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Staphylococcus aureus bacteraemia - Disease progression and prognosis

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Academic Dissertation
To be presented, with permission of the Faculty of Medicine,
University of Helsinki, for public examination in Auditorium 3,
Biomedicum Helsinki, Haartmaninkatu 8, on September 6 th, 2014,
at 12 noon.

Helsinki 2014

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ISBN 978-951-51-0079-5 (pbk)
ISBN 978-951-51-0080-1 (pdf)
UNIGRAFIA
HELSINKI 2014

To my family, friends and colleagues

Contents

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following four original publications. The publications are referred to in the text by Roman numerals I – IV.

I Forsblom E, Ruotsalainen E, Mölkänen T, Ollgren J, Lyytikäinen O, Järvinen A. Predisposing factors, disease progression and outcome in 430 prospectively followed patients of healthcare- and community-associated *Staphylococcus aureus* bacteraemia. *J Hosp Infect* 2011; 78:102-107.

II Forsblom E, Aittoniemi J, Ruotsalainen E, Helmijoki V, Huttunen R, Jylhävä J, Hurme M, Järvinen A. High cell-free DNA predicts fatal outcome among *Staphylococcus aureus* bacteremia patients with intensive care unit treatment. *PLoS One* 2014; 10:e87741.

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III Forsblom E, Ruotsalainen E, Ollgren J, Järvinen A. Telephone consultation cannot replace bedside infectious disease consultation in the management of *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2013; 56:527-535.

IV Forsblom E, Ruotsalainen E, Järvinen A. Improved outcome with early rifampicin combination treatment in *Staphylococcus aureus* bacteremia with a deep infection fo cus. Submitted.

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
APACHE	Acute Physiological and Chronic Health Evaluation
ARDS	adult respiratory distress syndrome
AUC	area under the curve
CA	community-associated
Cf-DNA	cell-free DNA
CI	confidence interval
CoNS	coagulase-negative staphylococci
CRP	C-reactive protein
CT	computed tomography
CVC	central venous catheter
DIC	disseminated intravascular coagulation
FDG-PET	fludeoxyglucose - positron emission tomography
HA	health care-associated
HIV	human immunodeficiency virus
hVISA	heteroresistant vancomycin intermediate *Staphylococcus aureus*
ICU	intensive care unit
IDS	infectious disease specialist
IDSC	infectious disease specialist consultation
IDU	injection drug use
MIC	minimum inhibitory concentration
MODS	multiple organ dysfunction syndrome
MRI	magnetic resonance imaging
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
OR	odds ratio
PCT	procalcitonin
PFGE	pulsed-field gel electrophoresis
RMP	rifampicin
ROC	receiver-operating characteristic
RR	relative risk
SAB	*Staphylococcus aureus* bacteraemia
SABU	*Staphylococcus aureus* bacteraemia and bacteruria
SIRS	systemic inflammatory response syndrome
SOFA	sequential organ failure assessment
TC^{99}	technetium-99 bone scan
TEE	transoesophageal echocardiography
TTE	transthoracic echocardiography

ABSTRACT

Introduction *Staphylococcus aureus* bacteraemia (SAB) is increasingly common in both community and health care settings. Clinical investigations have demonstrated some fundamental elements in the management of SAB. Intravenously administered antimicrobial therapy is indispensable and needs to be initiated without delay. The presence of deep infection foci dictate duration of therapy, and β-lactam antibiotics are superior in methicillin-sensitive *S. aureus* (MSSA) bacteraemia and should be preferred whenever possible. Deep infection foci, including infected foreign devices (both permanent and non-permanent), should be meticulously sought and eradicated whenever possible. However, despite the use of effective antistaphylococcal antibiotics, radiological investigations, intensive care unit surveillance and invasive or surgical interventions, the overall mortality in SAB has remained high and has ranged from 14% to 32% in recent studies. No substantial reduction in overall SAB mortality has been observed in the past two decades.

The present studies were performed to evaluate factors that may affect progression and prognosis in SAB. The following studies were undertaken: 1) evaluation of predisposing factors, severity of illness, clinical picture and outcome of methicillin-sensitive health care-associated (HA-) and community-associated (CA-) SAB in disease progression and prognosis, 2) comparison of the prognostic value of cell-free DNA (cf-DNA) and C-reactive protein as biomarkers among ICU and non-ICU SAB patients, 3) evaluation of the impact of bedside (formal) infectious disease specialist consultation (IDSC), telephone (informal) IDSC and no IDSC on disease progression and prognosis, 4) investigation of the impact of rifampicin (RMP) combination therapy on outcome.

Study population The studies were based on 430 prospectively followed SAB patients in a nationwide multicentre study in 1999-2002 and retrospectively collected data from all SAB patients (n=187) in Helsinki University Central Hospital in 2000-2002 and 2006-2007. For studies on comparison of HA- and CA-SAB and cell-free DNA as a prognostic marker alone the prospective patient cohort was used. IDSC and RMP combination therapy were analysed from the retrospective patient cohort.

Main results CA-SAB cases represented 46% of all SAB cases and differed from the HA-SAB cases in many respects. CA-SAB patients, as compared with HA-SAB cases, were younger (52.9±19.5 vs. 62.4±15.2 years, ±SD, $p < 0.0001$), had less chronically ill cases (12% vs. 41% of patients, $p < 0.0001$, in McCabe's classification) and presented higher prevalence of deep infection foci within three days of *S. aureus*-positive blood culture (84% vs. 69%, $p < 0.0001$). No significant difference in mortality was observed between CA- and HA-SAB at 28 days (11% vs. 14%), whereas at three months the mortality difference was

significant (13% vs. 22%, p=0.023). Factors independently predicting outcome were age (p <0.0001), alcoholism (p=0.020), immunosuppressive treatment (p=0.007), underlying diseases (p=0.002), severe sepsis at positive blood culture (p=0.022), *S. aureus* pneumonia (p<0.0001) and endocarditis (p=0.007). High Pitt bacteraemia scores and ICU treatment presented high cf-DNA values at both days 3 and 5. At day 3, cf-DNA cut-off value >1.99 µg/mL among ICU SAB patients predicted mortality with a sensitivity of 67% and a specificity of 77%. High Pitt bacteraemia scores and day 3 cf-DNA were the strongest factors significantly predicting outcome in ICU patients when accounting for all prognostic factors. Cf-DNA at day 5 was more dependent on patient age and underlying diseases and did not predict outcome. CRP had no mortality predictive value for either ICU or non-ICU SAB patients. Most SAB patients received bedside IDSC (72%). Bedside (formal) IDSC, as compared with telephone (informal) IDSC, was associated with more localized deep infection foci (78% vs. 53%, p<0.0001) and lower mortality at seven days (1% vs. 8%, p=0.001), at 28 days (5% vs. 16%, p=0.002) and at three months (9% vs. 29%, p <0.0001). When all prognostic factors were controlled, the three-month mortality for telephone IDSC patients was higher (OR, 2.31) as than that for bedside IDSC patients. Adjunctive rifampicin therapy for at least 14 days was received by 47% of SAB patients, and among 88% of them the therapy was initiated within seven days of *S. aureus*-positive blood culture. Early onset adjunctive rifampicin therapy for at least 14 days was linked to significantly reduced risk for fatal outcome (OR 0.38), and the risk was even lower in patients with a deep infection focus (OR 0.29). Late-onset rifampicin therapy or rifampicin therapy for less than 14 days did not have any prognostic impact.

Conclusions The overall prevalence of deep foci exceeded those reported in previous studies. As in many previous reports, CA- and HA-SAB patients differed regarding patient characteristics, severity of illness, deep infection foci prevalence and outcome. CA- and HA-SAB should be viewed as two different entities. The prospective study design and IDS surveillance contributed to a high number of deep infection foci diagnosed already within three days of *S. aureus*-positive blood culture and to overall low mortality rates. The prognostic value of cf-DNA in SAB patients with ICU treatment was evident. The study clearly indicated that cf-DNA was associated with high Pitt bacteraemia scores and ICU treatment, and ICU non-survivors present high cf-DNA values irrespective of time of death. Bedside (formal) IDSC appeared to be superior to telephone (informal) IDSC with respect to radiological investigations provided, deep foci localization, appropriate antibiotic therapy and outcome. SAB cases should be provided with formal bedside IDSC whenever possible. SAB patients, especially those with deep infection foci, seemed to gain from adjunctive rifampicin therapy initiated within seven days of *S. aureus*-positive blood culture and continued for at least 14 days. A positive prognostic impact of early initiation of rifampicin adjunctive therapy on MSSA bacteraemia was demonstrated for the first time.

1. INTRODUCTION

Staphylococcus aureus is a major pathogen of both community- (CA-) and health care-associated (HA-) bacteraemias and results in considerable morbidity and mortality [1,2,3]. Throughout the last decades, the incidence of *S. aureus* bacteraemia (SAB) has increased both worldwide and in Finland [4,5,6,7]. Today, *S. aureus* is responsible for 11-20% of bacteraemias worldwide [8,9,10,11].

Mortality associated with SAB is remarkably high, ranging from 14% to 32% in recent studies [2,3,12,13,14,15,16,17,18]. High mortality is encountered despite the availability of antistaphylococcal antimicrobial therapy [19], high-standard radiological investigations such as transthoracal and transoesophageal echocardiographies [20,21], improved accessibility to intensive care unit surveillance [3] and better possibilities for surgical interventions and deep infection foci eradication [22]. Prognosis of SAB is impacted by patient-specific background characteristics such as age and underlying diseases [22,23], severity of illness at *S. aureus*-positive blood culture [24,25], development of complications e.g. endocarditis or persistent SAB and uneradicated deep infection foci [3,26] and clinical management such as appropriate antimicrobial therapy [19,22].

Several aspects of SAB have received more attention in recent years. CA- and HA-SAB are increasingly recognized as completely different entities. CA-SAB patients are younger, healthier, more often injection drug users (IDUs) and in 20-61% of cases present with no port of entry or no primary focus (i.e. primary SAB) [2,7,19,27,28,29]. In addition, they have a high occurrence of deep infection foci and endocarditis [2,3,7,19,28] and an increased risk for persistent SAB (positive blood cultures subsequent to onset of appropriate antibiotic therapy) [30]. In HA-SAB, the source of bacteraemia is mostly iatrogenic with catheter-related aetiology in 21-64% of patients and wound or surgery related infections are common [2,3,7,19,28,29] and a low occurrence of deep infection foci is observed [2,7,28].

The standard antimicrobial therapy of methicillin-sensitive SAB is a β-lactam, with semisynthetic penicillin as the drug of choice [15,31,32], whereas first- or second-generation cephalosporins or clindamycin are the choice for patients with non-anaphylactic semisynthetic penicillin allergy [31,33,34]. However, the bacteriostatic nature of clindamycin is associated with an increased relapse risk, and hence, clindamycin is not recommended for treatment of endocarditis in SAB [31,34,35]. For SAB patients with severe penicillin-cephalosporin allergy, vancomycin constitutes the standard treatment [36,37], although several reports connect vancomycin therapy to treatment failures as compared with β-lactams [22,38,39, 40,41]. For methicillin-resistant SAB, vancomycin is the drug of choice [36]. Short par-

enteral antibiotic therapy for 10-14 days is usually sufficient for uncomplicated SAB and for most cases of catheter-related SAB when the catheter has been removed [42,43,44,45], whereas parenteral therapy for 4 (-6) weeks is the standard practice for patients with deep or metastatic infection foci, left-sided endocarditis and non-eradicable primary foci. This expert opinion is, however, backed by only limited scientific evidence [31,46,47,48].

The impact of methicillin-resistant *S. aureus* (MRSA) bacteraemia, delayed onset of appropriate antimicrobial therapy and infectious disease specialist consultation (IDSC) -guided SAB management are topics intensively debated in recent years [3,49,50]. Several reports connect MRSA bacteraemia to both poor prognosis and delay in onset of appropriate antimicrobial therapy [49,51,52]. Delayed effective antimicrobial therapy is known to be a major risk for poor prognosis in SAB [50]. Vancomycin, the first-line therapy for MRSA, results in a higher risk for persistent and recurrent SAB than the standard staphylococcal penicillin cloxacillin [53]. However, bacteraemic infections due to MRSA are rare in Finland, with MRSA prevalence remaining near 3% in SAB [54]. IDSC-guided SAB management has recently been shown to enhance proper antibiotic selection [55] and improve diagnostics [56], with more deep foci localized [57], reducing mortality [3,57]. The nature of the IDSC are, however, mostly undescribed [3,16,18,56,58].

The antimicrobial agent rifampicin and its role in invasive and bacteraemic *S. aureus* infections have received much attention, as rifampicin possesses potentially valuable antimicrobial characteristics, such as bactericidal and high antistaphylococcal activity for both methicillin-sensitive *S. aureus* (MSSA) and MRSA, biofilm-penetrating features and capability of achieving high intracellular concentrations [59,60,61,62,63,64,65]. Monotherapy with rifampicin results in rapid resistance development, and thus, combination therapy is a prerequisite for this agent [36,66,67,68]. However, *in vitro* studies with rifampicin have presented conflicting results. *In vitro* studies on the efficacy and interaction of combining rifampicin and oxacillin (semisynthetic penicillin) have reported antagonistic or indifferent interactions [69], no antagonism [70] or antagonism (at high oxacillin concentrations) and synergy (at low oxacillin concentrations) [71]. A recent review concluded that *in vitro* studies are heavily method-dependent and have limited relevance in clinical practice [72]. Animal studies with rifampicin combination therapy have been more encouraging, with reports of e.g. standard therapy (nafcillin or vancomycin) and rifampicin in chronic osteomyelitis leading to non-significantly [73] and significantly [74] improved results compared with monotherapy.

Clinical studies with rifampicin combination therapy in invasive and bacteraemic *S. aureus* infections have been small-sized and underpowered [66,75,76,77,78,79], although in two reports the patient number is considerable (93 and 381) [15,80]. Rifampicin combination therapy in patient cohorts with low MRSA prevalence have improved clinical progression

and outcome. However, patient cohorts with high MRSA prevalence have reported prolonged SAB [81,82], development of rifampicin resistance [82,83,84] and poorer clinical outcome [82,83,84]. The optimal time-point for rifampicin adjunctive therapy remains a matter of debate, as no prospective studies have investigated this topic. However, a retrospective report [82] and general guidelines [36] for high MRSA prevalence recommend rifampicin onset after bacteraemia clearance. These recommendations apply, however, only to patient populations with a high MRSA prevalence, and no recommendations are available for low MRSA or solely MSSA bacteraemia. The exact role of rifampicin in SAB management remains to be elucidated.

2. REVIEW OF THE LITERATURE

2.1. Incidence of *Staphylococcus aureus* bacteraemia

The current, precise incidence of SAB remains a matter of debate, as most SAB studies are limited to specific hospitals [2,3,19] and few population-based surveillance studies reporting incidence trends are available. Several reports indicate increased incidence of SAB and SAB-related complications, e.g. deep infection foci such as endocarditis, over the past decades [4,5,6,7]. Countries with low MRSA prevalences, such as Finland, the Scandinavian countries and Canada, have generally reported a low annual SAB incidence of 14-28 episodes /100 000 person-years [6,7,29,85,86,87], whereas countries with high MRSA prevalence, such as England, Wales, Northern Ireland, United States and Australia, have reported much higher SAB incidences of 32-39 episodes /100 000 person-years [88,89, 90,91]. Several factors, including advanced age [6,7,85], male gender [6,85,92] and MRSA [92], have been associated with increased incidence of SAB.

However, a multinational population-based surveillance study from 2012 [93] concluded that the overall SAB incidence is not increasing, although MRSA bacteraemia incidence is rising, and a very recent Danish rapport [94] from 2014 observed a decreasing incidence of SAB, from 30.8 episodes /100 000 person-years in 2000-2002 to 24.4 episodes /100 000 person-years in 2006-2008. Further research is required to establish whether a change in the incidence trend of SAB is occurring.

2.2. Predisposing factors for *Staphylococcus aureus* bacteraemia

2.2.1. Microbiology and carriage of *Staphylococcus aureus* bacteria

2.2.1.1. Microbiological aspects of *Staphylococcus aureus*

S. aureus is a facultative anaerobic Gram-positive coccal bacterium and the most virulent among the staphylococci family. *S. aureus* is visualized as grape-like cocci clusters under the microscope [1]. Various biochemical characteristics distinguish *S. aureus* from the other Gram-positive bacteria. The catalase-positive nature of *S. aureus* distinguishes staphylococci from enterococci and streptococci, whereas positive coagulase tests, mannitol-fermentation and deoxyribonuclease tests separate *S. aureus* from other *staphylococcal* bacteria [1,95]. The production and secretion of enzymes and exotoxins represent essential virulence factors for *S. aureus*, e.g. coagulase that enables clotting of plasma and coating of bacterial cells to prevent phagocytosis, hyaluronidase that breaks hyaluronic acid, facilitating spread of *S. aureus* exotoxins that demonstrate superantigen activity capable of in-

ducing toxic shock syndrome or exfoliative toxins capable of initiating staphylococcal scalded-skin syndrome [1,95,96,97].

2.2.1.2. Colonization with Staphylococcus aureus

Colonization of skin and mucous membranes with *S. aureus* is increasingly recognized as a major predisposing factor for SAB and invasive *S. aureus* infections (ISA infections). Several body areas, such as the perineum or the throat, are viewed as potential colonization areas, although the anterior nasal region is regarded as the primary ecological and endogenous reservoir site [98]. Within the healthy population, approximately 20% of individuals are persistent nasal carriers, whereas 30% are viewed as intermittent carriers and 50% as non-carriers [99,100,101]. The prevalence of nasal carriage varies according to age, gender, living habitats and underlying conditions, with higher occurrence associated with young age [102,103], male gender [102], imprisonment and crowded living conditions [104], hospitalization [102,103] and chronic diseases such as HIV/AIDS [105] and diabetes mellitus [102].

2.2.1.3. Clinical impact of nasal Staphylococcus aureus carriage

Nasal carriage of *S. aureus* has appeared to be a major predisposing factor for both community- and health care-associated SAB as well as *S. aureus* infections related to invasive or surgical procedures. Nasal *S. aureus* carriers have a two- to nine-fold higher risk for surgical-site infections as compared with non-carriers [100,106,107,108,109]. The risk for *S. aureus* infections concerns persistent carriers. Intermittent carriers have an infection risk similar to that of non-carriers, and the infection risk is significantly lower than that of persistent carriers [110]. In HA-SAB, up to 80% of *S. aureus* blood culture isolates have been identical to the ones from the anterior nasal region of the patient, proclaiming an endogenous origin of SAB [100,108]. In superficial skin infections, up to 100% of the *S. aureus* skin culture isolates have matched the anterior nasal *S. aureus* isolates [109]. However, among patients with HA-SAB the all-cause mortality and SAB-related mortality have been significantly lower in *S. aureus* nasal carriers than in non-carriers [100].

2.2.1.4. Decolonization of Staphylococcus aureus carriage

Eradication and decolonization of nasal *S. aureus* carriage through the use of an antistaphylococcal agent, usually mupirocin, has been explored as one method of preventing SAB and ISA infections in various patient populations [98,111,112,113,114]. So far, only

specific patient groups seem to significantly gain from mupirocin. The most encouraging results have been achieved among dialysis patients, where mupirocin application reduced both *S. aureus* peritonitis in peritoneal dialysis patients and SAB occurrence in haemodialysis [113,114]. However, mupirocin failed to reduce surgical-site *S. aureus* infections in general surgical or orthopaedic patients [111], although it significantly reduced overall health care-associated and endogenous *S. aureus* infections among carriers [112]. Among non-surgical patients, no significant impact of mupirocin was observed for health care-associated *S. aureus* infections [98]. Two studies from 2007 and 2009 investigated the impact of body washing with the antiseptic chlorhexidine among ICU patients to reduce MRSA colonization and MRSA bacteraemia and reported significantly reduced MRSA colonization, whereas no significant change in MRSA bacteraemia occurred [115,116].

2.2.2. Patient-related underlying factors for *Staphylococcus aureus* bacteraemia

2.2.2.1. Impact of age and gender

High age and male gender are generally viewed as major risk factors for SAB. Patients younger than 1 year or older than 60 years and males are reported to be at increased risk for SAB in several recently performed population-based studies [6,29,87,117,118]. Epidemiological studies have demonstrated a rising incidence of SAB in recent decades in both Finland [6] and worldwide [7,92], and this increase has occurred predominantly in patients with higher age and male gender [6,87,92,118].

2.2.2.2. Impact of underlying diseases

The vast majority of SAB patients have some underlying disease such as cardiovascular disease, chronic pulmonary disease, chronic liver disease, chronic renal failure and dialysis need, malignancy, diabetes mellitus and autoimmune disease [2,7,14,15,17,19,29, 85,120,121,122,123,124]. In studies from the 1990s, only 3-5% of SAB patients had no underlying diseases [27,119], whereas in the 2000s, almost one-third of SAB patients had no underlying diseases [120] and over half of SAB patients, depending on SAB acquisition, had no chronic illnesses [7]. Very recently, 41% of SAB patients were reported to be healthy [23].

Haemo- and peritoneal dialysis have been observed to increase the risk of SAB (RR 150-360) along with several other much weaker predisposing factors such as rheumatoid arthritis (RR 2.2-2.6), diabetes (RR 7.0-10.6), malignancy (RR 12.9-13.6) and HIV infection (RR 17.1-23.7) [85,87]. Immunosupression has been shown to be a major predisposing factor for SAB in connection with radiation therapy or immunosupressive medication, e.g. chemotherapy or corticosteroid treatment [14,17,121,123]. The most commonly reported immuno-

suppressive conditions among SAB patients have been HIV/AIDS (1-5%) [3,12,14,15,122] and neutropenia (1-16%) [22,40,123].

2.2.2.3. Impact of substance abuse

Among SAB patients, alcohol abuse and alcoholism have been identified in 5-14% and IDU in 2-31% [2,14,15,19,29,87,120,121,122,123,124]. The alcohol-related increased infection risk is multifactorial. Several studies associate alcohol abuse with malnutrition, poor dental hygiene and aspiration risk [125]. Alcoholism is known to impair the immune system through modifications in macrophage and neutrophil function and to cause dysfunction of lung surfactant and cilia [125,126]. Alcohol abuse is viewed as a significant risk factor for severe bacteraemic infections [127], and alcoholism is a risk factor for sepsis among ICU patients [128]. *S. aureus* is viewed as the most relevant IDU bacterial pathogen [129,130]. Several factors are proposed to contribute to the high incidence of *S. aureus* infections in IDUs such as higher *S. aureus* colonization rates than in non IDUs [131], increased infection risk due to poor hygiene and unsterile injection habits [132,133] and *S. aureus* transmission through sharing of injection equipment [134].

2.2.3. Destruction of skin and mucous membrane

The presence of intact skin provides an excellent defence against *S. aureus*. However, any damage, such as trauma, invasive or surgical procedures, implantation of foreign body material or injection drug use, enables *S. aureus* to penetrate the skin barrier and disseminate to deeper tissues or the bloodstream, with bacteraemia as a consequence [135]. Factors destroying intact skin and mucous membranes, are well-recognized predisposing factors, especially among HA-SAB patients [2,12,15,122,123]. Catheter-related SAB is present in 21-64% of HA-SAB patients [2,3,28,29], whereas wounds and surgical infections occur in 6-16% [7,19,28]. Some studies report that 11-23% of SAB patients have undergone surgery in the previous one to three months [15,136]. Chronic dialysis as a predisposing factor is reported in 7-19% of SAB cases [7,19]. Several studies report trauma as a risk for SAB, with up to 26% of SAB patients having experienced some trauma two months prior to *S. aureus*-positive blood culture [15,87].

2.3. Clinical picture of *Staphylococcus aureus* bacteraemia

2.3.1. *Staphylococcus aureus*-positive blood culture

The clinical characteristics and symptoms at SAB presentation may be non-specific, with the clinical picture varying from afebrile to critically ill with high fever, septic shock, adult

respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) and need for ICU treatment [2,3,31,57].

Fever is the most common symptom among 72-96% of patients on or before hospital admission [19,29]. Fever commonly persists, as 39% of patients have been reported to have fever 72 hours after onset of appropriate antimicrobial therapy [121]. Among patients with SAB and endocarditis, up to 100% present with fever [137]. Other common symptoms are chills, nausea and fatigue [29].

When patients seek medical treatment for SAB, haemodynamic complications, sepsis and severe organ complications are commonly observed. In SAB, lack of sepsis has been reported in 2% of patients [58], whereas 10-25% of patients may suffer from haemodynamic instability such as severe sepsis or septic shock [2,3,19,28,58,121,138]. Up to 20% of patients present with some degree of organ failure [28], with 3% suffering from adult respiratory distress syndrome (ARDS) [2,28], 6% needing mechanical ventilation [2], 5-22% having acute renal failure [2,3,28,138] and 1-2% having disseminated intravascular coagulation (DIC) [2,28]. ICU treatment is needed in 16-32% of patients [2,18,57].

Various scoring systems for assessment of severity of illness and outcome prediction have been developed, e.g. the Acute Physiology and Chronic Health Evaluation II (APACHE II) or Sequential Organ Failure Assessment (SOFA) score [139,140]. However, although the APACHE II is efficient in determining the severity of illness among critically ill patients, it is challenging to apply in clinical practice due to the complex score calculation procedure and the requirement for altogether 12 parameters, including 6 laboratory tests (arterial pH, serum sodium and potassium, creatinine, haematocrit and white blood cell count) [141]. As with APACHE II, the SOFA score calculation is complex and requires several laboratory parameters (platelet count, bilirubin and creatinine) [140]. The Pitt bacteraemia score is, however, an alternative to APACHE II and SOFA scores, and it requires only 5 clinical parameters (fever, presence of hypotension, need for mechanical ventilation, cardiac arrest event and altered mental status) [142]. Among SAB patients, the Pitt bacteraemia score system reflects severity of illness [13,14] and among ICU patients with sepsis it predicts mortality better than APACHE II [141].

2.3.2. Methicillin-resistant *Staphylococcus aureus* bacteraemia

Methicillin, a semisynthetic penicillin derivative, was presented in 1959 against penicillin-resistant *S. aureus* strains. However, in 1961 the first MRSA strains emerged in the United Kingdom [143], after which MRSA has steadily become more common and is now encountered worldwide [1]. The mechanism for methicillin resistance in *S. aureus* is the mecA

gene, located on an DNA region named the staphylococcal cassette chromosome mec (SCCmec element, found also in coagulase-negative staphylococci), encoding the penicillin-binding protein 2a (PBP2a) [144,145]. PBPs are required for bacterial cell wall synthesis. However, PBP2a differs from regular PBPs as it does not bind methicillin or other β-lactam antibiotics, hence, PBP2a can function despite the presence of methicillin or other β-lactam antibiotics. The mechanism for MRSA is the expression of PBP2a, which is not inhibited by methicillin or other β-lactam antibiotics at concentrations that inhibit other PBPs [146,147].

MRSA was for decades regarded as a health care-associated challenge, but in the 1990s community-associated MRSA spread rapidly [148,149]. During the past decades the overall incidence of SAB due to MRSA has increased without any corresponding decline in MSSA bacteraemia [150,151]. Hence, the overall impact of SAB has increased. In the United States, health care-associated MRSA bacteraemia increased from 35% in 1991 to 45% in 1997-1999 [152,153], whereas the overall MRSA bacteraemia incidence in the United Kingdom and Wales increased from 4% in 1993 to 43% in 2002 [154]. The aetiology behind rising MRSA rates is complex and probably multifactorial. However, the increase in MRSA prevalence is associated with underlying diseases and comorbidity, prolonged hospitalization and poor adherence to infection control precautions [5,155].

Patients with MRSA bacteraemia have been reported to be older and to more often have previous MRSA colonization and a longer duration of hospitalization than patients with MSSA bacteraemia [156,157,158]. Higher mortality in MRSA relative to MSSA bacteraemia is a common observation – a topic discussed more in detail in section 2.7.3. As community-associated MRSA infections emerged in the 1990s, several studies reported that CA-MRSA frequently causes severe skin and soft tissue infections and severe necrotising pneumonia linked to Panton-Valentine leukocidin (PVL) toxins [159,160,161,162, 163]. CA-MRSA bacteraemias are increasing and have been associated with necrotizing pneumonia and cutaneous abscesses, although no mortality difference relative to MSSA bacteraemias has been observed [120].

2.3.3. Classification, characteristics and prevalence of infection foci

2.3.3.1. Categorization of infection foci

Infection foci in SAB are mostly defined as primary (i.e. cutaneous or portal of entry) or secondary (i.e. deep or metastatic) [164,165,166] (see Tables 1 and 2a-b). Moreover, an unknown portal of entry is defined as primary SAB [27]. Some authors classify SAB simply as complicated or uncomplicated [21]. Furthermore, some authors have used definitions

such as deep-seated foci [120], whereas others have only mentioned the foci with most clinical relevance, e.g. endocarditis [167], and some report infection foci when they are eradicable and eradicated [19,22,168]. Categorization as primary, secondary, cutaneous, deep or metastatic foci are the most common categorization types [164,165,166], whereas complicated and uncomplicated SAB are seldom used [21,30,136]. Many reports list the occurrence of primary SAB, i.e. cases where the portal of entry or primary source of SAB is unknown [27] (Table 1). The variable classification of infection foci in the literature makes comparison of different patient materials cumbersome.

2.3.3.2. Primary infection foci

Various body locations may function as the primary site of infection. The recognition of SAB foci as either primary or secondary was first introduced in 1976 [164], and SAB patients were divided according to recognizable primary infection lesions. The primary *S. aureus* infection focus was viewed as a potential portal of entry for the SAB if the clinical picture of the primary focus preceded SAB, whereas secondary foci were viewed as metastatic infections. When no primary focus is found, the presence of an intravascular catheter or a postoperative wound may be the primary focus [19]. Also the urinary tract may serve as a portal of entry and a primary focus for SAB. Patients with urological challenges, such as long-term care patients with frequent urine catheterization, often have *S. aureus* isolated from urine samples. However, although the urinary tract may function as a primary focus, in most cases simultaneous *S. aureus* bacteruria is a result of haematogenous spread and is secondary to SAB [169,170,171,172]. The respiratory tract is identified as a primary source in many reports [12,19,40].

2.3.3.3. Secondary, metastatic or deep infection foci

Due to haematogenous spread in SAB, virtually any organ may be infected [173] and the infections are defined as secondary, metastatic or deep foci. However, *S. aureus* infections are seldom the result of bacterial inoculation due to trauma or an iatrogenic process, e.g. joint puncture, surgery or arthroscopy [12,44,173,174]. Foreign body infections and deep-seated abscesses are often of haematogenous origin, although foreign body infections may be postoperative without a bacteraemia phase [121,136,175,176]. Prosthetic joint infections are commonly classified as early (i.e. development within 3 months of surgery), as delayed (i.e. development within 3-24 months of surgery) and as late (i.e. development later than 24 months after surgery) [177,178]. Early and delayed prosthetic joint infections are mostly achieved during the prosthesis implantation process, whereas late prosthetic joint infections are commonly of haematogenous origin where the skin, dental region or respiratory or

urinary tract are frequent sources of SAB [179]. *S. aureus* pneumonia is predominantly of haematogenous origin and may be due to release of infected tricuspidal vegetations or release of infected thrombotic material in the venous system [19,180]. *S. aureus* meningitis is most often postoperative and on rare occasions haematogenous (due to massive *S. aureus* bacteraemia load and usually a high number of other deep foci present) [181,182]. Most studies report endocarditis, osteomyelitis, abscesses and pneumonia or respiratory infection, whereas septic arthritis and foreign body infections are rarely described (Table 2a). Some report bone and joint infections together (Table 2a), whereas some mention only specific abscesses, e.g. epidural [29,183], psoas [184] or abdominal abscesses [16].

Table 1. Frequency of *Staphylococcus aureus* bacteraemia (SAB) with unknown portal of entry, deep foci, persistent bacteraemia and relapse or recurrence of bacteraemia.

Study	Unknown entry portal [1]	Deep foci (reported) [2]	Deep foci (estimated) [2]	Persistent SAB [3]	Recurrent SAB
Chong et al. 2013	-	19%	-	26%	4%
Khatib et al. 2013	17%	13%	-	36%	-
Robinson et al. 2012	24%	13%	-	56% [4]	-
Choi et al. 2010	16%	-	66%	-	5%
Walker et al. 2009	8%	-	32%	-	2%
Kim et al. 2008	31%	19%	-	-	2%
Ruotsalainen et al. 2006	-	87%	-	-	1%
Kaech et al. 2006	-	24%	-	-	-
Khatib et al. 2006	19%	9%	-	38%	6%
Fowler et al. 2003	13%	74%	39%	-	16%
Chang et al. 2003	15%	-	16%	7%	-
Jensen et al. 2002	-	16%	-	-	12%
Blyth et al. 2002	26%	26%	-	-	6%
Ringberg et al. 2000	33%	53%	-	-	-

[1] Primary SAB. [2] Reported: Reported in original article. Estimated: Summary of all deep foci. [3] Positive blood cultures ≥ 3 days past the onset of antibiotic therapy. [4] Positive blood cultures ≥ 1 day past the onset of antibiotic therapy.

2.3.3.4. Definitions of complicated and uncomplicated bacteraemia

Some reports classify SAB as complicated or uncomplicated, but the definitions used have not been uniform [21,30,136]. Complicated SAB has been regarded as the presence of secondary foci or recurrence of SAB within three months. Furthermore, any event requiring ICU treatment or careful monitoring or follow-up of the SAB patient, e.g. severe sepsis, septic shock, ARDS, DIC, thromboembolic event or septic embolization, have been regarded as complicated SAB [28,121,185,186]. Uncomplicated SAB has been defined as catheter-related bacteraemia or when there is no suspicion of secondary foci or SAB recurrence [121,136]. Furthermore, some have included defervescence within 72 hours of onset

of appropriate antibiotic therapy in the criteria [30]. A very recent report form 2013 defined complicated SAB as persistent bacteraemia (duration ≥ three days), SAB relapse and/or secondary foci, whereas uncomplicated SAB was defined as bacteraemia duration ≤ two days, no foreign device and/or secondary foci [21].

2.3.3.5. Risk factors associated with complicated bacteraemia

Persistent bacteraemia or fever for longer than 72-96 hours have been identified as risk factors for complicated SAB [14,40,121,187]. Furthermore, CA-SAB, underlying diseases and especially haemodialysis and unremoved infected catheters have been connected to complicated SAB [42,136,166]. Several reports have failed in connecting MRSA bacteraemia to complicated SAB [121,188], although one report associated intravascular catheter-related MRSA bacteraemia with complicated SAB [136]. Vancomycin therapy in SAB, regardless of MSSA or MRSA, has been recognized as an independent risk factor for recurrence, treatment failure and mortality [37,41,189,190], whereas high vancomycin minimum inhibitory concentration (MIC) (>1.5 ug/mL) has been presented as an independent predictor for complicated bacteraemia [186]. Some reports associate primary SAB with higher occurrence of secondary deep foci [2]. A thorough prospective report from 2003 identified four risk factors to be significantly associated with the risk for complicated SAB: persistent bacteraemia and persistent fever (positive blood cultures > 72-96 hours and fever > 72 hours) subsequent to onset of appropriate antibiotic therapy, CA-SAB and presence of skin lesions suggesting acute systemic infection. The lack of any of these risk factors gave a probability of 16% for complicated SAB, whereas the presence of three risk factors had a probability of 70% for complicated SAB [121].

2.3.3.6. Prevalence of infection foci

The prevalence of infection foci in SAB differs widely in studies due to usage of various definitions and likely underdiagnosis [191] (Table 1).

SAB with unknown portal of entry or unknown primary source (i.e. primary SAB) [27] varies between 8% and 33% (Table 1), whereas some report up to 50% [192]. The occurrence among HA-SAB patients is much less frequent [2,192]. A primary focus (i.e. cutaneous or portal of entry) has been identified in 37-88% of SAB cases [2,3,19,136]. The term secondary foci is used by a few studies, with an occurrence of up to 16% [19,21], whereas the term metastatic or deep foci is commonly applied (9-87%) (Tables 1 and 2a).

The occurrence of endocarditis has varied from only 2% to up to 39% (Table 2a). Most studies present endocarditis *en bloc* [3,12,21,57,121] and a few specify left- or right-sided or prosthetic valve endocarditis [2]. Osteomyelitis is often reported and the frequency varies between 2% and 14%. It is noteworthy that many authors present osteomyelitis and septic arthritis together, whereas deep-seated abscesses have been reported in only a few studies, with an occurrence of 1-24% in SAB patients. The prevalence of SAB pneumonia has varied from 5% to 30% (Table 2a).

Table 2a. Frequency of deep infection foci in *Staphylococcus aureus* bacteraemia (SAB).

Study	Endo-carditis	Osteo-myelitis	Deep-seated abscesses	Pneumonia	Septic arthritis	Foreign body infection	Deep foci
Robinson et al. 2012	10%	11% [1]	-	9%	-	-	13%
Nagao et al. 2010	5%	5% [7]	1%	6%	1%	-	10%
Choi et al. 2010	2%	10% [1]	7%	30%	-	-	-
Walker et al. 2009	2%	2%	-	6%	2%	-	-
Lahey et al. 2009	12%	10%	3% [6]	15%	5%	2%	-
Rieg et al. 2009	11%	10% [1]	15%	-	-	-	36%
Wang et al. 2008	26% [3]	20% [1]	24%	13%	-	-	-
Jacobsson et al. 2008 [3]	-	12%	1% [4]	5%	14%	-	-
Jacobsson et al. 2007 [3]	-	14%	1% [4]	5%	15%	-	-
Ruotsalainen et al. 2009	18%	34%	44%	40%	13%	18%	87%
Kaech et al. 2006	17%	6% [2]	-	-	-	12%	24%
Khatib et al. 2006	8%	10%	-	5%	-	-	9%
Fowler et al. 2003	39%	10%	10%	-	24%	-	74%
Jensen et al. 2002	8%	7%	-	15%	-	-	16%
Blyth et al. 2002	4%	11%	10%	5%	5%	-	26%
Mylotte et al. 2000	6%	2%	1%	14%	-	-	15%
Ringberg et al. 2000	33%	-	-	-	-	-	53%
Fowler et al. 1998	13%	6%	2% [5]	-	6%	-	-

[1] Bone and joint infections together. [2] Endocarditis or mycotic aneurysm. [3] Patients with invasive *Staphylococcus aureus* infections. [4] Only epidural abscesses. [5] Only psoas abscesses. [6] Abdominal abscesses. [7] Only vertebral osteomyelitis.

2.3.4. Characteristics of the most common deep infection foci

2.3.4.1. Endocarditis

Historically, endocarditis has been found predominantly in community-associated bacteraemia cases, with rheumatic heart disease as a common predisposing valvular abnormality, and streptococcal bacteria has accounted for up to 60-80% of the microbiological aetiology [193,194]. However, in recent decades the characteristics of endocarditis have changed; the prevalence of rheumatic heart disease has decreased and new risk factors, such as degenerative valve diseases among the elderly population, prosthetic valves, intravascular catheters, nosocomial bacteraemia and an increasing IDU incidence, have emerged [19,195, 196,197]. Moreover, *S. aureus* is replacing streptococcal bacteria as an aetiological pathogen in endocarditis [198,199], and several reports indicate increasing incidences of SAB and *S. aureus*-related endocarditis over the last decades [4,5,7]. A thorough prospective study reported a nearly 7-fold increase in *S. aureus*-related endocarditis in the 1990s in the United States [198]. However, the absolute incidence of infective endocarditis has not increased [20].

The presence of SAB, in combination with some predisposing cardiac disease, constitutes the basis for endocarditis development [191]. The combination of improved diagnostics, increasing incidence of nosocomial SAB, the ever-increasing usage of invasive procedures and intravascular catheters as well as more frequent injection drug abuse are presented as explanations for the increase of *S. aureus* endocarditis [5,20,34,199]. Major cardiac risk factors are degenerative valve sclerosis associated with older age, prosthetic valves, mitral valve prolapse, valvular diseases in general, previous endocarditis and injection drug abuse [14,31,166, 191,198,200]. Furthermore, risk factors predisposing to SAB endocarditis are persistent bacteraemia, fever [14,121,200,201] CA-SAB [14,121] and unknown source of bacteraemia [14,200].

2.3.4.2. Pneumonia

Pneumonia due to *S. aureus* constitutes 1-10% of cases of community-acquired pneumonia and up to 50% of cases of health care-associated pneumonia [202]. The aetiology of *S. aureus* pneumonia may be aspiration or haematogenous spread due to release of infected thrombotic material from the venous system or from tricuspidal vegetations (tricuspidal endocarditis) [19,180]. *S. aureus* pneumonia may eventually become complicated leading to lung abscess in 19% [203] or pleural empyema in 11-15% of cases [204,205].

Community-acquired necrotizing pneumonia due to the Panton-Valentine leukocidin (PVL) toxin secreted by *S. aureus* is highly fatal, affecting previously healthy individuals and young people [163]. The association between PVL toxin secreting *S. aureus* and necrotizing pneumonia was demonstrated in 1999 [206], and the clinical picture involves leuko- and thrombocytopenia, severe respiratory distress, airway haemorrhage, multilobar necrosis and rapid septic shock development with a high mortality ranging from 40% to 60% [163,207,208,209,210]. However, two recent studies presented outcome and mortality rates for health care-associated *S. aureus* pneumonia that were irrespective of PVL even after adjusting for confounding factors [211,212]. A recent meta-analysis associated PVL-positive *S. aureus* strains more commonly with skin and soft tissue infections than with pneumonia and demonstrated either no evidence or an uncertain indication (due to confounding factors) that PVL-positive *S. aureus* strains were associated with poorer outcome [213].

2.3.4.3. Septic arthritis

S. aureus is the most common causative organism, accounting for 40-60% of all cases of septic arthritis [214,215,216,217]. In specific patient subgroups, e.g. diabetes or rheumatoid arthritis, *S. aureus* is found in as many as 80% of cases [218]. Risk factors for septic arthritis are rheumatoid arthritis, gout, osteoarthritis and HIV infection [218].

Throughout recent decades, the predominance of *S. aureus* as the leading cause of septic arthritis has remained unchanged [219]**.** In SAB, the occurrence of septic arthritis has been up to 24% (Table 2a) [2,13,16,121]. A prospective study, including *S. aureus* as a causative organism in 44% of cases, investigated the source of infection in septic arthritis and concluded that 67% were of haematogenous origin and 33% non-haematogenous origin [220]. Septic arthritis is rarely the result of an iatrogen joint intervention and has been estimated to occur in < 0.5% of arthroscopies [221]. Most septic arthritis affects a single joint, with 50% afflicting the knee and most of the rest the hip or shoulder [218], whereas the pubic symphysis or sacroiliac joint is affected in only 5% of cases [222]. Septic arthritis due to *S. aureus* is an emergency due to the high risk of non-reversible and rapid joint destruction [223].

2.3.4.4. Osteomyelitis

S. aureus as a causative pathogen accounts for more than 50% of osteomyelitis cases. The classical picture of osteomyelitis involves infection, destruction and necrosis of bone and potentially new bone formation [224]. Osteomyelitis is encountered in 2-34% of SAB patients (Table 2a). Many reports apply the Waldvogel classification of osteomyelitis ac-

cording to aetiology: 1) haematogenous osteomyelitis (due to haematogenous spread), 2) contiguous focus osteomyelitis (infection spreading from nearby structures, e.g. joint or soft tissue infections or infection spread due to *S. aureus* implantation as a result of trauma or surgery) and 3) osteomyelitis due to vascular insufficiency (most commonly diabetics or patients with peripheral vascular disease) [225]. Haematogenous osteomyelitis predominates among paediatric patients, and 85% of haematogenous osteomyelitis is diagnosed among children < 17 years of age [226]. A study from 2003 investigating osteomyelitis due to various pathogens (54% *S. aureus*) reported 6% haematogenous osteomyelitis, 90% contiguous osteomyelitis, 2% vascular osteomyelitis and 2% other forms [224]. Some studies use the categorization of acute and chronic osteomyelitis, but there is no strict time reference for the separation of these two [227]. The clinical presence of a new bone infection in combination with the lack of bone necrosis and devascularized bone are viewed as acute osteomyelitis. Histopathologically, acute osteomyelitis correlates with clinical symptoms that have been present for less than 10-14 days [228]. Chronic osteomyelitis is defined as long-term bone infection, including low-grade inflammation in pathological analysis and possible presence of devascularized necrotic bone and new bone formation [229]. Specific osteomyelitis sites are associated with certain SAB patient subgroups, e.g. clavicular or sternal osteomyelitis, and are reported more frequently among IDUs than non-IDUs [230].

S. aureus osteomyelitis of the vertebral column (i.e. spondylitis) with or without intervertebral disc space affision (i.e. spondylodiscitis) is a continuous clinical challenge. A thorough Danish nationwide report concluded that 82% of *S. aureus* spondylitis patients were related to CA-SAB. Only 39% of the patients had a diagnosis at admission that suggested an active vertebral column process, such as back pain, prolapse suspicion or fracture, and only 5% were admitted due to suspicion of osteomyelitis. Altogether 53% had an unknown portal of entry (primary SAB) for the SAB. The spondylitis in 70% of patients was located in the lumbar part of the vertebral column [231].

2.3.4.5. Foreign body infection

S. aureus is presently ranked as the second most common causative pathogen after coagulase-negative staphylococci in foreign body infections [227], accounting for 12-23% of prosthetic joint infections [232,233,234]. Patients with foreign body devices are at high risk of device-related infections in SAB; these are encountered in 2-18% of patients (Table 2a). Two prospective studies investigating patients with a foreign body device and SAB concluded that over 42% of orthopaedic devices, 34% of prosthetic joints and 45% of cardiac devices became infected [175,235]. Another prospective study observed that 45% of pa-

tients with permanent pacemakers or implantable cardioverter-defibrillators developed cardiac device infections as a result of SAB [236].

During the last decades increasingly more foreign body devices, e.g. orthopaedic or cardiac devices, are inserted [178,237] and device-related infections are receiving more attention [168,175,236]. Foreign body infections and especially prosthetic joint infections are categorized according to the time-point of infection onset after insertion as early, delayed, late or acute haematogenous [178,238]. However, the exact time references vary in different reports. According to Zimmerli et al. [178], early infections develop within 3 months of surgery, delayed infections within 3-24 months of surgery and late infections 24 months or more after surgery [178]. The categorization according to Zimmerli et al. is the one most commonly used in clinical practice (discussion with Dr. Kaisa Huotari, Helsinki University Central Hospital). Some authors use different time references and define early infections (onset within one month) and haematogenous infections (rapid onset after one month) as mostly caused by *S. aureus,* whereas late infections (onset after one month) are usually caused by coagulase-negative staphylococci [227,238].

2.3.4.6. Meningitis

S. aureus meningitis as a result of SAB is rare and encountered in 0.1-5% of cases [21,29,121]. SAB with subsequent meningitis mostly reflects a very complicated situation, with vast infection spread and high probability of other secondary foci. The prognosis is often poor [239]; one report states a mortality rate of 56% [181].

2.3.4.7. Role of bacteruria

S. aureus bacteruria is very uncommon among the healthy population, except for patients with urological challenges, such as catheterization or urologic procedures, and usually represents secondary haematogenous spreading for patients with bacteraemia symptoms. *S. aureus* seldom causes urinary tract infections [240]. *S. aureus* bacteraemia and bacteruria are observed in 7-10% of patients [241,242]. A case-controlled study including 58% MSSA bacteraemia cases found that patients with *S. aureus* bacteraemia and bacteruria (SABU) had an almost 3-fold increased mortality risk (OR 2.9) as compared with SAB patients without bacteruria even after adjusting for factors known to increase the risk for *S. aureus*-positive urine cultures (e.g. bladder catheters, recent urologic surgery, urinary tract symptoms) [170]. Two retrospective studies, one of which included solely MSSA bacteraemia cases [171], concluded that SABU was a significant risk factor for ICU admission (OR 2.5)

and an independent predictor for both in-hospital mortality (OR 2.18) [171] and septic shock and mortality [172].

2.3.4.8. Time-point for diagnosis of deep infection foci

The time-point for diagnosis of deep foci differs, with some authors reporting 74% of SAB patients having a complicated infection present at the time of initial hospitalization [121] and others concluding that 84% of SAB patients have deep foci within one week [15] or metastatic foci within two weeks of SAB diagnosis [185].

2.3.5. Diagnostics of deep infection foci

2.3.5.1. Clinical examination

The clinical status, physical examination and symptoms of the patient constitute the basis for the search for deep infection foci in SAB patients. A thorough clinical status may reveal signs of various deep infection foci.

There are several well-documented clinical signs of endocarditis. New heart murmur is heard in up to 45% of patients during the initial phase of native valve endocarditis [33]. Systemic thromboembolic events [243] and embolization due to mitral valve endocarditis or large-sized vegetations (>10 mm) may result in acute neurological symptoms, e.g. hemiparesis [236,244], necessitating a search for endocarditis [245]. Peripheral thromboembolic events and embolizations may present as skin petechiae, Janeway lesions (haemorrhagic spots on soles and palms) and Roth´s spots (haemorrhagic spots on retina) [246], whereas endocarditis-related immunological complications may result in renal insufficiency [33] and Osler´s nodes (nodules in the subcutis of fingers and toes) [246]. Clinical signs lead to diagnosis of endocarditis in 7% of cases [247], and radiological methods have been shown to substantially increase the odds for diagnosis [248].

The diagnostic criteria for endocarditis have been modified repeatedly in recent decades. Originally, autopsy was a prerequisite for endocarditis diagnosis. However, the first criteria for prediction of endocarditis in SAB were established in 1976 and these included CA-SAB, lack of primary focus of infection and presence of metastatic infection [164]. These criteria were improved in 1981 [249] and the introduction of echocardiography further improved the diagnostics, with the Duke criteria established in 1994. The Duke criteria take into account echocardiographic imaging, histopathological findings and microbiology and classify the probability of endocarditis as definite, possible or rejected [250]. The Duke criteria were further developed in 2000 (modified Duke criteria), with proposed modifications to the cate-

gory ´´possible endocarditis`` [248]. The modified Duke criteria are described in detail in the ´´Definitions`` section (2.3.7.1.).

2.3.5.2. Echocardiography

Transthoracic (TTE) and transoesophageal echocardiography (TEE) are the foremost radiological investigations for diagnosis and follow-up of endocarditis [20,34]. TTE is rapid, non-invasive, widely available in hospitals and may easily be performed bed side; however, despite excellent specificity (up to 98%) for infective vegetations, a negative TTE does not exclude *S. aureus* endocarditis due to its poor sensitivity (40-80%) [247,251,252].

TEE is invasive and requires a patients complete perfect cooperation. However, TEE is superior to TTE in revealing infective vegetations with a sensitivity of 87-100% and a specificity of 89-100% [251,253,254]. Furthermore, TEE is indispensable for prosthetic valve endocarditis [255] and detection of small vegetations [256]. In a thorough prospective study of patients with definite *S. aureus* endocarditis (with over 50% of patients having an infected intravascular device as the source of bacteraemia), TTE revealed findings leading to diagnosis of endocarditis in 34% of cases; the corresponding figure for TEE was 94% [137]. Clinical use of TTE is fairly common, with reports of up to 23-60% of SAB patients receiving TTE [12,19,21,121], whereas 6-42% are provided with TEE [12,21,121] and 13% are investigated with both TTE and TEE [21]. Moreover, patients receiving an infectious disease specialist consultation are significantly more often provided with TTE or TEE [3,18,56,257] than patients managed without an infectious disease specialist consultation.

2.3.5.3. Radiological investigations

Radiological investigations in SAB constitute a cornerstone, alongside clinical physical examination, for diagnosing deep infection foci [31,34,227]. However, there are no generally accepted algorithms or guidelines for the use of radiological investigations. The choice and time-point for radiology and possible subsequent control imaging should be assessed for each SAB patient individually. The foremost radiological imaging techniques for SAB-related joint and bone infections, deep-seated abscesses and pneumonia are x-ray, ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) [31,227,258].

In septic arthritis, the specific diagnosis is based on joint fluid aspiration analysis, including cultures [259]. However, various radiological investigations are occasionally indispensable. Ultrasound may be needed for demonstrating joint effusion and for guidance of needle aspiration [260]. Ordinary x-ray, especially in the early phase of septic arthritis, usually shows

regular bone structures and periarticular soft tissue oedema. However, in septic arthritis of sternoclavicular or pubic symphysis, x-ray reveals adjacent osteomyelitis in 60-90% of cases [222,261]. Therefore, MRI may be useful in unclear cases of deep joint septic arthritis, e.g. in the hip region, and MRI may reveal joint changes even within 24 hours of infection onset [262].

In acute osteomyelitis, ordinary x-ray imaging may reveal bone destruction, periosteal reactions, soft tissue oedema as well as joint alterations (narrowing or widening of joint area). However, these transformations, including bone destruction, are not visible with ordinary x-ray until 10-21 days after infection onset [263,264,265]. In chronic osteomyelitis, the typical features are periosteal new bone formation, bone sclerosis and sequestrae and sinuses [227]. In osteomyelitis, CT is superior to MRI for detection of sequestrae and intraosseus gas, whereas MRI is superior in localizing vertebral osteomyelitis and epidural and soft tissue abscesses [227]. Regarding vertebral osteomyelitis, bone scintigraphy, i.e. technetium-99 (Tc^{99}), may localize and confirm the infection at an early stage, although the accuracy of MRI is higher [266]. A thorough nationwide Danish study concluded that within one week bone scintigraphy results were abnormal in 80% and CT scanning in 50%, whereas MRI results were abnormal in 100% of cases of *S. aureus* spondylitis [231]. Compared with conventional radiography (x-ray), both scintigraphy and CT scanning produced positive radiological results significantly earlier [231]. A study comparing diagnostic methods for osteomyelitis demonstrated a sensitivity of 72% for MRI, 68% for bone scans and 45% for leukocyte scintigraphy [267].

Effective use of traditional radiological imaging techniques, such as x-ray, ultrasound, CT or MRI, is heavily dependent on guidance provided by of localized symptoms. Recently, it was proposed that a combination of CT scanning and whole-body positron emission tomography (FDG-PET/CT) might be superior in localization of infection foci relative to x-ray, ultrasound, CT or MRI [268,269]. FDG-PET/CT effectively localizes infection foci and improves diagnostics in bacteraemia patients and patients with fever of unknown origin [270,271]. A retrospective study observed that conventional radiological techniques (x-ray, ultrasound, CT or MRI) localized 75% of infection foci among bacteraemic patients (including 35% of SAB). However, subsequent to this, FDG-PET/CT managed to localize clinically relevant new infection foci in 45% of cases, although a median of four tests had already been performed [270]. A prospective study from 2012, including altogether 115 Gram-positive bacteraemic patients (74% of SAB) demonstrated deep foci in 73% of patients with at least one risk factor for metastatic infection (community acquisition, treatment delay, persistently positive blood cultures >48 hours and persistent fever >72 hours after initiation of treatment). However, only in 41% of cases did the infection foci produce local signs or symptoms, and hence, symptom-guided x-ray, ultrasound, CT or MRI revealed few metas-

tatic foci. FDG-PET/CT was much better, revealing 69% of all metastatic infection foci. Moreover, FDG-PET/CT revealed at least one clinically silent metastatic focus in approximately 30% of patients [203]. The use of FDG-PET/CT has been observed to reduce relapses of SAB compared with conventional radiology [272].

2.3.6. Persistent and recurrent bacteraemia

Persistent bacteraemia

Persistent bacteraemia in SAB is generally defined as ongoing positive blood cultures ≥ one day [57], ≥ three days [40] or ≥ seven days [84,273,274] after onset of proper antibiotic therapy. Persistent bacteraemia has varied from 7% to 56% in different studies [14,18,21, 30,40,273,275,276] (Table 1). Methicillin resistance is a major risk factor for persisting bacteraemia [276,277], and the inferior capability of vancomycin relative to β-lactams in eradicating SAB is viewed as one probable explanation [40,53,278]. Infected prosthetic devices [40] and deep infection foci (especially endocarditis) [199] have been described as other risk factors for persistent bacteraemia. Two studies have demonstrated a significant association between persistent SAB and development of metastatic foci [40,275].

Recurrent bacteraemia

SAB recurrence is defined as a second episode of SAB subsequent to appropriate antistaphylococcal medication and documentation of negative blood cultures and/or clinical improvement [41], with some reports subgrouping SAB recurrence as a relapse (i.e. identical pulsed-field gel electrophoresis, PFGE, pattern) or reinfection (non-identical PFGE pattern) [41]. Recurrent episodes of SAB have ranged from 1% to 16% in published studies [3,15,19,22,28,30,40,41,58,121,123] (Table 1). Most SAB recurrences are relapses and several factors are recognized as independent risk factors for SAB recurrence or relapse: endocarditis [41], vancomycin or other glycopeptide therapy for MSSA [37,41,123], secondary foci [19], a total daily dose of dicloxacillin less than 3 g [19], unremoved infected central venous catheter [123] and duration of bacteraemia longer than 3 days [37].

2.3.7. Community- or health care-associated *Staphylococcus aureus* bacteraemia

2.3.7.1. Definitions

SAB is categorized as community-associated (CA-SAB) or health care-associated (HA-SAB) according to the time-point of collection of the first positive blood culture [7,15,28], although some reports apply a third category of community-onset health care-associated SAB (COHA-SAB) [3]. The criteria for CA-, HA- and COHA-SAB are mostly standardized in

the literature. CA-SAB is defined as the first positive *S. aureus* blood culture taken within 48 hours of hospital admission without any preceding hospitalization within seven days. HA-SAB defined as the first positive *S. aureus* blood culture taken ≥ 48 hours after admission to hospital or within two days of admission in cases with a hospital discharge within the preceding seven days [3,15,19,40]. One report viewed long-term care facility residency during the previous two months or preceding haemodialysis treatment as HA-SAB [15]. COHA-SAB is defined as the first positive *S. aureus* blood culture within 48 hours of admission for outpatients with 1) previous healthcare contact, e.g. wound care, intravenous therapy or haemodialysis within 30 days, 2) any hospitalization for at least 48 hours within the past three months or 3) long-term care facility or nursing home residency prior to hospitalization [3,279]. However, some reports deviate from the time criteria mentioned above and categorize CA-SAB and HA-SAB according to whether the first positive blood culture for *S. aureus* was obtained within or subsequent to 72 hours of hospital admission [12,21,37,121].

2.3.7.2. Impact of clinical presentation

For decades, SAB was interpreted primarily as a health care-associated infection [118,136, 280], although in recent years the overall occurrence of both acquisition categories has increased [7,155], with reports of CA-SAB in 11-58% [2,3,6,15,18,21,37], HA-SAB in 30-81% [2,3,6,15,18,21,37,58] and COHA-SAB in 28-57% of cases [3,18,21].

Evident trends have emerged regarding age, underlying conditions and disease progression related to SAB acquisition. CA-SAB patients are younger and more often IDUs [2,7], whereas HA-SAB patients are older [2,7,28] and more often chronically ill [2,7,19]. Most reports present no differences in gender [19]. MRSA bacteraemia has been encountered more frequently in HA cases [23], with occurrences of 0.4-57% for HA-SAB [3,7,23] 0-18% for CA-SAB and 16% for COHA-SAB. Severity of illness at *S. aureus*-positive blood culture is reported to be more serious among CA-SAB patients with a higher occurrence of septic shock [2,28], ARDS [2,28], DIC [2,28], ICU treatment [2], mechanical ventilation [2] and renal failure [2,28].

Primary SAB (i.e. unknown portal of entry or unknown focus) is more common among community-associated cases as compared with HA-SAB, but one report presented the opposite results, with primary SAB in 12% of CA-SAB cases and 57% of HA-SAB cases [85] (Table 2b). When comparing occurrences of primary foci, CA-SAB presents more often with skin infections and soft tissue infections and IDU. Skin infections were present as a primary focus in 13-40% of CA-SAB [2,7,19,28] and in 3-4% of HA-SAB, whereas soft tissue infections were observed in 53% of CA-SAB and 23% of HA-SAB [29], and catheter-related

SAB occured in only 1-17% of CA-SAB [2,3,28,29], 21-64% of HA-SAB [2,3,28,29] and 37% of COHA-SAB [3]. The same trend applies to wounds and surgical infections, with occurrences of 6-16% for HA-SAB and 0-2% for CA-SAB [7,19,28]. Hence, the primary foci of HA-SAB are mostly iatrogenic and related to invasive procedures or catheter use, whereas the primary foci for CA-SAB are often unknown or related to IDU or skin and soft tissue infections.

Metastatic, secondary or deep foci are observed more often in CA-SAB than in HA-SAB (Table 2b). Generally, all deep foci occur more frequently in CA-SAB, with the exception of foreign body infections. Endocarditis is diagnosed in 7-29% of CA-SAB, 0-5% of HA-SAB and 10% of COHA-SAB, and both native and artificial valve endocarditis are more common in CA-SAB (Table 2b). Moreover, CA-SAB patients have been reported to receive more echocardiography than HA-SAB patients [21]. The occurrence of osteomyelitis is 13-16% for CA-SAB and 2-4% for HA-SAB [7,19,28], and many studies report septic arthritis and osteomyelitis together under the term bone and joint infections, with a presence of 11-47% in CA-SAB and 0-17% in HA-SAB [2,85]. Pneumonia and respiratory infection are reported in 4-18% of CA-SAB and 1-16% of HA-SAB [7,12,19,28], whereas some report explicitly more respiratory infections among HA-SAB [29,85]. Furthermore, deep-seated abscesses, *S. aureus*-related meningitis and CNS infections are reported more often among CA-SAB [7,28,85]. However, foreign body infections occur more frequently in HA cases, with frequencies of 0% for CA-SAB and 11% for HA-SAB [2], whereas surgical site infections with no foreign body are reported in 0% of CA-SAB and 9-20% of HA-SAB [2,28]. Persistent SAB is reported more often in CA-SAB [30], whereas recurrent SAB is seen in 5% of CA-SAB and 11% of HA-SAB [19]. However, no significant difference in recurrence prevalence with respect to acquisition was seen in one report [40].

Several studies have reported no significant difference in mortality between CA-SAB, HA-SAB and COHA-SAB at 28-day or 30-day [12,18] or three-month follow-up [3,19]. However, discrepant results have also been presented, with higher mortality in CA-SAB [2] or HA-SAB [37]. A thorough Danish study reported overall declined trends in mortality for both CA- and HA-SAB during the last decades [7]. The impact of SAB acquisition on mortality is discussed in more detail in Section 2.7.2.

Table 2b Frequency of *Staphylococcus aureus* bacteraemia (SAB) with unknown portal of entry, various primary infection foci and deep foci according to community-associated (CA) and healthcare-associated (HA) acquisition.

Study	Unknown portal of entry [1]		Primary foci (reported) [2]		Deep foci (reported) [2]		Endocarditis		Mortality [3]	
	CA	HA	CA	HA	CA	HA	CA	HA	CA	HA
Laupland et al. 2008	12%	57%	88%	43%	-	-	-	-	-	-
Jacobsson et al. 2007	44%	36%	56%	64%	-	-	-	-	-	-
Benfield et al. 2007	61%	53%	39%	47%	31%	6%	12%	2%	-	-
Kaech et al. 2006	52%	3% ¤¤	48%	97% ¤¤	43%	5% ¤¤	29%	5% ¤¤	26%	13% ¤
Johnson et al. 2003	-	-	-	-	-	-	-	-	24%	43% ¤
Jensen et al. 2002	20%	4% ¤¤	80%	96% ¤¤	29%	5% ¤¤	14%	3% ¤¤	40%	29%
Blyth et al. 2002	22%	3%	78%	97%	35%	12% ¤	7%	0	-	-
Mylotte et al. 2000	42%	44%	58%	56%	-	-	-	-	23%	23%

[1] Primary SAB. [2] As reported in the original article. [3] Mortality at 3-month follow-up. ¤ p<0.05 and ¤¤ p<0.001.

2.4. Treatment of *Staphylococcus aureus* bacteraemia

2.4.1. Standard antibiotic therapy

Countries with low MRSA prevalence, such as Finland, use semisynthetic penicillin (i.e. cloxacillin) as the standard antimicrobial therapy in SAB [15] and for patients with penicillin allergy either clindamycin or first, or second-generation cephalosporins [31,33,34]. Several older reports observe that semisynthetic penicillin might be superior to cephalosporines such as cefazolin (first-generation cephalosporin) [281,282], cefonicid (second-generation cephalosporin) and ceftazidime (third-genertaion cephalosporin) [283,284]. In contrast, a recent study concluded that cefazolin and cloxacillin therapy did not differ with respect to outcome in MSSA bacteraemia and both were associated with a lower 30-day mortality than second- (cefuroxime) and third-generation cephalosporins (ceftriaxone and cefotaxime) [285]. However, the bacteriostatic nature of clindamycin may increase the risk for relapses, and there are recommendations to avoid clindamycin in SAB with endocarditis, whereas in osteomyelitis clindamycin is often recommended due to its excellent tissue penetration [35,165,286]. Alternatively, MSSA bacteraemia patients with severe allergy to penicillins or cephalosporins may be treated with vancomycin [36,37].

For MRSA, vancomycin is viewed as the drug of choice, although newer antibiotics like daptomycin or linezolid have been presented as alternatives (with the exception of left-sided endocarditis). Daptomycin has been reported to be non-inferior to standard antistaphylococcal therapy in SAB and in right-sided endocarditis due to MSSA or MRSA

[276], whereas one meta-analysis found no outcome difference between linezolid and vancomycin therapy [287] and another meta-analysis showed higher success with linezolid, ableit without improved survival compared with β-lactam or glycopeptide therapy [288].

2.4.2. Duration of antimicrobial therapy and aminoglycoside combination

Short duration of therapy

Short parenteral antibiotic therapy (10-14 days) is usually regarded as sufficient for uncomplicated SAB and, in particular, for most cases of catheter-related SAB [44]. Several studies have demonstrated that in uncomplicated catheter-related SAB the risk of secondary foci is low and 10-14 days of parenteral therapy is sufficient when the catheter is removed [42,43,44,45], whereas two reports show rising complications among patients receiving shorter than 14 days parenteral therapy [19,289,290]. However, in catheter-related SAB with persistent bacteraemia, prolonged fever (> 72 hours), predisposing factors for endocarditis, e.g. valvular abnormalities, and in some subgroups of patients e.g. rheumatologic diseases or malignancies, the risk for complicated SAB is increased and long parenteral therapy may be needed [121,290,291,292]. For uncomplicated non-catheter-related SAB, the recommendation has been 14 days of parenteral therapy with subsequent 14 days of oral therapy [293,294]. Moreover, some reports indicate that 14 days of parenteral therapy may be sufficient for uncomplicated cases of right-sided endocarditis [295,296,297,298].

Long duration of therapy

Patients with deep or metastatic infection foci, left-sided endocarditis, non-eradicable primary focus or signs of a complicated catheter-related SAB after catheter removal (e.g. persistent bacteraemia, prolonged fever, predisposing factors for endocarditis and some patients with severe underlying diseases) are considered to need parenteral therapy for 4 (-6) weeks [46,47,48]. Most SAB-related deep infection foci (i.e. septic arthritis, osteomyelitis, deep-seated abscesses and foreign body infections) require at least 4 or even 6 weeks of standard parenteral antibiotic therapy [299,300,301]. However, there is scant evidence to support the standard parenteral antibiotic therapy of 4 (-6) weeks.

One randomized controlled trial investigated the impact of 2 versus 4 weeks of intravenous antimicrobial therapy for adult SAB patients. Endocarditis developed in one patient in the 2-week group, whereas the 4-week group no endocarditis was observed [294]. Recommended antimicrobial therapy differs considerably for left-sided native valve, prosthetic valve and right-sided endocarditis. For left-sided native valve endocarditis, standard parenteral therapy of (4) -6 weeks in uncomplicated cases [20,48] and 6 weeks in complicated cases [48] is recommended (IA strength of recommendation according to the Infectious

Diseases Society of America, IDSA) [48]. In both cases, a combination with the first 3-5 days on an aminoglycoside is suggested in most guidelines [1,20,48], although no recommendation strength has been established according to IDSA [48]. The antimicrobial therapy for prosthetic valve endocarditis resembles that of left-sided native valve endocarditis, although the standard parenteral therapy is recommended to continue ≥ 6 weeks (IB strength of recommendation according to the IDSA) [48] with initial aminoglycoside therapy continued for 14 days [1,20,48]; no strength of recommendation has been established by to the IDSA, however [48].

The pathophysiology of right-sided endocarditis differs from other forms of endocarditis and is frequently encountered among IDUs. The recommendation is a standard antibiotic therapy and aminoglycoside combination, and in uncomplicated right-sided endocarditis 14 days of parenteral therapy may be sufficient [295,296,297,298,301]. However, in complicated right-sided endocarditis, including extracardiac infections, vegetations of considerable magnitude (>2 cm), MRSA cases, immunosuppression or slow response to initial therapy, 4 weeks of parenteral therapy is recommended [301,302,303].

The current role of aminoglycosides in SAB endocarditis is controversial. Experimental settings have demonstrated β-lactam and gentamicin synergy [304], although only one clinical study has reported reduced defervescence and reduced duration of bacteraemia (by one day) when 2 weeks of gentamicin was combined with nafcillin in SAB endocarditis [305]. In 2006, a meta-analysis observed no improved treatment success and no mortality reduction as a result of β-lactam and aminoglycoside combination relative to β-lactam alone for native valve SAB endocarditis [306]. However, aminoglycoside combination therapy was associated significantly with nephrotoxicity. In 2009, one study concluded that addition of low-dose gentamicin in native valve SAB endocarditis is an independent predictor for renal toxicity and should not be routinely used [307]. The recommendation not to routinely add gentamicin to SAB endocarditis treatment has been supported by other authors [46].

Continuous debate exists as to whether parenteral and oral therapy are equally sufficient in some subgroups of SAB patients. Two reports, one comparing *per os* rifampicin and ciprofloxacin with standard parenteral therapy for right-sided endocarditis in IDUs [80] and the other comparing *per os* rifampicin and fleroxacin with standard parenteral therapy for SAB patients with bone, joint or catheter-related infections [308] presented equal clinical cure rates in both groups.

2.4.3. Role of rifampicin adjunctive therapy

The role of rifampicin in SAB and, in particular, in deep infections has been debated for decades. Recommendations suggest combining rifampicin with standard therapy in foreign body infections [178], osteomyelitis [77] and deep-seated abscesses [308]. Rifampicin has potentially valuable antimicrobial characteristics such as high intracellular concentrations, bactericidal and high antistaphylococcal activity for MSSA and MRSA, penetration of biofilms [59,60,61,62,63,64,65] and eradication of *S. aureus* in both non-phagocytic cells [309] and cells in sessile and planktonic growth phases [310]. Monotherapy with rifampicin results in rapid resistance development, and thus, combination therapy is a prerequisite for rifampicin use [36,66,67,68]. However, the exact role of rifampicin in SAB management remains to be elucidated.

2.4.3.1. Rifampicin studies in vitro

In vitro studies have investigated the efficacy and interactions of rifampicin combined with other antimicrobial agents – with contradictory results. Rifampicin combined with oxacillin has shown antagonistic or indifferent interactions [69], antagonistic (at high oxacillin concentrations) and synergistic (at low oxacillin concentrations) interactions [71] or no antagonism [70]. Rifampicin and ciprofloxacin *in vitro* combinations have demonstrated antagonism [311], indifference [60,312] or synergistic effects [313]. Corresponding conflicting results have been presented also for rifampicin and vancomycin combinations, with reported indifference [314], antagonism [315] or synergy [316]. Several reports have noted that changes in antibiotic concentrations affected the interaction [70,317,318]. Some reports have suggested that the interaction between rifampicin and other antimicrobial agents may be method-dependent, e.g. time-kill curve assay versus checkerboard microdilution assay [71,319,320,321]. However, contradictory results have been achieved also in cases where the same research methodology has been applied, e.g. time-kill curve assay (rifampicin-oxacillin combinations) [69,70,71]. A recent thorough review summarizing the results of altogether 72 reports concludes that *in vitro* studies are heavily method-dependent and questions whether *in vitro* studies have any relevance in exploring the efficacy of rifampicin combination therapy for clinical infections [72].

2.4.3.2. Rifampicin studies with animal models

Animal models have investigated monotherapy versus rifampicin combination therapy in various study settings. Mouse models with penicillin-susceptible SAB have demonstrated higher ($p < 0.001$) survival rates for rifampicin in combination with penicillin or methicillin

than for penicillin or methicillin alone [322]. Rat and rabbit models of osteomyelitis have demonstrated reduced colony-forming units in pefloxacin-rifampicin [73] and clindamycin-rifampicin [323] and higher sterile bone cultures in vancomycin-rifampicin ($p < 0.01$) [74], cephalothin-rifampicin ($p < 0.001$) [75] and trimethoprim-rifampicin [324] combinations compared with pefloxacin, clindamycin, vancomycin, cephalothin or trimetophrim alone. Rabbit and rat models of endocarditis treated with monotherapy versus rifampicin combination therapy have demonstrated enhanced valve sterilization or reduced colony-forming units as a result of cloxacillin-rifampicin [325] or vancomycin-rifampicin [326] versus non-rifampicin monotherapy alone. However, contradicting these are reports of an indifferent impact of vancomycin-rifampicin therapy versus vancomycin alone in rat endocarditis models [327,328].

2.4.3.3. Clinical studies with rifampicin combination therapy

During 1983-2011 the clinical effect of rifampicin was evaluated in 16 reports. The vast majority of these studies were prospective, whereas three of the most recent ones were retrospective [82,83,176]. The studies differ widely with respect to MRSA occurrence. Some report no MRSA [15,77], whereas others report high (76-100%) MRSA prevalence [81,82,83,329,330]. In addition, definitions and inclusions of deep infection foci vary considerably. Some studies report only endocarditis [81] or right-sided endocarditis [80], whereas others report only osteomyelitis [75,77], and one study presented various deep infection foci [15]. The main results of the clinical rifampicin combination studies are summarized in Table 3. These studies compare the clinical outcome of rifampicin combination therapy against standard therapy alone. Most studies with low MRSA occurrence report some degree of improved clinical outcome due to rifampicin combination therapy as compared with standard therapy alone, whereas studies with high MRSA occurrence mostly report adverse effects and negative prognostic impact of rifampicin combination therapy.

Several small prospective studies with 14-65 patients from the 1980s report higher cure rate or lower mortality with rifampicin combination therapy than with standard therapy alone, although statistical significance is not achieved in many studies due to small sample size [66,75,76,77]. The end-points, the MRSA prevalence and the deep focus classification differ between these studies. One study with right-sided endocarditis among IDUs reported a 100% cure rate of rifampicin combination therapy among patients who managed to complete the study, but no control group was included [78]. Some studies have either failed to observe resistance development to rifampicin [75,76] or rifampicin resistance is not mentioned [66,77].

During the 1990s and 2000s several prospective studies of varying size (33-381 patients) and mostly low MRSA occurrence (0-11%) have reported positive results with rifampicin combination therapy relative to standard therapy (Table 3). One study of right- sided endocarditis among IDUs compared oral rifampicin-ciprofloxacin with intravenous oxacillin or vancomycin (in addition to gentamicin) and noted no difference in clinical failures in the rifampicin combination group relative to the standard therapy group (5% vs. 12%) [80]. Another study compared oral rifampicin-ciprofloxacin with ciprofloxacin-placebo in foreign body infections and showed significantly higher cure rates among patients with rifampicin combination therapy (100% vs. 58%, $p < 0.05$) [79].

A *post hoc* analysis of 331 MSSA bacteraemia patients, including various deep infection foci patients but no MRSA bacteraemia cases, demonstrated improved three-month outcome for adjunctive rifampicin therapy [15]. A prospective randomized trial with MSSA bacteraemia (2% MRSA) and a high number of various deep infection foci compared fleroxacin and rifampicin combination against conventional intravenous monotherapy with flucloxacillin or vancomycin [308]. The study observed similar cure rates for both therapies, although rifampicin therapy resulted in several adverse reactions such as hepatitis. Furthermore, a retrospective study with 17% MRSA cases concluded that rifampicin-fluoroquinolone therapy, compared with other antimicrobial regimens, was associated with improved outcome in patients with total hip or knee prosthetic infections, with no differences in outcome between MSSA and MRSA infections [176]. Altogether, four studies with high (51-100%) prevalence of MRSA bacteraemia have investigated rifampicin combination therapy in endocarditis [81,82], osteomyelitis [331] or various deep infection foci [83] or in persistent MRSA bacteraemia [329]. Of these studies, one included 10% heteroresistant vancomycin-intermediate *S. aureus* (hVISA) cases [83].

Development of rifampicin resistance in *S. aureus* is a well-known disadvantage [67,68] and has been observed in studies with high MRSA prevalence [82,83,84,329,331]. These studies have reported development of rifampicin resistance in 5-56% of cases [82,83, 84,329], whereas one study reported unspecified rifampicin resistance [331]. All of these studies have reported poorer clinical outcome with rifampicin combination therapy. However, studies with MSSA cases only [15,75] or low (1-17%) MRSA occurrence [176,308] have reported no rifampicin resistance. Moreover, one study with mixed MSSA and MRSA cases (percentages not provided) [76] reported no rifampicin resistance.

Conflicting results have been obtained with rifampicin combination therapy for prolonged bacteraemia. A prospective randomized study with 42 native valve endocarditis patients compared vancomycin-rifampicin combination with vancomycin only and observed a non-significantly prolonged bacteraemia rate due to vancomycin-rifampicin combination therapy

(7 days vs. 9 days) [81]. In another study, rifampicin combination therapy was observed to lead more often to prolonged bacteraemia than vancomycin or nafcillin treatment alone [82]. In the latter study, each rifampicin resistance case was associated with rifampicin initiation during the bacteraemia phase. A study comparing MRSA and hVISA bacteraemia treated with a vancomycin-rifampicin combination demonstrated prolonged bacteraemia and higher rifampicin resistance for hVISA cases [83]. The authors proposed that due to hVISA the vancomycin serum concentration was below the required hVISA MIC, and hence, rifampicin therapy might be viewed as monotherapy resulting in rifampicin resistance. In the fourth study, 19 elderly patients with prolonged MRSA bacteraemia were treated with a glycopeptide-rifampicin combination. Patients who developed rifampicin resistance (30%) showed no higher mortality [329]. A retrospective study from 2009, including 35 patients with persistent MRSA bacteraemia and various deep infection foci, compared the effect of linezolid (with or without carbapenem) against vancomycin (with or without aminoglycoside or rifampicin) and reported significantly more rapidly achieved early microbiological response in the linezolid group than in the vancomycin-rifampicin group. Moreover, significantly higher (80%) mortality rate for the vancomycin and aminoglycoside-rifampicin combination therapy as compared with vancomycin alone (40% mortality) or linezolid alone (0% mortality) or linezolid and carbapenem (22% mortality) was observed [84].

Table 3. Impact of standard antimicrobial treatment versus rifampicin combination therapy on outcome and development of rifampicin resistance in *Staphylococcus aureus* bacteraemia.

Study	MSSA/ MRSA	Infection foci	Antimicrobial treatment [1]	Cure rate with RMP vs. non-RMP	RMP-R
Norden et al. 1983	MSSA	osteomyelitis	nafcillin	70% vs. 30% (NS)	no
Van der et al. 1983	Mixed	various	oxacillin [2]	67% vs. 41% (**p <0.01**)	NR
Van der et al. 1985	Mixed	various	oxacillin [2]	61% vs. 56% (NS)	no
Norden et al. 1986	MSSA	osteomyelitis	nafcillin	80% vs. 50% (NS)	NR
Levine et al. 1991	MRSA	endocarditis	vancomycin	90% vs. 82% (NS)	NR
Heldman et al. 1996	5% MRSA	endocarditis [6]	oxacillin vs. ciprofloxacin [3]	95% vs. 88% (NS)	NR
Zimmerli et al. 1998 [8]	Mixed	foreign body [7]	flucloxacillin + ciprofloxacin[4]	100% vs. 58% (**p <0.05**)	no
Ruotsalainen et al. 2006	MSSA	various	cloxacillin vs. cloxacillin+levofloxacin [5]	† 17% vs. 38% (**p <0.001**)	no
Schrenzel et al. 2004 [9]	<1% MRSA	various	flucloxacillin vs. fleroxacin p.o.	86% vs. 84% (NS)	no
Daver et al. 2007	51% MRSA	osteomyelitis	vancomycin	43% vs. 84% (**p <0.02**)	yes
Riedel et al. 2008	76% MRSA	endocarditis	nafcillin or vancomycin	† 79% vs 95% (**p <0.05**)	56%
Jang et al. 2009 [11]	100% MRSA	various	linezolid [12]	† 80% vs. 0-43% (**p=0.03**)	9%
Maor et al. 2009	10% hVISA	various	vancomycin	---	5-44%
Senneville et al. 2011	17% MRSA	foreign body [7]	various	75% vs. 47% (**p=0.01**) (remission rate)	no

†=Mortality. RMP=Rifampicin. RMP-R=Rifampicin resistance. NR=Not reported. [1] Parenteral if not otherwise specified. [2] Oxacillin for MSSA and vancomycin for MRSA. [3] Ciprofloxacin orally vs. oxacillin and gentamicin. [4] Ciprofloxacin orally after parenteral therapy. [5] Rifampicin combination for deep foci only. [6] Right-sided endocarditis. [7] Foreign body infections. [8] *S. aureus* 79% and *S. epidermidis* 21%. [9] *S. aureus* 82% and *S. epidermidis* 18%. [10] Percentages and p-value not available. [11] Persistent MRSA bacteraemia (positive blood cultures despite appropriate antibiotic therapy ≥ 7 days). [12] Linezolid ± carbapenem vs. vancomycin.

2.4.4. Drainage or surgical treatment

The importance of deep infection foci localization and eradication is emphasized in several studies [19,168,173]. Eradication and possible surgical intervention are dictated by the nature and accessibility of the deep focus. Abscesses are mostly eradicated through percutaneous or surgical drainage, although small abscesses may be managed with antibiotic therapy alone [165,258]. However, a recent study of over 120 ileopsoas abscesses with more than 40% of *S. aureus* origin did not report an association between abscess drainage and improved outcome [258]. Both acute and chronic osteomyelitis may require surgical in-

tervention such as surgical decompression, debridement of the infected area and revascularization [333].

The requirement for surgical intervention is high in endocarditis, with up to 45% of left-sided native valve and virtually 100% of prosthetic valve endocarditis cases [129,334,335], whereas only a small proportion of right-sided endocarditis requires surgery [34]. For left-sided native valve endocarditis, the following conditions are generally considered to require surgical intervention: valvular regurgitation of haemodynamic significance (New York Heart Association stage III-IV congestive heart failure), mobile and/or large-sized vegetations, vegetations > 1 cm on the anterior mitral valve area, vegetation causing mechanical obstruction of valves, sinus Valsalva rupture, infection extending to the paravalvular area or paravalvular abscess formation and persistent SAB (≥7 days) despite appropriate antimicrobial therapy [336,337]. For right-sided endocarditis, persistent and recurrent SAB or continuous septic embolic complications are indications for surgical intervention [34].

2.5. Infectious disease specialist consultation (IDSC)

2.5.1. Formal and informal consultations

The role of IDSC-guided management in infectious diseases has received increasing attention in the last decades. Attempts to evaluate the complexity, prognostic impact, error avoidance and economic cost of IDSC have been made in several studies [184,338,339, 340,341,342].

IDSC is generally categorized as formal or informal (or ´´curbside``) [338]. In formal consultations, the infectious disease specialist (IDS) makes his decision based on information received from communication with the patient and from physical examination of the patient as well as retrieval of patient records. In informal consultations, the IDS provides information via telephone or other informal discussion and gives advice on disease management without meeting the patient or retrieving the patient´s medical records [340,343,344]. As a result of the ever-deepening specialization in clinical medicine, IDS consultations, and especially informal IDS consultations, have become common [340,345,346,347]. IDSs are among the physicians most frequently consulted [344]. Already in 1998, a study concluded that informal IDSC was more common than formal IDSC [344]. Most informal consultations occur via telephone conversation (30-64%) [338,344,346], whereas a much smaller proportion are made up of informal curbside discussions (19%) [338], or e-mail communication (5%) [344].

Potential advantages and disadvantages of formal versus informal consultations have been investigated in several reports. Many studies view informal curbside consultations as time-saving [344,348], improving the quality of care and reducing hospital admissions [349]. There are concerns that insufficient information may be provided or important information missed in informal consultations [344]. A recent study concluded that informal or curbside consultations are associated significantly more often with inaccurate or incomplete information being presented, which may result in inappropriate advice [350]. Potential medicolegal aspects and risks of informal curbside consultations have also been debated [351], with one report questioning the overall legality of informal curbside consultations [352].

Regarding IDSC, only two studies have evaluated the impact of formal versus informal consultations on disease progression and prognosis. A prospective *post hoc* study, including altogether 627 patients with various infections, observed no significant difference between formal and informal IDSC regarding compliance with recommendations for treatment, performing of diagnostic or monitoring tests, early clinical improvement, in-hospital mortality or length of hospital stay [343]. However, only 3% of all patients received ICU treatment and only 7% were defined as bacteraemic or septic. Moreover, no causative pathogens were reported for bacteraemic or septic patients [343]. Another study that included altogether 233 retrospectively followed SAB patients found that informal consultations were not associated with poorer outcome (i.e. no more SAB relapses). However, improved survival with informal IDSC as compared with formal IDSC was observed, although very few patients (6/179) received informal IDSC, which almost certainly affected the results [353].

2.5.2. Impact of IDSC on clinical management

IDSC has been shown to enhance proper antibiotic selection [16,18,55,58,257,342,354], appropriate duration of therapy [18], proper route of delivery of antibiotic therapy and proper patient monitoring for minimizing adverse drug reactions [341,355]. A positive impact of IDSC on disease management, progression and prognosis has been established for a large number of specific infectious diseases and clinical situations. As a result of IDS involvement and following of IDS recommendations, patients receive more often correct diagnoses [356], more proper therapies [342,357] and less complications [342]. Improved clinical outcome as a result of IDS-guided management has been shown in HIV and AIDS patients [358], in *candida* bloodstream infection [359] and in community-acquired pneumonia [360] and osteomyelitis [361]. Four studies in the 1990s demonstrated reduced morbidity, mortality and cost as a result of IDSC in management of bacteraemic patients [184,357,362,363]. A recent study including various infectious diseases reported IDS intervention, compared with non-IDS intervention, to be significantly associated with lower mor-

tality rates, less readmissions, less ICU treatment and shorter hospital length of stay as well as reduced payments and costs [364].

2.5.3. IDSC in *Staphylococcus aureus* bacteraemia

A total of 11 studies during 1998-2012 investigated the impact of IDSC on SAB [2,3,12,16, 18,56,57,58,184,257,353]. Study design, setting and study population differed in these studies. Most studies were retrospective, two were are prospective [18,184] and one combined prospective and retrospective patient data [3]. The patient number (100-599), MRSA frequency (2-76%) and the proportion of patients with IDCS (27-82%) also varied widely. However, common in these studies was a positive impact of IDSC on either disease progression or prognosis. Altogether, 9 studies reported significantly improved clinical management comprising 1) an increased number of follow-up blood culture collections [16,56,257], 2) more radiological investigations, i.e. echocardiography and bone scans [3,18,56] resulting in more endocarditis and deep infection foci diagnosed [3,56,57, 184,257], 3) more appropriate selection and duration of antibiotic therapy, 4) more appropriate timing regarding MRSA therapy as well as use of β-lactam antibiotic whenever possible [3,18,56,57,58,257,353], 5) more removal of infected prosthetic devices and intravascular catheters, drainage of pus or infection foci removed [16,257,353] and more admissions to surgical ward [58] and 6) longer hospital treatment duration [57].

As a result of improved clinical management, significant reductions in SAB relapses [184,353] and in both 28-day and three-month mortality (both SAB-related and in-hospital mortality) were reported in most studies [2,3,12,16,18,56,57,58] with no significant mortality reduction described in three studies [184,257,353]. The main results for these studies of IDSC in SAB are listed in Table 4.

Table 4. Impact of infectious disease specialist consultation (IDSC) on *Staphylococcus aureus* bacteraemia management and mortality.

Study	N	IDSC [1]	TTE TEE [2]	Radio-logy	Deep foci	IE [3]	Follow-up cultures	Anti-biotics [4]	Focus removal	SAB relapse [5]	†
Fowler et al. 1998	244	45%	-	-	↑	↑	-	-	-	↓	↔
Mylotte et al. 2000	293	36%	-	-	-	-	-	-	-	-	↓
Kaech et al. 2006	308	82%	-	-	-	-	-	-	-	-	↓
Jenkins et al. 2008	234	53-90%	↑	↑	↑	↑	↑	↑	↑	↔	↔
Lahey et al. 2009	240	51%	-	-	-	-	↑	↑	↑	-	↓↓
Rieg et al. 2009	521	67%	↑	↑	↑	↑	-	↑	-	↔	↓↓
Nagao et al. 2010	346	---	↑	-	↑	↑	↑	↑	-	-	↓
Honda et al. 2010	341	33%	↑	-	-	-	-	↑	-	-	↓↓
Choi et al. 2010	100	42%	↔	-	↔	↔	↑	↑	↔	-	↓
Robinson et al. 2012	599	27%	-	-	↑	↑	↔	↑	-	-	↓
Pragman et al. 2012	233	77%	-	-	-	-	-	↑	↑	↓	↔

† Mortality. ↑ Significant increase in univariate analysis. ↓ Significant decrease in univariate analysis. ↓↓ Significant decrease in multivariate analysis. ↔ No significant effect. --- Not reported. [1] Percentage of patients receiving IDSC. [2] Transthoracic or oesophageal echocardiography. [3] Infective endocarditis. [4] Appropriate choice and/or duration. [5] Relapse or recurrence of SAB.

2.6 Biomarkers in *Staphylococcus aureus* bacteraemia

Symptoms of serious infections such as bacteraemia can be non-specific, resulting in challenging clinical diagnostics and evaluations of disease severity [365]. Biomarkers have been explored as a tool for risk stratification or as a surrogate marker for patient outcome, to identify a patient with increased probability of having a disease or a pathologic process or to follow the treatment response [366]. Vast numbers of biomarkers, e.g. C-reactive protein (CRP) or procalcitonin (PCT), have been either used or proposed for clinical practice [367,368], although the predictive value of both has been questioned [369,370]. A review from 2010 evaluated altogether 178 different sepsis biomarkers from 3370 studies, reflecting the wide scope of the biomarker field in infectious diseases. The review concluded that none of these biomarkers demonstrate the sensitivity or specificity required for faultless routine clinical diagnostics. The most widely used are CRP and PCT, although it is well-

recognized that these have limited prognostic value and insufficient capability to distinguish sepsis from various inflammatory conditions [371]. Despite extensive research, the specific role of biomarkers in infections, bacteraemias and sepsis remains undefined [372].

2.6.1. Biomarker candidates

Serological diagnostic assays

Although *S. aureus*-positive blood culture is generally a prerequisite for SAB diagnosis, there are situations with clinical suspicion of SAB when blood cultures are not taken or remain negative due to foe example earlier antibiotic therapy. In clinical situations with SAB suspicion, but unavailable blood cultures, the SAB serological response to *S. aureus* antigens may be valuable when evaluating the clinical situation and *S. aureus* as a causative agent. Furthermore, serology has been suggested as a tool for differentiating between complicated and uncomplicated SAB [373] and for evaluating the effect of treatment [374]. The healthy adult population presents circulating antibodies for most *S. aureus* antigens [375,376], and elevated antibody levels or seroconversion (alteration in antibody titres) constitutes the basis for serological diagnosis.

Two serological tests, antistaphylolysin, i.e. antibody against staphylolysin (ASTA), and teichoic acid antibody (TAA), are frequently used in clinical practice. Most studies report a TAA response to infection within 14-28 days [377], with titer elevations of 1:2 - 1:4 suggesting *S. aureus* infection [378], 1:4 indicating active *S. aureus* infection [379] and 1:8 encountered in SAB patients with endocarditis [379,380]. The predictiveness of TAA varies widely, with complicated SAB cases presenting TAA response in 80% [381]. One study presented TAA response in 91% of endocarditis, 86% of complicated SAB and 68% of uncomplicated SAB [382]. However, other studies have reported elevated TAA levels also in 44% of healthy controls [383].

ASTA has been more applicable among dermatological patients than among invasive infections due to its correlation with skin barrier function [384]. High ASTA titers are encountered among patients with dermatoses and atopic dermatitis [384,385]. However, complicated SAB or endocarditis have presented ASTA responses in only 32-62% of cases [386,387], and hence, due to low sensitivity the predictiveness of ASTA is regarded as limited [379]. Although extensive research with TAA, ASTA and other serological parameters has been performed, no serological diagnostic test alone has managed to present titers positive for all SAB patients or titer elevations separating uncomplicated and complicated SAB cases [388].

A study comparing serological characteristics of *S. aureus* endocarditis in addicts and non-addicts concluded that serological tests were not helpful for identification of deep infection foci. Surprisingly, among addicts with no diagnosed endocarditis, ASTA titers were more often positive than among addicts with endocarditis [389].

Soluble urokinase plasminogen activator

Soluble urokinase plasminogen activator receptor (suPAR) has been presented as an interesting new biomarker in SAB [390,391,392]. Several cell types, e.g. neutrophils, macrophages and monocytes, express on the cell surface suPAR (uPAR/CD87) [393,394]. The suPAR is encountered in various body fluids, such as plasma and urine [395,396], and elevated suPAR levels manifest in inflammation and immune activation [397]. A prospective study including 59 SAB patients found that suPAR was prognostic for mortality, with a sensitivity of 79% and a specificity of 68%, although suPAR did not predict the presence of deep infection foci [390]. SuPAR levels were significantly higher among non-surviving bacteraemia patients, with a sensitivity of 83% and a specificity of 76% for fatal outcome [392]. However, a study including 55 patients with bacteraemia due to various pathogens, including 18% SAB cases, demonstrated that suPAR was a better predictor for Gram-negative than Gram-positive bacteraemia [391].

Interleukin-10

The cell wall of *S. aureus* consists of peptidoglycan, which has the capability of macrophage stimulation, cytokine release and endotoxin-like activity [1,398]. It has been suggested that circulating peptidoglycan may elevate interleukin-10 levels [399] and this may hamper the T-helper adaptive immunity for *S. aureus* bacteria. Thus, interleukin-10 may present a potential harmful effect on SAB patients [400]. A prospective study including 59 SAB cases (35% of MRSA) identified elevated interleukin-10 as an independent mortality predictor, whereas survivors seemed to often have normal interleukin-10 levels [401].

Procalcitonin

Procalcitonin (PCT), the precursor for calcitonin hormone, has been suggested as a biomarker for bacteraemia and sepsis [391,402,403,404]. PCT might have a role in distinguishing between Gram-positive and Gram-negative bacteraemia among critically ill patients [405] and in differentiating bacteraemia from contaminated blood cultures [406]. However, no studies have investigated the predictive value of PCT solely among SAB patients, although SAB patients are included in several reports with PCT [391,403,407]. Moreover, PCT has been proposed as a predictor for endocarditis in general [407] and in SAB [408]. Two thorough prospective reports including bacteraemic patients with 9-27% of SAB investigated the relationship between PCT and endocarditis. The first study demonstrated high PCT levels among SAB patients and significantly higher PCT levels in SAB-

related endocarditis compared with endocarditis due to other bacteraemias [408]. The second study demonstrated high PCT levels in SAB patients relative to patients with bacteraemia due to other pathogens and even higher PCT levels in patients with SAB and endocarditis. Moreover, the study showed significantly higher PCT levels in patients with confirmed endocarditis than in patients with rejected endocarditis [407]. A retrospective report including 119 Gram-positive (20% of SAB) and 44 Gram-negative bacteraemic patients concluded that PCT among SAB patients was significantly higher than in patients with bacteraemia due to coagulase-negative staphylococci (CoNS). It was postulated that PCT might be useful in differentiating *S. aureus* from CoNS [403]. A prospective study, originally designed to investigate suPAR, included 55 bacteraemic patients fulfilling systemic inflammatory response syndrome (SIRS) criteria (18% of SAB) and reported significantly higher PCT levels among Gram-positive bacteraemia than among Gram-negative bacteraemia. However, PCT did not predict mortality [391].

2.6.2. Cell-free DNA

Cell-free DNA (cf-DNA) has been viewed as a potential biomarker for bacteraemic and septic patients as well as critically ill patients [402,409,410,411,412,413,414]. As a result of necrosis, and due to apoptotic cells, free cellular DNA fragments (i.e. cell-free DNA) are released into plasma [415]. Among healthy individuals, low levels of plasma cf-DNA are encountered [416] due to removal of deceased cell debris by phagocytes [417]. Specific clearance processes of cf-DNA remains unestablished, although experimental studies indicate that liver and kidneys play a key role [418].

Sepsis in both hospitalized [402] and critically ill patients [409] is reflected by elevated cf-DNA levels, and sepsis has been reported to amend cell necrosis [419] and apoptosis [420] and result in rising cf-DNA levels [417,421]. Cf-DNA is known to independently predict mortality in patients with bacteraemia due to *S. aureus, Streptococcus pneumoniae, ß-haemolytic streptococcae* or *Escherichia coli* [412]. In patients with severe sepsis and septic shock, the plasma cf-DNA demonstrates an independent correlation with serum lactate elevation at ICU admission. This is suggested to demonstrate sepsis-related hypoxaemia in apoptosis [411]. ICU non-survivors have been shown to present higher cf-DNA levels than ICU survivors [414,422]. Cf-DNA has been demonstrated to predict mortality among ICU non-survivors with severe sepsis more accurately than Acute Physiology and Chronic Health Evaluation (APACHE) II scores, Multiple Organ Dysfunction Syndrome (MODS) scores, age or gender [414].

Two studies have evaluated the prognostic value of cf-DNA in bacteraemic patients with positive blood cultures for various bacteraemic pathogens. They demonstrated higher cf-

DNA levels in ICU patients than in non-ICU patients [412], and in ICU non-surviving patients with severe sepsis than in those surviving ones [414]. The prognostic use and cut-off values of cf-DNA regarding bacteraemic ICU patients with only one causative pathogen have not been studied.

2.7. Prognosis and mortality in *Staphylococcus aureus* bacteraemia

During the pre-antibiotic era, very high mortality rates of 75-82% in SAB were reported [423,424]. From the 1950s until the 1980s, much lower mortality rates, varying from 24% to 58% were seen [425,426,427,428,429]. From the 1980s to the beginning of the 2000s, as a result of improved SAB management, the mortality rates fell significantly from 36% to 21% from 1981-1985 to 1996-2000 for HA-SAB and from 34% to 26% for CA-SAB during the corresponding time-period [7]. At the end of the 1990s several studies reported even lower overall mortality rates of 7-39% [51,137,184]. However, in the 2000s and 2010s the mortality seems to have stabilized around 14-32% for both in-hospital and SAB-related mortality [2,3,12,13,14,15,16,17,18]. A vast number of factors may affect the prognosis, and these may roughly be categorized as host-related, pathogen-related, acquisition-related, clinical picture (pathogen-host interaction related) -related and treatment-related [430].

2.7.1. Impact of host-related factors

Age

High age is viewed as one of the strongest predictors for both overall and SAB-related mortality. Numerous studies, including both MSSA and MRSA, apply high age, > 60-65 years, as a statistical parameter and report high age as an independent predictor of fatal outcome in multivariate analysis for overall mortality at 30 days [12,23,431,432], for 60-day [168] and three-month SAB-related mortality [156] and for in-hospital mortality related to SAB [22,433] as well as for overall in-hospital mortality [434]. Recently, also a case-controlled study where patient characteristics and clinical management were controlled found age over 65 years to be an independent predictor for fatal outcome [435].

Gender

Gender is not viewed as an independent mortality factor in SAB. Although several studies report higher SAB incidence among males [85,117], a 2-fold higher overall mortality at 30 days was reported in MRSA bacteraemia in females [436,437]. Different hormonal characteristics and health-seeking behaviour have been proposed as explanations for the higher mortality in women [438]. However, some studies have not identified any significant connection between SAB outcome and gender [3,7,23]. A large-sized (n > 9000) report of MRSA

bacteraemic episodes in England presented similar age-adjusted mortality among females and males, concluding that gender differences may be explained by age differences [439].

Socio-economic status

Although social deprivation and socio-economic status are known to be associated with higher risk of infection [440], a connection between SAB mortality and socio-economic status has been reported in only one study and socio-economic status had no impact on SAB outcome [441].

Underlying diseases

The presence of underlying diseases has a strong impact on SAB outcome. Several studies have listed cardiac disease [17], chronic liver disease [18,23,442], acute or chronic renal disease [2], dialysis [2,442], malignancy [23,431,442], alcoholism [2], immunosuppression [2,13], diabetes [12] and multiple comorbidities [7,443] as independent predictors of mortality. Many studies have applied the McCabe and Jackson criteria to categorize the severity and prognosis of underlying diseases and comorbidity as healthy, non-fatal, ultimately fatal or rapidly fatal [444]. Ultimately or rapidly fatal underlying diseases have been shown to predict fatal outcome in many reports [3,22,188]. However, two studies with small population sizes failed to detect an impact of comorbidities on outcome in SAB [120, 445].

2.7.2. Impact of community or health care acquisition on mortality

The impact of SAB acquisition on outcome has been controversial, with a trend in the last decade of no significant association with mortality. SAB has traditionally been divided according to acquisition into health care- (nosocomial) and community-associated cases. Several studies in 1970-1990 found HA-SAB to be associated with higher mortality [51,427, 446], and the association of HA-SAB with higher age and comorbidity has been presented as an explanation for the higher mortality. However, the majority of studies after 2000 have not managed to detect any significant prognostic impact of SAB acquisition on outcome [12,17,19,23,183,188,190,447], with the exception of one study connecting HA-SAB to lower mortality [2]. HA-SAB has been observed to carry higher mortality than CA-SAB in only two recent studies [442,448]. Hence, it appears that there might be a trend of diminishing impact of SAB acquisition on outcome.

2.7.3. Impact of methicillin resistance on mortality

The relationship between MRSA and SAB prognosis has been thoroughly investigated, but with conflicting results. Several studies have associated MRSA with a significantly higher

mortality rate in multivariate analyses [3,23,138,156,431]. Two meta-analyses in 2000 presented a significantly higher mortality rate in MRSA bacteraemia than in MSSA bacteraemia [49,449]. However, some studies have failed to connect MRSA bacteraemia to higher mortality rates than MSSA bacteremia [12,168,188,450]. Hence, the results of the two meta-analyses have been questioned due to lack of knowledge of hospital duration prior to SAB in the original studies; when length of hospital stay was adjusted for, bacteraemias with MRSA and MSSA presented similar mortality rates [451].

Several factors have been proposed to explain the higher mortality in MRSA bacteraemia. Some reports have suggested that in patients with MRSA bacteremia higher mortality is due to higher age [51,157], more severe underlying diseases [188], more severe illness at bacteraemia onset (e.g. septic shock) and more complications such as pneumonia [51] as compared with MSSA bacteraemia. One report states that higher mortality in MRSA bacteraemia than in MSSA bacteraemia is evident only in critically ill patients after adjustment for disease severity and acute illness [138]. Various factors in MRSA treatment may be associated with poorer outcome. MRSA has been connected to a delay in effective antibiotic therapy onset [52,157], and vancomycin therapy has been regarded as having weaker efficacy and a less effective blood-sterilizing effect, increasing the risk for persistence of SAB relative to semi-synthetic penicillin or other β-lactams [40,53,274,452,453]. Thus, although vancomycin is the drug of choice, it has repeatedly been connected to treatment failure and higher mortality than β-lactams [22,38,39,40,41]. A prospective study of considerable size (n=1865) presented glycopeptide (mostly vancomycin) therapy as an independent significant mortality predictor [190]. Both pro- and retrospective reports have demonstrated a connection between high vancomycin MIC and worse prognosis in patients with MRSA bacteraemia [454,455], with MIC values ≥ 1.5-2 mg/L representing independent parameters for treatment failure [454] and mortality [455]. Recently, a retrospective study reported high vancomycin MIC (≥ 1.5 mg/L) as the only independent risk factor for complicated bacteraemia when MSSA bacteraemia patients were treated with vancomycin. However, MIC ≥ 1.5 mg/L was not associated with higher mortality [186]. Moreover, pathogen-related virulence factors common in MRSA strains have been demonstrated, such as SCC*mec* type I or agr (accessory gene regulator) group II polymorphism, which might be associated with higher mortality or vancomycin treatment failure [456,457].

2.7.4. Impact of clinical manifestations on mortality

Severity of illness at Staphylococcus aureus bacteraemia onset

The severity of illness, particularly the presence of severe sepsis, septic shock or multi organ failure, at onset of SAB are factors strongly predicting mortality [2,19,24,25,183,445].

Different scoring systems for assessment of severity of illness and outcome prediction have been developed, e.g. the APACHE II, SOFA and PITT scores [139,140,142]. Severity of illness at *S. aureus*-positive blood culture, as evaluated by APACHE-scores [139], has been shown to be significantly correlated with mortality [12,13,458,459]. However, among ICU patients with sepsis, the Pitt bacteraemia score system has been observed to predict mortality better than APACHE II with respect to sensitivity-specificity (67% and 74% for APACHE II versus 68% and 82% for PITT scores) [141]. Other clinical conditions connected to higher mortality have been acute organ dysfunction, need for mechanical ventilation [431], acute renal failure [2], neutropenia [442] and thrombocytopenia [460]. Need for ICU treatment [3,25,188,436] or ICU admission [3,443] has been observed to independently predict weaker outcome as compared with non-ICU patients.

Deep infection foci

The prognostic impact of deep infection foci in SAB has varied widely depending on the type of deep focus. Several studies have presented pneumonia (OR 5.8-17.0) [12,17,51] and endocarditis (OR 2.8-12.1) [3,24,456] as independent predictors for mortality. However, among IDUs endocarditis has been associated with significantly better outcome than among non-IDUs [199]. In native valve infective endocarditis, factors such as age, periannular abscess, heart failure, lack of surgical intervention and thromboembolic central nervous system event, have been associated with significantly weaker outcome [129]. One study observed no association between deep infection foci and outcome [2], in contrast to another that found metastatic foci to lead to weaker outcome [24].

Dosing and onset of antibiotic therapy

Several studies have demonstrated an adverse impact of delayed empiric antibiotic therapy in both MSSA and MRSA bacteraemia [40,50,188,437]. The delay in time between *S. aureus*-positive blood culture and initiation of empiric appropriate antibiotic administration, after which mortality rises, has varied from 24 to 72 hours [40,50,188,461]. Contrary to this, there are studies demonstrating a non-significant association between correctly timed appropriate antibiotic therapy and survival rates in both MSSA and MRSA bacteraemia [24,25,157]. One study concluded that only severely ill SAB patients with APACHE II points > 15.5 gained from early onset of antibiotic therapy, whereas for SAB patients with APACHE II < 15.5 delayed antibiotic therapy had no impact on mortality [50]. Some studies have demonstrated the significance of appropriate dosing of antibiotic therapy. A prospective study of 278 cases of MSSA bacteraemia, including various deep infection foci, demonstrated that a total daily dose of penicillinase-stable penicillin < 4 g was an independent predictor for mortality and a total daily dose < 3 g an independent predictor for SAB recurrence [19]. Another study that included 87 cases of MRSA bacteraemia demonstrated in-

creased survival when vancomycin initiation took place within 48 hours of *S. aureus*-positive blood culture results and the dose was ≥ 2.0 g/day [462].

Surgery and focus removal

SAB patients with non-eradicated and non-eradicable foci had higher mortality than patients who had their focus surgically (or by another intervention) removed (OR 4.17 vs. OR 3.75) [168]. An uneradicated focus was associated with significantly weaker outcome (OR 6.7) also in a study that included only 1% of patients with MRSA bacteraemia [19]. A large retrospective study comparing vancomycin and β-lactam therapy on outcome in solely MSSA bacteraemia patients found eradicated infection foci to be an independent prognostic factor for improved outcome (OR 0.3) [22]. Antibiotic therapy combined with early surgery had significantly better outcome in native valve endocarditis in SAB as compared with antibiotic therapy alone [463]. A study investigating the prognostic impact of IDS recommendations on outcome of 244 SAB patients found unremoved, infected intravascular devices to be significantly associated with SAB relapse and mortality (OR 6.5) [184]. A very recent prospective study, including 58% of patients with MRSA bacteraemia, found a three-day delay in removing eradicable foci to be associated significantly with persistent SAB (OR 2.18) [30]. However, no direct connection between delayed eradication and mortality was presented.

3. AIMS OF THE STUDY

Specific objectives of this study were as follows:

I To compare predisposing factors, disease progression and outcome of health care- and community-associated methicillin-sensitive *Staphylococcus aureus* bacteraemia.

II To evaluate the prognostic value of the biomarker cell-free DNA in methicillin-sensitive *Staphylococcus aureus* bacteraemia patients with early intensive care unit treatment.

III To investigate the impact of formal bedside infectious diseases specialist consultation, informal telephone consultation and no consultation on disease progression and prognosis of methicillin-sensitive *Staphylococcus aureus* bacteraemia.

IV To evaluate the impact of early and late adjunctive rifampicin therapy onset on disease progression and prognosis in methicillin-sensitive *Staphylococcus aureus* bacteraemia patients with deep infection foci.

4. MATERIALS AND METHODS

4.1. Study populations

The study populations consisted of prospectively collected patient data (Studies I and II) and retrospectively collected data (Studies III and IV).

Study I was a prospective, multicenter study carried out in all five university central hospitals and in seven central hospitals in Finland throughout two time periods: January 1999 to May 1999 and January 2000 to August 2002. Adult patients with at least one blood culture positive for *Staphylococcus aureus* were prospectively followed from a median of three days after blood culture collection. In total, 1226 SAB patients were identified during the study period and after controlling for exclusion criteria and excluding patients unable to provide an informed consent or patients who refused participation, altogether 430 cases were included. The exclusion criteria were age < 18 years, imprisonment, pregnancy (suspected or proven), breastfeeding, epilepsy, bacteraemia during previous 28 days, polymicrobial bacteraemia (≥ 3 microbes), history of allergy to any quinolone antibiotic, previous tendinitis during fluoroquinolone therapy, prior fluoroquinolone use for more than five days before randomization, positive culture for *S. aureus* only from a central intravenous catheter, neutropenia (<0.5 x 10^9/L), patients with bacteraemia due to MRSA (n=6) and a *S. aureus* strain resistant to any fluoroquinolone.

Study II included the same prospectively collected patient data as in Study I, although due to missing study samples (n=12), only 418 SAB cases were included in the analysis.

Study III was retrospective with 342 SAB cases representing all adult patients from Helsinki University Central Hospital in Finland with at least one blood culture positive for *Staphylococcus aureus* during two time periods: 2000–2002 and 2006–2007. The earlier time period 2000-2002 included the patients from Studies I and II. Through the use of a unique personal number provided to all Finnish residents, *S. aureus* isolates and patients were matched. The patient data were collected from written (2000–2002) and electronic (2006–2007) patient records. Due to missing patient records, 7 patients had to be excluded. Two time periods were included in order to exclude the possible effect of unidentified temporal differences regarding personnel, treatment practices or any other factors difficult to control. All cases with MRSA bacteraemia were excluded (5 cases during 2000-2002, but none during 2006-2007).

Study IV included all patient data collected for Studies I and III. Cases with MRSA bacteraemia were excluded (n=6)

4.2. Study designs

Data collection included basic patient characteristics: age, gender, underlying diseases and predisposing factors. SAB acquisition, infection focus and antibiotic treatment were registered. Surgical procedures, duration of hospitalization and infection foci confirmed through radiological, bacteriological or pathological research or clinical suspicion only were documented. Radiological investigations and time to defervescence (axillary temperature < 37.5 ^{o}C) were recorded. Laboratory findings included plasma cf-DNA and CRP concentrations at days three and five from the positive blood culture sampling. IDSC during the first week after the first blood culture positive for *S. aureus* was documented. SAB relapse within three months was documented.

Study I was a prospective study. An IDS followed up each SAB patient for three months. SAB cases were categorized according to acquisition into CA- and HA-SAB. The differences of CA- and HA-SAB regarding patient characteristics, underlying conditions, predisposing factors and prevalence of deep infection foci within three days and three months were analysed with univariate analysis. Three-month survival of CA- and HA-SAB were estimated with the Kaplan-Meier method and prognostic factors analysed with multivariate analysis. The primary end-point was case fatality at 28 days and at three months. Secondary end-points were the time to defervescence, decrease in serum CRP concentration and number of deep infection foci within three days and three months.

Study II was a prospective study. Plasma cf-DNA at days three and five from the positive blood culture were stratified and compared according to patient demographics, underlying conditions, severity of illness, deep infection foci, treatment in ICU and mortality for 1) the whole patient population and 2) patients receiving ICU treatment within seven days of *S. aureus*-positive blood culture. Receiver-operating characteristic (ROC) analyses for cf-DNA and CRP were performed, and cut-off values for day three and five cf-DNA were calculated. The patient demographics, underlying conditions, severity of illness, deep infection foci, treatment in ICU and mortality were then stratified and compared according to the cf-DNA cut-off values of days three and five. Prognostic factors were analysed according to the Cox regression model. The primary end-point was mortality at seven days, 28 days or three months, and secondary end-points were deep infection foci localized during the three-months follow-up.

Study III was a retrospective study. The SAB cases were categorized according to bedside (formal), telephone (informal) or no IDSC within one week of *S. aureus*-positive blood culture. Patients with fatal outcome within three days after *S. aureus*-positive blood culture were excluded to allow for the possibility of death occurring before IDSC, as the mean time lapse between blood culture collection and IDSC was three days. Patient demographics, underlying conditions, severity of illness, deep infection foci, treatment in ICU and mortality were stratified and compared according to IDSC. Multinomial logistic regression analyses were performed to simultaneously compare the three consultation groups. The Kaplan-Meier method was used to compare the impact of various IDSC groups on mortality and defervescence. Prognostic factors were analysed according to the Cox regression model in order to determine the prognostic impact of IDSC. The primary end-point was case fatality at 28 days and three months. Secondary outcome measures were the time to defervescence, any inadequate antibiotic therapy, duration of hospitalization, number of deep infection foci and any relapse of SAB within three months.

Study IV was a retrospective study. The patient population was categorized according to whether rifampicin therapy was received, whether it was initiated within seven days (early) or seven days past (late) positive blood culture and whether it was continued for at least 14 days. The main analyses were performed by excluding cases with a fatal outcome within three days as well as excluding patients with alcoholism and acute or chronic liver disease to allow for death before positive blood culture results (the mean time-lapse between blood culture collection and positive blood culture results was three days) and to account for rifampicin therapy contraindications (alcoholism and liver disease). Moreover, as a parallel analysis, the patient population was analysed by excluding cases with a fatal outcome within 14 days of blood culture collection to allow for death before completing 14 days of rifampicin therapy.

Patient demographics, underlying conditions, severity of illness, deep infection foci, treatment in ICU, antibiotic therapy and mortality were stratified and compared according to rifampicin therapy ≥ 14 days or < 14 days. Cox regression analysis was performed to evaluate the prognostic value of early and late rifampicin therapy for 1) the whole patient population, 2) patients with a deep infection foci. The prognostic impact of early and late rifampicin therapy for ≥ 14 days or < 14 days in the whole patient population and among patients with deep infection foci was analysed using the Kaplan-Meier method. The primary end-point was mortality at three months and the secondary end point deep infection foci during the three-month follow-up. The rifampicin dose was 450 mg (< 50 kg of body weight) or 600 mg (> 50 kg of body weight) given once daily.

4.3. Definitions of terminology

Prognosis or severity of underlying conditions was classified as healthy (no approximated time period to death), non-fatal (no approximated time period to death), ultimately fatal (approximated death within 6 months - 5 years) or rapidly fatal (approximated death within 6 months) according to the McCabe and Jackson criteria [444]. SAB was considered HA if the first positive *S. aureus* blood culture was received ≥ 48 hours after admission to hospital or within two days of admission when the patient had been discharged from a hospital within seven days precedingly or when the patient was a long-term care facility resident during the previous two months or was attending haemodialysis. SAB was considered CA when the first positive *S. aureus* blood culture was received within 48 hours of hospital admission without any preceding hospitalization within seven days. Deep infection foci comprised deep-seated abscesses, endocarditis, foreign body infection, meningitis, mediastinitis, osteomyelitis, pneumonia and septic arthritis. Central venous catheter (CVC) infections were defined according to the guidelines of the IDSA [42]. The modified Duke criteria were used to define endocarditis as definite, possible or rejected according to the presence of major and minor criteria [248]. SAB relapse was defined as the same pattern of resistance and PFGE typing for the two *S. aureus* strains. Severe sepsis was regarded as sepsis including 1) hypotension, i.e. systolic blood pressure < 90 mmHg, mean arterial blood pressure < 70 mmHg, or a systolic blood pressure decrease > 40 mmHg in adults or < 2 SD below normal for age or 2) hypoperfusion, i.e. hyperlactataemia (>1 mmol/L) or decreased capillary refill or 3) organ failure, i.e. arterial hypoksaemia Pao_2 / $F10_2$ < 300, acute oliguria with urine output < 0.5mL/kg/h, creatinine increase > 0.5 mg/dL, coagulation abnormalities of INR > 1.5 or APTT > 60, ileus with absent bowel sounds, thrombocytopenia (< 100) or hyperbilirubinaemia (> 70 mmol/L) [464]. Corticosteroid therapy comprised systemic prednisone at a dose of at least 10 mg/day or the equivalent for at least one month. Immunosuppressive treatment was defined as any immunosuppressive treatment received within 6 months of SAB. IDU was considered any information from the patient or the patient records on injection drug use within 6 months of the first positive *S. aureus* blood culture. Pitt bacteraemia score was calculated based on fever, presence of hypotension, need for mechanical ventilation, cardiac arrest event and altered mental status. The exact criteria are listed below [142].

Pitt bacteraemia score criteria.

Criterium		Score
I	Mental status	
	Alert	0
	Disoriented	1
	Stuporous	2
	Comatose	4
II	Fever	
	≤ 35°C or ≥ 40°C	2
	35.1 - 36.0°C or 39.0 - 39.9°C	1
	36.1 - 38.9°C	0
III	Hypotension	
	Systolic blood pressure < 90 mmHg, Intravenous vasopressor requirement, or acute drop in blood pressure: > 30 mmHg (systolic), > 20 mmHg (diastolic)	2
IV	Mechanical ventilation requirement	2
V	Cardiac arrest	4

Complicated SAB (Study II) was defined as SAB in combination with deep infection foci, severe sepsis, septic shock or high Pitt bacteraemia score of 4. Hence, the definition of ´´complicated SAB`` (Study II) is not identical to the definition used by other authors who have defined complicated SAB as the presence of secondary foci, recurrence of SAB within three months, any event requiring ICU treatment or careful monitoring or follow-up of the SAB patient for any of the following: severe sepsis, septic shock, ARDS, DIC, thromboembolic event or septic embolization [28,121,185,186].

IDSC were categorized into three groups: bedside (formal) IDSC, telephone (informal) IDSC or no IDSC. Bedside (formal) IDSC was documented when the IDS had included written directives in the patient records regarding patient status based on careful patient record review and physical investigation. Telephone (informal) IDSC was defined when the treating physician documented in the patient records the directives given by the IDS and the name of the IDS. Cases where data or any documentation of IDSC were lacking were categorized as no consultation. IDSC was considered to have occurred only when it had taken place within one week after the first positive blood culture of *S. aureus*. Antibiotic therapy was regarded as appropriate if administered intravenously for at least 28 days for deep infection foci and at least 14 days otherwise.

4.4. Laboratory methods

During 2000-2002 blood cultures were performed with the Bactec system (BD Diagnostic Systems, Sparks, MD, USA) in five hospitals and the BacT Alert System (Organon-Teknika, Boxtel, The Netherlands) in seven hospitals, whereas during 2006-2007 the BacT Alert System was applied. Gram-staining and subculturing on chocolate agar plates were carried out for aliquots of bottles with a positive signal. Standard laboratory methodology with Gram-staining, colony morphology, production of urease and DNAase and application of mannitol and trehalose were used to identify *S. aureus* isolates. The disk diffusion method (guidelines of the Clinical and Laboratory Standards Institute, CLSI) was applied for testing of antimicrobial drug susceptibility. Tested antibiotics were oxacillin, cephalexin, clindamycin, levofloxacin, rifampicin, and vancomycin. E-test (AB Biodisk, Solna, Sweden) was applied to determe of MICs of oxacillin (according to manufacturer`s instructions).

Via automatic immunoturbidometric analysis with the 917 analyser or Modular PP-analyser (Hitachi Ltd, Tokyo, Japan) and Tina-quant CRP reagents (Roche Diagnostics, Tina-quant CRP), the serum or plasma C-reactive protein (CRP) (Studies I-IV) was determined. CRP concentration <10 mg/L was defined as normal for both methods. Plasma cf-DNA (Study III) was analysed with Quant-iT^{TM} high-sensitivity DNA assay kit and QubitH fluorometer (Invitrogen, Carlsbad, CA, USA). The directives provided by the manufacturer were followed during each laboratory analysis step. At mean cf-DNA levels of 0.734 mg/mL, 1.377 mg/mL and 4.954 mg/mL the intra-day variation coefficients were 1.8%, 4.3% and 1.7%, respectively, and the corresponding inter-day variation coefficients were 3.8%, 5.0% and 3.2% [412].

4.5. Statistical methods

The primary end-point in all statistical analyses was mortality at 28 days or three months. In Study III, any cases with a fatal outcome within three days of *S. aureus*-positive blood culture were excluded from statistical analyses (except cases in Table 1 of Study III) in order to allow for the possibility of death before any IDSC. In Study IV, for the main analyses, cases with a fatal outcome within three days of *S. aureus*-positive blood culture, alcoholism and acute or chronic liver disease were excluded. As a parallel analysis, cases with a fatal outcome within 14 days of *S. aureus*-positive blood culture were excluded.

Data are presented as either absolute values including percentages (Studies I, III and IV) or medians and interquartile ranges (IQR, 25th and 75th percentiles) (Study II). The Pearson $\chi 2$ test to compare categorical variables and Student´s t-test was used for non-categorical variables. Mann-Whitney U-test was used for non-parametric data (Study II). Odds ratios

(ORs) with 95% confidence intervals (CIs) were calculated. Univariate factors with $p < 0.1$ were entered into binary logistic regression analysis (multivariate analysis) (Study I) or into proportional hazards regression (Cox regression model) (Studies II-IV) to estimate factors predicting three-month mortality. Multinomial logistic regression allowed simultaneous comparison of the three different IDSC processes (Study III).

Receiver-operating characteristic (ROC) curves were produced for cf-DNA and CRP to estimate the discriminative power of these two in predicting three-month mortality. For each ROC curve, the area under the curve (AUC) was calculated. The Youden index was defined from the ROC curves as either the ROC-curve point maximizing both sensitivity and specificity values or the sensitivity and specificity sum with the highest value in order to locate the cut-off point.

Survival estimates and time to defervescence were presented with the Kaplan-Meier method using the Log-Rank test to compare the graphs (Studies I, III and IV). The Kaplan-Meier method was used to present survival estimates and time to defervescence (Studies I, III and IV). The ROC curve cut-off points were used for the Kaplan-Meier survival estimation (Study II). All tests were two-tailed and $p < 0.05$ was considered significant. All analyses were performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA).

4.6. Ethical aspects

The study protocols were approved by the ethics committees of all study sites and by the Institutional Review Board and the Ethics Committee of Helsinki University Central Hospital. In Study I, patients provided a signed informed consent. Severely ill patients, e.g. patients in an unconscious state with assisted ventilation, were included as well without a signed informed consent, as these patients were presumed to gain from the study medication. A signed informed consent was provided by the patient or a representative as soon as possible.

5. RESULTS

5.1. Community- and health care-associated bacteraemia (Study I)

5.1.1. Patient characteristics

The 430 SAB cases included 198 (46%) CA-SAB and 232 (54%) HA-SAB patients.

CA-SAB patients, as compared to HA-SAB patients, were significantly more often HIV-positive (4% vs. <1%, p=0.018) and had more often chronic alcoholism (16% vs. 7%, p =0.002), liver disease (25% vs. 7%, p <0.0001) and injection drug abuse (21% vs. 1%, p <0.0001). HA-SAB patients underwent significantly more frequently a foreign body implantation within one year preceding SAB (41% vs. 9%, p <0.0001), surgical procedures within three months (44% vs. 8%, p <0.0001) or CVC application (23% vs. 1%, p <0.0001). Wounds and chronic skin diseases were significantly more common among CA-SAB patients than among HA-SAB patients (59% vs. 46%, p=0.006) (Study I; Table I).

HA-SAB patients, as compared to HA-SAB patients, were significantly older (62.4 ± 15.2 vs. 52.9 ± 19.5 years, mean ± SD, p <0.0001), had more often an ultimately or rapidly fatal underlying disease (41% vs. 12%, p <0.0001) and were more chronically ill with cardiovascular disease (55% vs. 27%, p <0.0001), chronic renal failure (24% vs. 3%, p <0.0001), dialysis care (20% vs. <1%, p <0.0001), heart valve disease (22% vs. 7%, p <0.0001), malignancy (21% vs. 7%, p <0.0001), complicated diabetes (21% vs. 11%, p=0.003), haematological malignancy (6% vs. <1%, p=0.001) or connective tissue or rheumatic disease (15% vs. 8%, p=0.021) and more often received immunosuppressive treatment (19% vs. 7%, p <0.0001).

5.1.2. Clinical aspects

At *S. aureus*-positive blood culture and within the first week of treatment, no significant differences between the patients with CA-SAB or HA-SAB were seen in severe sepsis (7% vs. 6% at positive blood culture and 13% during the first week for both groups, respectively) septic shock (3% for both groups and 7% vs. 4%, respectively) or need for ICU treatment (16% vs. 15% and 23% vs. 21%). During the first three days of SAB, less deep infection foci were localized among patients with HA-SAB relative to CA-SAB (69% vs. 84%, p <0.0001), and throughout the three-month follow-up a slight increase was observed in the number of patients with identified deep foci (80% vs. 87%, p=0.045) (Table 5). HA-SAB patients presented significantly more often mediastinitis or infection of CVC or peripheral catheter or permanent foreign bodies, whereas osteomyelitis and deep-seated abscesses were seen significantly more frequently in CA-SAB. No difference was observed in the oc-

currence of pneumonia, septic arthritis, endocarditis or cutaneous infections between CA- and hA-SAB patients. The prevalence of CA- and HA-SAB patients without any diagnosed infection focus was low at three days (6% vs. 3%) and remained low throughout the three-month follow-up (5% vs. 2%); however, the difference at both time-points was non-significant (Table 5).

Table 5. Comparison of infection focus and mortality in community- (CA-) and health care- (HA) associated *Staphylococcus aureus* bacteraemia at day three and at three months. Data are number (%) of patients.

	All SAB episodes (n=430)	HA-SAB (n=232, 54%)	CA-SAB (n=198, 46%)	OR (95%CI)	p-value
From blood culture to day 3					
SAB without foci	21 (5)	9 (3)	12 (6)	0.63 (0.26-1.52)	NS
Cutaneous foci	268 (62)	151 (65)	117 (59)	1.29 (0.87-1.91)	NS
Deep foci	325 (76)	159 (69)	166 (84)	0.42 (0.26-0.67)	<0.0001
Mortality	3 (1)	1 (<1)	2 (1)	0.42 (0.04-4.71)	NS
From blood culture to 3 months					
SAB without foci	14 (3)	5 (2)	9 (5)	0.46 (0.15-1.40)	NS
Cutaneous foci	283 (66)	160 (69)	123 (62)	1.36 (0.91-2.02)	NS
Deep foci	359 (83)	186 (80)	173 (87)	0.58 (0.34-0.99)	0.045
Mortality	76 (18)	50 (22)	26 (13)	1.82 (1.08-3.05)	0.023

OR, odds ratio; CI, confidence interval; NS, non-significant; SAB, *Staphylococcus aureus* bacteraemia.

5.1.3. Antimicrobial treatment

From the first day of the positive blood culture, all patients with CA- and HA-SAB were treated with an antibiotic effective against the isolated *S. aureus* strain. The vast majority of patients received a β-lactam antibiotic whereas only 12% were treated with vancomycin.

5.1.4. Outcome

The case fatality at 28 days did not differ between CA- and HA-SAB (11% vs. 14%), whereas at three months it was significantly higher among the HA-SAB patients (22% vs. 13%, p=0.023). Overall, the case fatality at 28 days was 13% and at three months 18%. The mortality difference between CA- and HA-SAB remained significant in Kaplan-Meier analysis. However, no significant difference between CA- and HA-SAB was observed in time to defervescence (Figure 1). Prognostic factors for a fatal outcome within 28 days according to binary logistic regression analysis were age > 60 years, ultimately or rapidly fatal

disease, immunosuppressive treatment within six months, chronic alcoholism, pneumonia and endocarditis (Study I; Table III). On the day of *S. aureus*-positive blood culture, the mean serum CRP was significantly lower among HA-SAB patients. As described in Section 5.1.2., the overall prevalence of deep infection foci among CA- and HA-SAB differed during the first three days (84% vs. 69%, p <0.0001) and throughout the three-month follow-up (87% vs. 80%, p=0.045).

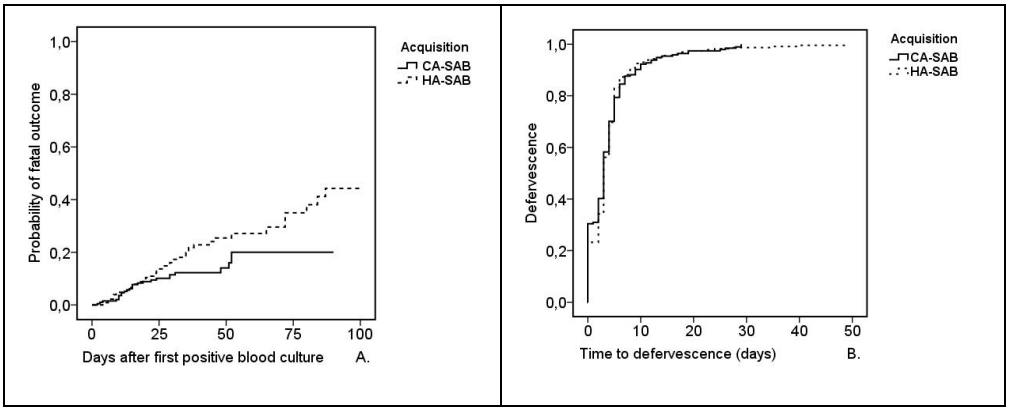

Figure 1. Kaplan-Meier estimation of survival (Log-Rank <0.05) (**A**) and time to defervescence (Log-Rank non-significant) (**B**) for health care-associated (HA) versus community-associated (CA) *Staphylococcus aureus* bacteraemias (SAB).

5.2. Cell-free DNA and *Staphylococcus aureus* bacteraemia (Study II)

5.2.1. Patient characteristics

The study included 418 SAB patients. Initially, 430 SAB cases were identified, but as a result of missing plasma samples 12 SAB cases were not found. Cf-DNA was determined at days three and five and the median cf-DNA results were stratified according to patient characteristics. No significant differences were seen in the day three cf-DNA levels when stratified and compared according to various underlying factors. At day five, male sex (p <0.0001), age > 60 years (p <0.05), alcoholism (p <0.05), coronary artery disease (p <0.01) and complicated diabetes (p <0.05) were associated with significantly higher cf-DNA concentrations, whereas McCabe's healthy and non-fatal classification was associated with significantly lower (p <0.01) values of cf-DNA (Study II; Table I). Patients with a deep infection focus (p <0.001), ICU treatment at *S. aureus*-positive blood culture or within three to seven days (p <0.0001) or with a fatal outcome irrespective of death time (p <0.0001) presented significantly higher cf-DNA concentrations at both days three and five (Study II: Table I). From the day of positive blood culture onwards, an effective antibiotic against the cultured *S. aureus* strain *in vitro* was provided to all patients. Vancomycin was received by

a minority (2%) of the patients and vancomycin as the only antibiotic was given to only 1%, whereas the vast majority of patients were treated with a β-lactam antibiotic (76%).

5.2.2. Treatment in intensive care unit and cell-free DNA

Within three days of *S. aureus*-positive blood culture, 87 patients (21%) and within seven days 99 patients (24%) needed ICU treatment. Regarding demographics and underlying conditions, the only difference was that ICU patients significantly more often suffered from alcoholism ($p < 0.05$) than non-ICU patients. However, deep infection foci and mortality at seven days, 28 days and three months were significantly higher among ICU patients relative to their non-ICU counterparts. These results were similar when ICU treatment during the first three days only or during the fiest seven days after positive blood culture were taken into account (data not shown).

SAB patients who needed ICU treatment within seven days of positive blood culture were analysed as a subgroup according to three-month survival. At day 3, the non-survivors in the ICU group presented significantly higher cf-DNA values for age ($p < 0.01$), healthy or non-fatal disease, alcoholism and cardiovascular disease ($p < 0.05$), ICU-specific characteristics such as severe sepsis, need for mechanical ventilation or inotropia support or Pitt bacteraemia scores ≥ 4 ($p < 0.05$) as well as deep infection foci and complicated SAB ($p < 0.01$), whereas no difference was seen between CA- and HA-SAB acquisition. However, at day 5, no significant difference between ICU survivors and non-survivors was observed (Study II; Table II) and Table 6 (below).

Table 6. Plasma cf-DNA concentration (µg/mL) at days 3 and 5 from the positive blood culture in 99 patients with *Staphylococcus aureus* bacteremia (SAB) in the intensive care unit (ICU) within 7 days of positive blood culture. Patients are divided according to survivors and non-survivors at the three-months follow-up. Values are given as median (quartiles). NS=non significant.

	cf-DNA at day 3			cf-DNA at day 5		
	ICU survivor	ICU non-survivor	p-value [x]	ICU survivor	ICU non-survivor	p-value [x]
Demographics						
Age >60 years	1.62(1.37-2.16)	3.97(2.55-9.46)	<0.01	1.57(1.31-2.46)	2.27(1.59-3.01)	NS
HA-SAB [1]	1.60(1.37-2.13)	2.01(1.59-4.84)	NS	1.53(1.31-1.89)	2.27(1.14-2.26)	NS
Underlying conditions						
Healthy or nonfatal [2]	1.67(1.40-2.12)	2.37(1.80-7.92)	<0.05	1.53(1.29-1.93)	2.27(1.52-3.01)	NS
Cardiovascular	1.65(1.38-2.13)	3.97(2.32-3.63)	<0.05	1.72(1.38-2.44)	2.30(1.19-2.26)	NS
Alcoholism	1.68(1.37-2.30)	5.96(2.37-11.0)	<0.05	1.81(1.34-1.93)	2.96(1.70-2.73)	NS
ICU characteristics						
Severe sepsis	1.69(1.40-2.11)	3.94(1.91-7.22)	<0.05	1.64(1.31-1.88)	2.66(1.72-3.48)	NS
Mechanic ventilation	1.82(1.43-2.30)	3.60(1.91-7.22)	<0.05	1.90(1.48-2.66)	2.64(1.71-3.48)	NS
Inotropic support	1.77(1.50-2.23)	3.05(2.01-5.96)	<0.05	1.63(1.29-1.97)	2.31(1.89-3.33)	NS
Pitt scores ≥4	1.83(1.42-2.42)	3.05(2.01-5.96)	<0.05	1.76(1.45-2.45)	2.31(1.89-3.33)	NS
Infection focus						
Any deep infection	1.66(1.40-2.15)	3.05(2.01-4.84)	<0.01	1.60(1.29-2.18)	2.27(1.56-2.66)	NS
Complicated SAB [3]	1.66(1.39-2.14)	3.07(2.09-5.68)	<0.01	1.59(1.29-2.12)	2.31(1.89-3.33)	NS

[x] Mann-Whitney *U-test*. [1] Health care-associated. [2] Classified according to McCabe and Jackson [444].

[3] Defined as SAB and deep infection focus, severe sepsis, septic shock or Pitt bacteraemia scores ≥ 4.

5.2.3. Sensitivity and specificity of cell-free DNA

Receiver-operating characteristic (ROC) curves were applied to estimate predictive value of cf-DNA and CRP on the 28-day and three-month outcome in patients treated in the ICU within 7 days of *S. aureus*-positive blood culture (n=99) and in non-ICU patients (n=319). For ICU and non-ICU patients, the ROC analysis revealed that both the 3-day and the 5-day cf-DNA significantly predicted fatal outcome. This finding held when the data were analysed separately for the 28-day and the three-month follow-up. CRP, however, had no predictive value for outcome.

The 3-day AUC for cf-DNA in ICU patients was 0.71 (95% CI 0.57-0.84, p <0.01) with a cut-off value of 1.99 µg/mL and a corresponding sensitivity of 67% and specificity of 77% in predicting fatal outcome within three months. The corresponding 5-day cf-DNA AUC was 0.71 (95% CI 0.58-0.84, p <0.01) with a cut-off value of 1.69 µg/mL and a sensitivity of 63%

and a specificity of 60%. For the 28-day mortality, the 3-day cf-DNA AUC was 0.66 (95% CI 0.52-0.81, p <0.05) with a cut-off value of 1.99 µg/mL and a sensitivity of 60% and a specificity of 73% (data not shown). For CRP, the 3-day AUC was 0.46 (95% CI 0.32-0.59, p=0.55) and the 5-day AUC 0.47 (95% CI 0.34-0.61, p=0.67). Among non-ICU patients, AUC for 3-day cf-DNA was 0.64 (95% CI 0.55-0.74, p <0.01) with a cut-off value of 1.57 ug/mL and a sensitivity of 62% and a specificity of 61% in predicting a fatal outcome in the three-month follow-up. The corresponding 5-day cf-DNA AUC was 0.68 (95% CI 0.59-0.77, p <0.01) with a cut-off value of 1.49 µg/mL and a sensitivity of 65% and a specificity of 61%. For CRP, the 3-day AUC was 0.46 (95% CI 0.36-0.57, p=0.48) and the 5-day AUC 0.51 (95% CI 0.41-0.62, p=0.80) (Study II; Figure 1a-b)

ROC analysis was applied to predict seven days mortality for day three and five cf-DNA in patients with SAB divided according to Pitt bacteraemia scores and intensive care unit treatment (see Figure 2a-d below).

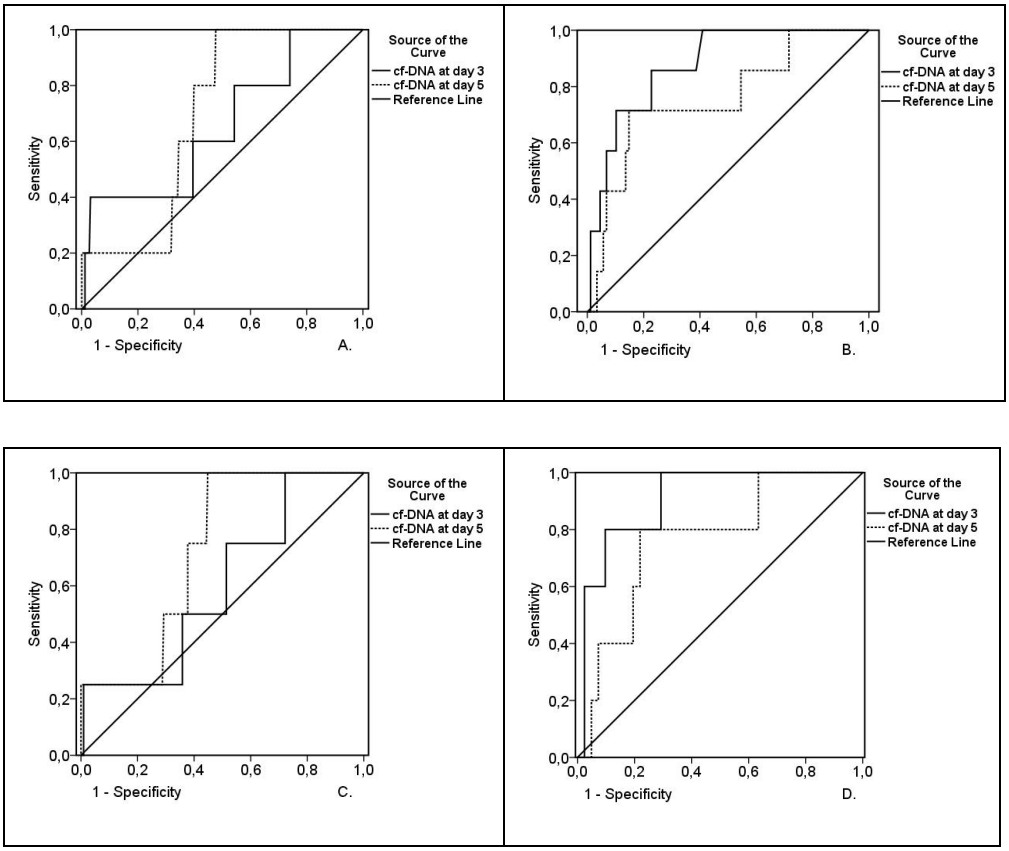

Figure 2.
Receiver-operating characteristic (ROC) curves for cf-DNA predicting 7-day mortality in patients with *Staphylococcus aureus* bacteraemia (SAB) divided according to Pitt bacteraemia scores and intensive care unit treatment.

A. Pitt bacteraemia score=0. The AUC for day 3 cf-DNA was 0.66 (95% CI 0.40-0.91) (p-value non-significant) with a cut-off value of 1.59 mg/mL and a sensitivity of 60% and a specificity of 61%. The AUC for day 5 cf-DNA was 0.69 (95% CI 0.54-0.84) (p-value non-significant) with a cut-off value of 1.49 mg/mL and a sensitivity of 80% and a specificity of 60%.

B. Pitt bacteraemia scores >0. The AUC for day 3 cf-DNA was 0.88 (95% CI 0.77-0.98) (p <0.01) with a cut-off value of 2.01 mg/mL and a sensitivity of 86% and a specificity of 77%. The AUC for day 5 cf-DNA was 0.76 (95% CI 0.56-0.95) (p <0.05) with a cut-off value of 1.96 mg/mL and a sensitivity of 71% and a specificity of 81%.

C. Pitt bacteraemia score=0 and no intensive care unit. The AUC for day 3 cf-DNA was 0.59 (95% CI 0.34-0.86) (p-value non-significant) with a cut-off value of 1.45 mg/mL and a sensitivity of 75% and a specificity of 48%. The AUC for day 5 cf-DNA was 0.72 (95% CI 0.55-0.89) (p-value non-significant) with a cut-off value of 1.49 mg/mL and a sensitivity of 75% and a specificity of 63%.

D. Pitt bacteraemia scores >0 and intensive care unit treatment. The AUC for day 3 cf-DNA was 0.91 (95% CI 0.81-1.00) (p <0.01) with a cut-off value of 2.35 mg/mL and a sensitivity of 80% and a specificity of 91%. The AUC for day 5 cf-DNA was 0.78 (95% CI 0.58-0.97) (p <0.05) with a cut-off value of 2.14 mg/mL and a sensitivity of 80% and a specificity of 79%.

5.2.4. Prognostic value of cell-free DNA relative to other prognostic factors

The prognostic value of cf-DNA was analysed with proportional hazards regression (Cox regression model) for ICU and non-ICU patients by applying the cf-DNA cut-off values (from Study II; Figure 1a-b) as one statistical parameter. In univariate analysis, factors among ICU patients that were associated with three-month mortality were age > 60 years ($p < 0.01$), inotropia need ($p < 0.05$), mechanical ventilation ($p < 0.05$), Pitt bacteraemia scores ≥ 2-4 ($p < 0.01$) and cf-DNA > 1.99 µg/mL (at day 3) ($p < 0.0001$), whereas healthy or non-fatal underlying disease had a protective prognostic impact ($p < 0.05$). For non-ICU patients, age > 60 years ($p < 0.01$), corticosteroid use ($p < 0.0001$), previous dialysis treatment ($p < 0.01$), diabetes and complications ($p < 0.05$), haematological malignancy ($p < 0.05$), chronic pulmonary disease ($p < 0.01$), presence of a deep infection focus ($p < 0.05$) and cf-DNA > 1.57 µg/mL (at day 3) ($p < 0.01$) were all associated with fatal outcome within three months, whereas healthy or non-fatal underlying disease had a protective prognostic impact ($p < 0.0001$) (Table 7).

In the Cox regression model for ICU patients only Pitt bacteraemia scores ≥ 4 (OR 4.47, CI 1.94-10.3) ($p < 0.0001$) and cf-DNA > 1.99 µg/mL (at day 3) (OR 3.56, CI 95% 1.69-7.59) ($p < 0.001$) predicted a fatal outcome whereas, healthy or non-fatal underlying disease (OR 0.34, CI 95% 0.15-0.77) ($p < 0.05$) had a protective prognostic value. Among non-ICU patients, corticosteroid use (OR 2.89, CI 95% 1.39-6.07) ($p < 0.01$), chronic pulmonary disease (OR 2.45, CI 95% 1.21-4.96) ($p < 0.05$) and healthy or non-fatal underlying disease (OR 0.26, CI 95% 0.11-0.63) ($p < 0.01$) had a significant impact on prognosis (Table 7).

Table 7. Prognostic factors for three-month mortality in *Staphylococcus aureus* bacteraemia patients with (n=99) or without (n=319) intensive care unit treatment within 7 days of *S. aureus*-positive blood culture. CI, confidence interval; OR, odds ratio; NS, non-significant.

	Univariate analysis OR (95% CI)	p-value	Cox regression analysis OR (95% CI)	p-value
Intensive care unit				
Age > 60 years	3.64 (1.43 - 9.29)	<0.01	-	-
Healthy or non-fatal [A]	0.33 (0.13 - 0.81)	<0.05	0.34 (0.15 - 0.77)	<0.05
Chronic alcoholism	1.80 (0.64 - 5.06)	NS	-	-
Corticosteroid use [B]	3.21 (0.67 - 15.3)	NS	-	-
Chronic renal failure [C]	0.71 (0.14 - 3.75)	NS	-	-
Dialysis (haemo- or peritoneal)	0.87 (0.16 - 4.75)	NS	-	-
Diabetes (and complications)	1.01 (0.35 - 2.98)	NS	-	-
Haematological malignancy	1.10 (0.09 - 12.1)	NS	-	-
Any deep infection	1.89 (0.20 - 17.5)	NS	-	-
Endocarditis	1.63 (0.67 - 3.99)	NS	-	-
Inotropia need	3.19 (1.18 - 8.64)	<0.05	-	-
Mechanical ventilation	2.67 (1.04 - 6.86)	<0.05	-	-
Pitt bacteraemia scores ≥ 4	3.19 (1.18 - 8.64)	<0.01	4.47 (1.94 - 10.3)	<0.0001
Pitt bacteraemia scores ≥ 3	3.14 (1.43 - 9.79)	<0.01	-	-
Pitt bacteraemia scores ≥ 2	3.03 (1.29 - 7.58)	<0.01	-	-
cf-DNA cut-off 1.99 µg/mL [D]	5.24 (2.03 - 13.5)	<0.0001	3.56 (1.69 - 7.59)	<0.001
Non-intensive care unit				
Age > 60 years	3.04 (1.49 - 6.18)	<0.01	-	-
Healthy or non-fatal [A]	0.13 (0.06 - 0.26)	<0.0001	0.26 (0.11 - 0.63)	<0.01
Chronic alcoholism	1.93 (0.73 - 5.09)	NS	-	-
Corticosteroid use [B]	7.91 (3.66 - 17.1)	<0.0001	2.89 (1.39 - 6.07)	<0.01
Dialysis (haemo- or peritoneal)	3.70 (1.70 - 8.07)	<0.01	-	-
Diabetes (and complications)	2.17 (1.01 – 4.66)	<0.05	-	-
Haematological malignancy	3.54 (1.02 - 12.3)	<0.05	-	-
Chronic pulmonary disease	3.17 (1.58 - 6.39)	<0.01	2.45 (1.21 - 4.96)	<0.05
Any deep infection	3.67 (1.09 - 12.3)	<0.05	-	-
Endocarditis	2.24 (0.98 - 5.14)	NS	-	-
cf-DNA cut-off 1.57 µg/mL [D]	2.86 (1.45 - 5.64)	<0.01	-	-

[A] Classified according to McCabe and Jackson [444]. [B] In the 6 months preceding the positive blood culture. [C] Chronically elevated plasma creatinine (>180 mol/L). [D] Cut-off value at day 3 as in Figure 1a-b in Study II.

The Cox regression model above was performed using a day 3 cf-DNA cut-off value. When performed with a day 5 cf-DNA cut-off value of 1.69 µg/mL for ICU patients or 1.49 µg/mL for non-ICU patients, the prognostic value in univariate analysis was significant for both ICU patients and non-ICU patients ($p < 0.05$). However, in Cox regression model the 5-day cf-DNA cut-off values had no significant prognostic value for either ICU or non-ICU patients.

The Kaplan-Meier method was applied to estimate the survival for ICU and non-ICU patients according to 3-day and 5-day cf-DNA cut-off values. According to the Kaplan-Meier Log-Rank test, both 3-day and 5-day cf-DNA cut-off values presented significant differences in three-month survival both for ICU patients at day three ($p < 0.0001$) and at day five ($p < 0.05$) and for non-ICU patients at days three and five ($p < 0.01$) (data not shown).

5.3. Impact of infectious disease specialist consultation (IDSC) on *Staphylococcus aureus* bacteraemia outcome (Study III)

5.3.1. Patient characteristics

The vast majority of the SAB patients received formal bedside IDSC (72%), whereas informal telephone conversation-based IDSC was given much less often (18%) and a small proportion of patients had no IDSC (10%). No significant differences emerged in sex, age, nosocomial acquisition or underlying diseases between patients with formal bedside and informal telephone IDSC. However, significantly more ultimately or rapidly fatal underlying diseases were seen among SAB patients managed without any IDSC (p=0.008). The mean time-lapse between blood culture collection and IDSC was 3 days for both formal bedside and informal telephone groups (Study III; Table I). No significant differences in the occurrence of severe sepsis at *S. aureus*-positive blood culture were seen between the formal bedside (7%) and the informal telephone consultation (10%) groups, whereas patients without IDS consultation (31%) suffered from severe sepsis significantly more often than bedside IDS consultation patients (p <0.0001). The need for ICU treatment among informal telephone IDSC patients (34%) was more common than among formal bedside IDSC patients (21%) during the first 3 days after *S. aureus*-positive blood culture (p=0.037), whereas within the first week the difference was non-significant (37% for informal telephone IDSC and 29% for formal bedside IDSC, p=0.22) (Study III; Table I).

5.3.2. Impact on radiological diagnostics

The use of echocardiography - transthoracic (TTE) or transoesophageal (TEE) - did not differ between formal bedside and informal telephone IDSC (77% vs. 71% for TTE and 11% vs. 3% for TEE). The use of whole-body computed tomography was less common among informal telephone IDSC (55%) (p=0.049) and no IDSC (29%) (p <0.0001) than among formal bedside IDSC (68%). The use of leukocyte indium-111 scintigraphy was more common in formal bedside IDSC (43%) than in informal telephone (13%) or no IDSC (9%) (p <0.001) (Study III; Table I). However, after adjustment for mortality before IDSC, leukocyte indium-111 scintigraphy was the only radiological investigation provided less often to informal telephone IDSC patients than to formal bedside IDSC patients (p=0.011) (Study III; Table II).

5.3.3. Impact on deep infection focus localization

Formal bedside IDSC resulted significantly more often in identification of a deep infection focus (78%) than to informal telephone IDSC (53%) or no IDSC (29%), and this was seen in all deep infection focus types, except endocarditis, for which the difference between for-

mal bedside (16%) and informal telephone IDSC (7%) was non-significant (p=0.055) (Study III; Table I). According to multinomial logistic regression analysis, the odds ratio (OR) for deep infection focus localization was only 0.15 (95% CI 0.06-0.38, p <0.0001) for informal telephone IDSC and 0.13 (95% CI 0.03-0.54, p=0.005) for no IDSC relative to formal bedside IDSC (Study III; Table II).

5.3.4. Impact on antibiotic treatment

From the first day of positive blood culture, each patient received an antibiotic effective against *S. aureus*. Most patients received a β-lactam antibiotic (93%), whereas only 3% were treated with vancomycin. Patients with formal bedside IDSC (85%) received significantly more often proper length of antibiotic therapy relative to informal telephone IDSC (63%) (p=0.008) or no IDSC patients (54%) (p=0.004) (Study III; Table I). However, after adjustment for mortality before IDSC, no difference in number of patients with proper antibiotic length was observed (Study III: Table II).

5.3.5 Impact on outcome

Formal bedside IDSC resulted in longer mean duration of hospitalization (38.7 ± 21.7 days) than either informal telephone IDSC (30.6 ± 23.0 days) (p=0.014) or no IDSC (24.9 ± 24.8) (p=0.001) (Study III; Table I). Mean time to defervescence was shorter for formal bedside IDSC (6.7 ± 9.7 days) than for informal telephone IDSC (12.6 ± 13.4) (p=0.001) or no IDSC (13.4 ± 14.7) (p =0.003) (Study III; Table I). Similar results were obtained after early 3-day mortality was omitted (Figure 3b) (Study III; Table II and Figure 1a). No difference in mortality was seen between formal bedside and informal telephone IDSC within the first 3 days. However, mortality was lower among patients with bedside IDSC at 7 days (1% vs. 8%, p=0.001), at 28 days (5% vs. 16%%, p=0.002), and at three months (9% vs. 29%, p <0.0001) than among patients treated with informal telephone IDSC (Study III; Table I). Patients who received no IDSC had a high mortality already at 3 days (26%) and had a higher mortality throughout the three-month study period than patients receiving formal bedside IDSC (9% vs 46%%, p <0.001) (Study III; Table I). After adjustment for early death, the 28-day mortality for formal bedside IDSC (5%) as compared with informal telephone IDSC (15%) (p=0.08) and no IDSC (12%) (p=0.68) became non-significant. However, the three-month mortality remained significantly lower for formal bedside IDSC (9%) than for the other two IDSC groups (28% for informal telephone IDSC and 27% for no IDSC) (Figure 3a) (Study III; Table II and Figure 1b).

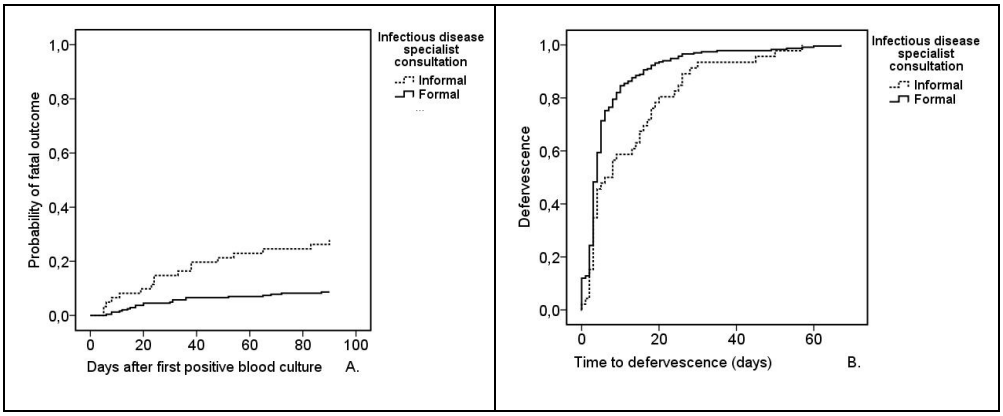

Figure 3. Kaplan-Meier analysis for fatal outcome (**3a**) and time to defervescence (**3b**) in patients with *Staphylococcus aureus* bacteraemia according to telephone (informal) (n=62) or bedside (formal) (n=245) infectious disease specialist consultation. Log-Rank test for fatal outcome in informal versus formal consultation (p <0.0001) and for defervescence in informal versus formal consultation (p=0.001). Patients who died during the first 3 days of *S. aureus*-positive blood culture (n=11) were excluded.

Prognostic factors for three-month mortality were analysed with proportional hazards regression (Cox regression model). When all prognostic determinants were taken into account, the factors associated with fatal outcome were informal telephone IDSC (p=0.01), no IDSC (p=0.002), pneumonia (p=0.001), ICU within three days of *S. aureus*-positive blood culture (p =0.012) and corticosteroid therapy (p=0.01), whereas healthy or non-fatal underlying disease (p <0.0001), leukocyte indium-111 scintigraphy (p=0.021) and whole-body computed tomography (p=0.022) had positive prognostic value (Table 8). When compared with formal bedside IDSC, the odds ratio (OR) for mortality in informal telephone IDSC was 2.31 (95% CI 1.22-4.38) (Study III; Table II).

Table 8. Prognostic factors for three-month mortality in 331 patients with *Staphylococcus aureus* bacteraemia. Patients with a fatal outcome within 3 days of *S. aureus*-positive blood culture were excluded. CI, confidence interval; OR, odds ratio.

	Univariate analysis OR (95% CI)	p-value	Cox regression analysis OR (95% CI)	p-value
Positive prognostic impact				
Healthy or non-fatal disease [A]	0.11 (0.05 - 0.022)	<0.0001	0.18 (0.09 - 0.35)	<0.0001
Leukocyte indium-111 scintigraphy	0.41 (0.19 - 0.87)	0.018	0.40 (0.19 - 0.87)	0.021
Whole-body computed tomography	0.43 (0.23 - 0.80)	0.007	0.49 (0.26 - 0.90)	0.022
Negative prognostic impact				
Pneumonia	2.31 (1.23 - 4.33)	0.008	2.74 (1.49 - 5.05)	0.001
ICU within 3 days	1.96 (1.00 - 3.83)	0.046	2.28 (1.19 - 4.15)	0.012
Corticosteroid therapy[B]	5.48 (1.93 - 15.6)	<0.0001	2.98 (1.29 - 6.85)	0.01
Telephone IDS within 1 week	3.21 (1.63 - 6.33)	<0.0001	2.31 (1.22 - 4.38)	0.01
No IDS consultation within 1 week	2.51 (0.99 - 0.37)	0.045	3.56 (1.59 - 7.94)	0.002

[A] Underlying Diseases characterized according to McCabe and Jackson [444]. [B] Systemic prednisone >10 mg/day or equivalent for >1 month.

During the first 7 days altogether 31% of patients needed ICU treatment. Of these patients treated in ICU, survivors and non-survivors were compared separately and prognostic factors for three-month mortality were analysed. Survival was significantly associated with formal bedside IDSC (p <0.0001), healthy or non-fatal underlying diseases (p=0.003) and performed whole-body computed tomography (p=0.027). However, ultimately or rapidly fatal diseases (p=0003), informal telephone IDSC (p=0.001) and no IDSC (p=0.008) were associated with fatal outcome among ICU patients (Study III; Table IV).

Factors difficult to control in retrospective studies are hospital-related temporary differences in treatment or personnel practices, and thus, in order to exclude the effect of unidentified differences, two different time periods for data collection were included in Study III.

Moreover, two study periods were regarded as mandatory as most patients from the first study period had participated in our previous prospective study [15]. This naturally raises the question of whether any differences are present in the results when the data of the two time periods are analysed separately. As a parallel investigation (results not mentioned and data not shown in Study III) the data were analyzed and prognostic factors for the three-month mortality according to Cox regression were presented I) by including only patients from the later time period in 2006-2007, II) by including only patients from the earlier time period in 2000-2002 and III) by excluding the patients from the previous study [15]. Table 9 presents the results.

Table 9. Prognostic factors for three-month mortality according to Cox regression analysis in patients with *Staphylococcus aureus* bacteraemia from different time periods. Patients with a fatal outcome within 3 days of *S. aureus*-positive blood culture were excluded. CI, confidence interval; OR, odds ratio.

	n (342 total)	Cox regression analysis OR (95% CI)	p-value
2006 - 2007	139		
Healthy or non-fatal disease [A]		0.35 (0.15 - 0.80)	< 0.05
Corticosteroid therapy[B]		---	---
Telephone IDSC within 1 week [C]		3.33 (1.17 - 9.49)	< 0.05
Whole-body computed tomography		0.42 (0.18 - 0.96)	< 0.05
Pneumonia		2.80 (1.28 - 6.13)	=0.01
2000 - 2002	48		
Healthy or non-fatal disease [A]		Insufficient	Insufficient
Corticosteroid therapy[B]		statistical	statistical
Telephone IDSC within 1 week [C]		power	power
Whole-body computed tomography			
Pneumonia			
2000 - 2002 & 2006 - 2007 [X]	187		
Healthy or non-fatal disease [A]		0.24 (0.10 - 0.53)	<0.0001
Corticosteroid therapy[B]		3.13 (0.99 - 9.89)	=0.052
Telephone IDSC within 1 week [C]		3.48 (1.30 - 9.27)	<0.05
Whole-body computed tomography		---	---
Pneumonia		---	---

[A] According to McCabe and Jackson [444]. [B] Systemic prednisone >10 mg/day or equivalent for >1 month. [C] Infectious disease specialist consultation. [X] Patients from previous prospective study [15] excluded (n=155).

When analysing the earlier time period (2000 – 2002), the statistical power was insufficient for Cox regression due to the low number of telephone consultations relative to formal bedside consultations; however in univariate analysis the factors predicting three-month mortality were McCabe healthy or non-fatal classification (OR 0.09, 95% CI 0.03-0.25, p <0.0001), pneumonia (OR 2.62, 95% CI 1.08-6.39, p <0.05) and formal bedside IDSC (OR 0.35, 95% CI 0.12-1.07, p=0.057).

By performing separate analyses that take into account only the 2000-2002 or the 2006-2007 time periods or that exclude data from the earlier study [15], we receive results that strongly resemble those of Study III. Thus, the two different time periods or the usage of data from our earlier study [15] do not significantly alter the results.

5.4. Adjunctive rifampicin treatment in *Staphylococcus aureus* bacteraemia (Study IV)

5.4.1. Patient characteristics

Altogether 617 SAB patients were included, 291 (47%) of whom received rifampicin for at least 14 days, with a mean (± SD) duration of 45.1 ± 24.7. Rifampicin for 1-13 days were received by 72 patients (12%) and no rifampicin at all by 254 patients (41%).

The main analyses were then performed by excluding patients with a fatal outcome within three days (n=8) of *S. aureus*-positive blood culture, alcoholism and acute or chronic liver disease (n=128). When taking into account these exclusion criteria the patient cohort decreased to 475 patients of whom 240 (51%) received rifampicin for at least 14 days, 58 (12%) for 1-13 days and 177 (37%) received no rifampicin therapy. When the two groups (rifampicin ≥ 14 days and rifampicin < 14 days) were compared, no difference in age > 60 years, underlying diseases including McCabe´s rapidly fatal disease classification, ICU treatment or severe sepsis were seen. Male sex, however, associated to longer rifampicin therapy ($p<0.01$) (Study IV; Table I).

As a parallel analysis, the patient population was analysed by excluding patients with a fatal outcome within 14 days. When comparing patients with rifampicin ≥ 14 days and rifampicin < 14 days the patients with rifampicin ≥ 14 days were more likely to be of male gender ($p <0.05$) and to be healthier and have less underlying diseases (more McCabe's healthy or non-fatal classification) ($p <0.01$), whereas alcoholism ($p <0.01$) and dialysis treatment ($p <0.05$) were more common among patients with rifampicin < 14 days. No difference in ICU treatment or severe sepsis was seen between the groups (data not shown in Study IV).

5.4.2. Deep infection foci and *Staphylococcus aureus* bacteraemia relapse.

Deep infection focus was significantly more often found in patients with rifampicin ≥ 14 days than in patients with rifampicin < 14 days (88% vs. 62% $p <0.0001$), and they more often had various kinds of deep foci visualized e.g. pneumonia (40% vs. 28%, $p<0.01$) and endocarditis (18% vs. 9%, $p<0.01$). However, no difference in SAB relapse within 90 days follow-up was seen between the two groups (Study IV; Table 1).

5.4.3. Antibiotic therapy

No significant differences were observed in standard background antibiotic therapy for patients receiving rifampicin ≥ 14 days or rifampicin < 14 days. The vast majority (99%) were treated with a standard antibiotic, with *in vitro* efficacy against the cultured *S. aureus* strain.

Most patients received staphylococcal penicillin cloxacillin (52%) or cefuroxime (20%). Vancomycin was given to only 2% of patients (Study IV; Table 1).

5.4.4. Effect of rifampicin treatment on outcome

The total case fatality at 90 days was 17%. A fatal outcome was significantly lower in patients who received rifampicin ≥ 14 days than in patients with rifampicin < 14 days. The case fatality was at 28 days (6% vs. 15%, p <0.01) and at three months (11% vs. 22%, p <0.01).

The patient population (n=475) was analysed with proportional hazards regression (Cox regression model) to address factors predicting three-month mortality. Early rifampicin therapy onset for at least 14 days associated to improved prognosis (OR 0.38, p<0.01). Factors connected to poor prognosis were age > 60 years (OR 3.02, p<0.001), rapidly fatal underlying diseases (OR 6.84, p<0.001), corticosteroid therapy (OR 4.45, p<0.001), severe sepsis at time of sampling of positive blood culture (OR 2.11, p<0,01), pneumonia (OR 3.13, p<0.001) and endocarditis (OR 2.32, p<0.01) (Study IV; Table 2). When only patients with a deep infection focus (n=357) were included in the Cox regression model, the factors associated with prognostic impact were early rifampicin therapy onset for at least 14 days (OR 0.29, p<0.01), age > 60 years (OR 2.61, p<0.01), rapidly fatal underlying conditions (OR 4.19, p<0.01), corticosteroid therapy (OR 5.29, p<0.001), severe sepsis at positive blood culture (OR 2.43, p<0,01), pneumonia (OR 3.44, p<0.001) and endocarditis (OR 2.34, p<0.01) (Study IV; Table 3). Table 10 presents these results of Study IV.

Kaplan-Meier analysis of the effect of onset time point of rifampicin treatment for at least 14 days on outcome demonstrated a significant survival benefit with early onset therapy as compared to late onset among all SAB patients (Log Rank 0.001) and this difference was even more accentuated among patients with a deep infection focus (Log-Rank 0.0001) (Study IV; Figure 2a-b). Figure 4a-b below presents these results.

Lack of rifampicin therapy or rifampicin therapy for 1-7 days or 8-13 days had no positive prognostic impact in Cox regression analysis. These results were achieved for both the whole patient cohort and when analysing patients with a deep infection foci separately (data not shown).

As a parallel analysis, patients with a fatal outcome within 14 days of *S. aureus*-positive blood culture (n=49) were excluded, and the patient population (n=568) was analysed with proportional hazards regression (Cox regression model) to address factors predicting three-month mortality. In the Cox regression model, a significant positive prognostic impact

was seen for early onset of rifampicin therapy ≥ 14 days (OR 0.55, p <0.05) and for McCabe's healthy or non-fatal disease (OR 0.29, p <0.0001), whereas pneumonia (OR 3.99, p <0.0001), corticosteroid therapy (OR 2.91, p <0.01) and age > 60 years (OR 1.97, p <0.05) had a negative prognostic impact. When only patients with a deep infection focus (n=429) were included in the Cox regression model a positive prognostic impact was seen for early onset of rifampicin therapy ≥ 14 days (OR 0.38, p <0.01) and for McCabe's healthy or non-fatal disease (OR 0.43, p <0.01), whereas pneumonia (OR 3.88, p <0.0001), corticosteroid therapy (OR 3.55, p <0.0001) and age > 60 years (OR 1.94, p <0.05) once again predicted poor outcome (data not shown).

Table 10. Cox regression analysis for prognostic factors according to three-month mortality of 475 patients (all patients) and of 357 (patients with a deep infection focus) with *Staphylococcus aureus* bacteraemia. Patients with a fatal outcome within the first 3 days (n=8), alcoholism and acute or chronic liver disease (n=128) were excluded. Data are given as number (%) of patients in each parameter and odds ratio for fatal outcome within three months. NS, non significant.

			Univariate analysis		Cox regression	
	Died	**Survived**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
All patients						
Positive prognostic value						
Male sex	44(56)	237(60)	0.84(0.52-1.37)	NS	---	---
Rifampicin ≥14 days early onset [A]	22(28)	188(47)	0.43(0.25-0.73)	<0.01	0.38(0.23-0.64)	<0.01
Rifampicin ≥14 days late onset [A]	4(5)	26(7)	0.76(0.26-2.28)	NS	---	---
Negative prognostic value						
Age > 60 years	60(76)	187(47)	3.53(2.03-6.13)	<0.0001	3.02(1.79-5.11)	<0.001
Rapidly fatal disease [B]	13(16)	3(1)	25.8(7.16-93.0)	<0.0001	6.84(3.65-12.8)	<0.001
Cardiovascular disease	23(29)	76(19)	1.69(0.94-3.05)	NS	---	---
Corticosteroid therapy [C]	23(29)	20(5)	7.72(3.98-14.9)	<0.0001	4.45(2.65-7.48)	<0.001
Severe sepsis [D]	9(11)	16(4)	3.05(1.29-7.18)	<0.01	2.11(1.24-3.59)	<0.01
Intensive care unit [D]	20(25)	57(14)	2.02(1.13-3.59)	<0.05	---	---
Pneumonia [E]	48(61)	112(28)	3.93(2.38-6.49)	<0.0001	3.13(1.98-4.96)	<0.001
Endocarditis [E]	18(23)	44(11)	2.36(1.28-4.35)	<0.01	2.32(1.36-3.97)	<0.01
Patients with a deep focus						
Positive prognostic value						
Male sex	37(56)	181(62)	0.75(0.45-1.33)	NS	---	---
Rifampicin ≥14 days early onset [A]	18(27)	167(57)	0.28(0.15-0.50)	<0.01	0.29(0.17-0.52)	<0.01
Rifampicin ≥14 days late onset [A]	4(6)	22(8)	0.79(0.26-2.37)	NS	---	---
Negative prognostic value						
Age > 60 years	49(74)	142(49)	3.02(1.66-5.49)	<0.0001	2.61(1.49-4.59)	<0.01
Rapidly fatal disease [B]	8(12)	3(1)	13.2(3.41-51.4)	<0.0001	4.19(1.92-9.16)	<0.01
Cardiovascular disease	21(32)	60(21)	1.63(0.88-3.02)	NS	---	---
Corticosteroid therapy [C]	22(33)	16(5)	8.59(4.19-17.6)	<0.0001	5.29(3.05-9.19)	<0.001
Severe sepsis [D]	9(14)	14(5)	3.12(1.29-7.56)	<0.01	2.43(1.37-4.32)	<0.01
Intensive care unit [D]	18(27)	42(14)	2.22(1.18-4.19)	<0.05	---	---
Pneumonia [E]	48(73)	111(38)	4.32(2.39-7.81)	<0.0001	3.44(1.98-5.97)	<0.001
Endocarditis [E]	18(27)	44(15)	2.11(1.12-3.59)	<0.05	2.34(1.34-4.08)	<0.01

[A] Rifampicin for at least 14 days initiated early (within 7 days of *S. aureus*-positive blood culture) or late (7 days past *S. aureus*-positive blood culture). [B] According to McCabe and Jackson [444]. [C] Systemic prednisone >10 mg/day for > 1 month. [D] Intensive care unit treatment or severe sepsis at time-point of *S. aureus*-positive blood culture. [E] Pneumonia or endocarditis diagnosed within 90 days follow-up.

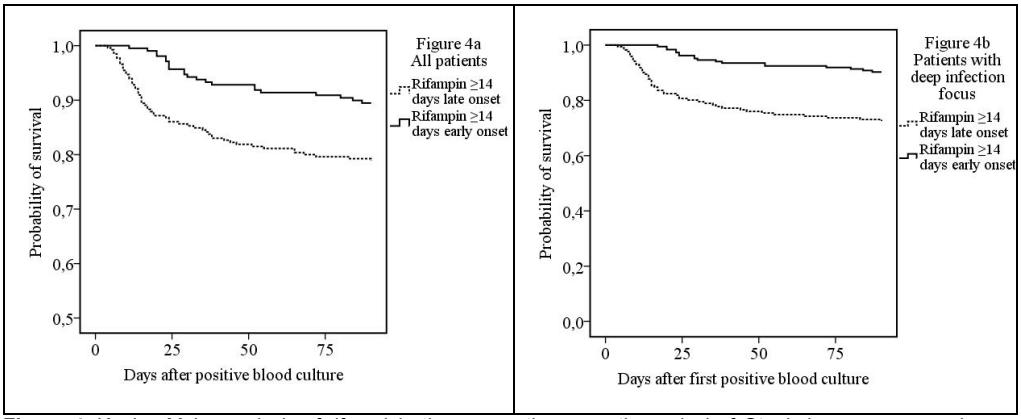

Figure 4. Kaplan-Meier analysis of rifampicin therapy on three-month survival of *Staphylococcus aureus* bactaeremia. Rifampicin therapy was continued ≥ 14 days and divided according to onset time point: early onset (i.e. onset within 7 days of positive blood cultures) and late onset (i.e. onset 7 days after positive blood cultures). **4a** Including all study patients (N=475). Log-Rank 0.001. **4b** Including patients with a deep infection focus (N=357). Log-Rank 0.0001.

6. DISCUSSION

6.1. Health care- and community-associated *Staphylococcus aureus* bacteraemia

Disease progression and prognosis of SAB are positively impacted by various factors. IDSC has been shown to improve identification of deep infection focus and endocarditis [3,56,57,184,257], resulting in fewer episodes of persistent SAB [18] and reduced probability for SAB relapse [184,353]. Some reports have viewed deep focus localization as a prerequisite for appropriate management of SAB and improved survival rate [3,16,18,167].

We observed that CA-SAB patients were significantly younger (52 vs. 62 years) and less often chronically ill with significantly more McCabe's healthy or non-fatal disease classifications (88% vs. 59%) than HA-SAB patients. Regarding underlying conditions, only alcoholism, IDU and chronic liver disease were significantly more common among CA-SAB. These observations are in agreement with previous reports [2,7,19,28]. Also consistent with an earlier study is the lack of gender difference [19].

In most patients, deep infection foci were evident already within 3 days of positive blood culture. Deep infection foci were diagnosed more frequently among CA-SAB than among HA-SAB both within three days (84% vs. 69%) and at three months (87% vs. 80%). In Study I, each SAB patient was treated and followed up by an IDS, which might explain the higher number of infection foci localized in our study than in previous studies, but not the early acquisition. The time-point for deep infection identification has not been reported in most studies, but one older study observed that metastatic foci were verified within the initial two weeks of SAB [185], whereas another more recent study concluded that 74% of patients had a complicated infection (deep infection foci, including septic thrombophlebitis) at the time of hospitalization [121]. The higher frequency of deep, metastatic or secondary infection foci among CA-SAB than among HA-SAB has been well-documented [2,19,28,29], with overall deep focus prevalence of 31-43% for CA-SAB and 5-12% for HA-SAB [2,7,28].

Regarding various deep infection foci, the trend with higher prevalence among CA-SAB than among HA-SAB, was consistent - except for foreign body infections. CA-SAB patients compared with HA-SAB patients were observed to have significantly more osteomyelitis at three days (36% vs. 24%) and at three months (41% vs. 28%), both exceeding figures in previous reports with overall osteomyelitis occurrence of 13-16% in CA-SAB and 2-4% in HA-SAB [7,19,28]. The frequency of septic arthritis in CA-SAB and HA-SAB patients at three days was 13% vs. 9% and at three months 17% vs. 11%, respectively, whereas previous reports have localized septic arthritis in only 5% of CA-SAB and 0% of HA-SAB patients [28]. Many authors report septic arthritis and osteomyelitis together with an overall

presence of 11-47% in CA-SAB and 0-17% in HA-SAB [2,85]. Occurrence of pneumonia among CA-SAB and HA-SAB at three days was 31% and 25% and at three months 38% and 39% in our patient population, which is more common than reports in earlier studies of 4-18% in CA-SAB and 1-16% in HA-SAB [7,12,19,28]. Endocarditis has been found in 7-29% in CA-SAB and 0-5% in HA-SAB, with both native and artificial valve endocarditis being more common in CA-SAB [2,3,7,19,28]. We diagnosed endocarditis within three days in 15% and 11% and at three months in 20% and 15% of CA-SAB and HA-SAB patients, respectively. In conclusion, Study I localized deep infection foci among 83% of patients altogether, which is high considering that some studies report deep or secondary foci in only 5-6% of HA-SAB and 29-31% of CA-SAB patients [7,19].

In Study I, the CA-SAB patients presented significantly higher mean CRP level on the day of positive blood culture than HA-SAB patients. A probable explanation for this could be longer bacteraemia duration, and thus, more time for metastatic spread (as seen in Table 5) and deep infection focus development in CA-SAB patients before arrival to hospital.

We observed that infection foci related to prior surgical interventions, or other invasive procedures were significantly less common among CA-SAB than among HA-SAB at both three days and three months (data not shown here). Infections related to a foreign body were more common in HA-SAB than in CA-SAB at three months for PVCs (peripheral venous catheters) (2% vs. 14%, $p < 0.0001$), for central venous catheters (0 vs. 19%, $p < 0.0001$) and for permanent foreign bodies (11% vs. 25%, $p < 0.0001$). Various penetrating foreign bodies are reported as common predisposing factors for HA-SAB, and catheter-related infection has been reported behind 21-64% of HA-SAB cases [2,3,28,29] and 1-22% of CA-SAB cases [2,12,19,28]. Surgical infections or infected wounds have been observed in 0-2% of CA-SAB and 6-16% of HA-SAB patients [7,19,28]. In our study, surgical procedures during three months preceding SAB were less common in CA-SAB (8%) than in HA-SAB (44%). A previous study reported 11% of SAB patients have undergone surgery in the previous month, although a clear division between CA-SAB and HA-SAB was not provided [136]. The prevalence of permanent foreign body infections was lower for CA-SAB (11%) than for HA-SAB (25%), but both exceeded an earlier report of 0% for CA-SAB and 11% for HA-SAB [2].

No difference was seen in the occurrence of cutaneous infection foci throughout the three-month study period (69% vs. 62%). Skin infections as primary foci have been reported in earlier studies among 13-40% of CA-SAB and 3-4% of HA-SAB. Hence, Study I presents a higher prevalence of skin foci in HA- and CA-SAB patients than in previous reports [7,19,28].

Prospective studies have reported higher numbers of serious SAB cases [183], and fewer patients (12-27%) have been classified as pirmary SAB or without infection focus [19,137,183]. In contrast, retrospective studies have reported 3-61% of SAB patients to present with primary SAB or without any infection focus [2,7,12,192]. Primary SAB is reported among 20-61% of CA-SAB and 3-53% of HA-SAB cases [2,7,19,28,29]. All patients in Study I were followed up by an IDS, which have been reported to result in more radiological examinations, more echocardiography and more bone scans [3,18,56,257]. The patient population in Study I was examined by numerous radiological investigations, e.g. echocardiography, whole-body computed tomography and leukocyte indium-111 scintigraphy. This is demonstrated by Study III, in which up to 45% of the patient population was the same as in Study I. Thus, the prospective study design and IDS follow-up resulted in more investigations performed and higher numbers of deep infection focus localization.

In Study I, no difference emerged between HA- and CA-SAB patients suffering from severe sepsis (13% for both groups), septic shock (4% vs. 7%) or need for intensive care unit surveillance (21% vs. 23%) within one week of *S. aureus*-positive blood culture. Likewise, no difference was seen in severity of illnesses on the day of positive blood culture (data not shown). However, the severity of illness reported here (severe sepsis and septic shock) was far lower than in previous studies of septic shock (11-24% of CA-SAB and 7-26% of HA-SAB) [2,19,28] or ICU stay (29% of CA-SAB and 18% of HA-SAB) [2].

Meticulous deep infection search has been shown to result in optimized treatment and improved survival due to deep infection foci often needing eradication and longer antibiotic treatment [19,22,167]. Our study revealed that most infection foci were present already early during the first week of SAB and that a thorough search for deep infection foci is warranted in most SAB patients.

The overall mortality of SAB in recent studies has varied between 14% and 32% for both in-hospital and SAB-related mortality [2,3,12,13,14,15,16,17,18]. The overall mortality in Study I, 13% at 28 days and 18% at three months, lies within the lower range of previous findings. The mortality at three months was high for HA-SAB patients, which is in accordance with several previous studies conducted in the 1970s - 1990s [51,427,446].

Due to the prospective nature of Study I, the possibility exists that critically ill patients may have been missed. Possible reasons for this may be difficult recruitment processes among very ill patients and severely ill patients with an expected rapid disease progression and fatal outcome may be rejected. Originally, 1226 SAB episodes were identified during the study period [15], and 430 patients were finally included.

Studies from the 1970s - 1990s have connected HA-SAB to higher mortality and explained this link by higher age and more comorbidities in HA-SAB patients than in CA-SAB patients [51,427,446], whereas more recent reports in the 2000s have failed to detect any significant mortality difference between HA- and CA-SAB [12,17,19,23,183,188,190,447]. However, one study connected HA-SAB to significantly lower mortality [2] and two studies to higher mortality relative to CA-SAB [442,448]. Higher mortality in CA-SAB has been explained by earlier detection of bloodstream infections among HA-SAB cases [2]

The higher 28-day mortality rate of HA-SAB than CA-SAB in our patient material was probably due to severe underlying conditions and higher age among HA-SAB cases. These factors have outweigted the negative prognostic impact of later hospital admission and higher deep focus occurrence among CA-SAB patients. Further support for this interpretation is the similarity in occurrence of unstable haemodynamic status and ICU surveillance between CA-SAB and HA-SAB; thus, severity of illness has not influenced the mortality trend. This observation of Study I is in contradiction with many previous studies associating CA-SAB with more severe illness at *S. aureus*-positive blood culture with higher occurrence of septic shock [2,28], ARDS [2,28], DIC [2,28], ICU need [2], mechanical ventilation [2] and renal failure [2,28].

6.2. Cell-free DNA as a biomarker in *Staphylococcus aureus* bacteraemia

Cell-free DNA (cf-DNA) is formed from DNA fragments released into the circulation from apoptotic cells [415]. Cf-DNA has been observed to serve as a biomarker for fatal outcome in septic and critically ill patients [402,409,410,411,412,413,414,422]. However, only two reports have evaluated the predictive value of cf-DNA in bacteraemic patients [412,414], and no studies with *S. aureus* as the only causative bacteraemia pathogen have been performed. High cf-DNA values in ICU-treated patients were reported in a previous study that included solely bacteraemic patients with various causative pathogens (*S. aureus*, *Streptococcus pneumoniae*, ß-haemolytic streptococcae or *Escherichia coli*). However, in this study, separate prognostic cf-DNA cut-off values were not evaluated for individual pathogens and no specific comparison of predictive values of cf-DNA between ICU and non-ICU patients on outcome was performed [412].

We investigated the prognostic value of cf-DNA among SAB patients treated in the general ward and in ICU (Study II). Plasma cf-DNA levels were found to be significantly higher at both day 3 and day 5 among ICU patients than among non-ICU patients, and the high cf-DNA levels predicted fatal outcome, especially in ICU patients, during the first week, 28 days or three months, i.e. irrespective of death time. When accounting for all prognostic markers, Pitt bacteraemia scores ≥4 points, the day 3 cf-DNA cut-off value and McCabe's

healthy or non-fatal classification were observed to have the strongest association with fatal outcome among ICU patients. However, day 5 plasma cf-DNA was not a significant prognostic marker and was more dependent on patients age and underlying diseases.

In Study II, in 99 ICU patients cf-DNA predicted fatal outcome with a sensitivity of 67% and a specificity of 77% and AUC of 0.71 in ROC analysis at day 3 from positive blood culture. These results are comparable with those seen in studies with other types of patient cohorts, but are clearly lower than the highest reported ones [402,409,411,412,413,422]. In previous studies, cf-DNA as a predictor of mortality has been determined within 0 - 72 hours of ICU admission and the sensitivity and specificity have ranged from 60% to 92% and 67% to 93%, with ROC analysis AUC values of 0.70 - 0.97 [409,411,414,422]. It seems that larger studies may reveal a lower specificity and sensitivity since cf-DNA determined at admission and at 48 hours in 580 mechanically ventilated critically ill patients had a predictive value for fatal outcome with sensitivity of 53% and specificity of 69% and a ROC analysis AUC of 0.62 [413]. Furthermore, in a recent study, cf-DNA predicted the presence of infection among febrile patients with AUC of 0.99 and 95% sensitivity and 96% specificity and among sepsis patients with AUC of 0.95 and 77% sensitivity and 94% specificity [402].

The lower sensitivity and specificity observed in Study II relative to earlier studies maybe explained by several factors. Most previous cf-DNA studies have determined cf-DNA at ICU admission or subsequent to ICU admission, whereas in Study II cf-DNA was measured in relation to positive blood culture [409,411,413,414,422]. As Study II correlated cf-DNA measurement with a specific time-point of disease progression, and not with clinical deterioration (i.e. ICU admission), the patients who deteriorated later certainly presented with lower cf-DNA levels. In Study II, only 4% of ICU patients presented with severe sepsis, whereas in previous studies much higher percentages of severe sepsis have been reported, with one study reporting 100% of patients suffering from severe sepsis with high APACHE points and high sensitivity and specificity for the prognostic value of cf-DNA [414]. Thus, it is evident that some of the most severely ill SAB patients have been missed, which is substantiated by a 28-day mortality of 12%, a much lower figure than in previous SAB studies and clearly lower than the mortality of 25-34% in ICU studies only [51,137,184, 409,422]. Furthermore, 24% of the SAB patients in Study II required ICU surveillance within one week and 8% of these ICU patients did not survive. Although these factors clearly reduced the prognostic value of cf-DNA in our study, the cut-off value of day 3 cf-DNA together with Pitt bacteraemia scores ≥4 points were the strongest factors predicting fatal outcome among ICU patients when all prognostic markers were accounted for. The results of Study II suggest that early apoptosis in SAB patients requiring ICU surveillance might

contribute to fatal outcome, as ICU non-survivors had significantly higher cf-DNA levels at day 3, although non-significant differences were observed at day 5.

More studies on the use of cf-DNA as a biomarker in serious infections and in septic patients are needed before it can be used in everyday clinical practice. Different cf-DNA measurement scales applied in various reports complicates the clinical usefulness of cf-DNA, with some authors using cf-DNA qPCR quantification with results presented as genome equivalents per millilitre (GE/mL) [402,411,413,422] and others measuring cf-DNA straight from plasma in micro- or nanograms per millilitre (µg/mL or ng/mL) [409,412].

6.3. Bedside and telephone infectious diseases specialist consultation in *Staphylococcus aureus* bacteraemia

Several studies with varying study settings and study populations have demonstrated a positive impact of IDSC on SAB management and prognosis [2,3,12,16,18,56,57,58, 184,257,353]. IDSC and especially informal IDSC have become more common as a result of the ever-deepening specialization in clinical medicine [340,345,346,347], and IDSs are among the specialities most frequently consulted [344]. The value of informal consultations in SAB was investigated in one study with a power of only six informal consultations out of all 233 studied IDSC. This study came to the conclusion that informal consultations were not associated with more SAB relapses or lower survival rate [353]. Furthermore, a prospective *post hoc* study of 627 patients with various infections observed no significant difference between formal and informal IDSC regarding compliance with recommendations for treatment, performing of diagnostic or monitoring tests, early clinical improvement, in-hospital mortality or length of hospital stay. However, only 3% of the patients had received ICU treatment and only 7% were defined as bacteraemic or septic. No causative pathogens were reported for the bacteraemic or septic patients [343].

In Study III, the impact of formal bedside IDSC, informal telephone IDSC and no IDSC on disease progression and prognosis of MSSA bacteremia was investigated. The main result was a significantly poorer prognosis of SAB patients treated with informal telephone IDSC as compared with formal bedside IDSC. Informal telephone IDSC was associated with an over twofold higher mortality than formal bedside IDSC when all prognostic factors were adjusted for. Altogether, informal telephone IDSC, as compared with formal bedside IDSC, was associated with less frequently performed radiological investigations, fewer deep infection focus localized, fewer patients with proper duration of antibiotic therapy, prolonged duration of fever and shorter hospitalization time. Moreover, the poorer outcome among in-

formal telephone IDSC patients was not explainable by differences in underlying conditions or severity of illness as compared with patients provided with formal bedside IDSC.

Delayed onset of appropriate antibiotic therapy impairs prognosis in both MSSA and MRSA bacteraemia [40,50,188,437]. MRSA bacteraemia has been connected to poorer prognosis and delayed onset of correct antibiotic therapy [49,51,52,58]. Vancomycin, the first-line drug for MRSA infections, has been associated with higher occurrence of persistent and recurrent SAB than the staphylococcal penicillin cloxacillin [53]. However, invasive and bacteraemic MRSA infections are rare in Finland, with a recent prevalence below 3% [54], and no MRSA cases were included in Study III. Moreover, effective empiric antibiotic therapy was provided to each patient on the day of first positive blood culture, and only 3% of SAB patients were treated with vancomycin. Hence, the study setting enabled analyses of impact of various IDSC types on disease progression and prognosis without any bias or disturbance from MRSA or differences in antibiotic selection prior to the IDSC.

The nature of Study II was retrospective, which has been linked to risk for various biases, such as failing to detect serious *S. aureus* cases, as compared with prospective studies [183]. In Study III, no significant difference was observed in underlying diseases (McCabe's classification) or severe sepsis at *S. aureus*-positive blood culture in patients receiving formal bedside IDCS and informal telephone IDSC. Thus, underlying diseases and severity of illness at *S. aureus*-positive blood culture have most probably not influenced the results between formal bedside and informal telephone IDSC.

Factors associated with poor prognosis that were identified in Study III have been reported previously: need for ICU surveillance [3,18], corticosteroid therapy (>10 mg/day for > 1 month) [58] and pneumonia [7,12,51]. Informal telephone IDSC was linked more often to ICU treatment within the first three days, but not within the first week, than formal bedside IDSC. This might have contributed to a higher mortality rate in the informal telephone IDSC group. ICU treatment within the first three days was significantly more common among patients presenting with other factors associated with poor prognosis, such as severe sepsis at *S. aureus*-positive blood culture (OR 10.2, $p < 0.001$) and acute congestive heart failure (OR 5.94, $p < 0.001$), than among patients managed outside the ICU. When the patients with ICU treatment were analysed separately, informal telephone IDSC remained one of the strongest prognostic factors for poor outcome (OR 4.87, $p = 0.001$).

IDSC has been observed to result in more diagnosed endocarditis and deep infection focus [3,56,57,184,257] and improved selection and duration of antibiotic therapy. Furthermore, IDSC has resulted in more appropriate timing of MRSA therapy and in use of β-lactam antibiotics whenever possible [3,18,56,57,58,257,353], longer mean duration of therapy [16,

257] and longer hospital treatment than non-IDSC cases [57]. Interestingly, these differences were observed also between formal bedside and informal telephone IDSC in our study. Formal bedside IDSC resulted more often in proper duration of antibiotic therapy and longer hospitalization duration than informal telephone IDSC. However, in multinomial logistic regression analysis, when the various prognostic factors were adjusted for, no differences were seen between formal bedside and informal telephone IDSC with regard to proper duration of antibiotic treatment or duration of hospitalization. The presence of deep infection foci was linked to proper length of antibiotic therapy. Less radiological examinations were made based on informal telephone IDSC, which resulted in fewer deep infection foci localized. Therefore, one key factor behind better outcome among formal bedside IDSC than among informal telephone IDSC seemed to be the more thorough search for deep infection foci.

Previous reports have concluded that IDSC results in more radiological investigations, including both echocardiography and bone scans [3,18,56,257]. Study III presented, surprisingly, leukocyte indium-111 scintigraphy as an independent positive prognostic factor, which has not been reported previously. Leukocyte indium-111 scintigraphy is uncommon in the clinical management of bacteraemic infections, but it was provided significantly more often to formal bedside IDSC patients than to the other two SAB patient groups. Despite the independent nature in statistical analysis, the independent prognostic nature of leukocyte indium-111 scintigraphy must be considered carefully and further validation and investigation of its prognostic nature are needed.

The number of potentially missed informal telephone IDSC cases was low as only 10% of all SAB patients had no mention of any IDSC in their patient records and all *S. aureus* blood culture isolates could be linked to patient identification, verifying that no SAB patients were missed. Furthermore, patient records were unavailable for only seven patients. During office hours the same IDS consultant performed informal telephone and formal bedside IDSC. However, outside office hours and on weekends, the IDS or resident on call received the informal telephone consultation calls. The specific time-point of the consultations could not be retrieved from the patient records and might potentially explain the high number of informal telephone IDSCs among ICU patients, and to some extent also provide a reason for the improved outcome of formal bedside IDSC relative to informal telephone IDSC. The risk of insufficient information being provided or important information being missed in informal consultations [344] or inaccurate or incomplete information being presented in informal consultations resulting in inappropriate management advice [350] has been reported previously. Hence, it is advisable that informal telephone IDSC in SAB should be complemented by formal bedside IDSC as soon as possible.

Study III demonstrated that formal bedside IDSC, compared with informal telephone IDSC, associated with improved outcome. This trend was observed also in Study IV, as telephone IDSC patients had higher risk for fatal outcome in univariate analysis, but not in Cox regression analysis. However, Study IV analysed a much larger patient number than Study III (617 vs. 342), and patients with a fatal outcome within 14 days were excluded to allow for a fatal outcome before completing at least 14 days of rifampicin therapy. When Cox regression analysis was performed excluding only patients with a fatal outcome within 3 days as in Study III, the negative prognostic value of telephone consultation became significant (OR 1.72, $p<0.05$) (data not shown).

Hospital-related temporary differences in treatment or personnel practices are factors difficult to control for in retrospective studies, and thus, to exclude the effect of unidentified differences, two different time periods for data collection were included in Study III. Moreover, two study periods were regarded as mandatory, as most patients from the first study period had participated in our previous prospective study [15]. When the two study periods were analysed separately, no significant difference in the results was observed. The electronic patient records available in the later study period enabled retrieval of patient records and laboratory results during the ongoing informal telephone IDSC. However, the degree to which electronic patient records were retrieved during consultations could not be assessed and further investigations on possible benefits of electronic patient records during the consultation process are necessary.

The results of Study III differ from those of an earlier study [353] and are completely opposite to those demonstrated by another study [343], in which no significant difference was observed between formal and informal IDSC. However, only 7% of the patients were bacteraemic or septic and only 3% required ICU in the latter report. Hence, it is reasonable to assume that a positive prognostic impact of formal IDSC, as compared with informal IDSC, is not observed until the patient population reaches a considerable size and a certain degree of severity of illness.

6.4. Rifampicin in *Staphylococcus aureus* bacteraemia with deep infection foci

Few clinical studies have evaluated the impact of rifampicin combination therapy on disease progression and prognosis in SAB and *S. aureus*-related deep infection foci. In general, studies with low MRSA bacteraemia occurrence have reported some degree of improved clinical outcome due to rifampicin combination therapy [15,66,75,76,77,78, 79,80,176]. Studies with high MRSA occurrence, however, have reported such adverse ef-

fects as prolonged bacteraemia, rifampicin resistance development and negative prognostic impact to be associated with rifampicin combination therapy [82,83,84,329]. Rifampicin resistance development in *S. aureus* infections has been well-characterized [67,68] and has especially been described in studies with high (76-100%) MRSA prevalence, where rifampicin resistance has developed in 5-56% of cases [82,83,84,329,465]. Most authors conclude that initiation of rifampicin therapy during the MRSA bacteraemic phase [82,329] and especially with suboptimal vancomycin effect creates a setting resembling rifampicin monotherapy against MRSA [83,465].

The Infectious Diseases Society of America guidelines regarding management of MRSA infections recommend rifampicin and vancomycin combination therapy with level A-II evidence for device-related osteoarticular infections, level B-III evidence for prosthetic valve infective endocarditis, osteomyelitis, meningitis and abscesses and level C-III evidence for recurrent skin and soft tissue infections [36]. Recently, rifampicin-vancomycin combination therapy was associated with higher cure rates than vancomycin alone for HA MRSA pneumonia [330].

However, no studies have evaluated the optimal onset time-point for rifampicin therapy in MRSA bacteraemia. Some studies recommend rifampicin initiation after clearance of bacteraemia based on observations that rifampicin onset during ongoing MRSA bacteraemia results in significantly prolonged bacteraemia and significantly poorer outcome [82]. No reports are available regarding the optimal rifampicin therapy onset time-point for MSSA bacteremia.

Study IV included only MSSA bacteraemia cases, and 99% of patients had appropriate empirical antibiotic therapy initiated at blood culture collection. Vancomycin therapy was received by only 2% of patients. MRSA has been associated with delayed effective antimicrobial therapy, which in turn has been identified as a major risk factor for poor prognosis [49,50,51,52]. In Study IV, the impact of rifampicin adjunctive therapy on disease progression and prognosis could be evaluated without disturbance from delayed empirical antibiotic therapy.

Study IV investigated the optimal time-point for onset of adjunctive rifampicin therapy and the minimal rifampicin therapy duration needed for a positive impact on prognosis in 475 patients. Originally, the patient cohort included 617 patients, however, after taking into account exclusion criteria, 475 patients were included in the study. The primary finding of this study was the positive prognostic impact of rifampicin adjunctive therapy on SAB patients with a deep infection focus. The positive impact was seen when rifampicin was initiated within one week of *S. aureus*-positive blood culture and continued for at least 14 days. As a

result of early adjunctive rifampicin therapy for at least 14 days, the risk of fatal outcome decreased (OR 0.38) in the whole patient population and this positive prognostic impact was further accentuated in patients with a deep infection focus (OR 0.29). Study IV is the first to demonstrate a positive prognostic impact of early initiation of rifampicin adjunctive therapy on MSSA bacteraemia.

Adjunctive rifampicin therapy has had a positive impact on disease progression and prognosis in SAB patients with a deep infection focus and a low (0-13%) MRSA prevalence [15,66,75,76,77,78,79,80,176,308]. In two prospective studies, a mainly orally given combination of a fluoroquinolone, ciprofloxacin, together with rifampicin has been compared with intravenous conventional antistaphylococcal therapy (oxacillin, flucloxacillin or vancomycin) [79,80]. In these studies, a tendency for less clinical failures in endocarditis (5% vs. 12%, non-significant) [80] and a higher cure rate in foreign body infection (100% vs. 58%, $p<0.05$) [79] were observed with the combination therapy. However, the cure rate was not significantly different in a prospective randomized trial with a fleroxacin-rifampicin combination compared with conventional intravenous monotherapy in patients with mostly MSSA bacteraemia and a high number of deep infection foci [308]. Recently, a retrospective report that included patients with total hip or knee prosthetic infections and 17% MRSA associated rifampicin-fluoroquinolone therapy with improved outcome relative to other antimicrobial regimens, with no outcome difference between MSSA and MRSA infections [176]. No prospective studies with rifampicin as an adjunctive therapy to standard β-lactam-based intravenous therapy in MSSA bacteraemia have been done. We observed in a *post hoc* analysis of our previous prospective study in MSSA bacteraemia that patients with a deep infection focus who also received rifampicin had lower three-month mortality than patients treated without rifampicin. However, no randomization for rifampicin was performed and the rifampicin therapy onset time-point was not analysed [15].

Most commonly, the combination of vancomycin and rifampicin has been investigated. Many studies involving a high MRSA prevalence (76-100%) have reported poorer clinical outcome due to rifampicin combination therapy in endocarditis, various deep infection foci or persistent bacteraemia [81,82,83,84,329]. A retrospective randomized cohort study of 42 native valve endocarditis patients compared rifampicin-vancomycin with vancomycin only; the combination treatment group demonstrated prolonged bacteraemia [81]. Resistance development to rifampicin is a common finding, occurring in 56% of cases in a retrospective study in 2008 that investigated the benefit of rifampicin adjunctive therapy in native valve infective endocarditis with high (76%) MRSA prevalence and showed prolonged bacteraemia with rifampicin treatment [82]. Another retrospective report followed rifampicin-vancomycin therapy in MRSA and hVISA bacteraemia patients and demonstrated prolonged bacteraemia and higher rifampicin resistance development (5% vs. 44%) for hVISA

cases [83]. A recent study investigated prolonged MRSA bacteraemia in elderly (> 65 years) patients and demonstrated rifampicin resistance in 36% of cases due to glycopeptide-rifampicin combination therapy and patients with rifampicin resistance presented higher, although not significant, MRSA-related and 30-day mortality [329]. Thus, it is reasonable to assume that resistance development to rifampicin may de due to prolonged bacteraemia during vancomycin therapy rather than to the methicillin resistance of the staphylococcal strain [53]. No prospective studies evaluating the optimal onset of rifampicin therapy in SAB are available in the literature. Some reports recommend onset of rifampicin adjunctive therapy after clearance of bacteraemia [36,82]. These recommendations, however, are based on reports including high MRSA occurrence. No guidelines exist regarding adjunctive rifampicin therapy in MSSA bacteraemia.

In Study IV, the median time from blood culture to clinical report of *S. aureus* as the causative bacteraemic pathogen was three days. Hence, rifampicin adjunctive therapy was initiated at the earliest at three days subsequent to onset of appropriate empirical antibiotic therapy, i.e. mostly a β-lactam antibiotic. As Study IV did not observe a single case of rifampicin resistance during follow-up, it is reasonable to assume that the three days of appropriate β-lactam antibiotic therapy prior to rifampicin onset prevented resistance development among the MSSA bacteraemia patients. Moreover, only 1% of patients experienced a SAB relapse during the three-month follow-up, which is far lower than reported in previous studies of 2-16% of SAB patients suffering from SAB recurrence, reinfection or relapse [3,19,22,41,121,123]. The low SAB relapse percentage supports the observation of successful antimicrobial therapy for each SAB patient and the lack of any rifampicin resistance development. However, repeated blood culture during antimicrobial therapy is not a routine procedure in Finland, and thus, some cases of prolonged bacteraemia or even rifampicin resistance might have been missed.

Study IV clearly indicates that rifampicin adjunctive therapy should be initiated within the first week of positive blood culture. A subanalysis (Study IV; Table 3) included 26 SAB patients with various deep infection foci with adjunctive rifampicin therapy initiated 7 days after *S. aureus*-positive blood culture and with rifampicin therapy duration for at least 14 days. Interestingly, no positive impact on prognosis was observed among these patients as a result of rifampicin adjunctive therapy. Rifampicin adjunctive therapy is recommended to be continued for several weeks [36]. Study IV did not investigate the optimal duration of rifampicin treatment, however, patients with < 14 days of rifampicin therapy received rifampicin with a mean treatment period of 7.68 ± 3.8 days (mean ±SD). This short rifampicin treatment was not associated with improved prognosis.

Patients with a history of alcoholism or patients suffering from acute or chronic liver diseases were excluded in Study IV. These exclusion criteria reduced the patient number by 128 cases. Alcoholism and acute or chronic liver failure were viewed as contraindications for rifampicin therapy as the risk for liver failure, as a complication of rifampicin therapy, is accentuated in patients with these conditions. Patients with alcoholism and liver diseases unavoidably contributes to a statistical bias due to the fact that patients with these conditions are unlike to be treated with rifampicin.

When comparing age, gender, underlying conditions, severity of illness at blood culture time point and antibiotic therapy, no difference was seen between patients with rifampicin ≥ 14 days and patients with rifampicin < 14 days with the exception of male sex associating to longer rifampicin therapy. Similar patterns were seen when comparing the patient groups receiving shorter duration of rifampicin therapy. Thus, rifampicin therapy duration could be compared without disturbance from confounding differences in age, underlying diseases, severity of illness or standard antibiotic therapy. These are parameters difficult to control for in statistical analyses.

It is well documented that rifampicin is a potent CYP3A4 liver enzyme inducer (including other hepatic CYP- liver enzymes such as CYP2C8, CYP2C9 and 2C19 as well). Thus, the metabolism of drugs administered simultaneously may be enhanced. Furthermore, rifampicin carries the risk of hepatitis and this risk is increased by alcoholism [61,62]. Underlying conditions (as well as rapidly fatal McCabe´s classification), did not differ between patients receiving shorter and longer rifampicin therapy, and thus, it may be assumed that background medication of the different patient groups were approximately the same. Hence, the risk and possibility for pharmacological interactions with rifampicin as a potential contraindication for rifampicin therapy was not accentuated in any patient group.

Several factors associated with poor prognosis in Study IV have been reported earlier, e.g. McCabe's healthy or non-fatal diseases [3,7], high age [7,12,19] and pneumonia [7,12]. Rifampicin adjunctive therapy for at least 14 days with early onset within one week of bacteraemia remained a favourable prognostic factor with lower likelihood for fatal outcome (OR 0.38, 95% CI 0.23-0.64) ($p<0.01$) when controlling for all of these factors. Furthermore, when only patients with a diagnosed deep infection focus were included, the positive prognostic value was intensified (OR 0.29, 95% CI 0.17-0.52) ($p<0.01$).

The overall mortality in Study IV was 13% at 30 days and 17% at three months, which is at the lower end compared to the mortality of 14-32% reported in several recent studies from the 2000s and 2010s [2,3,12,13,14,16,17,18]. The power to detect a positive prognostic effect of rifampicin has most probably been reduced by the low overall mortality rate.

6.5. Limitations due to designs in Studies III and IV

Several notable limitations in the designs of Study III and IV warrant discussion.

Study III

Study III lacked documentation of the precise timing and content of the telephone IDSC provided. The study design could not determine the extent to which the informal IDSC was telephone-based or an informal sidewalk discussion between the treating physician and the IDS. However, outside office hours and during the weekend, the on-call IDS is physically located at the Division for Infectious Diseases at Aurora Hospital, which is located separately from Helsinki University Central Hospital in Meilahti - this setting enables only telephone consultations outside office hours, and thus, it is reasonable to assume that the vast majority of informal IDSCs were in fact telephone-based. We could not document I) whether the advice given by the IDS via the telephone conversation was correct, II) whether the treating physician requesting advice understood the advice received and III) whether the advice received was properly documented by the treating physician. Hence, misconceptions taking place during the telephone conversation were not documented. Moreover, Study III did not document the type of physician requesting an informal IDSC, and thus, we do not know the proportions of internists, neurologists, surgeons and intensivists requesting informal IDSC. This information would have been valuable as it might have enlightened the fact that ICU patients, during the initial 3 days, received more telephone IDSCs.

In Study III, rigorous adjustments were made for confounding factors, however, the adjustments may have been insufficient. High age [12,22,23,156,168,431,432,433,434, 435], ultimately or rapidly fatal underlying diseases [3,22,188] and severe sepsis at *S. aureus*-positive blood culture [25,183,445] were associated independently with higher mortality. When comparing background patient characteristics and severity of illness at *S. aureus*-positive blood culture in patients who received bedside and telephone IDSC, the differences regarding age, McCabe's classification and severe sepsis were statistically non-significant. However, the telephone IDSC patients were somewhat older (54.8 ± 16.5 vs. 53.2 ± 17.7 years, mean ±SD), had somewhat more ultimately or rapidly fatal underlying diseases (42% vs. 29%) and slightly more severe sepsis at *S. aureus*-positive blood culture (10% vs. 7%).

Moreover, telephone IDSC patients, as compared with bedside IDSC patients, received significantly more ICU treatment (34% vs. 21%) during the initial 3 days. Previous reports have independently associated need for ICU treatment [3,25,188,436] and ICU admission [3,443] with weaker outcome relative to non-ICU patients. However, when analysing

separately patients treated in the ICU by survival rate, telephone IDSC was one of the most important prognostic markers for fatal outcome, with an OR of 4.87 (p=001).

Study III was retrospective. Retrospective design, compared with prospective design, may fail to detect serious *S. aureus* cases [183]. A prospective study could have been designed to document the time, content and type of treating physician requesting a consultation, and thus, many of the limitations discussed above would have been avoided.

The limitations of Study III, especially the lack of documentation of exact timing, content and possible misconceptions about informal IDSC, make it difficult to understand the precise reason for the poorer outcome of informal telephone IDSC patients. However, these limitations do not invalidate the major finding of Study III, the significantly better prognosis of bedside IDSC, relative to telephone IDSC, in the management of SAB. This conclusion was reached also by Chu and Sexton in the editorial commentary connected to Study III [466].

Study IV

The major limitation of Study IV was its retrospective design. Randomized controlled trials are viewed as the gold standard for clinical trials and medical interventions. Hence, the patient cohort in Study IV could not be randomized and controlled with respect to rifampicin.

Study IV applied dichotomization of rifampicin therapy duration, according to rifampicin therapy ≥ 14 days or < 14 days, which may appear artificial and arbitrary. However, approximately on third of the patients received no rifampicin therapy and more than half of patients received rifampicin ≥ 14 days. Few received rifampicin for only a few days. Categorization of rifampicin therapy, according to duration ≥ 14 days or < 14 days, in the present study was performed enable statistical analyses. A valid continuous statistical analysis estimating specific cut-off values (in days) after which rifampicin therapy would impact the prognosis positively was not possible with the present patient cohort. The present study, however, shows a positive prognostic impact in Cox regression for patients with early onset of rifampicin therapy for at least 14 days. This observation was not seen among patients receiving shorter durations of rifampicin therapy.

Study IV excluded cases with a fatal outcome within three days. This was made to allow for death prior to positive blood culture results and the possibility for rifampicin therapy. One may argue that patients with early death (e.g. within 14 days) were sicker and thus more likely to be treated without rifampicin. Thus, a parallel analysis was performed by excluding patients with a fatal outcome within 14 days - with results very similar to those reported

above. When excluding patients with a fatal outcome within 14 days, early onset of rifampicin therapy for at least 14 days was associated to a positive prognostic impact in Cox regression when taking into account only patients with a deep infection foci (OR 0.54, $p < 0.01$).

7. CONCLUSIONS

Results of Studies I - IV summarized as follows:

Study I

The community-associated (CA) SAB patients, compared with the health care-associated (HA) SAB patients, were younger and more often classified as healthy or non-fatal due to underlying conditions (88% vs. 59%, $p < 0.0001$). Regarding underlying conditions, only alcoholism, IDU and chronic liver disease were significantly more common among CA-SAB than among HA-SAB. Deep infection foci were more common than reported in previous studies, and they were diagnosed more frequently in CA-SAB than in HA-SAB both within three days (84% vs. 69%, $p < 0.0001$) and at three months (87% vs. 80%, $p < 0.05$). At 28 days, no difference was present in mortality between CA- and HA-SAB (11% vs. 14%), but at three months the mortality among HA-SAB patients was significant higher (13% vs. 22%, $p < 0.05$). Factors independently predicting outcome were higher age, alcoholism, immunosuppressive treatment, ultimate or rapidly fatal underlying diseases, severe sepsis at *S. aureus*-positive blood culture, *S. aureus* pneumonia and endocarditis. The prospective study design and infectious disease specialist (IDS) surveillance of each patient and the intensive deep infection focus search resulted in a high percentage of SAB patients having deep infection focus diagnosed already within three days of *S. aureus*-positive blood culture and overall low mortality rates.

Study II

SAB patients with high Pitt bacteraemia scores and need for ICU surveillance had significantly higher plasma cf-DNA levels at both day 3 and day 5 after positive blood culture as compared with non-ICU SAB patients. Moreover, significantly higher cf-DNA values among ICU non-survivors than among ICU survivors were observed at days 3 and 5. When all prognostic factors were controlled for, Pitt bacteraemia scores ≥ 4 and day 3 cf-DNA were the strongest mortality predictors among ICU patients, whereas day 5 cf-DNA was not a significant prognostic marker and it depended more on patients age and underlying diseases.

Study III

Formal bedside IDSC significantly improved patient outcome in SAB as compared with informal (telephone) IDSC. Patients treated according to formal bedside IDSC more often had proper length of antibiotic treatment, more often radiological investigations performed, more frequently localized deep infection focus (78% vs. 53%) and shorter duration of fever. Formal bedside IDSC was associated with significantly lower mortality at seven days (1% vs. 8%), 28 days (5% vs. 16%) and three months (9% vs. 29%). When all prognostic mark-

ers were noted, patients receiving informal telephone IDSC had over twofold higher odd ratio of fatal outcome relative to formal bedside IDSC. Our results indicate that informal telephone IDSC was inferior to formal bedside IDSC and that it might be reasonable to complement and complete informal telephone IDSC either with thorough patient record retrieval or formal bedside consultation.

Study IV

Adjunctive rifampicin therapy initiated within seven days of *S. aureus*-positive blood culture and continued for at least 14 days had a significant positive prognostic impact on SAB patients and this positive impact was further accentuated when taking into account only patients with a deep infection focus. As a result of early rifampicin adjunctive therapy for at least 14 days, the risk of a fatal outcome was more than halved in the whole patient population. When continued for less than 14 days or, initiated seven days past *S. aureus*-positive blood culture, adjunctive rifampicin therapy demonstrated no positive impact on prognosis.

The results of Study IV encourage a recommendation of rifampicin adjunctive therapy already during the first week in bacteraemia due to MSSA and with a suspicion of a deep infection focus. However, these recommendations apply solely to MSSA bacteraemia and are not applicable to MRSA bacteraemia and MRSA infections.

8. ACKNOWLEDGEMENTS

These studies were carried out at the Department of Medicine, Division of Infectious Diseases, Helsinki University Central Hospital, during 2009 - 2014.

My sincere gratitude is owed to my supervisor Docent Asko Järvinen, Head of Division of the Infectious Diseases, for encouraging supervision, great enthusiasm and constructive criticism throughout this work. His professionalism and vast experience in the field of infectious diseases, both clinically, and academically, have made a lasting impression on me. I look forward to future collaboration with him.

I am very grateful to my other supervisor, Dr Eeva Ruotsalainen, for her friendly support and scientific advice. This work is partly a continuation of the prospective studies performed by Dr Eeva Ruotsalainen, Docent Asko Järvinen and Professor Ville Valtonen in 1999-2006. Hence, I am deeply thankful for having been provided with the opportunity and the privilege of continuing the work with the largest prospective *S. aureus* bacteraemia patient cohort database in Finland - originally gathered by Dr Eeva Ruotsalainen.

This work would not have been possible without the advice and efforts of the other members of our study group. My warmest thanks go to each co-author and collaborator: Professor Outi Lyytikäinen, MD Tomi Mölkänen, Statistician Jukka Ollgren, Professor Mikko Hurme, Docent Janne Aittoniemi, Docent Reetta Huttunen, Dr Juulia Jylhävä and medical student Visa Helmijoki.

Professor Outi Lyytikäinen, at the National Institute for Health and Welfare, offered valuable scientific advice regarding the epidemiological aspects of *S. aureus* bacteraemia. MD Tomi Mölkänen, at the Division of Infectious Diseases, generously assisted in the statistical analyses at the initial phase of this work. Statistician Jukka Ollgren, at the National Institute for Health and Welfare, provided invaluable statistical advice on several occasions.

The second article, the cell-free DNA biomarker work, was done in co-operation with the following institutions connected to the University of Tampere: the Department of Microbiology and Immunology, the Department of Clinical Microbiology, the Department of Internal Medicine, the Fimlab Laboratories and the School of Medicine at Tampere University. I am grateful to Professor Mikko Hurme and Docent Janne Aittoniemi for providing scientific advice, financial support and laboratory facilities that enabled the analyses of the cell-free DNA biomarker. My warmest thanks go to medical student Visa Helmijoki for carrying out the laboratory procedures and to Dr Juulia Jylhävä and Docent Reetta Huttunen for insightful advice and constructive criticism.

I warmly thank Docent Anu Kantele for providing important guidance in practical matters connected to this thesis and Docent Pentti Kuusela for his contribution regarding the methodology of bacterial blood cultures.

I am deeply grateful to my reviewers, Docent Timo Hautala and Docent Pertti Arvola, for their thorough review and insightful comments that markedly improved the manuscript.

My author-editor Carol Ann Pelli is thanked for editing the language of the manuscript.

My colleagues at the Division of Infectious Diseases at Aurora Hospital I thank for their friendly, warm and supportive attitude throughout the study period. Special thanks go to secretary Minna Ollikainen for invaluable help with practical matters.

Finally, heartfelt thanks go to my dear family, my parents, my sister and, most of all, my wife Jutta and our son lilla Wilton.

This study was financially supported by grants from the following foundations: Finska Läkaresällskapet (The Medical Society of Finland), Biomedicum Helsinki Säätiö (Biomedicum Helsinki Foundation), Suomen Infektiolääkärit ry, Stiftelsen Perkléns Minne, Suomen Lääketieteen Säätiö (The Finnish Medical Foundation), Maud Kuistilan Muistosäätiö (The Maud Kuistila Memorial Foundation), Nylands Nation, Wilhelm och Else Stockmanns Stiftelse, Svenska Kulturfonden (The Swedish Cultural Foundation in Finland), Infektiotautien tutkimusyhdistus (The Finnish Society for Study of Infectious Diseases), Medicinska Understödsföreningen Liv och Hälsa, Oskar Öflunds Stiftelse, Paulon Säätiö (The Paulo Foundation) and The SSAC Foundation (Scandinavian Society for Antimicrobial Chemotherapy).

The study also received financial support from the Valtakunnallinen Kliininen Tutkijakoulu (The National Graduate School of Clinical Investigation) and the Faculty of Medicine at University of Helsinki.

Helsinki,
Erik Sebastian Forsblom
August 2014

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