to universal tax-supported health care provided by general practitioners and seven hospitals (8), of which Aalborg Hospital (600 beds) serves as both a district hospital for the greater Aalborg area (~200,000 inhabitants) and as a referral hospital. Bacteriological services for the entire county, including blood cultures, were provided by the Department of Clinical Microbiology, Aalborg Hospital. Since 1968, all residents in Denmark have been registered in the Civil Registration System and given a unique identification number that is used in all national registries to identify that person. Linkage between registries can be made on the basis of this number (9;10).

Blood culture data

Blood culture data were obtained from the electronic laboratory information system (ADBakt, Autonik, Ramsta, Sköldinge, Sweden) at the Department of Clinical

Microbiology, Aalborg Hospital. A blood culture set comprised three blood culture bottles that were inoculated bedside using one needle. Two different systems for blood culture were used during the study period: the Colorbact system in 1995 (Statens Serum Institut, Copenhagen, Denmark) (11) and the BacT/Alert system (bioMerieux, Marcy l'Etoile, France) in 1996-2006. The nominal volume per blood culture was 20-22 mL for the Colorbact system and 28-32 mL for the BacT/Alert system. Positive blood cultures were unloaded at fixed times between 8 a.m. and 8.30 p.m. and examined immediately by a technician (12). On the basis of the microscopy results, a first notification was made by telephone to the attending physicians; antibiotic treatment was adjusted if deemed inappropriate. As soon as the antibiotic susceptibility pattern of the isolate was obtained, a second contact was made to either confirm or adjust antibiotic treatment. Negative blood cultures were incubated for a total of 6.7 days, after which a written report was sent to the attending physicians.

Data on community-acquired bacteremia

We defined bacteremia as a clinical entity associated with the presence of viable bacteria or fungi in the bloodstream, as evidenced by blood cultures in which contamination has been effectively ruled out (2). We regarded coagulase-negative staphylococci, *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* as contaminants unless they were isolated from two or more separate blood culture sets (13). Community-acquired bacteremia was defined as an infection present or incubating at the time of hospital admission (14); in this study, positive blood cultures obtained within the first two days of admission were considered evidence of community-acquired bacteremia. Patients with community-acquired bacteremia were identified in the microbiological North Denmark Region Bacteremia Research Database, in which all episodes of bacteremia in North Jutland County have been registered since 1992 concurrently with the clinical episode (15). The recorded information

includes the following: the patient's civil registration number, age, sex, date of admission, specialty and ward on the date of venipuncture, origin and focus of infection, data on bacterial isolates and susceptibility patterns, and empirical antibiotic treatments. We excluded patients who had regular hospital visits or a hospital stay in the 30 days prior to admission; these cases were considered to constitute a separate healthcare-related bacteremia group (16). The bacteremia episodes were further categorized into three categories according to the isolated pathogens: Gram-positive, Gram-negative, and polymicrobial or fungemia.

Data on comorbidity and marital status

The level of comorbidity was classified according to the Charlson Comorbidity Index (17;18). We computed this index based on International Classification of Diseases (ICD codes) for all previous diagnoses recorded in the National Registry of Patients since 1977, defining three levels of comorbidity; low (score of 0), medium (scores of 1-2), and high (score of ≥3) (19). As a marker of social status (20;21), we obtained information through the Civil Registration System about marital status (married, never married, divorced or widowed) on the date of blood culture.

Statistical analysis

Mortality was the primary outcome and was determined using the Civil Registration System (9;10). Because our definition of patients with community-acquired bacteremia was based on positive blood cultures obtained within the first two days of admission, our follow-up started on the third day of admission and extended for 180 days or until death or migration, whichever occurred first (22). To compare mortality in patients with positive and negative blood cultures over different time-periods, we categorized the follow-up time into three intervals: early mortality (within the first 3 to 7 days of admission), short-term

mortality (within 8 to 30 days), and long-term mortality (within 31 to 180 days). Kaplan-Meier curves and product limit estimates were calculated for these outcomes. To compare mortality in patients with at least one positive blood culture obtained within the first two days of admission and patients with negative cultures, we used Cox regression analysis to compute crude and adjusted MRRs for each time period with 95% confidence intervals (CI). All models included adjustment for calendar periods (1995-1998, 1999-2002, and 2003-2006), age at the date of blood culture (15-39, 40-59, 60-79, 80 years and older), sex, marital status, and level of comorbidity. We were concerned that the evidence for infection might differ among patients with blood cultures, and specifically that some cultures might have been performed to rule out bacteremia rather than in response to clinical suspicion of bacteremia. To address this, we also conducted an analysis restricted to patients with a primary or secondary infectious disease discharge diagnosis (ICD-10 codes A00-B99, G00-G02, I32, I33, I41, J00-J06, J10-J18, J20-J22, J36-37, J85-J86, K65, L00-L03, L080, L088-L080, M00-M01, N10, N12, N30, N39.0). We conducted a Cox regression analysis with categorization of bacteremia into different types (Gram-positive, Gram-negative, and polymicrobial or fungemia). Furthermore, we stratified the analysis by comorbidity level. We assessed the assumption of proportional hazards in the Cox model graphically, and it was appropriate for each follow-up interval. Statistical analyses were performed using Stata Statistical Software v. 9.2 (Stata Corp., College Station, TX, USA). This study was approved by the Danish Data Protection Agency (record no. 2006-41-7413).

Results

During the period from January 1, 1995, to December 31, 2006, we identified 179,917 patients admitted to medical departments in North Jutland County, Denmark, of whom 35,673 had at least one blood culture taken within the first two days of admission. After excluding patients hospitalized within the preceding 30 days (n=6,084), patients with non-community-acquired bacteremia (n=316), and patients who died before the third day of admission (n=663), our study population included 28,610 medical patients (Figure 1). Of these patients, 2,520 (8.8%) had positive blood cultures conducive to the diagnosis of bacteremia (Figure 1). Table 1 shows the discharge diagnosis of patients in the study population.

Patient characteristics

Patients with positive blood cultures were older (median age, 72; interquartile range (IQR), 59-81 years) than patients with negative blood cultures (median age, 68; IQR, 50-79 years). Compared with patients who had negative blood cultures, the prevalence of patients with congestive heart failure, peripheral vascular disease, peptic ulcer disease, and diabetes was slightly higher in patients with community-acquired bacteremia, whereas patients with negative blood cultures had a higher prevalence of chronic pulmonary disease (14.9% vs. 10.6%) (Table 2). Medium or high comorbidity index scores were assigned to 53.0% of patients with community-acquired bacteremia compared with 49.8% of patients with negative cultures.

In the 2,520 patients with positive blood cultures, 1,091 (43.3%) had Gram-positive bacteremia, 1,281 (50.8%) had Gram-negative bacteremia, and 148 (5.9%) had polymicrobial bacteremia or fungemia (there were only 3 fungemias). *Streptococcus pneumoniae* accounted for 55.5% of the Gram-positive pathogens, and *Staphylococcus*

aureus and beta-hemolytic streptococci accounted for 16.8% and 12.8%, respectively. The Gram-negative bacteremias were predominantly Enterobacteriacae (90.1%), with *Escherichia coli* accounting for 71% of all Gram-negative bacteremias.

Blood culture status and mortality

Figure 2 shows mortality curves for patients during the 180 days after admission, stratified according to blood culture result and type of bacteremia. The 3-7 day mortality was 5.1% in patients with community-acquired bacteremia and 3.7% in patients with negative cultures, resulting in an adjusted MRR of 1.2 (95% CI: 1.0-1.4). The strongest confounder was age, which changed the estimate by approximately 14% when included in the analysis. The highest mortality rates were observed among patients with polymicrobial bacteremia or fungemia (3-7-day mortality, 10.1%), followed by patients with Gram-positive bacteremia (3-7-day mortality, 5.8%). Compared with patients with negative cultures, the adjusted 3-7-day MRR was 2.0 (95% CI: 1.2-3.4) for patients with polymicrobial bacteremia or fungemia, 1.5 (95% CI: 1.2-1.9) for patients with Gram-positive bacteremia, and 0.8 (95% CI: 0.6-1.1) for patients with Gram-negative bacteremia (Table 3).

Death during days 8-30 days after admission occurred in 4.9% of patients with community-acquired bacteremia vs. 4.5% of patients with negative cultures. The adjusted MRR was 0.9 (95% CI: 0.8-1.1) for patients with community-acquired bacteremia compared with patients with negative blood cultures. Mortality was 20% higher in patients with Gram-positive bacteremia than in patients with negative cultures (adjusted MRR = 1.2, 95% CI: 0.9-1.6), whereas mortality in patients with Gram-negative bacteremia and patients with polymicrobial bacteremia or fungemia was slightly lower than in patients with negative cultures. However, the mortality estimates for patients with polymircobial bacteremia or fungemia were imprecise (Table 3).

Long-term mortality

We extended follow-up to 180 days after admission for patients who were alive on day 30. During days 31-180, 8.4% of patients with community-acquired bacteremia died compared with 9.3% of patients with negative blood cultures (Table 3). The adjusted MRR for days 31-180 days for patients with community-acquired bacteremia was 0.9 (95% CI: 0.8-1.1) compared with culture-negative patients. Only polymicrobial bacteremia or fungemia was associated with increased long-term mortality when compared with culture-negative patients (adjusted MRR 1.4, 95% CI: 0.9-2.2).

As shown in Table 4, mortality increased with the level of comorbidity. Within each level of comorbidity, community-acquired bacteremia was associated with increased early mortality. We then restricted the analysis to patients with a primary or secondary infectious disease discharge diagnosis and found that mortality in patients with community-acquired bacteremia compared with patients with negative cultures was 4.7% vs. 3.1% after 3-7 days, 4.3% vs. 3.6% after 8-30 days, and 8.3% vs. 6.6% after 31-180 days, respectively, equivalent to adjusted MRRs of 1.5 (95% CI: 1.0-2.2) within 3-7 days, 0.8 (95% CI: 0.5-1.4) within 8-30 days, and 0.9 (95% CI: 0.6-1.3) within 31-180 days of admission, respectively.

Discussion

In this large cohort study of approximately 28,000 medical ward patients, we found slightly increased short-term mortality in patients with community-acquired bacteremia compared with culture-negative patients. The mortality increase conferred by bacteremia was highest during the first week of infection but persisted for at least 180 days among patients with polymicrobial bacteremia or fungemia. Gram-positive bacteremia, polymicrobial bacteremia, and fungemia were associated with the highest mortality whereas mortality in patients with Gram-negative bacteremia was similar to patients who had negative blood cultures. The level of comorbidity did not materially influence the association between blood culture status and mortality.

Because of universal health care coverage in Denmark and our use of data from population-based registries with complete follow-up, the opportunities for selection bias and recall bias were minimized. In order to define a source population of patients with suspected community-acquired bacteremia, we restricted the study to patients with no hospital contact during the preceding 30 days who had blood cultures taken within two days of admission to a medical ward. We may, however, have missed some bacteremias if the patient did not have blood cultures taken (i.e. did not seek medical attention, died before blood cultures were taken, or were treated empirically without cultures). Furthermore, some patients with negative cultures may have had bacteremia that was undetected, especially if they received antimicrobial therapy prior to cultures being obtained (23). Such information bias would cause an underestimation of bacteremia-related mortality and thereby lead to more conservative relative mortality estimates. Unfortunately, there is no gold standard test for bacteremia, and false-negatives cannot currently be identified. Because we used routine hospital discharge data to identify comorbidities, some coding errors may have occurred. The validity of discharge diagnoses registered in the National Patient Registry is variable

but is generally high for the most prevalent diseases including diabetes, myocardial infarction, chronic obstructive pulmonary disease, and cancer (24). Nonetheless, the accuracy of the discharge diagnosis from previous admissions is unlikely to be affected by the results of blood cultures obtained during the current admission. Thus, any misclassification should bias the observed mortality estimates toward unity.

We are aware of only two studies that compared the prognosis of patients with positive and negative blood cultures (4;5). In agreement with our findings, a Canadian study (4) found that patients with bacteremia had a considerably higher in-hospital mortality in the 30 days after a positive blood culture than patients with a negative blood culture. Within this time window, the difference in mortality was higher than in our study, possible because only in-hospital mortality was measured and patients with negative cultures were matched only by age and sex, but not comorbidity. Similarly, Bates et al (5) found that short-term mortality was higher among 142 bacteremia patients (adjusted 30-day MRR = 2.3, 95% CI: 1.2-4.4), whereas long-term mortality was less affected by the presence of bacteremia (adjusted 1-year MRR = 1.3, 95% CI: 0.76-2.1) when compared with 142 patients with negative blood cultures (matched by age, sex, severity of underlying disease, and the presence of major comorbidity). In comparison, long-term survival was severely curtailed in 1,991 patients with bacteremia at Beillinson Medical Center, Israel, who were compared with 1,991 controls without infectious diseases matched by age, sex, underlying diseases, department, and date of admission (3); mortality within 6 months of follow-up was 43% in the bacteremia patients compared with 19% in the controls.

Mortality from bacteremia is the result of several pathophysiological mechanisms. Recently, Laupland et al (7) reported that bacteremia was associated with a 60% increase in in-hospital mortality (crude OR = 1.6, 95% CI: 1.1-2.2) among Canadian ICU patients with

systemic inflammatory response syndrome. When they adjusted for variables that reflected the acute systemic response to infection, the adjusted OR was 1.1 (95% CI: 0.7-1.8). This suggests that the effect of bacteremia to a large extent is mediated by the severity of the systemic inflammatory response. Likewise, a study based on data from 170 French ICUs showed that bacteremia was associated with mortality within 3 days of ICU admission (OR = 1.7, 95% CI: 1.1-2.8) but not at 28 days after admission in patients with severe sepsis and septic shock (6). In that study mortality within 3 days of ICU admission was associated with an increased number of failing organs and with variables reflecting the acute response to infection (6). Mortality within 28 days of admission was also associated with the severity of the underlying disease(s) and with pre-existing organ insufficiency (6). We used overall mortality as the primary outcome measure because we find that attempts to distinguish attributable and non-attributable fractions of mortality is at best a reflection of the current understanding of the contributions of the septic process and underlying disorders. However, the use of patients with negative blood cultures as our reference is an important premise for our findings. We cannot grade the level of sepsis on the basis that a blood culture was obtained, but we surmise that patients with negative cultures had presented with several essential signs of sepsis and not just pyrexia. Our MRRs may, therefore, reflect the impact of bacteremia per se on mortality. However, it is possible that underlying disorders and comorbidities can have influenced the physician's decision to obtain blood cultures. Still, the association between blood culture status, and early, short-term, and long-term mortality remained robust in analyses restricted to patients with a primary or secondary infectious disease discharge diagnosis and in analyses stratified according to the level of comorbidity.

The predominant Gram-positive pathogens, *S. pneumoniae*, *S. aureus*, and beta-hemolytic streptococci, are all primary pathogens that possess an array of virulence factors, which may have caused the greater and longer-lasting influence on mortality in patients with

Gram-positive or polymicrobial bacteremia and fungemia. In addition, the systemic inflammatory response in sepsis is a consequence of activation of pro-inflammatory and counteracting anti-inflammatory immune reactions. It has been suggested that the balance between the beneficial and deleterious effects of the systemic inflammatory immune response may differ between Gram-positive and Gram-negative infections (25). Polymicrobial infections and fungemia are observed most commonly in immunocompromised, chronically ill, or elderly patients (26), and though we adjusted for age and comorbidities, we cannot preclude residual confounding from underlying disorders. The almost identical mortality in patients with Gram-negative bacteremia and patients with negative blood cultures is intriguing and needs further investigation. It may be related to the utility of blood cultures; positive blood cultures can provide a microbiological diagnosis, guide antibiotic treatment, and also alert clinicians to underlying sites of infection (27;28). This information is not available for patients with negative cultures.

In conclusion, to the best of our knowledge, our study is the first to compare mortality in patients with community-acquired bacteremia with mortality in patients with negative blood cultures. We found that community-acquired bacteremia was associated with an increased risk of mortality in the first week of medical ward admission. This increase was mediated by Gram-positive bacteremia and polymicrobial bacteremia or fungemia, since patients with Gram-negative bacteremia had the same or a slightly lower risk of death as patients with negative blood cultures.

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Table 1. Discharge diagnoses of 28,610 medical ward patients who were not hospitalized in the preceding 30 days, had blood cultures taken within the first two days of admission, and who were alive on the third day of admission.

Discharge diagnosis	Blood culture	
	Negative (%)	Positive (%)
Total	26,090	2,520
Infectious diseases		
Primary discharge diagnosis	11,306 (43.3)	1,924 (76.4)
Primary or secondary discharge diagnosis	13,688 (52.5)	2,157 (85.6)
Pneumonia	6,923 (26.5)	644 (25.6)
Urinary tract infection	2,548 (9.8)	531 (21.1)
Intestinal infectious disease	1,032 (4.0)	76 (3.0)
Fever of unknown origin	766 (2.9)	20 (0.8)
Neoplasm	1,104 (4.2)	36 (1,4)
Anemia	279 (1.1)	9 (0.4)
Diabetes	401 (1.5)	11 (0.4)
Neurological or mental disease	597 (2.3)	10 (0.4)
Cardiovascular disease	2,692 (10.3)	60 (2.4)
Respiratory disease	1,733 (6.6)	27 (1.1)
Gastrointestinal disease	1,052 (4.0)	79 (3.1)
Skin, connective tissue, or musculoskeletal disease	739 (2.8)	21 (0.8)
Renal and urinary tract disease	265 (1.0)	23 (0.9)
Symptoms without a specific diagnosis*	2,158 (8.3)	54 (2.1)
Medical poisoning	313 (1.2)	5 (0.2)
Other†	303 (1.2)	8 (0.3)

*Includes patients under evaluation for suspected diseases and conditions (~50%), patients with dehydration (~12%), patients with disorientation, dizziness, headache, syncope or collapse (~12%), and patients with pain, nausea, or vomiting.

† Endocrine and metabolic disorders (other than diabetes), as well as diseases of the eye and ear

Table 2. Descriptive characteristics of 28,610 medical ward patients who had one or more blood cultures taken within two days of hospital admission and who were alive on the third day of admission.

	Blood culture	
	Negative (%)	Positive (%)
Total	26,090 (91.2)	2,520 (8.8)
Calendar period		
1995-1998	8,525 (32.7)	876 (34.8)
1999-2002	8,227 (31.5)	801 (31.8)
2003-2006	9,338 (35.8)	843 (33.5)
Marital status		
Married	12,148 (46.6)	1,153 (45.8)
Never married	5,029 (19.3)	333 (13.2)
Divorced or widowed	8,640 (33.1)	997 (39.6)
Unknown	273 (1.1)	37 (1.5)
Sex		
Male	13,267 (50.9)	1,161 (46.1)
Female	12,823 (49.1)	1,359 (53.9)
Age group		
15-39	4,390 (16.8)	204 (8.1)
40-59	5,304 (20.3)	469 (18.6)
60-79	10,626 (40.7)	1,109 (44.1)
80 and older	5,770 (22.1)	738 (29.3)
Comorbidity		
Previous myocardial infarction	1,672 (6.4)	169 (6.7)
Congestive heart failure	1,865 (7.2)	222 (8.8)
Peripheral vascular disease	1,395 (5.4)	170 (6.7)
Cerebrovascular disease	2,623 (10.1)	266 (10.6)
Dementia	410 (1.6)	40 (1.6)
Hemiplegia	115 (0.4)	5 (0.2)
Chronic pulmonary disease	3,898 (14.9)	266 (10.6)
Connective tissue disease	1,077 (4.1)	124 (4.9)
Peptic ulcer disease	1,736 (6.7)	199 (7.9)
Mild liver disease	347 (1.3)	59 (2.3)
Moderate to severe liver disease	84 (0.3)	20 (0.8)
Diabetes without end-stage organ damage	1,804 (6.9)	242 (9.6)
Diabetes with end-stage organ damage	862 (3.3)	96 (3.8)
Moderate to severe renal disease	82 (0.3)	19 (0.8)
Solid cancer	2,752 (10.6)	293 (11.6)
Metastatic solid cancer	362 (1.4)	46 (1.8)
Leukemia	170 (0.7)	16 (0.6)
Lymphoma	308 (1.2)	24 (1.0)
AIDS	28 (0.1)	3 (0.1)
Charlson Comorbidity Index Score		
Low score (0)	13,108 (50.2)	1,185 (47.0)
Medium score (1-2)	9,434 (36.2)	940 (37.3)
High score (>2)	3,548 (13.6)	395 (15.7)

Table 3. Crude and adjusted risk of death within 3-7, 8-30, and 31-180 days of admission among medical patients with one or more blood cultures taken within two days of hospital admission.

Blood culture status	n	3-7 days of admission			8-30 days of admission†			31-180 days of admisson‡		
			MRR			MRR			MRR	
		Mortality (95% CI)	Crude (95% CI)	Adjusted* (95% CI)	Mortality (95% CI)	Crude (95% CI)	Adjusted (95% CI)	Mortality (95% CI)	Crude (95% CI)	Adjusted (95% CI)
Negative	26,090	3.7 (3.5-3.9)	1.0 (reference)	1.0 (reference)	4.5 (4.3-4.8)	1.0 (reference)	1.0 (reference)	8.4 (8.1-8.8)	1.0 (reference)	1.0 (reference)
Positive	2,520	5.1 (4.3-6.0)	1.4 (1.1-1.6)	1.2 (1.0-1.4)	4.9 (4.1-5.8)	1.1 (0.9-1.3)	0.9 (0.8-1.1)	9.3 (8.2-10.5)	1.1 (1.0-1.3)	0.9 (0.8-1.1)
Gram-positive	1,091	5.8 (4.6-7.4)	1.6 (1.2-2.0)	1.5 (1.2-1.9)	5.6 (4.3-7.1)	1.2 (0.9-1.6)	1.2 (0.9-1.5)	8.9 (7.2-10.8)	1.1 (0.9-1.3)	1.0 (0.8-1.3)
Gram-negative	1,281	3.8 (2.9-5.0)	1.0 (0.8-1.3)	0.8 (0.6-1.1)	4.3 (3.3-5.6)	1.0 (0.7-1.3)	0.8 (0.6-1.0)	8.2 (6.7-10.1)	1.1 (0.9-1.3)	0.9 (0.7-1.0)
Polymicrobial or fungemia	148	10.1 (6.2-16.3)	2.7 (1.6-4.4)	2.0 (1.2-3.4)	4.5 (2.1-9.8)	1.0 (0.5-2.3)	0.8 (0.3-1.7)	14.2 (9.2-21.6)	1.8 (1.1-2.9)	1.4 (0.9-2.2)

*Adjusted for age, sex, level of comorbidity, marital status, and calendar period.

†For patients alive on day 8.

‡For patients alive on day 31

Table 4. Crude and adjusted risk of death within 3-7, 8-30, and 31-180 days after admission among medical patients alive on the third day of admission stratified by level of comorbidity.

Comorbidity	Blood culture status	n	3-7 days of admission		8-30 days of admission†		31-180 days of admission‡	
			Mortality, % (95% CI)	Adjusted MRR* (95% CI)	Mortality, % (95% CI)	Adjusted MRR (95% CI)	Mortality, % (95% CI)	Adjusted MRR (95% CI)
Low (0)	Negative	13,108	2.5 (2.3-2.8)	1.0 (ref)	2.9 (2.7-3.2)	1.0 (ref)	4.8 (4.4-5.2)	1.0 (ref)
	Positive	1,185	3.4 (2.5-4.6)	1.0 (0.8-1.4)	3.3 (2.4-4.5)	0.8 (0.6-1.2)	6.4 (5.1-8.0)	1.1 (0.8-1.4)
Medium (1-2)	Negative	9,434	4.3 (3.9-4.8)	1.0 (ref)	5.3 (4.9-5.8)	1.0 (ref)	10.4 (9.7-11.0)	1.0 (ref)
	Positive	940	5.6 (3.3-7.3)	1.2 (0.9-1.5)	5.2 (3.9-6.9)	1.0 (0.7-1.3)	10.5 (8.6-12.7)	0.9 (0.7-1.1)
High (>2)	Negative	3,548	6.3 (5.6-7.2)	1.0 (ref)	8.3 (7.4-9.3)	1.0 (ref)	17.6 (16.3-18.9)	1.0 (ref)
	Positive	395	8.9 (6.4-12.1)	1.3 (0.9-1.9)	8.9 (6.4-12.3)	1.0 (0.7-1.5)	15.9 (12.3-20.3)	0.9 (0.7-1.2)

*Adjusted for age, sex, chronic diseases, marital status, and calendar period.

†For patients alive on day 8.

‡For patients alive on day 31

Figure 1. Flow diagram of the study population

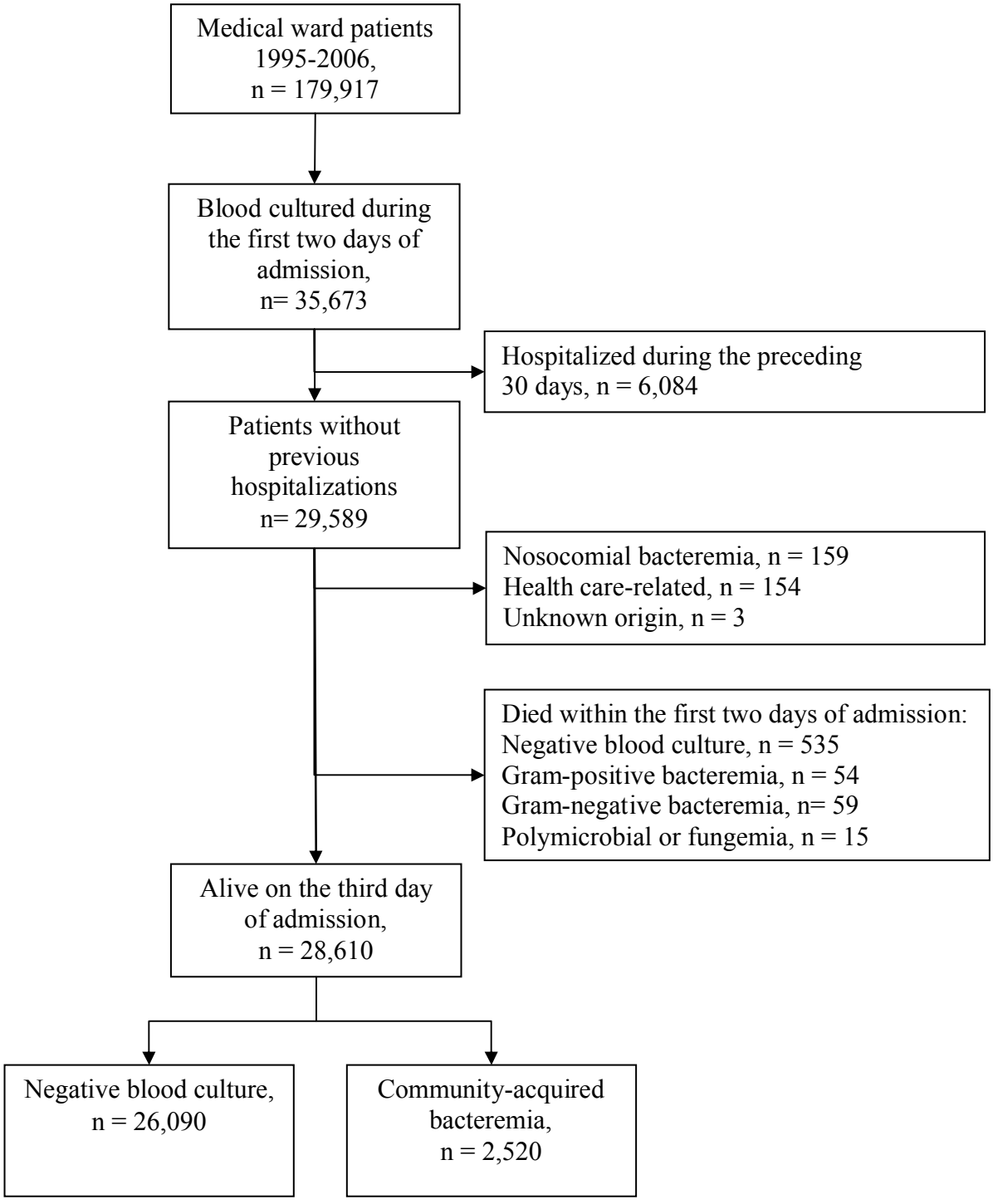
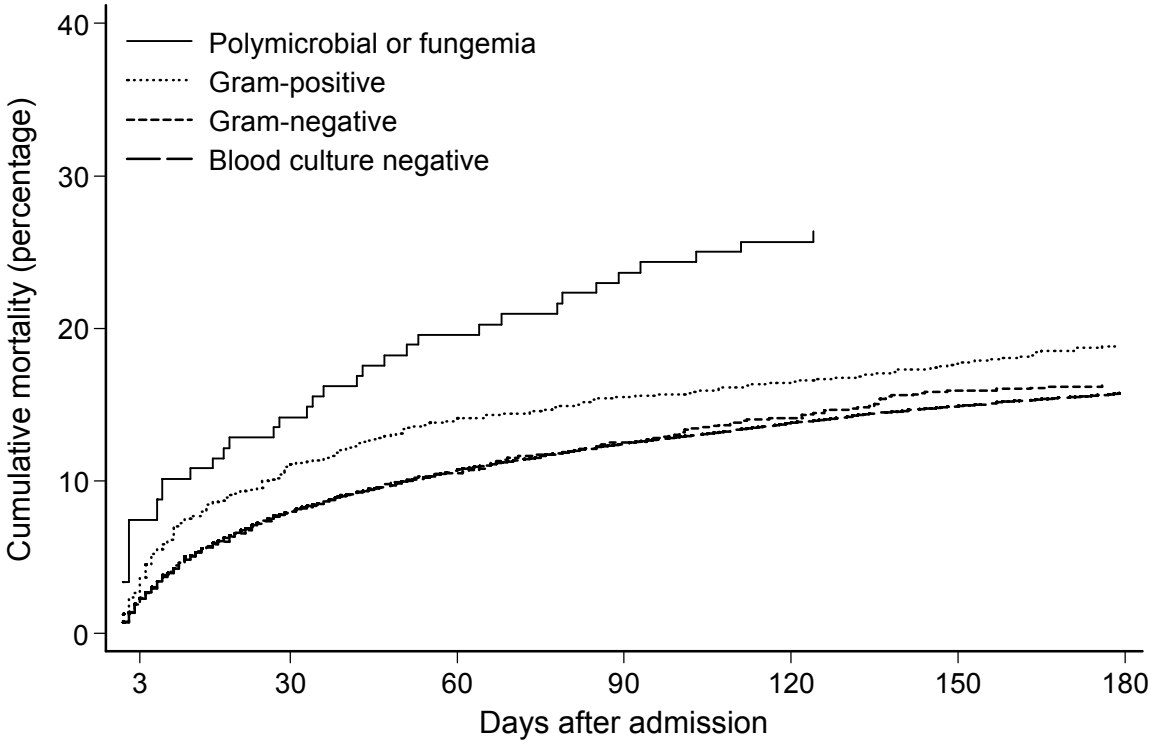


Figure 2. Mortality curves for the 28,610 medical patients alive on the third day of admission, stratified by blood culture result and type of bacteremia.



Short-Term Mortality in Relation to Age and Comorbidity in Older Adults with Community-Acquired Bacteremia: A Population-Based Cohort Study

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(Editorial Comments by Chistopher J. Crinch and David R. Zimmerman, 1750–1752)

OBJECTIVES: To assess 30-day mortality from bacteremia in relation to age and comorbidity and the association between age and mortality with increasing comorbidity.

DESIGN: Population-based cohort study.

SETTING: North Jutland County, Denmark.

PARTICIPANTS: Adults in medical wards with community-acquired bacteremia, 1995 to 2004.

MEASUREMENTS: Smoothed mortality curves and computed mortality rate ratios (MRRs) using Cox regression analysis.

RESULTS: Two thousand eight hundred fifty-one patients, 851 aged 15 to 64, 1,092 aged 65 to 79, and 909 aged 80 and older were included. Mortality increased linearly with age. Compared with patients younger than 65, adjusted MRRs in patients aged 65 to 79 and 80 and older were 1.5 (95% confidence interval (CI) = 1.2–2.0) and 1.8 (95% CI = 1.4–2.3), respectively. Mortality also increased with level of comorbidity. Compared with patients with low comorbidity, adjusted MRRs in patients with medium and high comorbidity were 1.5 (95% CI = 1.2–1.8) and 1.7 (95% CI = 1.4–2.2), respectively. Regardless of the level of comorbidity, MRRs were consistently higher in older than in younger patients.

CONCLUSION: Older age and greater comorbidity predicted mortality, and increasing age-related comorbidity did not explain the effect of age. **J Am Geriatr Soc 56:1593–1600, 2008.**

Key words: bacteremia; age; comorbidity; mortality

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Bacteremia is an important public health concern, with a reported rate of 7.6 to 16.9 per 1,000 hospital admissions in many countries and 30-day mortality greater than 20%.[1–3] The incidence increases markedly with age, and bacteremia is associated not only with greater short-term mortality, but also with long-term impairment of health and high risk of death.[4,5] As average life expectancy increases in most countries, bacteremia may become an even more common clinical problem.

Age is a strong predictor of mortality in patients with bacteremia, but the association between age and mortality is not clear. Former studies have shown a relative risk of death within 30 days ranging from 1.9 to 6.5 in patients aged 65 and older compared with younger patients,[2,6,7] but those studies were clinic based and investigated the effect of age in subpopulations such as patients in the intensive care unit or with cancer,[3,8] patients in geriatric hospitals,[9] and patients with infection caused by selected bacterial species.[7,10] The results may therefore have limited generalizability and are potentially biased because of incomplete follow-up,[9,11,12] lack of control groups,[9,12–14] and uncontrolled confounding from diseases other than bacteremia.[13–16]

Being closely related to older age, a greater burden of comorbidity (i.e., the presence of chronic diseases in addition to community-acquired bacteremia) may also partially explain the higher mortality in older patients. Nevertheless, no data exist on age-related levels of comorbidity influencing the effect of older age on mortality from bacteremia. Previous studies on the effect of comorbidity were also clinic based and restricted to selected patient groups such as critically ill patients[17] and patients with *Staphylococcus aureus*[18] or enterococcal bacteremia.[10]

Therefore, a population-based cohort study was conducted that aimed to assess in detail the association between older age and mortality from community-acquired bacteremia, whether the level of comorbidity affects mortality adjusted for

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age, and the association between age and mortality in patients with bacteremia with increasing levels of comorbidity.

METHODS

Setting and Study Population

The study was conducted in North Jutland County, Denmark (mean population 1995, 488,303; 2004, 495,669), using data from 1995 to 2004. The study was restricted to adult patients (aged $\geq$15) admitted to a medical department who were residents of North Jutland County at the time of admission. The Danish healthcare system provides universal tax-supported care, guaranteeing free access to primary and hospital care. Patients with bacteremia were treated in one of seven public hospitals, of which one (Aalborg University Hospital) served as district and referral hospital. The Department of Clinical Microbiology, Aalborg Hospital, provided bacteriological services, including blood cultures, for the entire county. During the study period, all clinical specialities were represented, with the exception of organ and bone marrow transplantations, plastic surgery, and dermatology.

The Department of Clinical Epidemiology, Aarhus University Hospital, runs a research record linkage database containing several datasets, including bacteremia data, hospital discharge data, and mortality data.

Patients with Community-Acquired Bacteremia

All episodes of bacteremia in North Jutland County since 1992 have been registered contemporaneously with the events in the County Bacteremia Registry.[19,20] The information includes the patient's civil registration number, age, sex, date of admission, speciality and ward on the date of venipuncture, origin and focus of infection, and data on bacterial isolates and susceptibility patterns and the empirical antibiotic treatment given.

All adult county residents who had their first episode of community-acquired bacteremia during January 1, 1995, to December 31, 2004, were identified. Community-acquired bacteremia was defined as an episode of bacteremia present or incubating at admission to the hospital.[21] Patients with regular contact with hospitals or a hospitalization within 30 days before admission with bacteremia were excluded, because these cases were considered to constitute a distinct group more affected by factors associated with nosocomial infections.[22]

Characteristics of Bacteremia

Blood cultures were obtained upon a physician's suspicion of infection. Two different systems for blood culture were used during the study period: the Colorbact system (Statens Serum Institut, Copenhagen, Denmark)[23] (1995) and the BacT/Alert system (bioMérieux, Marcy l'Etoile, France) (1996–2004). The nominal volume per blood culture for the two systems was 20 to 22 mL and 28 to 32 mL for adult patients, respectively.

Bacteremia was defined as bacterial or fungal growth in blood cultures in which the isolated pathogen was determined to have an etiological role based on clinical and microbiological assessment. Coagulase-negative staphylococci, *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* were regarded as contaminants unless they were isolated from two or more separate blood culture sets. Based on the isolated pathogens, three types of bacteremia were defined: gram-positive, gram-negative, and a third group encompassing polymicrobial bacteremia and fungemia. The focus of infection was assessed based on microbiological and clinical findings and categorized as urinary, respiratory, abdominal or hepatobiliary, miscellaneous, or unknown.

The empirical antibiotic therapy administered was recorded at first notification of the positive blood culture. It was regarded as appropriate if given intravenously (with the exception of fluoroquinolones and metronidazole) and if the blood isolate was susceptible to one or more of the antibiotic drugs given and as inappropriate if isolates were found to be resistant or if doses or the form of administration were insufficient.[1] In some cases the patients had already died, or the treatment had ceased, because the patients were terminally ill and a decision to withhold therapy had been made.

Comorbidity

To assess the prognostic effect of comorbidities, the Charlson Comorbidity Index[24] was computed for each patient based on the complete hospital discharge history in the county before the date of admission. The index has been adapted and validated for use with hospital discharge data for prediction of short- and long-term mortality.[25] It includes 19 major disease categories weighted according to their effect on patient survival, and the score is the sum of these weights. The score was computed based on all previous discharge diagnoses recorded in the Hospital Discharge Registry covering all hospitals in the county since 1977. Physicians coded the diagnoses at discharge according to the *International Classification of Diseases, Eighth Revision* (ICD-8) until the end of 1993 and the Tenth Revision (ICD-10) thereafter. For this study, three levels of comorbidity were defined based on Charlson index scores: 0 (low), corresponding to patients with no recorded underlying diseases included in the Charlson index; 1–2 (medium), and >2 (high).[8,26]

Marital Status and Mortality Data

The Central Office of Civil Registration assigned a 10-digit civil registration number to each Danish citizen shortly after birth. The number is used in all public records and contains embedded codes for age and sex. This civil registration system, which is updated daily, also contains information on vital status (dead or alive), date of birth, marital status, and the residence of all Danish citizens. Through the civil registration number, information was obtained on age and marital status (married, never married, divorced, or widowed) at the date of first positive blood culture. Follow-up began on the date the patient's first positive blood culture was drawn, and the vital status of all patients was followed until death or migration or for 30 days, which ever came first.

Statistical Analysis

The study outcome was death within 30 days. Kaplan-Meier curves and product limit estimates were computed for the main study variables: age (15–64, 65–79, and ≥80), sex, marital status, level of comorbidity (according to Charlson score categories), type of bacteremia, focus of infection, and whether the initial antibiotic treatment was appropriate. To assess graphically the relationship between age, or comorbidity, and bacteremia mortality, quadratic splines were used to smooth the crude 7- and 30-day mortality curves, with linear restrictions imposed on both tails.[27]

To compare the risk of death in the different age groups and levels of comorbidity, Cox proportional hazards analysis was used to compute the mortality rate ratio (MRR) for 7- and 30-day mortality with 95% confidence intervals (CIs), controlling for sex, marital status, type of bacteremia, focus of infection, and whether the initial antibiotic treatment was appropriate. Comorbidity and age were included in the Cox model as continuous variables when examining the effect of age and comorbidity, respectively. In the initial model, the variable "calendar time" was also included, comparing the first and second half of the study period (1994/99 vs 2000/04) as a covariate. This variable did not have any effect on the estimates of the other variables in the adjusted analysis and was excluded from the final model.

To assess the effect of age at different levels of comorbidity, bacteremia patients with low comorbidity in the youngest age group were chosen as the reference group against which mortality rates were compared. For each of the remaining combinations of age and comorbidity, a binary variable was created that indicated age and level of comorbidity, which were entered into the Cox regression model.[28]

The assumption of proportional hazards was assessed graphically and found to be appropriate.

Statistical analyses were performed using Stata Statistical Software version 9.0 (Stata Corp., College Station, TX). The Danish Data Protection Agency approved the study (Record no. 2006-41-7413).

RESULTS

Descriptive Data

Two thousand eight hundred fifty-one patients with a first hospitalization for community-acquired bacteremia and a median age of 74 (range 15–99, interquartile range 61–82) were identified. Of these, 1,374 (48%) were male and 1,477 (52%) were female. The distribution between the sexes varied with age; in patients younger than 65 52% were male, compared with 46% of patients aged 80 and older. The characteristics of the patients are shown in Table 1. The prevalence of patients with a medium or high comorbidity index was similar in the two oldest age groups (69% and 65%, respectively) but considerably higher than in the reference group of patients aged 15 to 64 (44%). The prevalence of patients with an unknown focus of infection increased from 14% in patients younger than 65 to 19% in patients aged 65 to 79 and 24% in patients aged 80 and older. Likewise, the prevalence of bacteremia with a urinary tract focus increased with age, whereas the prevalence of bacteremia with a respiratory tract focus decreased. This change was most pronounced in men, in whom the proportion with a urinary tract focus increased from 15% in the youngest age group to 41% in patients aged 80 and older (data not shown). For women, this increase was only 15%.

Appropriate empirical antibiotic therapy was given less often in patients aged 65 and older than in younger patients. With regard to comorbidity, the prevalence of patients

Table 1. Characteristics of 2,851 Patients with a First-Time Episode of Community-Acquired Bacteremia According to Age

Characteristic	15–64	65–79	≥80
	n (%)		
Total patients	851 (30)	1,091 (38)	909 (32)
Admitted to intensive care unit	72 (8)	40 (4)	29 (3)
Sex			
Male	444 (52)	512 (47)	418 (46)
Female	407 (48)	579 (53)	491 (54)
Comorbidity index			
Low (0)	477 (56)	335 (31)	322 (35)
Medium (1–2)	259 (30)	482 (44)	381 (42)
High (>2)	115 (14)	274 (25)	206 (23)
Marital status			
Married	457 (54)	560 (51)	275 (30)
Never married	223 (26)	83 (8)	66 (7)
Divorced or widowed	171 (20)	448 (41)	568 (62)
Isolated pathogen			
Staphylococcus aureus	77 (9)	93 (9)	55 (6)
Coagulase-negative staphylococci	5 (0.6)	7 (0.6)	2 (0.2)
Beta-hemolytic streptococci	43 (5)	69 (6)	46 (5)
Streptococcus pneumoniae	267 (32)	218 (20)	136 (15)
Other gram-positive organisms	51 (6)	64 (6)	48 (5)
Escherichia coli	206 (24)	406 (37)	387 (43)
Other enterobacteria	83 (10)	117 (11)	100 (11)
Pseudomonas aeruginosa	3 (0.4)	8 (0.7)	9 (1)
Other gram-negative organisms	45 (5)	19 (2)	15 (2)
Anaerobes	28 (3)	24 (2)	19 (2)
Yeasts	2 (0.2)	3 (0.3)	2 (0.2)
Polymicrobial	41 (5)	63 (6)	90 (10)
Focus			
Urinary tract	185 (22)	395 (36)	387 (43)
Respiratory tract	254 (30)	205 (19)	132 (15)
Abdominal and hepatobiliary tract	105 (12)	112 (10)	99 (11)
Miscellaneous*	184 (22)	167 (15)	75 (8)
Unknown	123 (14)	212 (19)	216 (24)
Antibiotic treatment at first notification			
Appropriate	558 (66)	679 (62)	549 (60)
Inappropriate	221 (26)	311 (29)	259 (28)
Treatment ceased†	47 (5)	65 (6)	74 (8)
No information	25 (3)	36 (3)	27 (3)

* Central nervous system, muscles, skin, joint and bones, genital system, and intravascular devices.

† The patients had died or were in terminal stages of their disease.

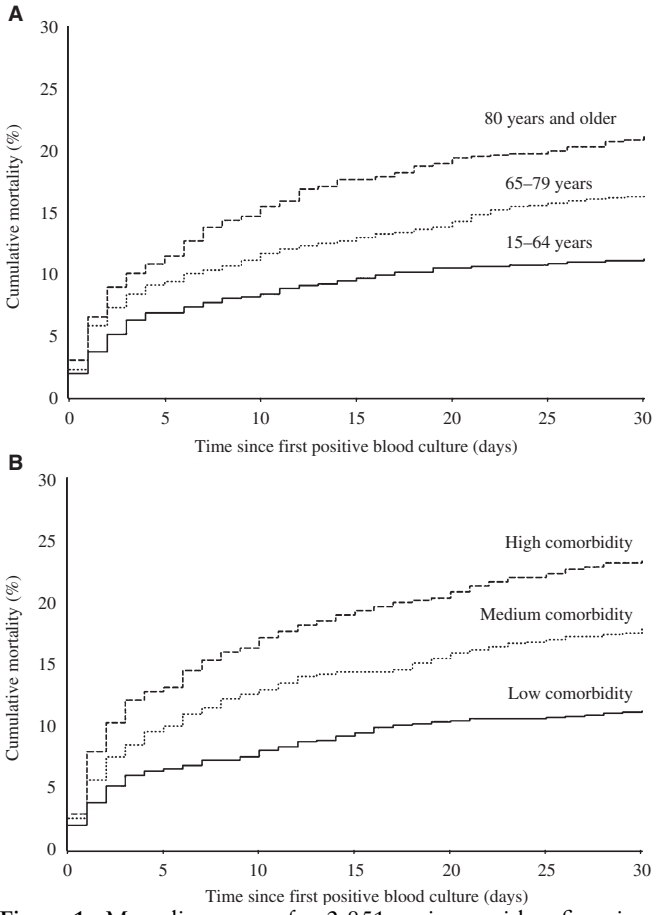

Figure 1. Mortality curves for 2,851 patients with a first-time episode of community-acquired bacteremia stratified according to age group (A) and level of comorbidity (B).

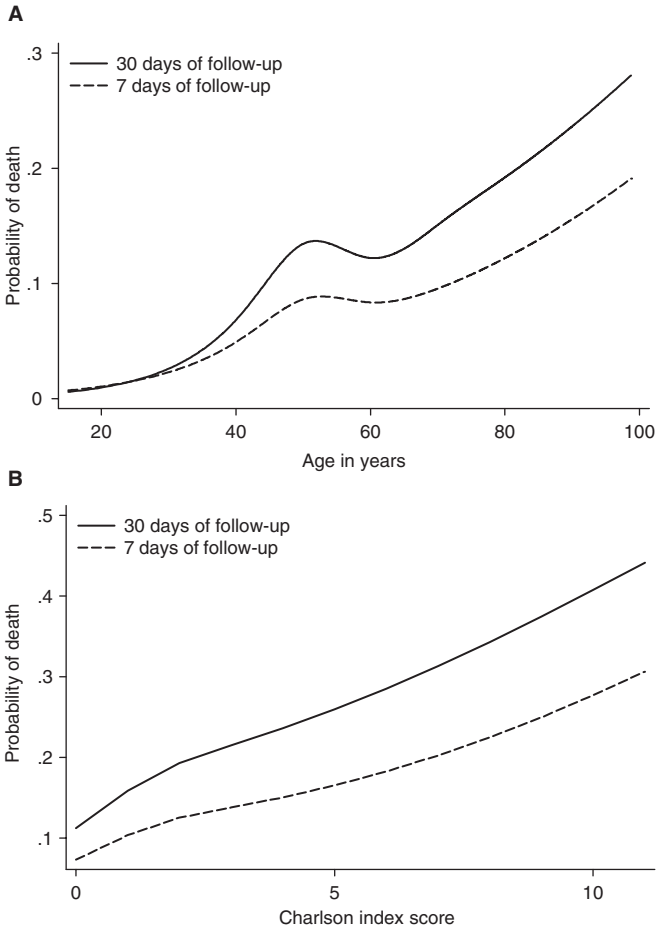

Figure 2. Estimated 7- and 30-day mortality from community-acquired bacteremia related to age (A) or comorbidity (B).

given appropriate treatment decreased from 68% in patients with low comorbidity to 60% in patients with medium comorbidity and 56% in patients with high comorbidity.

Age and Bacteremia Mortality

Figure 1 presents Kaplan-Meier curves displaying 30 days of follow-up for bacteremia patients stratified according to age group and level of comorbidity. The curves diverged early after the positive blood culture had been obtained; patients aged 65 and older and patients with medium or high comorbidity were at greater risk of dying throughout the observation period. The smoothed age–mortality curve indicated that 7- and 30-day mortality increased linearly except for a plateau between the ages of 50 and 65 (Figure 2).

Seven-day mortality was 8% in patients younger than 65, 10% in patients aged 65 to 79 years, and 14% in patients aged 80 and older. When compared with patients younger than 65 years, the crude 7-day MRR was 1.4 (95% CI = 1.0–1.8) for patients aged 65 to 79 and 1.8 (95% CI = 1.4–2.5) for patients aged 80 and older. Adjustment for potential confounders, including level of comorbidity, did not change the respective MRR estimates substantially (Table 2).

Thirty-day mortality was 11% in patients younger than 65, 16% in patients aged 65 to 79, and 21% in patients aged 80 and older. When compared with patients younger than 65, 30-day MRR adjusted for potential confounders, including level of comorbidity, was 1.5 (95% CI = 1.2–2.0) for patients aged 65 to 79 and 1.8 (95% CI = 1.4–2.3) for patients aged 80 and older.

Comorbidity and Bacteremia Mortality

The smoothed comorbidity–mortality curve showed that 7- and 30-day mortality increased almost linearly with increasing levels of comorbidity (Figure 2). Seven-day mortality was 7% in patients with low comorbidity, 12% in patients with medium comorbidity, and 15% in patients with high comorbidity (Table 2). Compared with patients with low comorbidity, the MRR was 1.6 (95% CI = 1.2–2.1) for patients with medium comorbidity and 2.2 (95% CI = 1.6–2.9) for patients with high comorbidity. After controlling for possible confounders, these 7-day MRRs decreased to 1.4 (95% CI = 1.0–1.8) and 1.5 (95% CI = 1.1–2.0), respectively. Thirty-day mortality was 11% for patients with low comorbidity, 18% for patients with medium comorbidity, and 23% for patients with high comorbidity. The corresponding 30-day MRR estimates were equivalent to the 7-day MRR estimates.

Table 2. Crude and Adjusted Risk of Death Within 7 or 30 Days in Patients with a First-Time Admission for Community-Acquired Bacteremia, According to Age and Level of Comorbidity

Risk Factor	n	Dead, n	Mortality % (95% CI)	Mortality Rate Ratio (95% CI)	
				Crude	Adjusted*
Age					
7-day					
15–64	851	65	8 (6–10)	1.0 (reference)	1.0 (reference)
65–79	1,091	112	10 (9–12)	1.4 (1.0–1.8)	1.4 (1.0–2.0)
≥80	909	125	14 (12–16)	1.8 (1.4–2.5)	1.6 (1.1–2.2)
30–day					
15–64	851	95	11 (9–13)	1.0 (reference)	1.0 (reference)
65–79	1,091	179	16 (14–19)	1.5 (1.2–1.9)	1.5 (1.2–2.0)
≥80	909	192	21 (19–24)	2.0 (1.6–2.5)	1.8 (1.4–2.3)
Level of comorbidity					
7-day					
Low (0)	1,134	82	7 (6–9)	1.0 (reference)	1.0 (reference)
Medium (1–2)	1,122	129	12 (10–14)	1.6 (1.2–2.1)	1.4 (1.0–1.8)
High (>2)	595	91	15 (13–18)	2.2 (1.6–2.9)	1.5 (1.1–2.0)
30-day					
Low (0)	1,134	127	11 (10–13)	1.0 (reference)	1.0 (reference)
Medium (1–2)	1,122	200	18 (16–20)	1.6 (1.3–2.1)	1.5 (1.2–1.8)
High (>2)	595	139	23 (20–27)	2.2 (1.7–2.8)	1.7 (1.4–2.2)

* Adjusted for sex, marital status, type of bacteremia, focus of infection, and appropriateness of empirical antibiotic treatment.
CI = confidence interval.

Effect of Age at Different Levels of Comorbidity

In a stratified analysis, it was found that, for patients with low comorbidity, 30-day mortality increased from 7.3% in those aged 15 to 64 to 16.5% in those aged 80 and older. This increase corresponded to an adjusted MRR of 2.2 (95% CI = 1.3–3.7). Similarly, for patients with medium comorbidity, 30-day mortality increased from 15.1% in those aged 15 to 64 to 21.8% in those aged 80 and older, corresponding to an adjusted MRR of 1.7 (95% CI = 1.1–2.5). For patients with high comorbidity, 30-day mortality increased from 18.3% in those aged 15 to 64 to 27.2% in those aged 80 and older, corresponding to an adjusted MRR of 1.2 (95% CI = 0.7–2.1).

An analysis was also performed stratified according to age that showed that, for patients aged 15 to 64, 30-day mortality increased from 7.4% in those with low comorbidity to 18.3% in those with high comorbidity. This increase corresponded to an adjusted MRR of 1.9 (95% CI = 1.0–3.3). Similarly, for patients aged 65 to 79 years, 30-day mortality increased from 11.7% in those with low comorbidity to 22.6% in those with high comorbidity, corresponding to an adjusted MRR of 1.9 (95% CI = 1.3–2.9). For patients with in the oldest age group, 30-day mortality increased from 16.5% in those with low comorbidity to 27.2% in those with high comorbidity, corresponding to an adjusted MRR of 1.6 (95% CI = 1.0–2.3).

The combined effects of age and comorbidity are shown in Table 3, with patients in the youngest age group and a low level of comorbidity serving as the reference. Judging from these data, there is no synergistic effect between age and comorbidity (i.e., the joint effects of age and comorbidity do not exceed the sum of their individual effects on mortality).[42]

Confounding Factors and Bacteremia Mortality

The 30-day adjusted MRRs were 1.3 (95% CI = 0.9–1.9) for unmarried persons and 1.3 (95% CI = 1.0–1.7) for divorced or widowed persons compared with married persons. No major differences were observed in mortality between men and women (30-day adjusted MRR 1.0, 95% CI = 0.8–1.2) or with regard to the appropriateness of empirical antibiotic treatment (30-day adjusted MRR 0.9, 95% CI = 0.6–1.3). The adjusted 30-day MRR for gram-negative bacteremia compared with gram-positive bacteremia was 0.9 (95% CI = 0.7–1.2). For polymicrobial bacteremia or fungemia, the adjusted 30-day MRR was 1.5 (95% CI = 1.0–2.2). In comparison with bacteremia with a urinary tract focus, the adjusted 30-day MRR was 2.5 (95% CI = 1.5–4.0) for bacteremia with a respiratory tract focus. The corresponding adjusted 30-day MRRs were 1.7 (95% CI = 1.0–2.7) for bacteremia with an abdominal or hepatobiliary tract focus; 2.1 (95% CI = 1.2–3.5) for bacteremia originating from the central nervous system, muscles, the skin, joints and bones, the genital system, or intravascular devices; and 2.8 (95% CI = 1.9–4.1) for bacteremia with an unknown focus.

DISCUSSION

This study found that increasing age was associated with increasing mortality in patients with community-acquired bacteremia. Although mortality also increased linearly with

Table 3. Risk of Death Within 30 Days for Patients with a First-Time Admission for Community-Acquired Bacteremia According to Age Group and Level of Comorbidity

	Charlson Comorbidity Index		
Age	Low (0)	Medium (1–2)	High (>2)
15–64			
N	477	259	115
Dead, n	35	39	21
Mortality, %	7.3	15.1	18.3
MRR (95% CI)			
Crude	1.0 (reference)	2.13 (1.35–3.36)	2.61 (1.52–4.48)
Adjusted*	1.0 (reference)	1.98 (1.24–3.15)	2.24 (1.29–3.88)
65–79			
N	335	482	274
Dead, n	39	78	62
Mortality, %	11.6	16.2	22.6
MRR (95% CI)			
Crude	1.62 (1.02–2.55)	2.29 (1.54–3.41)	3.31 (2.19–5.01)
Adjusted*	1.92 (1.20–3.08)	2.37 (1.56–3.60)	3.77 (2.44–5.83)
≥80			
N	322	381	206
Dead, n	53	83	56
Mortality, %	16.5	21.8	27.2
MRR (95% CI)			
Crude	2.33 (1.52–3.57)	3.18 (2.14–4.72)	4.10 (2.69–6.25)
Adjusted*	2.27 (1.44–3.57)	3.34 (2.19–5.11)	3.29 (2.10–5.16)

Note: Reference group = patients in the youngest age group and with low comorbidity.

* Adjusted for sex, marital status, type of bacteremia, focus of infection, and appropriateness of empirical antibiotic treatment.

MRR = mortality rate ratio; CI = confidence interval.

level of comorbidity, a greater burden of comorbidity in elderly people did not fully explain the differences in mortality. Thus, at each level of comorbidity, increasing age adversely affected the outcome.

Consistent with previous reports, the urinary tract was the most common source of bacteremia in all three age groups.[6,12–16,29,30] In contrast, the finding of an increasing prevalence of an unknown focus and a decreasing prevalence of a respiratory tract focus with age differed from previous reports. The most likely explanations for variations between studies are differences in hospital settings and study populations. The current study population was restricted to patients admitted to medical wards with a first-time episode of community-acquired bacteremia, and patients with a previous hospital contact within 30 days were excluded. Community-acquired bacteremia is rarely studied as a separate entity, and it is difficult to determine the patients' first episode without access to population-based health databases.[1,31]

Several studies have addressed the effect of age on bacteremia mortality, with conflicting results,[2,7,9,11–16,30,32,33] but the majority of studies finding no effect of age included patients only aged 65 and older and often made no distinction between old (65–79) and old-old (≥80), leading to a failure in demonstrating differences not only between younger and older people, but also within the older population itself.[9,12,13,16,32,33] Other studies divided patients into two groups: young (<65) and old (≥65),[2,7,15,34] but identifying older people at the cutpoint of age 65, generally corresponding to retirement age, is probably unsatisfactory. Thus, the current results extend those from previous studies. A linear association was found between age and mortality that corroborates the findings in subpopulations of patients with hematological malignancies,[8] sepsis,[35] and *S. aureus* bacteremia.[18] Comorbidity, functional status, and nutritional status rather than age itself have been suggested as risk factors for mortality,[6,16,36] but in many previous studies, differences in comorbidity were not taken into account in the statistical analysis.[13–16] Different levels of comorbidity between the age groups may therefore have confounded the observed association between age and mortality. In agreement with previous studies,[10,17,18,33,37] the current study found that comorbidity is a predictor of mortality.

The Charlson Comorbidity Index was used to control for confounding by comorbidity. The original investigation used to derive the Charlson index predicted 1-year mortality in medical inpatients at a New England teaching hospital in 1984, and the 19 diseases included were selected and weighted on the basis of the strength of their association with mortality. Plotting the 7- and 30-day mortality against the Charlson index score, a linear increase in mortality was observed, supporting the index's capacity to predict short-term mortality in the study population. In accordance with findings from a previous study,[15] the highest 30-day mortality was found in elderly subjects, although the relative effect of age on mortality was higher in patients with no comorbidity. Still, a 49% higher 30-day mortality was found in patients aged 80 and older with high comorbidity than in patients aged 15 to 64 with high comorbidity. Thus, even though comorbidities are highly relevant for predicting the outcomes, it is unlikely, according to these findings, that they fully account for the differences in mortality observed between age groups.

Because comorbidity cannot explain the greater mortality with age, other factors must play a role. Possible explanations are progressive deterioration of the immune system, diagnostic difficulties, and potential differences in treatment and clinical quality associated with old age. Aging is associated with complex changes in most parts of the immune system, including an altered acute phase reaction and thus a greater risk of a poorer outcome after severe infection.[38] The febrile response may be blunted or absent, and older patients with infections often present with vaguer symptoms than younger patients,[39,40] which may delay diagnosis and treatment. The medical databases used in this study lack clinical data on diagnostic and treatment delay, although it was found that the proportion of bacteremia with an unknown focus was 10% greater in the oldest than in the youngest age group and 4% greater with high than with low levels of comorbidity. Failure to determine the focus has been associated with greater mortality in patients with bacteremia[1,41] and may reflect nonspecific symptoms and greater likelihood that older patients die before further diagnostic investigations are undertaken. It was also found that patients aged 65 and older and patients with high comorbidity were less likely than younger patients to receive

appropriate empirical antibiotic treatment and less likely to be admitted to the intensive care unit. This suggests that older patients may also be at risk of receiving suboptimal supportive treatment, which could further influence the prognosis of bacteremia.

This study has several strengths. The uniformly organized Danish public healthcare system allowed for a population-based cohort design with complete follow-up, reducing the risk of several types of bias. Other strengths are the high quality and completeness of the bacteremia data and a large sample with a high prevalence of older patients. In addition, use of routinely recorded medical data, collected independently of the study aim, reduced the risk of information bias. To obtain a more homogeneous study population and thereby reduce confounding from underlying diseases and interventions,[42] the study was restricted to patients admitted to medical departments. The Charlson index is one of the most extensively validated comorbidity indices for predicting mortality,[25] including in bacteremia cases.[18,26,33] The index has been shown to have a high specificity but a more-variable sensitivity.[43] The calculation of the index was based on the entire hospital history, but the index cannot control for confounding from comorbidity as effectively as clinical data. Moreover, it cannot be precluded that comorbidity is recorded more accurately in younger than in elderly patients. It therefore cannot be excluded that residual confounding due to misclassification or possible confounding by severity of disease may have influenced the findings, although the stratified analyses found that restriction to patients without recorded comorbidity did not change the association between age and mortality. Thus, providing an argument against that residual confounding, not captured by the Charlson index, could explain the association between age and mortality.

Data on nursing home residence were not available, but the policy in Denmark is to keep even very frail elderly people in their own homes. They are provided with home-based care on a 24-hour basis, including visits by nurses and nursing assistants. The number of nursing homes has declined markedly during the last 20 years, with their use being limited to a large extent to neuropsychiatric or terminal care. Marital status was used to control for differences in social status. Admittedly, it is a crude marker of social support, and more-elaborate measures have been used in other studies, but because studies have shown that the risk of mortality for widowed, divorced, or single persons is 1.2 to 2.5 times as high as for married persons,[44–46] it is appropriate to include adjustment for marital status and changes in status in an epidemiological analysis.

Because administrative data lacking clinical details were used, there was no information to evaluate the clinical state of the patients at the time of admission or any delay in antibiotic treatment. It was therefore not possible to calculate a measure of disease severity, such as the Acute Physiology and Chronic Health Evaluation II score. Information on functional and nutritional status, which could contribute to mortality in the elderly population, was also lacking.[6,47–49] It is therefore not possible to give the exact reasons for the association between age and poor outcome of bacteremia. It also remains possible that some cases of bacteremia were missed if the patient died before blood cultures were taken. If this applied to older patients to a greater extent, mortality in the elderly group might have been underestimated, leading to more conservative mortality estimates. Because this study focused on community-acquired bacteremia, mortality may also have been underestimated if fewer blood cultures are taken in older patients or if they are postponed because of vaguer symptoms.

In conclusion, aging is a strong predictor of mortality in patients with community-acquired bacteremia admitted to a medical ward. Comorbidity also predicted a fatal outcome, but increasing levels of comorbidity with increasing age did not explain the effect of age on bacteremia mortality.

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