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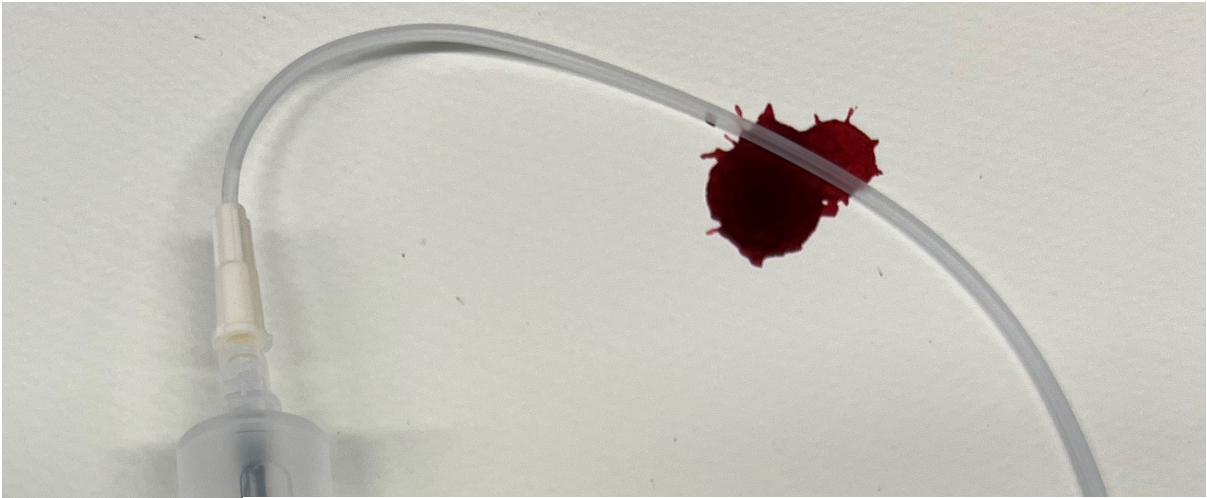
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Refining the use of blood cultures

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Refining the use of blood cultures

Refining the use of blood cultures

Karl Oldberg



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DOCTORAL DISSERTATION

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Abstract:

This thesis is built upon four research projects, in which we investigated different aspects of increasing the diagnostic yield of blood cultures. Blood culture sampling using a single venipuncture (single-sampling strategy, SSS) is non-inferior to sampling from two separate venipunctures when sensitivity is considered. The effect of SSS on blood culture contamination rate is less known. In Study I, we retrospectively evaluated the effect of introducing SSS and initial sample diversion in our region. The proportion of sampling events in which the recommended 4 bottles were collected, increased after the intervention, and sensitivity remained unchanged. There was a decrease in the proportion of sampling events in which coagulase-negative staphylococci grew in 1-2 out of 4 bottles, but an increase in the proportion of sampling events in which they grew in >2 bottles. This may indicate an increase in contaminations of multiple bottles, which can be hard for clinicians to identify.

Study II and III investigated whether persistent bacteraemia or blood culture time to positivity (TTP) were useful in the diagnosis of infective endocarditis caused by *Enterococcus faecalis*. Study II prospectively included patients with *E. faecalis* bacteraemia for repeated blood culture collection. Persistent growth in repeated cultures was associated with a high probability of infective endocarditis or other infectious foci that needed special therapeutic consideration. However, most patients with such complicated infections did not have persistent bacteraemia, which limits the use of repeated blood cultures. In study III, a retrospective observational study, we found that the median TTP among patients with infective endocarditis was shorter than in patients with other foci of infection. There was no difference in TTP between patients who died within 30 days and those who survived.

A major problem with using TTP is that it is shortened when the time elapsed from sampling to incubation start (TTI) increases. This makes comparisons between studies difficult and obscures the association between TTP and outcomes of interest. In Study IV, we used a large retrospective dataset to develop a method for correction of TTP for TTI, based on the linear correlation between these variables. Corrected TTP was more strongly associated with 7-day mortality than crude TTP.

Key words: Blood culture, bacteraemia, infective endocarditis, time to positivity, persistent bacteraemia

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Refining the use of blood cultures

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Preface

“The possibility has occurred to me that your symptoms were not necessarily to be referred to tuberculosis alone ... There must be some other source of trouble. In my view, you’ve got cocci” (1)

More than halfway into Thomas Mann’s novel *The Magic Mountain*, published in 1924, young Hans Castorp has already spent several years during the early 1900s in a sanatorium in Davos. His fevers won’t stop, and the doctor, Hofrat Behrens, has started to consider a modern disease concept at the time - streptococcal infective endocarditis. He knows just how to proceed with the diagnostic workup:

“If this little one has really settled down and made itself a home in you, the bacteriological blood-test will show it.”

Several days after an almost sacral procedure of drawing Hans’ blood into a glass container, and initial growth in only one culture, which Behrens thinks is of doubtful relevance, one day comes when Behrens informs Hans that

“... not only on the first culture, but on all the others as well, cocci had subsequently grown, in large quantities. It was not yet certain that they were all streptococci, but it was more than probable that they were the cause of the existing infection ...”

This short passage in one of my favourite novels, is the only description of blood cultures that I have ever encountered in fiction, and it remarkably touches upon several key questions of this thesis: What is the optimal sampling method for blood cultures? When should contamination be suspected? How should the number of positive blood cultures and the quantity of bacteria in blood be interpreted?

Blood cultures have been a large part of the everyday work of thousands or millions of professionals the past century, and though most aspects of blood culture have been improved, some questions remain. Blood cultures still hold a prominent position in the diagnostic toolbox for infectious diseases, but their hegemony might be seriously challenged within a decade or two. With that perspective, my research is not a revolutionising contribution, but rather a small refinement in the use of blood cultures. One could say that I, in disregard of an old recommendation, have filled new wine into old bottles.

List of papers

List of papers included in the thesis, referred to as Paper I – IV in the text. When referring to the underlying studies, they will be called Study I – IV.

Paper I

Oldberg K, Kahn F, Rasmussen M, Walles J. Detection of relevant pathogens and contaminants in blood cultures after implementation of single-sampling strategy and initial specimen diversion. *Eur J Clin Microbiol Infect Dis*. 2025;44(9):2275-82.

Paper II

Oldberg K, Rasmussen M. *Enterococcus faecalis* in blood cultures-a prospective study on the role of persistent bacteremia. *Diagn Microbiol Infect Dis*. 2021;101(1):115433.

Paper III

Oldberg K*, Thoren R*, Nilson B, Gilje P, Inghammar M, Rasmussen M. Short time to blood culture positivity in *Enterococcus faecalis* infective endocarditis. *Eur J Clin Microbiol Infect Dis*. 2021;40(8):1657-64.

*These authors contributed equally to the work

Paper IV

Oldberg K, Sunnerhagen T, Ljungquist O, Rasmussen M, Tverring J, Torisson G. Blood culture time to positivity corrected for delayed incubation start – a retrospective cohort study. Unpublished manuscript.

List of papers not included in thesis

Cederwall S, Ottander E, Björkhem D, **Oldberg K**, Pålman LI. Performance of quantitative PCR to distinguish *Pneumocystis jirovecii* pneumonia from colonisation in immunocompromised patients. *Mycoses*. 2025 Oct;68(10):e70120.

Oldberg K, Rasmussen M. Comment on: Bactericidal activity of antibiotic combinations against clinical isolates of *Enterococcus gallinarum* and *Enterococcus casseliflavus*. *J Antimicrob Chemother*. 2025 Oct 3;80(10):2882-2883.

Österhed E, **Oldberg K**, Gustafsson I. Aetiology and antibiotic susceptibility of bacterial keratitis at a referral centre in southern Sweden. *Sci Rep*. 2025 Jun 20;15(1):20123.

Ljungquist O, Tverring J, **Oldberg K**, Sunnerhagen T, Torisson G. Association of time to positivity with disease severity in bloodstream infections-a population-based cohort study. *Clin Microbiol Infect*. 2025 Sep;31(9):1532-1538.

Oldberg K, Stenmark J, Hammarström H. β -D-Glucan testing in candidemia: determinants of positivity and association with mortality. *Mycoses*. 2025 May;68(5):e70067.

Mohseni S, Abou-Chakra N, **Oldberg K**, Chryssanthou E, Young E. Terbinafine resistant *Trichophyton indotineae* in Sweden. *Acta Derm Venereol*. 2025 Feb 10;105:adv42089.

Hillstedt E, Dencker M, Nilson B, **Oldberg K**, Rasmussen M. The first report of *Staphylococcus argenteus* endocarditis with visualized valve vegetations. *IDCases*. 2024 Aug 14;37:e02058.

Oldberg K, Rasmussen M. Make modern microbiology matter more in the 2023 European Society of Cardiology guidelines for the management of infective endocarditis. *Clin Infect Dis*. 2024 Aug 16;79(2):336-338.

Lohikoski R, **Oldberg K**, Rasmussen M. Bacteraemia caused by non-*faecalis* and non-*faecium* *Enterococcus* species-a retrospective study of incidence, focus of infection, and prognosis. *Eur J Clin Microbiol Infect Dis*. 2024 Jan;43(1):45-53.

Nygren D, **Oldberg K**, Holm K. Short blood culture time-to-positivity in *Fusobacterium necrophorum* bacteremia is associated with Lemierre's syndrome. *Anaerobe*. 2022 Feb;73:102474.

Bläckberg A, Falk L, **Oldberg K**, Olaison L, Rasmussen M. Infective endocarditis due to *Corynebacterium* species: Clinical features and antibiotic resistance. *Open Forum Infect Dis*. 2021 Feb 4;8(3):ofab055.

Rasmussen M, **Oldberg K**. Febrile urinary tract infection in an immunocompetent male caused by *Burkholderia multivorans*- evidence of transmission in a home hot tub. IDCases. 2019 May 3;17:e00553.

Abbreviations

AST	Antimicrobial susceptibility testing
CFU	Colony-forming units
CI	Confidence interval
CIED	Cardiac implantable electronic device
CoNS	Coagulase-negative staphylococci
CT	Computed tomography
DNA	Deoxyribonucleic acid
MSS	Multi-sampling strategy
NPV	Negative predictive value
OR	Odds ratio
PCR	Polymerase chain reaction
PET-CT	¹⁸ F-fluorodeoxyglucose positron emission tomography-computed tomography
PPV	Positive predictive value
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SSS	Single-sampling strategy
TOE	Transoesophageal echocardiography
TTE	Transthoracic echocardiography
TTI	Time to incubation-start
TTP	Blood culture time to positivity

Introduction

This thesis is based on four papers that relate to different aspects of blood culture, a method that is extensively used for the laboratory diagnosis of severe infectious diseases. In Study I, we investigated the effects of a newly introduced sampling method for blood cultures on their sensitivity and on contamination rate, while Studies II – III tested the utility of repeated blood cultures and blood culture time to positivity, in the diagnosis of infective endocarditis caused by *Enterococcus faecalis*. In study IV, we aimed at improving the performance of time to positivity as a diagnostic and prognostic test, by developing a method for correcting this time for a disturbing variable – time to incubation start. To describe the context of these papers, I will begin by defining and reviewing the key diseases and syndromes that they concern, followed by a description of how blood cultures can be used in the diagnosis of these entities, focused on the subjects of Papers I-IV.

Bacteraemia, bloodstream infection and sepsis

Definitions

Bacteraemia means “the presence of viable bacteria circulating in blood” (2, 3), usually without requirement of an inflammatory response (3, 4). Asymptomatic and transient bacteraemia is well-known in situations where mucosal barriers are breached, *e.g.*, during dental procedures, oral surgery or when brushing the teeth (5, 6). Bloodstream infection is an entity that overlaps with bacteraemia, and that is poorly defined and often used interchangeably with bacteraemia in scientific literature (7). Some have argued that bloodstream infection, in addition to bacteraemia, requires an inflammatory response that gives rise to symptoms or signs of infection (3, 4). The concept furthermore includes both bacteria and fungi (4), but seldom other infectious agents such as Protozoa or viruses. Since the bacteria in the blood are required to be viable, the only way to prove bacteraemia and bloodstream infection, that is currently in clinical use, is blood culture. Blood cultures are practically only used in the setting of a suspected infection and therefore, all true cases of bacteraemia (excluding blood culture contaminations) that are found in clinical practice, can be assumed to be bloodstream infections. We have used both terms in the papers of this thesis, with the only intended difference being that

bloodstream infection also incorporates fungaemia – the less common fungal parallel to bacteraemia.

In contrast to bacteraemia and bloodstream infection, sepsis is a syndrome based on other criteria than a positive blood culture. The current definition of sepsis – “Sepsis-3” – is a “life-threatening organ dysfunction caused by a dysregulated host response to infection” (8). This dysregulated response consists of simultaneous hyperinflammation and immunoparalysis (9). The diagnostic criteria for sepsis are an acute increase in SOFA score (Sequential Organ Failure Assessment) by at least two points, caused by infection. The SOFA score (Table 1) was originally developed as a means to quantify multiple organ failure in sepsis, and it is based on the evaluation of the respiratory system, the cardiovascular system, the central nervous system, blood coagulation, and liver and renal function (10). During the derivation and validation of the diagnostic sepsis-3 criteria, patients with infection and a SOFA score ≥ 2 had a significantly higher in-hospital mortality compared to those with a SOFA score < 2 . The SOFA score is problematic since some of the data required is only available in an intensive care-unit setting, in particular the ratio between arterial partial oxygen pressure and the fraction of inspired oxygen. The requirement of obtaining the SOFA score variables also makes retrospective studies on sepsis more difficult, since certain data points are often missing.

Table 1. The sequential organ failure assessment score (SOFA score). Adapted from Vincent *et al.* (10).

SOFA score	1	2	3	4
Respiration PaO ₂ /FiO ₂ , mmHg ^a	< 400	< 300	< 200 (with respiratory support)	< 100 (with respiratory support)
Coagulation Platelets 10 ⁹ /l	< 150	< 100	< 50	< 20
Liver Bilirubin μ mol/l	20 – 32	33 – 101	102 – 204	> 204
Cardiovascular Hypotension	MAP ^b < 70 mmHg	Dopamine $\leq$ 5 or dobutamine any dose ^c	Dopamine > 5 or adrenaline $\leq$ 0.1 or noradrenaline $\leq$ 0.1 ^c	Dopamine > 15 or adrenaline > 0.1 or noradrenaline > 0.1 ^c
Central nervous system Glasgow Coma Scale	13 – 14	10 – 12	6 – 9	< 6
Renal Creatinine μ mol/l (or urine output)	110 – 170	171 – 299	300 – 440 (< 500 ml/day)	> 440 (< 200 ml/day)

^a Partial pressure of arterial oxygen / fraction of inspired oxygen. ^b Mean arterial pressure. ^c μ g/kg body mass / minute, administered for at least one hour.

Septic shock is defined as “a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase

mortality” (8). The diagnostic criteria for septic shock are sepsis with persisting hypotension, requiring vasopressors to maintain a mean arterial blood pressure ≥ 65 mm Hg and with a serum lactate concentration > 2 mmol/L despite adequate volume resuscitation. In the validation that was performed when constructing these criteria, the in-hospital mortality was over 40% (8).

Importantly, it is possible to have sepsis without bacteraemia or fungaemia. In a study on well-characterised patients fulfilling sepsis-3 criteria in an intensive care unit, 54% had bacteraemia, 16% were non-bacteraemic but had positive microbiology analyses on other sample materials than blood which explained their disease, while 30% had no detected pathogens (11). When excluding patients who had received antibiotics before cultures were obtained, the proportion of patients with pathogens detected in blood cultures increased to 63%, while those with no detected pathogens decreased to 22%. This points out the importance of obtaining blood cultures (and other cultures) before administration of antibiotics. Other explanations for not having positive blood cultures in sepsis might be low performance of the culture method (*i.e.*, bloodstream infection that is not detected), absence of viable bacteria in blood despite a bacterial cause of disease, an infection that is caused by agents (bacteria, fungi, viruses or protozoans) which cannot be cultured using standard culture methods, or misdiagnosis of another clinical condition.

Finally, bloodstream infection can be present without concomitant sepsis in diverse disease entities, *e.g.*, in infective endocarditis, in pyelonephritis or in appendicitis.

Pathogenesis

There are several ways for microorganisms to enter the circulation. Typically, symptomatic bacteraemia arises when bacteria have initiated a localised infection *e.g.*, pneumonia, urinary tract infection or skin- and soft tissue infection, and are thereafter translocated into the bloodstream. This process begins with adhesion and colonisation of a primary site, an epithelial surface or a wound, and it is facilitated by bacterial adherence factors, iron-acquisition and capsule (3). Thereafter the invasion through epithelium and underlying tissues can commence, dependent on numerous toxins, enzymes and other virulence factors, different for different species. Another mode of microbial entrance into the bloodstream is when the physical barriers of the body are breached in other ways, *e.g.*, due to mucositis during cancer therapy, ischaemic damage to intestinal mucosa, bacterial or fungal colonisation of intravascular catheters or injection of contaminated material in intravenous drug use (3). This might allow for more or less passive translocation to the vascular compartment, and it is reasonable to assume that this allows for less virulent organisms to cause bloodstream infection. Of course, there are intermediate variants of these two modes of invasion into the bloodstream. Sometimes the focus of primary infection remains unknown, a situation frequently described as “primary

bacteraemia”, but reasonably one of the already mentioned mechanisms must have led to the bacteraemia, although the local symptoms were not noticed (7).

Bacteria and fungi are attacked by the host immune response when they have ended up in the bloodstream, mainly by the complement system, antimicrobial peptides and leukocytes. The foreign cells are also filtered out of blood in the liver and spleen, where they are phagocytosed (3, 12). Pathogenic bacteria have multiple methods to survive the harsh environment in blood, *e.g.*, by complement inhibitors produced by the bacteria or recruited from the host, evading phagocytosis, intracellular persistence in host cells, evading antimicrobial peptides and acquisition of sequestered iron (12). It is not known whether bacteria proliferate in suspension in blood to a significant extent *in vivo*, or whether they are continuously leaking into the circulating blood from extravascular (or intravascular) foci of infection. If the bacteria survive the harsh environment, they can sometimes spread throughout the body and establish secondary foci of infection, *e.g.*, meningitis, arthritis or infective endocarditis.

Interestingly, the prevalence of bacteraemia differs by the type of primary focus of infection: Patients with pneumonia have a lower prevalence of bacteraemia than patients with pyelonephritis and bacterial meningitis (11, 13). This could be one of the reasons why *Escherichia coli*, the most common pathogen in urinary tract infections, is consistently the most common finding in blood cultures (14-16) although respiratory tract infections are frequently reported as the most common cause of sepsis and septic shock (11, 17, 18), only surpassed by diarrhoeal disease in a study on the global burden of sepsis (19). An explanation can partly be that respiratory tract infections are more commonly caused by viral pathogens, bacteria that cannot be isolated in standard blood cultures (*e.g. Legionella spp.* and *Mycoplasma pneumoniae*), and fungi, but there may be other reasons as well.

Epidemiology

When studying the incidence and mortality of bacteraemia and sepsis, it is a great advantage to use a population-based study design. This means that practically all incident cases of disease in a defined population can be registered, and that the size and composition of the population at risk is known. These criteria are often easier to fulfil in a geographically isolated region, where the inhabitants seldom consult health-care facilities outside of the region. Population-based designs avoid bias related to referral and hospital admission, and make it possible to calculate age- and sex-adjusted incidences (20). There is a massive over-representation of population-based studies on bacteraemia conducted in Nordic countries, arguably because the extensive use of personal identification numbers, that are represented in many high-quality databases, facilitates research.

Early population-based studies on bloodstream infection from the 1970s to 1980s reported incidences around 80 cases per 100 000 population per year (21, 22). This increased to between 150 – 200 per 100 000 per year in 1990s to early 2000s (14, 22-24), while Nordic studies from the 2010s have reported incidences around 300 per 100 000 per year (14, 15) and a Canadian study from 2017 150 per 100 000 (16). Only one Danish study did not report an increased incidence during its studied timespan (25). A feature seen in several studies is a higher incidence in males than in females, a markedly higher incidence in children under one year old, followed by a minimum incidence in older children and thereafter a continually increasing incidence in subsequent age groups (14, 21, 22, 24, 26).

There are several possible explanations for the increasing incidences over time: The culture methods have improved significantly from the 1970s and 1980s, and they are likely to contribute to increased detection (27). The blood volume that is sampled is also an important factor in blood culture sensitivity (see the following chapter on blood cultures), and the recommended volumes have increased over time as new evidence has emerged. The rate of blood culture sampling has also increased in several studies, which leads to increased incidence of bacteraemia (15, 24, 27, 28). Despite the increased culture rate, the proportion of positive cultures are often remarkably stable throughout the studied periods, which indicates that either the indications for sampling have widened to include more patients with true bacteraemia, or that the incidence of severe bacterial infections with bacteraemia have in fact increased. The latter possibility is supported by the fact that increase in incidence is highest in the oldest age groups (14, 15) and that the populations in the studied countries are progressively getting older. In addition, a Swedish study has reported an increase in age-standardised hospitalisation rate due to infectious diseases in 2015 – 2019 compared to 1998 – 2002, an increase that was highest in those aged > 80 years (29).

The proportion of people with bacteraemia that die within 30 days, *i.e.* the all-cause case fatality rate, is approximately 13% in recent population-based studies from Nordic countries (14, 30, 31).

It is more difficult to evaluate the burden of sepsis due to the lack of an easily interpreted gold standard, variability in definitions, low reliability of disease coding in electronic medical records and regional differences in health-care organisation. As often, there is also a relative lack of data from low- and middle-income countries (32). Furthermore, the attribution of mortality to sepsis is complicated, as it is often an intermediate cause of death or morbidity, *e.g.*, sepsis that is caused by stroke or traumatic injuries (19), of course this is true for bloodstream infections as well. Based on the Global Burden of Diseases, Injuries and Risk Factors Study from 2017 (19), the overall global age-standardised incidence of sepsis in 2017 was estimated to 677.5 (95% uncertainty interval [UI] 535.7 – 876.1) per 100 000 population per year, a decrease from year 1990. The sepsis-related mortality in 2017 was estimated to 148.1 (95% UI 136.4 – 161) deaths per 100 000, accounting for 19.7 % (95% UI

18.2 – 21.4) of all deaths. Both sepsis incidence and sepsis-related mortality was higher in countries with low income and education. In contrast, a meta-analysis that was mostly based on studies from high-income countries found an incidence of hospital-treated sepsis of 189 (95% confidence interval (CI) 133 – 267) per 100 000 population and year, and an increasing incidence (33). The case fatality rate in hospital-treated sepsis was 26.7% (95% CI 22.9 – 30.7%). The estimates of these studies are conflicting because of the reasons given previously, and neither one is representative for all settings. Two population-based studies, focused on the same region (Skåne) and the same period as Paper I-IV in this thesis, reported an incidence of hospital-treated sepsis according to sepsis-3 criteria of 780 (95% CI 633 – 926) per 100 000 (34) in adults only in 2015, and 747 (95% CI 663 – 832) per 100 000 in 2019 (35). The 30-day all-cause case fatality rate was 15.5% (95% CI 11.4 – 20.7) (35).

In conclusion, there is evidence from local studies of high quality for an increasing incidence of bloodstream infection and a high incidence of sepsis.

Infective endocarditis

Pathogenesis

Infective endocarditis is an infection of the endothelium on heart valves, the endocardium or on implanted foreign material in the heart, such as prosthetic heart valves or pacemaker leads. A healthy endothelium is usually resistant to infection, but when predisposing factors are present, such as damage due to congenital or acquired valve abnormalities, congenital heart disease, or previous infective endocarditis, bacteria and fungi can adhere and initiate local inflammation (36). In most cases, bacteria in the blood are thought to adhere to a thrombus that has been formed because of damage to the endothelium, but they may also interact more directly with the endothelial cells. Thereafter, platelets and monocytes are recruited to the infected site, and a so-called vegetation forms, built by fibrin and other plasma proteins, platelets and bacteria (Figure 1) (36). Foreign material can be colonised more easily by bacteria and fungi, which can establish a biofilm on its surface. The bacteraemia that initiates the infective endocarditis is thought to be transient and asymptomatic in many cases and may be caused by oral bacteria that gain access to the blood stream during *e.g.*, toothbrushing or during dental procedures (5). In other cases, the causative bacteraemia may be symptomatic and in turn caused by *e.g.*, a urinary tract infection with *Enterococcus faecalis* or a venous catheter infected by *Staphylococcus aureus* (37). Injection drug use is also a situation where there is a high risk of introducing microorganisms capable of causing infective endocarditis (36).

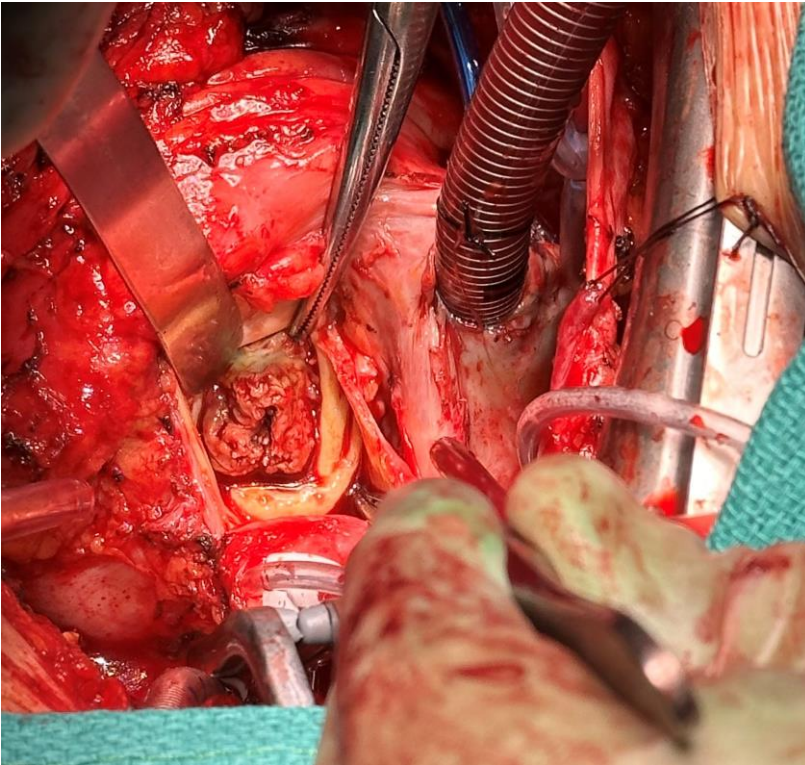


Figure 1. Aortic valve during surgery with pronounced vegetations. Photograph: Per Wierup.

The vegetations in infective endocarditis are most commonly located in the left side of the heart, on the mitral or aortic valves (38). Less commonly, the valves in the right side are affected and in those cases, the cause is often injection drug use, or infections in implanted devices such as pacemakers and implanted cardioverter-defibrillators, in common called cardiac implantable electronic devices (CIED) (38, 39). Importantly, prosthetic heart valves are a risk factor for infective endocarditis. In a relatively recent European study, 30.1% of all cases of infective endocarditis were infections in prosthetic valves (38). In addition, congenital shunts can be infected (36).

The clinical presentation of infective endocarditis is variable and non-specific. The most common symptom is fever, while dyspnoea, cough and sometimes chest pain can arise when valvular damage leads to congestive heart failure (38). In addition, parts of the vegetations can break off and embolise to different organs. This can cause symptomatic infarction (cerebral, myocardial, splenic *etc.*) and can also give rise to a secondary focus of infection (36, 38). Patients with infective endocarditis caused by *Staphylococcus aureus* often present with sepsis and a duration of symptoms of a few days at most (40-43). In contrast, patients with infective

endocarditis with non-beta-haemolytic streptococci (alpha-haemolytic streptococci and the *Streptococcus bovis* group combined) or *Enterococcus faecalis* often have a symptom duration at presentation of many days or even weeks (44, 45).

Microbial aetiology

The incidence of infective endocarditis is hard to establish with certainty, but an estimate for Europe of approximately 8 per 100 000 population per year in the year 2020 has been reported, with a significant increase observed from year 2000 (46). In the early 1900s, community acquired infective endocarditis caused by non-beta-haemolytic streptococci was the dominating type in the western world. Infective endocarditis was a disease that mostly affected young people, mainly with rheumatic heart disease as the underlying risk factor. With falling prevalences of rheumatic heart disease, the development of advanced healthcare with larger risks of bacteraemia due to medical procedures, an increase in injection drug use and an ageing population, the proportion of infective endocarditis caused by *S. aureus* and *E. faecalis* has risen (Table 2) (36, 47). In low-income countries, the typical patient with infective endocarditis is still relatively young, with non-beta-haemolytic streptococci as the aetiology and rheumatic heart disease as the risk factor (47).

Table 2. The microbial aetiology of infective endocarditis in Europe according to Habib *et al.* (38) and in Sweden in 2024 according to the annual report of the registry of infections of the Swedish society for infectious diseases (48).

Species or microbial group	Europe 2016 - 2018	Sweden 2024
<i>Staphylococcus aureus</i>	31.4%	35%
Coagulase-negative staphylococci	12.7%	5%
Non-beta-haemolytic streptococci	19.0%	28%
<i>Enterococcus</i> species	15.8%	12% ^a
Other or culture negative	21.1%	20% ^b

^a *E. faecalis* 90%, *E. faecium* 7%, other enterococci 3%. ^b Culture negative = 2%.

The proportion of people in the population who have prosthetic valves or a CIED has increased and consequently, so has the incidence of infection involving these foreign materials (49). In addition to the previously mentioned bacteria, skin commensals such as coagulase-negative staphylococci (CoNS), *Cutibacterium acnes* and *Corynebacterium* species are common in prosthetic valve infective endocarditis and CIED related infections (49-52). Transcatheter aortic valve implantation (TAVI) is a method for replacing a malfunctioning aortic valve in persons in whom the risks associated with open heart surgery are too high. Enterococci are a disproportionally common aetiology of infective endocarditis in infected TAVI valves (53).

Fungi cause 1 – 3% of all cases of infective endocarditis and *Candida* species are the most common aetiology in this group. Common risk factors are prosthetic

valves, immunosuppression and injection drug use. The vegetations in fungal infective endocarditis are often large, with a higher risk for embolisation (54).

In a proportion of infective endocarditis cases that ranges from 8% (55) to around 20 % in recent studies from Europe (38, 56), no organisms grow in blood. This is called blood culture negative infective endocarditis, and its most common reason is that the patient has received antibiotics prior to blood culture, so that most bacteria in the circulation are not viable (55). Another reason can be that the infection is caused by bacteria that grow slowly or not at all under standard culture conditions, *e.g.*, *Coxiella burnetii*, *Bartonella* species, *Tropheryma whippelii*, or *Mycobacterium* species. In these cases, other methods for microbiological diagnosis can be applied, such as polymerase chain reaction (PCR) targeting specific species, or amplification and sequencing of bacterial DNA coding for 16S ribosomal RNA, in blood, resected valves or biopsies from sites of metastatic infections (39, 55, 57). Detection of specific antibodies in serum is also used, and when fungal infection is suspected, the biomarkers β -D-glucan and *Aspergillus* galactomannan can be of value (54, 57). There is also a small proportion of cases with non-infective endocarditis, “non-bacterial thrombotic endocarditis”, that is often associated with cancer or autoimmune diseases, and that usually presents with embolic complications (58).

Complications

Infective endocarditis is considered a fatal disease if it is not treated (36, 59). There are several complications with fatal potential, both local and at distant sites in the body. The heart valves are ultimately weakened and degraded by the infection, which can lead to perforation and ruptures, and thereby regurgitation. Dehiscence of prostheses can lead to paravalvular leakage. Sometimes large vegetations can even obstruct blood flow (60, 61). Engagement of the tissues around the valves with abscess formation can lead to atrioventricular block and to fistulation with shunts or cardiac tamponade. All of these complications can lead to acute heart failure and in pronounced cases to rapid circulatory collapse (39). As already mentioned, systemic embolisation of vegetations can lead to ischemia in several organs, and in the case of right-sided infective endocarditis, to pulmonary embolism and abscess formation. The brain is most commonly affected by embolism, and in a prospective study on the risk for cerebrovascular complications, 35% of patients with infective endocarditis suffered a symptomatic stroke, while asymptomatic cerebral infarction or haemorrhage was detected in a further 30% using imaging and biomarkers for cerebral injury (62). Most cerebrovascular complications that occur after start of antimicrobial treatment, do so within the first week (62, 63). Moreover, septic micro-embolism can lead to mycotic aneurysms in the aorta and arteries. These are infections in the wall of the vessels, which weaken them and can lead to rupture and haemorrhage. Other metastatic infections due to micro-embolism are common, *e.g.*,

spondylodiscitis, septic arthritis and renal abscesses (36, 39). In other words, timely identification and treatment of infective endocarditis is important.

Treatment of infective endocarditis

The treatment of infective endocarditis is complicated by the environment within the vegetations, where low metabolic activity and slow proliferation of bacteria make them less susceptible to antimicrobial agents (36, 39, 64). An infection involving foreign material may be even harder to eradicate. For this reason, antimicrobial treatment administered in high doses and extended over several weeks (usually four to six) is recommended. In addition, combination therapy aiming at antibiotic synergy is frequently recommended for several microbial aetiologies, as well as the addition of rifampicin in prosthetic valve infective endocarditis (39, 64). However, the evidence for specific antimicrobial agents or the treatment length is low (39, 65). In the subset of patients with the most common bacterial aetiologies – *S. aureus*, *Streptococcus* species, *E. faecalis* or CoNS – and who respond to initial intravenous treatment for a minimum of 10 days with no signs of complications, it is safe to switch to oral antibiotics (66).

Heart surgery is of great importance in the management of infective endocarditis. The most common indication for surgery according to the guidelines of the European society for cardiology (39) is heart failure due to valvular regurgitation. Cardiogenic shock or pulmonary oedema are indications for emergency surgery. Another indication is the somewhat broadly defined “locally uncontrolled infection”. This includes local complications such as abscess formation, lack of clinical response on antimicrobials alone, persistently positive blood cultures for more than 7 days, or infection with organisms that are usually hard to treat with antimicrobials alone, *e.g.*, fungi and methicillin-resistant *S. aureus* (MRSA). Finally, prevention of embolism by surgical removal of the infected valves and vegetations is indicated when the risk is high, especially when vegetations are large and mobile, or when an embolisation has already occurred. A study in which patients were randomised to urgent surgery on the indication of prevention of embolism or to “conventional treatment”, found a significant decrease in the incidence of cerebrovascular complications in the group undergoing urgent surgery (67). The infected valves are often replaced by prosthetic valves during surgery, but sometimes a more conservative approach with repair of native mitral valves is chosen. When the infection involves the aortic root or other surrounding tissues, more complex reconstructions can be necessary (39). In Sweden in 2024, 40% of patients with infective endocarditis underwent heart surgery during the initial hospital stay (48).

Diagnostic criteria

Infective endocarditis can be a hard diagnosis to establish with certainty in a clinical context. The individual risk factors, symptoms, laboratory findings and imaging results must be weighted together to estimate the risk for an infection in a site that is not easily available for inspection or biopsy. In clinical practice, it might be sufficient in many cases to base decisions about treatment on these parameters without any strict criteria, but when it comes to statistics and research, consistent and reproducible categorisation of cases is important. In 1910 two publications by Schottmüller and by Libman and Celler concluded that the aetiology of subacute infective endocarditis was streptococci, based on the results of blood cultures (68). This enabled a more objective diagnostic procedure than the previous approach that was based on symptoms and clinical findings only. The first diagnostic criteria to include imaging of the heart valves with echocardiography were the so-called Duke criteria from 1994 (69). These criteria established a classification into proven, possible or rejected infective endocarditis based on major and minor criteria, that has largely persisted throughout later modifications. In paper II and III in this thesis, we used Duke criteria modified by Li *et al.* in 2000 (70), presented in detail in Table 3 and Table 4. There are more recent modifications as well: In 2015 the European Society of Cardiology (ESC) added the possibility to use computed tomography (CT) and ^{18}F -fluorodeoxyglucose positron emission tomography-computed tomography (PET-CT) as valid means to visualise infective lesions around the heart valves, and the possibility to use imaging tools for establishing embolism, even in the absence of symptomatic ischemia (71). In 2023, the ESC published another update that most notably added *E. faecalis* to the typical pathogens (39). Simultaneously, the International Society for Cardiovascular Infectious Diseases (ISCVID) proposed another modification of the Duke criteria, importantly incorporating the same imaging techniques as the 2015 ESC criteria, and a large set of new typical pathogens, including species that are only counted as typical in prosthetic valve infective endocarditis (Table 5). They further removed the criterion demanding that blood cultures must be obtained in different venipunctures and with separation in time and added detection in blood of DNA from certain non-culturable species as a new major criterion (72). Thus, somewhat unfortunately, researchers currently have two separate sets of criteria for diagnosing infective endocarditis to choose from.

Table 3. Definition of infective endocarditis according to the modified Duke criteria. Adapted from Li *et al.* (70)

Definite infective endocarditis

Pathologic criteria

1. Microorganisms demonstrated by culture or histologic examination of a vegetation that has embolised, or an intracardiac abscess specimen, or
2. Pathologic lesions: Vegetation or intracardiac abscess confirmed by histologic examination showing active endocarditis

Clinical criteria

1. 2 major criteria, or
2. 1 major and 3 minor criteria, or
3. 5 minor criteria

Possible infective endocarditis

1. 1 major and 1 minor criterion, or
2. 3 minor criteria

Infective endocarditis rejected

1. Firm alternate diagnosis explaining evidence of infective endocarditis, or
 2. Resolution of infective endocarditis syndrome with antibiotic therapy for ≤ 4 days, or
 3. No pathologic evidence of infective endocarditis at surgery or autopsy, with antibiotic therapy for ≤ 4 days, or
 4. Does not meet criteria for possible infective endocarditis
-

Table 4. Definition of major and minor criteria of the modified Duke criteria for infective endocarditis. Adapted from Li *et al.* (70)

Major criteria

1. Blood culture positive for infective endocarditis
 - (a) Typical microorganisms consistent with infective endocarditis from 2 separate blood cultures: Viridans streptococci, *Streptococcus bovis*-group, HACEK group, *Staphylococcus aureus*, community-acquired *Enterococcus* species in the absence of a primary focus, or
 - (b) Microorganisms consistent with infective endocarditis from persistently positive blood cultures, defined as follows: (1.) At least 2 positive cultures of samples drawn > 12 hours apart, or (2.) all of 3 or a majority of ≥ 4 separate cultures, with the first and last sample drawn at least 1 hour apart, or
 - (c) Single positive blood culture for *Coxiella burnetii* or antiphase I IgG antibody titer > 1:800
2. Echocardiogram positive for infective endocarditis, defined as follows
 - (a) Oscillating intracardial mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation, or
 - (b) Abscess, or
 - (c) New partial dehiscence of prosthetic valve
3. New valvular regurgitation

Minor criteria

1. Predisposition: Predisposing heart condition or injection drug use
 2. Fever: Temperature > 38°C
 3. Vascular phenomena: Major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhages, Janeway's lesions
 4. Immunologic phenomena: Glomerulonephritis, Osler's nodes, Roth's spots, rheumatoid factor
 5. Microbiological evidence: Positive blood culture that does not meet a major criterion^a, or serological evidence of active infection with an organism consistent with infective endocarditis
-

^a Excludes single positive cultures for coagulase-negative staphylococci, or species that do not cause infective endocarditis.

Imaging techniques

Echocardiography is the most important imaging technique in the diagnosis of infective endocarditis. Using ultrasound, the structures of the heart and their motility, as well as blood flow can be visualised. Not only can vegetations and valvular regurgitations be found and quantified, but parameters such as filling pressures and left ventricular ejection fraction can be estimated, which are important for evaluating the need for surgery (39, 64). There are two main types of echocardiography that are used in the initial diagnosis: transthoracic echocardiography (TTE) in which the probe is placed in different positions on the chest, and transoesophageal echocardiography (TOE), where the probe is inserted into the oesophagus to a position just posterior to the heart. A common problem with TTE is that it can be difficult to obtain adequate visualisation of all structures of the heart due to *e.g.*, obesity or pulmonary emphysema. TOE has a higher sensitivity for detecting infective endocarditis - the overall sensitivity of TTE is

around 50% in mixed populations, when using TOE as the reference standard (73). The results of a meta-analysis suggest that only when TTE gives optimal view, when it is conclusively negative, the patient has no prosthetic valves and the pre-test risk for endocarditis is low, can TTE be used to rule out infective endocarditis (73). TOE is especially superior in patients with prosthetic valves and for detecting paravalvular complications. Current guidelines recommend always performing TOE when infective endocarditis is suspected (39, 64). However, TOE demands more personnel to be performed, the patient must not have eaten within a certain time span, it is usually unpleasant for the patient and sedation can therefore be necessary. In contrast to the practically risk-free TTE procedure, there is a small risk for complications from the TOE procedure, such as dental damage, aspiration, and perforation or bleeding in the upper gastrointestinal tract (74).

Even TOE can be insufficient to detect infective endocarditis in certain cases. Cardiac CT can be more sensitive than TOE in detecting paravalvular abscess and prosthetic valve infections, and these two modalities can complement each other when it is needed (75, 76). In addition, CT can be of value in preoperative evaluation (39). PET-CT is a method that uses radiation emitted from glucose tagged with a fluorine isotope to visualise areas of increased metabolism, while simultaneously mapping the background anatomy with CT. It has a relatively high sensitivity for prosthetic-valve infective endocarditis and CIED-related infective endocarditis, but a low sensitivity for native-valve infective endocarditis (77). It is mostly used in cases that are difficult to diagnose, and a positive aspect is that it can simultaneously detect sites of metastatic infection (39) or give alternative explanations for the patient's symptoms. However, PET-CT is expensive, and access to it can be limited.

Risk stratification scores

Typically, infective endocarditis is not suspected until blood cultures, which were ordered because the patient had fever, become positive. In a study from Skåne, Sweden, an initial suspicion of infective endocarditis could be documented in only 23% of patients who presented at emergency departments, and who turned out to have infective endocarditis (78). This brings us into one of the main themes of this thesis: how can blood culture results be used to estimate the risk for infective endocarditis in an individual patient? Performing TTE and TOE on all patients with positive blood cultures would mean very poorly spent money. A reasonable start is to let the microbial species that is isolated, influence the grade of suspicion of infective endocarditis. Certain species are more associated with infective endocarditis than others, and many of the high-risk species have been included as typical pathogens in the Duke-ISCVID diagnostic criteria (Table 5) (72). Notably, there is a clear dominance of Gram-positive bacteria in this list, with some of the Gram-negative species which are most commonly isolated from blood *e.g.*, *E. coli* and *Klebsiella pneumoniae*, being extremely uncommon causes of infective

endocarditis. Some of the typical species are significantly more associated with infective endocarditis than others. *E.g.*, non-beta-haemolytic streptococci (here defined as viridans-streptococci, the *S. bovis*-group and excluding *S. pneumoniae*) can be stratified according to probability for infective endocarditis, when they are found in blood cultures (79). The *S. sanguinis*-group, *S. mutans*-group and *S. bovis*-group confers a high probability for infective endocarditis (20 – 40%) (44, 80), the *S. anginosus*-group a low probability (approximately 5%) (80), and the *S. mitis* and *S. salivarius*-groups an intermediate probability (5 – 19%)(44, 80). In a Danish nationwide retrospective study, Østergaard *et al.* found a prevalence of infective endocarditis of 10.1% in patients with *S. aureus* bacteraemia (81), which is similar to the 8 – 9% that has been reported in recent but smaller Swedish studies (40, 82). In patients with *E. faecalis* bacteraemia, Østergaard *et al.* found a prevalence of infective endocarditis of 16.7% (81). There is a possibility that many cases of enterococcal infective endocarditis remain undiagnosed, as Dahl *et al.* found a prevalence of 26.1% (95% CI 21.5 – 30.7%) in a prospective study where all consecutive patients with *E. faecalis* bacteraemia were examined with echocardiography (83).

Table 5. Microorganisms that commonly cause infective endocarditis, according to the 2023 Duke-ISCVID diagnostic criteria (72)

Typical pathogens	Additional typical pathogens in prosthetic valve infective endocarditis
<i>Staphylococcus aureus</i>	Coagulase-negative staphylococci
<i>Staphylococcus lugdunensis</i>	<i>Corynebacterium striatum</i>
<i>Enterococcus faecalis</i>	<i>Corynebacterium jeikeium</i>
All <i>Streptococcus</i> species except <i>S. pneumoniae</i> and <i>S. pyogenes</i>	<i>Pseudomonas aeruginosa</i>
<i>Granulicatella</i> species	<i>Serratia marcescens</i>
<i>Abiotrophia</i> species	<i>Cutibacterium acnes</i>
<i>Gemella</i> species	Non-tuberculous mycobacteria (especially <i>Mycobacterium chimaera</i>)
HACEK-group (<i>Haemophilus</i> species, <i>Aggregatibacter actinomycetemcomitans</i> , <i>Cardiobacterium hominis</i> , <i>Eikenella corrodens</i> and <i>Kingella kingae</i>)	<i>Candida</i> species

There are patient characteristics that influence the risk for infective endocarditis, several of which are already included in the predisposition minor criterion in the different diagnostic criteria *e.g.*, prosthetic valves, congenital heart disease and injection drug use (39, 69, 70, 72). Certain symptoms, clinical findings and laboratory results are also taken into account, such as evidence for embolisation and the number of positive blood cultures. The rationale for including the number of positive blood cultures in these criteria is of special interest for this thesis, and it will be discussed further under the heading “Blood culture”.

To facilitate clinical decision-making on when to proceed or not with imaging of the heart when bacteraemia is detected, risk stratification scores have been developed for three of the most common microbial aetiologies of infective endocarditis: PREDICT (41), VIRSTA (43), POSITIVE (40), LAUSTAPHEN (42) and SABIER (84) for *S. aureus*, HANDOC (44) and an unnamed score proposed by Chamat-Hedemand *et al.* (85) for non-beta-haemolytic streptococci, and NOVA (86), DENOVA (45) and MEFIER (87) for *E. faecalis*. Some important features of the risk stratification scores for *S. aureus* and non-beta-haemolytic streptococci will be given below, while the scores for *E. faecalis* are discussed in more detail, as they are the direct background against which the studies reported in Paper II and III of this thesis were planned. Notably, there are no risk stratification scores for CoNS, *Corynebacterium* species and *C. acnes*, which are relatively common findings in blood cultures, and which can pose diagnostic challenges in patients with prosthetic valves.

For *S. aureus* bacteraemia, the PREDICT score gives points for the presence of a CIED, for community-acquired or healthcare acquired bacteraemia, and for blood cultures persistently positive ≥ 72 hours after the index culture. The authors suggest that TOE can be obviated in patients obtaining a low score at an evaluation five days after the index culture, *i.e.* only TTE is necessary (41). The VIRSTA score aims at early identification of patients in need of an urgent TOE, but it does not suggest to routinely skip TOE based on any results. Patients with embolisation, meningitis, CIED, prosthetic valve, previous infective endocarditis, previous valve disease, injection drug use or persistent bacteraemia ≥ 48 hours are all qualified for the urgent TOE, while variables with a lower score have to be added together to fall into this category: vertebral osteomyelitis, non-nosocomial acquisition, severe sepsis or septic shock (roughly corresponds to sepsis or septic shock according to sepsis-3 criteria) or C-reactive protein (CRP) > 190 mg/L (43). POSITIVE tried to separate patients with high or low risk for infective endocarditis as early as possible, without having to wait for the results of repeated cultures, to direct TOE to those in highest need. The thing that makes this score especially interesting in the context of this thesis, is that the authors incorporated blood culture time to positivity (TTP, see a detailed description under the heading “Blood culture”) as a variable: When < 9 hours, TTP by itself is sufficient to indicate a need for TOE and so is different manifestations of embolism and predisposing heart disease. TTP between 9 and 13 in combination with injection drug use also results in a positive score. In the validation cohort of the study, the sensitivity and specificity were 93 % and 70 %, this specificity was higher than that of VIRSTA and PREDICT (40). The authors of LAUSTAPHEN aimed to make a simpler score, that could defer the need for TOE in low-risk patients. They incorporated the variables cardiac predisposing factors, CIED, persistent bacteraemia ≥ 48 hours, vascular phenomena and native bone and joint infection, and the presence of any of these variables means a positive score (42). Finally, the SABIER score was developed with a machine-learning approach and incorporates some of the classic infective endocarditis risk factors, but also

heavily relies on patient age, and gives a few points for sex and several non-microbiological laboratory parameters in a rather complex way. The reported sensitivity and specificity were lower than the earlier scores (84).

The HANDOC score gives guidance on whether echocardiography is needed or not in patients having bacteraemia with non-beta-haemolytic streptococci. Three variables are related to the blood cultures: microbial aetiology, with different scores given according to the different risks associated with different species, the number of positive cultures, and whether there is one or several species growing. The other variables are heart murmur on auscultation or known valvular risk factors, long duration of symptoms and community-acquired infection. The sensitivity and specificity for infective endocarditis in the validation-cohort of the study reached 100 % and 76 %, respectively (44). The score by Chamat-Hedemand *et al.* similarly uses the individual risk profile of different species of streptococci, together with valvular predisposition and CIED. Interestingly, it results in a multi-level recommendation: “wait and see”, TTE, or TTE plus TOE (85).

The first risk stratification score for bacteraemia with *Enterococcus* species was NOVA (86), which aims to identify patients in whom echocardiography is unnecessary. From a larger set of variables that were associated with infective endocarditis, the authors used multivariable logistic regression to select the variables Number of positive blood cultures – 5 points (all three positive, or the majority if more than three were obtained), unknown Oorigin of bacteraemia – 4 points, prior Valve disease – 2 points and Auscultation of heart murmur – 1 point. The cutoff for a positive score was set to ≥ 4 points, so that in practice, only the N- and O-variables are important for classification. The investigators reported a sensitivity for infective endocarditis of 100% and a specificity of 29%, *i.e.* most patients still had to undergo echocardiography. The authors specify that at least three blood cultures should be obtained, with at least one hour separating the first and the last. The main parameter that they were interested in thus seems to be continuous bacteraemia during this hour. In many settings, only two blood cultures are obtained in standard practice, and the importance of the concept of continuous bacteraemia can be questioned, see the separate discussion under the heading “Blood culture”. If continuous bacteraemia were a unique feature of infective endocarditis, it is interesting that 69% of patients without infective endocarditis also had continuous bacteraemia in this study (86). Some things are unclear from the article, *e.g.*, whether episodes with several bacterial species were included or not, and exactly what unknown origin of bacteraemia means.

The DENOVA score was also aimed at identifying patients with *E. faecalis* bacteraemia in whom echocardiography was not necessary, and the authors tried to improve the low specificity of the NOVA score (45). The score was generated in a cohort with monomicrobial *E. faecalis* bacteraemia, and several known risk factors for infective endocarditis were analysed, including all the NOVA-variables, with modification of the required number of positive blood cultures to ≥ 2 , and with a

specification of what unknown origin meant. The variables that were selected for the score were Duration of symptoms ≥ 7 days, Embolisation, Number of positive blood cultures ≥ 2 , unknown Organism of infection, Valve disease and Auscultation of heart murmur, with one point for each variable. There was no requirement for drawing blood cultures at different timepoints. With a cutoff for a positive score of ≥ 3 points, the sensitivity and the specificity of the score was 100% and 85% in the separate validation cohort of the study, compared to 100% and 35% for the NOVA score in the same cohort. The number of patients with a positive DENOVA score needed to test with TOE, in order to find a case of infective endocarditis was 2.4. In an external validation of both scores, in a cohort including polymicrobial episodes (more than one species), the sensitivity and specificity of DENOVA was largely retained, with NOVA having a slightly higher sensitivity and a lower specificity (88). Another smaller external validation also found similar high sensitivity and specificity of DENOVA (89).

Finally, the MEFIER score was reported by Lai *et al.* (87), the same research group that constructed the SABIER score. Again, this is a complex score, calculated in a cohort with a surprisingly low prevalence of infective endocarditis - 3.4% - and that incorporated many unspecific variables such as age, haemoglobin and albumin in addition to more traditional risk factors for infective endocarditis. The sensitivity and specificity were both around 70 %, which means that the utility of this score is very low.

Blood culture

The history of blood culture

Rudimentary procedures for the culture of blood from living people can be found in bacteriological handbooks from the 1890s (90, 91). One of them states that sampling of blood “is usually done by taking a small drop from the skin surface, *e.g.*, the lobe of the ear” after thorough washing and antisepsis (90). The drop of blood should be smeared on the surface of a solid culture medium. The author state that it is rare to find bacterial growth in human blood, and that if colonies appear, “the procedure should be repeated to exclude the possibility of accidental contamination”. If it is possible to obtain larger quantities of blood through venesection, “suitable animals may be inoculated with several cubic centimètres”. Another book reviews earlier cases where *Streptococcus pyogenes* was isolated from blood in puerperal fever, and cases with “*Staphylococcus pyogenes aureus*” (91).

The formulations of the blood culture media were gradually improved and standardised throughout the 20th century, and liquid media became the norm. Sodium polyanetholsulphonate (SPS), began to be added to media in the 1930s, to

inhibit blood coagulation and killing of bacteria by the complement system and phagocytosis, and it is still used in modern media (92). In 1947 Castañeda described a biphasic blood culture system that was to become a common method, with both liquid medium and a solid agar phase in the same bottle. This was to avoid laboursome subculturing from all bottles, and was also an attempt to improve the - obviously very poor - safety when culturing *Brucella* species: “The method ... also minimizes the handling of possibly infective material, since such handling has proved dangerous to the technician who sooner or later becomes infected with the melitensis strain.” (93). These bottles were inoculated with 10 ml of blood, and they were tilted regularly to flow the broth over the agar. The agar surface was inspected daily for growth of colonies.

A different method for blood culture is the lysis-centrifugation method: Blood is drawn into a medium containing agents that lyse blood cells and sodium polyanetholsulphonate to inhibit killing of bacteria. The tube is centrifuged and the pelleted material, including bacteria, is inoculated onto solid media. If the colonies are counted, the lysis-centrifugation method allows for quantification of the bacterial concentration in blood as colony-forming units per ml (CFU/ml). The method is seldom used today due to the labour-intensive processing and a high risk for contamination (94).

A revolution in blood culture technique was the advent of the automated and later continuously monitored blood culture systems, beginning in the 1970s (95, 96). The initial BACTEC systems detected radioactive CO₂ generated by the bacterial metabolism of substrates containing ¹⁴C. The gas atmosphere of the bottles was semi-automatically sampled with a needle, which limited the frequency of sampling. Later, non-radiometric methods for detecting an increase in CO₂ concentration were developed, that did not rely on penetration of the rubber stoppers of the bottles. Around 1990, systems for automatic incubation with continuous monitoring of CO₂ became available, the first one was BacT/Alert (96). In the two dominating blood culture systems currently used, measurements are made with intervals of 10 minutes. BACTEC (Becton Dickinson) measures fluorescence that increases with the CO₂ concentration, and BacT/Alert (Biomérieux) measures a colour change. The earliest systems could only detect when a predefined cutoff was reached, but as the measuring methods were improved and computers became more powerful, the rate of CO₂ concentration increase in individual bottles could be followed, *i.e.*, the start of the log-phase of bacterial growth could be identified earlier (95). Together, these methods have resulted in shorter times from sampling to the report of bacterial growth.

Timing of blood cultures

An old tradition is to try to obtain the blood sample during, or soon after, a spike of fever. This recommendation has been kept in British recommendations for the

diagnosis of sepsis and systemic or disseminated infections (97), as well as another old recommendation for spacing the samplings in time with the objective of increasing the sensitivity for intermittent bacteraemia. A frequently cited study by Li *et al.* (98) concluded that increasing the volume of sampled blood was important for increasing the sensitivity of blood cultures, but there was no difference in the increased yield whether the whole volume was sampled at once, or if the blood draws were spaced in time with up to 24 hours. Thus, the current recommendations from the Swedish society for clinical microbiology (99) and the American Clinical and Laboratory Standards Institute (100) is to collect all blood culture bottles simultaneously or within a short time span. A retrospective study on the importance of blood culture collection during fever, found that there was no statistically significant association in the distribution of positive blood cultures relative to the time of maximum temperature in the sampled patients (101). The authors' conclusion that blood cultures can be obtained based on convenience rather than waiting for a fever spike, is generally accepted, and this is stated in the Swedish and American guidelines (99, 100).

The mandatory separation in time of blood cultures has been retained in the current diagnostic criteria for infective endocarditis of the European Society of Cardiology (39). To fulfil a major criterion, microbes that are not typical but that are consistent with infective endocarditis, must be isolated from either at least two blood cultures sampled ≥ 12 hours apart, or in three out of three cultures (or in the majority of cultures if more than three were obtained) with a minimum timespan of one hour between the first and last culture. The reasons for this are not clearly stated, but probably the concept of continuous bacteraemia in infective endocarditis is one. A study that included cases of infective endocarditis during 1944 – 1960 reported a rather constant bacterial concentration over several days (102), which may support the idea of separating cultures in time. On the other hand, as continuously positive blood cultures can be found in many cases of bacteraemia without infective endocarditis (86, 98), the usefulness of this demand for separation in time can be questioned. Since most cases of infective endocarditis are not suspected of having infective endocarditis on presentation at an emergency department (78), blood cultures spaced in time are infrequently used in Sweden, and these diagnostic criteria for infective endocarditis are thus difficult to follow strictly.

Sample volume

As already mentioned, the sampled blood volume is important for the sensitivity of blood culture. Recent studies on the bacterial concentration in blood are rare, but a few old studies, using different methods, reported bacterial concentrations in blood roughly between 0.01 to 100 colony-forming units per ml (CFU/ml) (102-105). Approximately half of the cases of bacteraemia have a bacterial concentration between 0.01 and 1 CFU/ml. The lower and upper limits in these studies were

defined by the volume that was sampled, the methods for quantification and by the analytical sensitivity of the methods used. Cases with a high microbial concentration in blood can readily be detected even if a fraction of a millilitre is sampled (*e.g.*, the sampling procedure with a drop of blood from the ear, described in the 19th century handbook), while those with a concentration around 0.1 CFU/ml require at least 30-40 ml to be likely to have at least one positive 10-ml culture bottle. Several studies have accordingly provided evidence for an increasing sensitivity with an increased sample volume (98, 106-109) although the proportional increase in yield becomes progressively reduced with further additions beyond 40 – 60 ml (106, 107, 109). In addition to the number of obtained bottles, an adequate volume in each bottle is also of importance (110). Swedish, British and American guidelines recommend a sample volume of 40 – 60 ml (97, 99, 100).

An elaboration on the sample volume and the number of culture bottles, is that it can be used for estimating the concentration of bacteria in blood. At low concentrations, it is a random process whether a bottle filled with blood contains at least one viable bacterial cell or not. Jonsson *et al.* (105) used modelling based on Poisson distribution to calculate blood microbial concentrations. In the 29% of patients who had *E. coli* bacteraemia, and who had only one positive 5ml culture bottle out of six bottles, the blood bacterial concentration was estimated to 0.04 CFU/ml (95% CI 0.0008 – 0.2 CFU/ml). The authors also concluded that in all patients where less than all bottles were positive, the original blood concentration was < 1 CFU/ml. Using a similar method called “most probable number”, that was developed for estimating microbial concentration in environmental samples (111), I have calculated the 95% CI for blood bacterial concentration for each combination of the number of obtained 10 ml bottles and number of positive bottles (Table 6). These results differ slightly from the numbers of Jonsson *et al.* and according to chosen method for calculation of the confidence intervals. Still, in all cases where there is growth in less than all obtained bottles where the organism can physiologically grow, the blood microbial concentration is likely < 1 CFU/ml. There is some evidence for a higher bacterial concentration in blood during infective endocarditis, based on a shorter time to positivity and a higher DNA-concentration in blood (see separate sections). In my opinion, the correlation between the number of positive bottles or cultures, and blood microbial concentration, is probably why this variable has successfully been incorporated in the diagnostic criteria for infective endocarditis (39, 69, 70, 72) and in risk stratification scores (44, 86, 112, 113), rather than an effect of continuous bacteraemia.

Table 6. The theoretical bacterial concentration for each combination of number of sampled blood culture bottles and number of positive bottles. Concentrations are given in CFU/ml as 95% confidence intervals. The sample volume is assumed to be 10 ml in all bottles. Inf = infinity. Calculated with R package “MPN”.

Number of samples (10 ml)	0 positive	1 positive	2 positive	3 positive	4 positive	5 positive	6 positive
1	0 – 0.30	0.0051 – Inf					
2	0 – 0.15	0.0094 – 0.51	0.025 – Inf				
3	0 – 0.10	0.0056 – 0.29	0.026 – 0.47	0.046 – Inf			
4	0 – 0.075	0.0040 – 0.21	0.017 – 0.28	0.041 – 0.47	0.064 – Inf		
6	0 – 0.050	0.0026 – 0.13	0.010 – 0.16	0.022 – 0.22	0.039 – 0.31	0.066 – 0.49	0.093 – Inf

Blood culture contamination and the number of venipunctures

Blood cultures are easily contaminated by skin microbiota during sampling, most often by coagulase-negative staphylococci (CoNS), which is a bad thing for several reasons: The finding may lead to further diagnostic testing, *e.g.* new blood cultures, echocardiography or radiology. It can be erroneously interpreted as a true cause of bacteraemia, leading to antimicrobial treatment that in turn may cause side effects such as *Clostridioides difficile* infection or kidney injury, lead to acquisition of bacteria with antimicrobial resistance, and lead to increased length of hospital stay. A premature closure in the diagnostic process can also mean that the true cause of the patient’s symptoms remains unresolved. Taken together, this results in increased morbidity and a large waste of resources (114-117). One way to reduce this problem is not to order blood cultures for patients with very low risk of bacteraemia, where the positive predictive value of a positive blood culture is low, or when the results are unlikely to affect patient care (13, 117). Skin antisepsis using an alcohol-based disinfectant is important for reducing the risk for contamination. Ethanol, isopropanol, chlorhexidine gluconate - often in solutions containing an alcohol, and iodine are often used. Education and feedback to personnel that collect blood cultures in emergency departments has also proved effective in studies of interventions (118, 119).

Another way of decreasing the risk for blood culture contamination is the use of a so-called initial specimen diversion technique (ISDT) (119), first described by Patton and Schmitt as recently as 2010 (120). Their simple innovation was to draw the first portion of blood, up to two ml, into a standard vacuum blood collection tube, before continuing with filling the blood culture bottles. The hypothesis behind ISDT is that skin fragments with adherent bacteria become lodged in the lumen of

the needle after the penetration of the skin. These fragments might be sucked into the blood culture bottles and cause contaminant growth. To first draw blood through the needle into a tube that is discarded (or used for other analyses) might dislodge many of those fragments and thereby decrease the risk for contamination. The authors presented a reduction in contamination rate from 2.8 % to 1.4 % ($p = 0.005$) in the first bottle that was filled from the venipuncture (120). Several subsequent studies, with varying quality, have found significant reductions in contamination rate with using either simple vacuum tubes (121-123) or more specialised devices (124, 125).

The number of venipunctures has also been suggested to be of importance for the risk of blood culture contamination. In a study by Lamy *et al.* (126) the sensitivity and specificity of blood cultures was theoretically modelled. The authors assumed that each venipuncture was associated with a certain risk for contamination with skin microbiota. Thereby, each additional venipuncture increased the risk of a bottle being contaminated, and the authors suggested an optimal sampling procedure with six bottles collected from only one venipuncture. This has been called the single-sampling strategy (SSS) as opposed to the conventional multi-sampling strategy (MSS) with at least two separate venipunctures. Two prospective studies have since reported a sensitivity when using SSS that was at least as good as with MSS (127, 128), but neither study found a significant decrease in the proportion of contaminated sampling events when using SSS. The study by Yu *et al.* deserves special credit for its simple but efficient design, where each patient was simultaneously sampled with four bottles from one venipuncture and with two bottles from a second venipuncture. Bottle one to four from the first venipuncture represented the SSS and bottle one and two from the first venipuncture plus the two bottles from the second venipuncture represented the MSS. Thereby, many potential biases that would have been introduced if the two sampling events were separated in time or person, could be avoided (128). A retrospective study from the same group, comparing the period before and after the introduction of SSS at an emergency department, found an increase in blood culture sensitivity with SSS (129). The reason for this increase was a significant increase in the proportion of sampling events where at least four bottles were obtained, rather than only two bottles – a so-called “solitary set”. The authors also found an increase in the proportion of bottles filled with a sufficient blood volume after implementation of SSS. Another study using the study design of Yu *et al.* found an equal sensitivity of SSS and MSS, but also a significant reduction of contaminated sampling events when using SSS (12 / 337 with SSS and 19 / 337 with MSS, $p = 0.039$) (130). Other positive aspects of SSS are a reduced time spent on the sampling procedure (131) and a reduced number of painful venipunctures.

The current Swedish recommendations for blood culture collection are to use an ISDT and SSS (99). The American guideline recommends MSS and considers ISDT optional (100), whereas the British guideline recommend SSS in one section of the

document but MSS in another, and does not mention ISDT (97). As mentioned previously, the 2023 ESC criteria for infective endocarditis still require that blood cultures are spaced in time for the growth of certain species to be considered a major criterion. In addition, the 2023 Duke-ISCVID criteria strongly recommend separate venipunctures, although it is no longer strictly demanded for a major criterion (72). In a study published after our Paper I on SSS, Goehringer *et al.* compared SSS and MSS in a prospective study of patients with high suspicion of infective endocarditis, including 101 patients with this disease. SSS was non-inferior to MSS regarding sensitivity and specificity for infective endocarditis, and SSS resulted in more patients correctly fulfilling a microbiological major criterion than with MSS (131).

A final issue regarding blood culture contaminations, is the difficult task of identifying them in research and in clinical practice. As a general rule, the individual probability of having an infection with a low-virulence organism must always be evaluated when potential contaminants are found. A common practice in monitoring of contamination rate when using MSS, however, is to classify growth in only one out of several blood culture sets (each obtained through a new venipuncture) of CoNS, *Cutibacterium acnes*, *Micrococcus* species, viridans-streptococci, *Corynebacterium* species, *Aerococcus* species or *Bacillus* species as a contamination (100). It is recommended to have contamination rates under 3%. This definition for contamination is often used in research as well, if individual medical records cannot be reviewed. In my experience however, viridans-streptococci are often contaminants in small children but seldom in adults, while *Aerococcus urinae* and *Aerococcus sanguinicola* should be considered true pathogens and only *A. viridans* as a presumed contaminant (132). Similar to the rules for laboratory monitoring of contamination rate, the number of blood cultures (defined as all bottles from one venipuncture) or the number of positive bottles is often used as a method to stratify the risk for true bacteraemia with CoNS in our region. This is supported by some studies, although it is not a perfect method (133-135). Other studies have found a weak, if any, association between the number of positive bottles or cultures and true bacteraemia vs contamination, and their authors recommended against this use (136, 137).

A potential problem with SSS is that the previous rules of thumb of the number of positive blood cultures, might no longer be useful for estimating of the probability for a true infection vs contamination with CoNS: During the last decades, a blood culture has usually denoted two bottles obtained from one venipuncture, but with SSS all four to six bottles constitute one single blood culture. The term “blood culture set” or “set of bottles” is sometimes a synonym for blood culture, but in other instances, it is used more specifically for the aerobic and anaerobic bottles that are paired together. To add further potential for confusion, certain requisition systems for microbiological analyses and laboratory information systems (including the ones used in Skåne while the studies of this thesis were conducted), always pair two bottles together in this manner, even after the introduction of SSS. When using SSS

however, this special relationship between two bottles is an illusion - there is not a stronger relation between bottle one and two, than between bottle one and four. Furthermore, one frequently stated benefit of MSS is that it is easier to distinguish contaminations (100). It is not specifically stated, but perhaps assumed, that one reason for this is that a contamination during one venipuncture is less likely to occur in the other venipuncture as well. Leyssene *et al.* specifically studied whether the number of positive bottles can be used to distinguish between contaminations and true bacteraemia when using SSS (138). The reference classification was a reviewer's assessment based on all available information, and the authors found that for CoNS, the probability for a true infection increased with the number of positive bottles in a six-bottle culture. The positive predictive value (PPV) for true infection was 3.5 % when only one bottle was positive, and 100 % if ≥ 4 bottles were positive.

Blood culture logistics

After blood sampling, it is recommended to load the culture bottles into the blood culture incubator within two to four hours (97, 99, 100). If the bottles are kept at room temperature for up to 24 hours, the sensitivity is only slightly decreased. However, if the bottles are kept in a standard incubator at 37 °C before transport to a laboratory with blood culture incubators, there is a large drop in sensitivity (139, 140). The reason for this is likely that the log phase of bacterial growth has already passed when the bottle is loaded into the blood culture system, so that no sufficient increase in fluorescence or colour change is detected. Importantly, all delays of incubation start will also delay the report of a positive culture (141). In hospitals with no microbiological laboratory nearby, it can be difficult to start incubation within the recommended timespan. Transports from all hospitals to a central laboratory at 2-hour intervals, all times of the day, would be exceedingly expensive, at least in Sweden. A partial solution of this problem is to place blood culture incubators at external sites, *e.g.*, emergency departments or other hospital laboratories, and to only transport positive bottles to the central laboratory (142, 143). However, the positive bottles will still have to wait for a scheduled transport, which may cause much delay. An interesting alternative is to start incubation in a blood culture system at external sites, but to remove all bottles under ongoing incubation a few times per day and send them to the central microbiological laboratory for continued incubation (144, 145). It is possible to continue on the same fluorescence reading curve with the BACTEC blood culture systems, if the bottle is inserted again within 5 hours. This combines a short time to incubation start with already having most bottles at the central laboratory when they become positive, so that the time to reporting of a Gram-stain result is further shortened. Drawbacks with this are the risks for exceeding the maximum time out of the blood culture system, a need for more blood culture incubators, and more manual work as all bottles are loaded and unloaded twice.

Work with positive blood culture bottles

When a blood culture bottle becomes positive, a small volume of medium is drawn out of the bottle with a syringe and it is divided to several analyses (97, 99, 100): One portion is smeared onto a microscope slide for fixation, gram staining and microscopy. The report from the microscopy is often the first notification to the clinicians about a positive blood culture and it gives a rough idea about what microbial species that are probable. Another portion of medium is streaked on different agar plates that are incubated overnight in different gas environments to allow for growth of most relevant species. Additional specialised media can be added based on the result of the microscopy.

Simultaneously, efforts are made to identify the species growing in the bottle: A common method in high income countries is matrix-assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF MS) (146). After a relatively simple protein extraction process, a target plate with up to 96 sample positions, is loaded into the instrument. A laser combined with the added matrix ionises proteins, which subsequently fly over an electric field to hit a detector in the other end of a compartment under vacuum. The mass of the proteins can be estimated by the time it takes to reach the detector, so that the distribution of the frequency of proteins by mass can be plotted as a mass spectrogram. The mass spectrogram derived from the sample is thereafter automatically compared to mass spectrograms from known species in a database. The software returns a list of best hits in the database, together with a quality score for the matching. In this way a large majority of clinically relevant species of bacteria and yeasts can be identified within minutes to a relatively low cost, once the expensive instruments are in place. MALDI-TOF MS can either be performed directly on material from the positive blood culture bottle, after several manual steps of washing and extraction (147), or on colonies growing on an agar plate after an incubation of a few hours (148). Another approach that can be useful in some settings is to identify species and common antimicrobial resistance genes using PCR on material from the positive bottle (149). This method has a limited spectrum of species that can be detected and the cost per sample is high.

A final important analysis is antimicrobial susceptibility testing (AST) of the isolated microbe. A common method for this is phenotypic AST from colonies on an agar plate *e.g.*, using disk diffusion according to the method described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (150). Automated systems for AST are also used, such as the VITEK 2 (Biomérieux, France) or Phoenix (BD, USA). To return rapid AST results, there are automated methods for phenotypic AST using medium from a positive blood culture bottle (151), and EUCAST has developed a method for rapid disk diffusion AST using medium from positive bottles (152).

Time to positivity

The blood culture time to positivity (TTP) is usually defined as the time passed from start of incubation of a bottle in the blood culture system to the point where the bottle is flagged positive, and a common synonym is time to detection (TTD). Sometimes TTP is used in a broader sense, encompassing the time from the sampling to the detection of growth in a bottle, but in this thesis and in Paper III and IV, TTP is used in the narrow sense. As mentioned in the section about the history of blood cultures, the TTP decreased when continuous monitoring blood culture systems were introduced and TTPs for individual blood culture systems and bottle types can vary (153). Several other factors also influence TTP:

Different species have different generation times. Some common pathogens such as *E. coli*, *S. aureus* and *S. pneumoniae* generally have a shorter TTP than species such as *Pseudomonas aeruginosa*, obligate anaerobes and fungi, that have longer generation times (153, 154).

An increase in the blood microbial concentration leads to shorter TTP. This has been demonstrated in studies where bottles are inoculated with different amounts of bacteria *in vitro* (155-157). Furthermore, two studies have found that the TTP of blood cultures with growth of *E. coli* and *S. aureus* and the bacterial DNA concentration in blood were inversely correlated (158, 159). As bacteria multiply by binary division, their growth in the blood culture medium is ideally exponential with the base of 2, until growth becomes inhibited by a lack of substrates and an accumulation of metabolites. The bacterial concentration at time point x (c_x) during exponential proliferation can thus be described by the following formula, where c_0 is the starting concentration, Δt is the incubation time and t_g is the generation time of the isolate:

$$c_x = c_0 * 2^{\frac{\Delta t}{t_g}}$$

The time required to reach the final concentration c_x from the starting concentration c_0 is

$$\Delta t = t_g * \log_2 \frac{c_x}{c_0}$$

Here, Δt roughly corresponds to TTP, with the important difference that the blood culture system does not become positive when a certain concentration c_x is reached, but when CO_2 is starting to be accumulated at a sufficient rate. Thus, a doubled starting concentration in blood should theoretically lead to a TTP that is shortened by a time that approximately equals the generation time of the microbial species. The generation time of *E. coli* is approximately 20 minutes in optimal conditions (160) and that of *S. aureus* is 35 minutes (161). In a study performed in blood culture bottles, the corresponding generation times were 18 – 24 minutes for *E. coli* and 60 – 72 minutes for *S. aureus* (157). A blood culture bottle that flags positive with

Gram-negative bacteria contains roughly 6.4×10^9 CFU/ml (IQR $6.6 \times 10^8 - 7.26 \times 10^{10}$ CFU/ml) (162). With the simplistic model of blood culture growth dynamics described above, a bottle containing 30 ml medium, inoculated with one cell of *E. coli* in 10 ml of blood, would require 38 generation times, corresponding to 12 hours and 40 minutes, to become positive. As can be expected, this is not exactly what can be observed in reality, and an experimental study found an average TTP of 13 hours and 20 minutes when 5 CFU *E. coli* were inoculated in each blood culture bottle (157), but the general mechanism linking microbial growth and TTP is likely correct.

The association between blood bacterial concentration and TTP is the basis of different clinical applications of TTP. One of these is attempting to distinguish true CoNS bacteraemia from contamination, where a long TTP has been suggested as an indicator for contamination (163, 164), based on the hypothesis that contaminated bottles are in general inoculated with fewer CFUs (165, 166). A caveat concerning these studies is that the classification to true or false CoNS bacteraemia is partly based on the number of positive bottles, which may introduce a bias. Another use of TTP is the method of using the differential TTP of paired blood cultures, obtained by a peripheral venipuncture and from an intravenous catheter, to diagnose catheter-related bacteraemia. When a bottle collected from the catheter becomes positive more than two hours before a bottle collected simultaneously by peripheral venipuncture, a catheter related infection is probable (155, 167). This is likely the most widely used clinical application of TTP, and there have been numerous studies following the initial publications by Blot *et al.* Some of these, *e.g.*, one by Orihuela-Martin *et al.* found a lower diagnostic performance than expected and advice against its use (168). A meta-analysis of 28 studies reported an aggregated sensitivity of 81.3 % (95 % CI 72.8 – 87.7) and a specificity of 91.8 % (95 % CI 84.5 – 95.8 %) (169). Notably, the sensitivity for catheter-related bacteraemia with *S. aureus* is consistently lower.

TTP has also been studied as a diagnostic marker for infective endocarditis. There are several studies in which infective endocarditis caused by *S. aureus* was associated with a shorter TTP (40, 82, 170-172). One study, however, found an increased risk for infective endocarditis in both the shortest and longest TTP quartiles (173). As mentioned earlier, the POSITIVE score is a risk-stratification score that heavily relies on TTP as one of its variables (40). Another study has interestingly presented an inverse association between vegetation length and TTP in *S. aureus* infective endocarditis, which might be of interest in prediction of embolisation (174).

Except for a small study that found a significantly longer TTP in patients with *Acinetobacter baumannii* infective endocarditis than in patients without infective endocarditis (175), there were no studies on the association between TTP and infective endocarditis caused by other species of bacteria than *S. aureus*, when we started our research in this field in 2018. Apart from the study on TTP in *E. faecalis*

infective endocarditis, that is included in this thesis, members of our research group subsequently studied non-beta-haemolytic streptococci and found no association between TTP and infective endocarditis (176). Neither did a later study on non-beta-haemolytic streptococci that was also a validation of the HANDOC score (177).

A final major field of research on TTP, is its use as a marker of prognosis in bloodstream infection. In contrast to the situation regarding infective endocarditis and TTP, there are many studies in this field, on different microbial species (178). There are also several old studies, using different types of quantitative blood culture, that indicate an association between a high blood concentration of bacteria and a higher risk for death (179) – this is likely the mechanism explaining what we observe in studies on TTP. Again, *S. aureus* is the most studied species, and a shorter TTP has been associated with a higher mortality in many studies (170, 171, 173, 180). Kim *et al.* reported a higher mortality in patients with short or long TTPs, compared to those around the median (172). Examples of other studied species or groups of species include *Streptococcus pneumoniae*, with a study finding an increased in-hospital and 30-day mortality in patients with a TTP in the lower quartile (< 9.2 hours) (181), *E. coli* with a higher in-hospital mortality in patients with TTP in the lower quartile (< 10.25 hours) (182), *Klebsiella pneumoniae* with a higher 30-day mortality in patients with TTP < 7 hours (the median was 9.6 hours) (183), *Salmonella enterica* with a higher in-hospital mortality when TTP was < 10 hours (corresponding to the 18th percentile) (184), and *Streptococcus pyogenes* with an increased 30-day mortality rate when TTP was < 9 hours (the median was 10.4 hours) (185). A meta-analysis of 24 studies by Hsieh *et al.* reported an OR for death of 2.98 (95 % CI 2.25 – 3.96) when the TTP was short, compared to long, according to the individual cutoff in each study (178). Based on six studies reporting the prevalence of septic shock, the OR for developing septic shock was 4.06 (95 % CI 2.41 – 6.84) in patients with short TTP compared to those with long TTP.

Not all studies report congruent results *e.g.*, one study found a decreased mortality in patients with *Candida albicans* bloodstream infection and a short TTP, compared to those with a long TTP (186), while a later study on mixed *Candida* species found an increasing 30-day mortality with decreasing TTP for all species except for *Candida parapsilosis* (187). Also, two large studies with mixed bacterial and fungal species could find no statistically significant association between TTP and mortality (188, 189). However, both studies had unusual definitions of TTP, namely from incubation start to the first report of a microscopy result (188), and time from sampling to the positive signal (189), which may obscure the association. As a response to these studies, Laupland *et al.* (154) published a study on 84 941 bloodstream infection episodes, where there was an increased mortality in the lowest TTP quartile (< 10 hours) with an OR for death of 1.43 (95 % CI 1.35 – 1.50) when all microbes were grouped together. In sub-analyses on different microbial groups, a lower TTP was associated with an increased case fatality rate in bloodstream infection with the major pathogens *S. aureus*, *S. pneumoniae*, beta-

haemolytic streptococci, *Pseudomonas* species, *E. coli*, other Enterobacterales, other Gram-negative bacteria and anaerobes. For *Enterococcus* species, an increasing case fatality rate with increasing TTP was found instead. Ljungquist *et al.* also published a study in response to the conflicting results mentioned above (190). In contrast to previous studies, where TTP has predominantly been dichotomised or analysed as a continuous variable but with linear methods, the authors hypothesised that the exponential nature of bacterial growth makes non-linear statistical methods more appropriate. Studying 12 585 episodes of bloodstream infection, a non-linear model for the association between TTP and 30-day mortality demonstrated exponentially increasing mortality rates when TTP was below 10 hours. Again, the inverse association between TTP and mortality was only seen in bloodstream infection with some of the typical pathogens – beta-haemolytic streptococci, *S. pneumoniae*, Enterobacterales and *S. aureus*, while there was no association for *Enterococcus* species, CoNS and non-beta-haemolytic streptococci (190).

In addition to the factors that have already been mentioned as having a known influence on TTP (blood culture system and bottle type, microbial species and microbial concentration in blood), there are others that can be more difficult to measure and control for in studies and in clinical practice. Thereby, they can reduce the strength in the association between microbial blood concentration and TTP that we are usually interested in. The most important one of these is probably the time passed from sampling to start of incubation in the blood culture system – which will be called time to incubation start (TTI) in this thesis. When blood culture bottles are kept at ambient temperature awaiting transports or further handling, bacteria and fungi start proliferating, although more slowly than at the temperature of 35 – 37 °C in the incubator. When the bottles are subsequently loaded into the blood culture incubator, the TTP will be shorter than it would have been if they were loaded directly. This can reduce TTP by several hours when TTI approaches 12 – 24 hours, as has been demonstrated in several *in vitro* studies where bottles were inoculated with bacteria and fungi, and were either loaded into a blood culture incubator directly, or kept at room temperature for different lengths of time before being loaded (141, 156, 157, 191). The reduction of TTP is greater when the temperature that the bottles are kept at before loading is higher (139, 140). A shortening of TTP due to TTI, to a level that interferes with the analysis of the outcome of interest, has also been described in studies on the association between TTP and mortality (51, 190, 192). In a study on distinguishing true CoNS bacteraemia from contamination using TTP, Haimi-Cohen *et al.* (164) used a simple method for correcting TTP for TTI based on their previous *in vitro* studies on a strain of *Staphylococcus epidermidis* (156): If TTI was 3 – 8 hours, one hour was added to the TTP obtained from the blood culture instrument. If TTI was 9 – 15 hours, two hours were added, and if TTI was 16 – 23 hours, three hours were added to the measured TTP. The authors found no impact of this correction on the ability of TTP to categorise patients to high or low risk for true infection (164). Similarly, Turkeltaub *et al.* (157)

added 2 and 3 hours, respectively, to the measured TTP of blood cultures with *S. aureus* and *E. coli* if the TTI was > 12 hours. This did not have any effect on the categorisation of patients into levels of predicted bacterial blood concentration. Except for these small studies, I am not aware of other studies that have explored the possibility of correcting TTP for TTI, despite TTI frequently being described as an important disturbing factor (193, 194).

The blood volume that culture bottles are filled with is of importance for the overall sensitivity of blood cultures (98, 106-110), but it has also been suggested to affect TTP (193, 194). A study performed in Sweden reported that roughly half of all blood culture bottles were inadequately filled (110), so this effect might be of importance. A study by Weinstein *et al.* (195) on the effect of sampled blood volume on TTP in aerobic bottles, found that in paired bottles obtained at the same time, the bottles filled with 10 ml more often became positive before bottles filled with 5 ml, at least for Enterobacterales. However, the difference in TTP was very small. When only bottles that became positive within 72 hours were analysed, TTP was actually shorter (less than one hour shorter) in bottles filled with 5 ml when *S. aureus* and enterococci grew. A more recent study by Kim *et al.* (196) on anaerobic bottles filled with 5 and 10 ml simultaneously, included 1552 blood culture sets. Among the 93 blood cultures in which both bottles were positive, mean TTP was 13.5 hours in the 5 ml bottle and 12.9 hours in the 10 ml bottle ($p = 0.099$). Although these studies were small, it can be concluded that when a bottle is positive, the TTP is only affected by sample volume to a small extent. This effect is likely smaller than that of delayed incubation start in most situations. Theoretically, a reduction of the blood volume by half means that the number of inoculated CFUs is reduced by half. Thus, the difference in TTP should correspond roughly to one generation time of 20 to 60 minutes, which is fully in line with the results of the two studies. However, other mechanisms might also be of importance *e.g.*, the resulting blood to broth ratio and dilution effect on antibiotics in the blood, might both lead to faster growth in bottles with a low volume in some cases (195). As the modern blood culture system BacT/Alert VIRTUO can automatically measure the blood volume in individual bottles, there are good opportunities for further studies on the effect of sample volume on TTP.

Antibiotics in the blood inhibits the growth of bacteria and thereby increases the TTP (197). Some bottle types contain special resins or charcoal to adsorb antibiotics, but their effect is not perfect and the TTP may still be increased in the presence of antibiotics (198). This will probably interfere with the use of TTP for diagnostic or prognostic purposes, but the treating clinician will in most cases know that the patient has received antibiotics before blood cultures were collected.

There are a few other hypothetic factors that may affect TTP, but that have not been studied. *E.g.*, one can imagine that live bacteria and fungi in the bloodstream may be in different positions on a spectrum from a non-culturable state to rapid proliferation, and that the mean state of the microbial population can be shifted in

different directions. Focus of infection, individual traits of a microbial isolate and the host immune response might affect this. Studies have reported an association between a short TTP and liver disease (183, 199, 200), malignancy (183, 200) and neutropenia (199), but it is not possible to tell whether this was due to a higher bacterial concentration in blood, differences in the types of infectious foci, or a possible increased viability of bacteria in the bloodstream during immunosuppressed states.

Repeated blood cultures and persistent bacteraemia

Repeated blood cultures, also called follow-up or surveillance blood cultures *i.e.* new blood cultures that are obtained after the first blood cultures have become positive, are used differently in different countries or settings (201). The American CLSI guidelines on the procedures of blood cultures recommend repeated blood cultures in patients with *S. aureus* bacteraemia, infective endocarditis and catheter-related bacteraemia to guide management (100). The infective endocarditis guidelines from the American Heart Association recommend repeated blood cultures every 24 to 48 hours in established infective endocarditis, until they become negative, with the treatment length starting from that date (64), and the European Society of Cardiology has a similar, but less explicit recommendation (39). The rationale for this recommendation has been questioned on the basis of weak evidence (202) and the Swedish guidelines for infective endocarditis do not recommend repeated blood cultures on this indication (203). Furthermore, persistent bacteraemia in infective endocarditis despite > 5 – 7 days of active antimicrobial therapy, is an indication for heart surgery if there are no extra-cardiac foci of infection that can be treated (39, 64).

The other two widely endorsed situations in which repeated cultures are used, are in *Candida* bloodstream infection, where the rationale is again to control the efficacy of treatment, and to start counting the days of treatment from the first day with negative cultures (204, 205), and in *S. aureus* bacteraemia. Positive repeated blood cultures in *S. aureus* bacteraemia is associated with a higher risk for infectious foci needing special treatment (206) and, more specifically, a higher risk for infective endocarditis (40-43), which is why it has been incorporated in several risk stratification scores for infective endocarditis (41-43). The timepoint for obtaining the repeated cultures varies between 48 to 96 hours after the initial positive blood culture in the cited studies. A large study by Kuehl *et al.* has suggested that persistent bacteraemia should be defined as continued growth in cultures obtained the day after start of active therapy, and 32 % of the 987 patients had persistent bacteraemia according to this definition. The 90-day case fatality rate was 40 % in patients with persistent bacteraemia, compared to 22 % in patients without persistent bacteraemia (adjusted hazard rate 1.93, 95 % CI 1.51 – 2.46) (207).

There are also studies on the predictive value of persistent Gram-negative bacteraemia on mortality, with one of the largest ones showing a doubled risk for in-hospital mortality if repeated cultures were positive (208). In this retrospective study, repeated cultures were obtained in 68 % of cases with Gram-negative bacteraemia, and in 20 % of those cases, the repeated cultures were positive. An increased mortality in the setting of persistent bacteraemia has also been demonstrated in a meta-analysis of nine studies (209). In addition, several studies have reported a decreased mortality in patients with Gram-negative bacteraemia in whom repeated cultures were obtained, compared to those in whom no repeated cultures were obtained, and suggested that the mechanism behind this might be that the repeated cultures can help guide the management of complex patients (208-210). However, all these studies are retrospective observational studies, and there may be significant confounding involved, despite the attempts to adjust the analyses for multiple factors.

At the time that we planned our studies, no previous studies had specifically investigated the association between persistent bacteraemia with *E. faecalis* and infective endocarditis. In a Canadian retrospective study, repeated cultures were collected in 102 out of 212 patients with *Enterococcus* species bacteraemia, and 20 of those (20 %) were positive (211). The authors also reported an increased prevalence of endocarditis in patients with persisting bacteraemia, but this was for the whole group of patients with persisting bacteraemia, which was dominated by *S. aureus*. Moreover, it is not clear whether an active antimicrobial treatment had started or not at the time of repeated cultures, and there is a high probability for underlying bias in the selection of patients that had repeated cultures.

Novel diagnostic methods

Blood cultures are a relatively cheap and widely available method for detecting a wide variety of organisms in blood, and it makes phenotypic AST and epidemiological typing possible. However, blood cultures are still slow – even for quickly growing organisms, the results on aetiology are seldom reported within 12 hours from sampling, and seldom on the same day if the laboratory is not open 24-7. Slowly growing bacteria and fungi can take several days to be detected. In addition, administration of antibiotics before sampling can make it impossible to culture the organism from blood, and certain organisms cannot be recovered from blood using standard methods, although they can be detected by circulating DNA e.g., *Tropheryma whippelii*, *Legionella* species, *Bartonella* species and *Pneumocystis jirovecii* (212, 213).

In suspected bloodstream infection, bacterial or fungal DNA can be detected in blood with a variety of different methods, including real-time PCR, droplet digital

PCR, PCR followed by sequencing of the product (amplicon sequencing), or metagenomic sequencing of all DNA in the sample (214). The benefits of real-time PCR and droplet digital PCR are their relative simplicity and fast turn-around times. They can also be used to quantify microbial DNA, especially droplet digital PCR is well suited for that purpose (215). On the other hand, these methods are restricted to detection of a few species or genes encoding antimicrobial resistance. Furthermore, in order to result in significantly faster results than blood cultures, they have to be available at all hours of the day, and the instruments and personnel must be localised close to emergency departments and intensive care units to avoid lengthy waiting for transports. A meta-analysis of commercially available PCR kits has reported low sensitivities and moderate specificities compared to blood culture, but these aggregated statistics are difficult to interpret since blood culture is not a perfect reference test (216).

Bacterial DNA can be amplified using primers for the gene encoding 16S ribosomal RNA, a gene that is present in all bacteria. By sequencing the amplicon, the bacterial species or genus can be determined (217, 218). This method can detect a large variety of bacteria, but it is not possible to detect antimicrobial resistance. The process takes more time than directed PCR assays (and often more time than blood cultures), is labour intensive and more expensive than blood cultures. A promising method is shotgun metagenomic sequencing, where all DNA, and sometimes also RNA, in a sample is sequenced, without prior directed amplification of certain genes. This makes it possible to detect bacteria, viruses, fungi and other eucaryotic parasites in one analysis, and it may also be possible to detect genes that are associated with antimicrobial resistance. There are companies that perform these analyses on blood *e.g.*, the Karius test (Karius, USA) (212, 219) or the DISQVER test (Noscendo, Germany) (213). The time from sampling to result of these two analyses is around two days, including transports. Metagenomic analyses can also be set up at ordinary microbiology laboratories, and sequencing using Nanopore (Oxford Nanopore Technologies, United Kingdom) may reduce the turnaround time in the laboratory to six hours (220). Nevertheless, the price limits the use of these analyses in clinical practice, and one should not underestimate the complexity of validating them while taking into account different possible sample types and volumes, nucleic acid extraction methods, bioinformatics pipelines and varying levels of background microbial DNA (221).

Quantification of bacterial DNA has been studied as a parallel to the potential uses of TTP in the diagnosis of infective endocarditis and as a marker for more severe disease. In patients with *S. aureus* bacteraemia, a higher bacterial DNA concentration is associated with the presence of infective endocarditis and with increased mortality (215, 222). Furthermore, a study with repeated measurements of *S. aureus* DNA found that DNA persisted longer in patients with complex foci of infection, such as infective endocarditis and osteoarticular involvement (159). An association between high bacterial DNA concentration in blood and increased

disease severity (158, 223-225) and increased mortality (225) has also been demonstrated for other bacterial species.

Analyses for detection of microbial DNA in blood are currently used only in addition to blood cultures. My opinion is that in order to replace blood cultures as the basis in the diagnosis of sepsis and BSI, increased speed and ease of use, a comprehensive coverage of species and antimicrobial resistance genes, and a lower price is necessary. A complete replacement of blood cultures might not be a realistic goal in the short term; if most important species and antimicrobial resistance genes can be reliably detected within a few hours by non-culture methods, blood cultures that are obtained simultaneously can be handled at a lower priority to save costs *e.g.*, no work during evenings and nights and no rapid antimicrobial susceptibility testing or rapid species identification.

Aims and rationale

Paper I

1. To evaluate the impact of single-sampling strategy (SSS) of blood cultures and initial specimen diversion (ISDT) on the proportion of sampling events in which the recommended number of bottles were obtained.
2. To evaluate the impact of SSS and ISDT on the proportion of sampling events with relevant pathogens and with common contaminant species.
3. To study whether there was a change in the proportion of sampling events with growth of coagulase-negative staphylococci (CoNS) in 1-2 and 3-4 out of 4 bottles after introduction of SSS and ISDT

SSS is increasingly used in European countries and is now the recommended standard sampling method for blood cultures in Sweden. However, given the importance of blood cultures, disproportionately few studies have been published on the effects of SSS (127-130). Only one has reported a decrease in the contamination rate (130), although this has been an argument for using SSS (126). There are no studies on the combined effects of SSS and ISDT. There was accordingly a need for more data on SSS, the use of which is still discouraged by influential organisations (97, 100). Furthermore, the number of positive blood culture bottles with growth of CoNS is often used to estimate the probability of a true infection, and SSS might influence the probability of contaminating more than two bottles during sampling, compared to MSS. Finding problems related to this is important for directing further research and for introducing countermeasures.

Paper II

1. Is persistent *Enterococcus faecalis* bacteraemia associated with infective endocarditis and other foci of infection that are difficult to treat?

Persistent bacteraemia with *S. aureus* is a well-known risk factor for infective endocarditis, other infectious foci that need special treatment, such as osteomyelitis, and for death (40-43, 206, 207). At the time of planning our study, there was no data available on whether persistent *E. faecalis* bacteraemia could be used in the same

way, despite this species being one of the most common causes of infective endocarditis. Persistent bacteraemia with *E. faecalis* might be an additional tool that can be used in risk-stratification of patients with bacteraemia.

Paper III

1. Which factors are associated with blood culture time to positivity (TTP) in *E. faecalis* bacteraemia?
2. Can TTP predict 30- and 180-day mortality in *E. faecalis* bacteraemia?

TTP in *S. aureus* bacteraemia is associated with infective endocarditis, and most studies indicate a higher prevalence in patients with a short TTP (40, 82, 170-172). A short TTP is also a predictor of higher mortality in *S. aureus* bacteraemia (170, 171, 173, 180). TTP might be of use as a diagnostic and predictive test in *E. faecalis* bacteraemia, in analogy to its use in *S. aureus* bacteraemia.

Paper IV

1. Is there an association between TTP and time to incubation-start (TTI), that can be used for calculating a corrected TTP?
2. Is TTP corrected for TTI more strongly associated with 7-day mortality than crude TTP?

TTP is dependent on the microbial species growing in a blood culture bottle, and its concentration in blood at the time of sampling. Another factor with a large influence is TTI: With delayed loading of bottles into the blood culture system, TTP becomes progressively shorter (141, 156, 157, 191). This can obscure the association between TTP and mortality (190), and probably also between TTP and infective endocarditis. Since TTI can be highly variable within hospitals and between different hospitals, this is also a large problem when trying to compare results from different studies on TTP, or when trying to implement a TTP cutoff across different centres. Except for two small-scale studies that evaluated crude methods for correcting TTP for TTI without success (157, 164), there are no publications that have studied the correlation between TTP and TTI in detail. If TTP can be corrected for TTI, TTP could be used as a diagnostic and prognostic marker in settings where TTI is long or varies according to the time of day. It would also facilitate comparisons of studies from different centres.

Methods

Common methods and data sources

Geographical setting

All studies in this thesis were conducted in Skåne, a region in southern Sweden that had 1.29 million inhabitants in January 2015, when the first cases in Study III and IV were included, and 1.42 million in December 2023, when the last cases in Study IV were included (226). Emergency healthcare in Skåne is provided by 10 hospitals (Figure 3), the one in Simrishamn is run by a private company and the rest are publicly owned. During 2015, there were microbiological laboratories in both Malmö and Lund, to which all samples for standard microbiological analyses were sent, but during later years all microbiology was concentrated in Lund. The healthcare system in Skåne provides most types of care to its residents, except for liver transplantations. In addition, patients from several other regions in southern Sweden can be referred to hospitals in Lund and Malmö for *e.g.*, heart, lung, and kidney transplantations, highly specialised haematology and oncology, neurosurgery and thoracic surgery. Thus, the microbiological laboratory is almost ideal for population-based studies, although there is a small overrepresentation of more complex cases.

Microbiological methods

All studies in this thesis use data from blood cultures that were analysed with routine methods at the laboratory of clinical microbiology. Since January 2015, the laboratory uses the blood culture system BACTEC FX (BD, Franklin Lakes, USA) and this marks the starting point for two of the studies. The bottle types used during this period were Plus Aerobic/F, Lytic/10 Anaerobic/F, Peds Plus/F and Mycosis IC/F. The standard sampling recommendation was to obtain two aerobic and two anaerobic bottles from peripheral venipunctures, with a filling volume of 8 – 10 ml. The paediatric bottles are intended for small children, formally up to a weight of 10 kg, but they were frequently used in children of all ages, and even in adults in some cases. The Mycosis bottle is specialised for detection of fungi. It is seldomly used in Skåne, and in those cases only in addition to the aerobic and anaerobic bottles.

Cultures of blood in bottles specialised for mycobacteria were not registered as blood cultures.



Figure 3. Map of Skåne and its hospitals. Hospitals with on-site blood culture incubators in red and the smaller hospitals without blood culture incubators in blue. The central microbiological laboratory is located in Lund. The distance from Lund to Simrishamn, Ängelholm and Kristianstad is roughly 80 km. Adapted from "Location map of Skåne County, Sweden" by NordNordWest, CC BY-SA 3.0 DE.

Blood culture incubators were placed in local laboratories for clinical chemistry at the five largest hospitals (Lund, Malmö, Helsingborg, Kristianstad, Ystad, Figure 3), so that bottles could be loaded at all hours. Blood culture bottles obtained at the five smaller hospitals (Ängelholm, Trelleborg, Hässleholm, Landskrona and Simrishamn) were transported to another hospital with a blood culture incubator with scheduled transports. The number of transports was low, in weekends only one per day. The standard incubation time for blood cultures was five days, but this could be increased to 14 days at the discretion of the physician in care of the patient. When a bottle became positive in a decentralised blood culture incubator, it was

transported to the microbiological laboratory during its open hours – approximately 7.30 a.m. to 5.00 p.m. Positive blood cultures were handled according to what was outlined in the chapter on blood cultures in this thesis. Briefly, broth from the bottles was Gram-stained and the results reported immediately. Simultaneously, one bottle per patient was analysed with MALDI-TOF MS using the Sepsityper kit and instruments, software and databases from Bruker Daltonics (Bremen, Germany). Species identification using MALDI-TOF MS was also made on colonies growing on plates overnight, as well as sequencing of the bacterial 16S rRNA gene in rare cases. In addition, VITEK 2 (bioMérieux, Marcy-l'Etoile, France) was used for species identification and AST on a subset of Gram-negative bacilli up to 2022.

Up until October 6th, 2021, the multi-sampling strategy (MSS) for blood cultures, with at least two peripheral venipunctures, was recommended. The recommendation was to obtain both cultures at once, not waiting for the second venipuncture. On October 6th, 2021, the Department of Clinical Microbiology communicated a new recommended procedure for blood culture collection using only one peripheral venipuncture – single-sampling strategy (SSS) – and the use of a vacuum tube for blood collection for initial specimen diversion (ISDT). This corresponds to the intervention in Study I. Both before and after this change, the recommended skin disinfection agent was 5 % chlorhexidine gluconate in 56 % ethanol.

Microbiological databases

The basis for the three retrospective studies in this thesis was data produced during the routine work of the microbiological laboratory. Between 2015 and March 2021, blood culture data was registered in the laboratory information system (LIS) wwLab (previously Autonik, Sweden, now Mylab, Tampere, Finland) and thereafter in LabVantage LIMS (Software Point, Espoo, Finland). The structure of blood culture data differs between these systems, which has had practical implications on the design of our studies. In wwLab, the results of two paired bottles were integrated into one report. The positive/negative status of each bottle was registered, but in cases where more than one species was growing in a bottle pair with two positive bottles, it is not possible to know whether each individual species was growing in one or in both bottles. In LabVantage LIMS, this relationship is fully traceable. The databases of these two systems contain several important variables *e.g.*, patient-ID, laboratory-ID, patient sex and age at the sampling time, sampling time, requesting clinic, sample material, bottle type, TTP, the result of the analysis (positive/negative) and the reported species.

For Study IV in this thesis, we also collected data from Epicenter (BD, Franklin Lakes, USA), a software that controls the blood culture system and links it to the LIS. This data contains laboratory-ID, bottle type, result (positive/negative), timepoint for start of incubation and TTP.

Review of medical records

In Study II and III, data was retrieved through structured manual review of digital medical records. We only had access to records from the Skåne region, and only from the nine public hospitals, which included the absolute majority of the cases. Medical records from primary healthcare were not reviewed. I did this work for Study II and Rebecca Thorén for Study III, with assistance from Magnus Rasmussen when we deemed that there was a need for additional examination. A Windows Excel file with prespecified variables was used during the process.

Data for the modified Duke criteria (70) was recorded, and only episodes that fulfilled criteria for definite infective endocarditis were categorised as infective endocarditis. Other foci of infection were defined according to Berge *et al.* (45) *i.e.* at least two of the following criteria were required in addition to bacteraemia: 1. Typical signs or symptoms of infection. 2. Isolation of *E. faecalis* at the site. 3. Compatible imaging results. Episodes that did not fulfil criteria for localised infection or for definitive infective endocarditis were categorised as unknown origin. We collected data on current diseases for calculating the Charlson comorbidity index (227).

Other databases

For Study IV we needed data on hospital admission date, diagnoses coded according to ICD-10, medications that the patients had retrieved from pharmacies, and vital status including the date of death. This data was acquired from different regional and national databases, and it was assembled by staff at Clinical Studies Sweden, Forum South – an organisation providing support for research and development within healthcare in southern Sweden. Staff at this organisation also merged the clinical data with microbiological data before returning it in a pseudonymised form.

Statistical software

We used R version 4.4.2 for Study I and IV, SPSS Statistics version 26 (IBM) for study II, and SPSS Statistics version 26 plus Stata (StataCorp) for Study III.

Specific methods

Paper I

This was a retrospective observational study. We obtained anonymised data on all blood cultures that were analysed in Skåne from January 2016 to March 2023. The

dataset contained a patient pseudonym with no key, patient age, sample date, the name of the requesting clinic, the number and type of blood culture bottles, and the organisms growing in the cultures. We defined a “sampling event” as all blood culture bottles that were collected from a patient on one date and with a request from the same clinic. Only sampling events in the 10 emergency departments in Skåne were reviewed for inclusion, to decrease the proportion of sampling events with blood drawn from central venous catheters and other long-term intravascular catheters, and only samples obtained from adults (≥ 18 years), since a large part of the paediatric population would only have been sampled with one or two bottles from a single venipuncture. We thereafter excluded sampling events with single aerobic or anaerobic bottles, because of hesitations about whether these were correctly registered, and those with mycosis bottles since those clinical situations were out of the scope of this study. Moreover, the emergency department in Helsingborg had introduced SSS before 2016 and hence sampling events from that hospital were excluded from main analyses, but were used as a reference in one of the analyses.

We did not have data on how individual blood cultures were collected, and we therefore considered all sampling events before October 6th, 2021 as collected with MSS without ISDT, and those from that date as collected with SSS and ISDT. We classified several species and genera that often represent contamination as “contaminant species”, including all coagulase-negative staphylococci (CoNS) except *S. lugdunensis*, *Corynebacterium* species, *Cutibacterium* species, *Bacillus* species and a few other rare species. Other species were considered “relevant pathogens”. When we studied the proportion of sampling events with growth of CoNS in 1-2 and 3-4 out of 4 bottles, the differences in structure of blood culture data that is mentioned under the headline Microbiological databases, made special considerations necessary. We divided the data into two different but overlapping datasets, both only with sampling events consisting of four bottles, to cover both detail around the time of the routine change, and trends over a longer time span:

- Dataset CoNS A spanned the whole study period, January 2016 to March 2023, but only contained sampling events with only one species or negative sampling events (monomicrobial and negative).
- Dataset CoNS B included sampling events from March 2021 to March 2023 and contained all sampling events irrespective of the number of different species (monomicrobial, polymicrobial and negative).

We compared the proportion of outcomes with sampling time before and after the routine change (intervention) with the Chi-square test for categorical variables and the Mann-Whitney U test for continuous variables. As we saw a risk that other factors might have influenced the outcomes of interest, we also made additional multivariable logistic regression analyses where we adjusted for the following variables:

- Patient age categorised into quartiles.
- Waiting time for patients with a suspected infectious disease at the emergency department in question, as a proxy for workload. Data was collected from the database of the National Board of Health and Welfare (Socialstyrelsen). The data was available as the median waiting time per month, and the variable was categorised into quartiles. Sampling events with missing data in this variable were excluded from the multivariable analysis.
- The weekly total number of patients admitted to hospital wards in Skåne with COVID-19. Data was collected from the database of the National Board of Health and Welfare. This variable was categorised into percentiles with breaks at 5, 25, 50 and 75 %, and it was only included in the analysis of dataset CoNS B, where COVID-19 existed during the whole studied time frame.

Paper II

In this study we prospectively included adults with monomicrobial (only one species in the blood cultures) *E. faecalis* bacteraemia and collected repeated blood cultures, consisting of four bottles, 48 – 72 hours after initiation of effective antimicrobial treatment. The study was conducted June 2017 to November 2018. When growth of *E. faecalis* in a blood culture was identified at the microbiological laboratory, I was informed. I or a colleague contacted the staff in charge of the patient, to control whether the patient was in a state where he or she could understand information about the study. Patients who could not understand the information, or who declined participation, were not included. Several episodes affecting the same patient could be included, if more than 7 days had passed since the end of antimicrobial treatment for the previous episode.

We conducted a retrospective review of medical records covering 180 days after the index bacteraemia. Classification of the focus of infection and Charlson comorbidity index is described under the heading Review of medical records. We collected data for classifying the infection as nosocomial, healthcare-associated or community-acquired according to Friedman *et al.* (228). Complicated infection was defined as infective endocarditis, infection involving foreign material, undrained abscesses, septic arthritis or osteomyelitis. Hypothesis testing of differences between categorical outcomes were made using Fisher's exact test. The difference between TTP in initial and repeated cultures was tested with the paired Wilcoxon rank sum test.

Paper III

This was a retrospective study on adults with monomicrobial *E. faecalis* bacteraemia in Skåne in 2015 to 2018. We limited inclusion to the five hospitals with on-site blood culture incubators, as the long time to incubation start (TTI) in the other hospitals was thought to potentially disturb the analyses. Data on the number of positive blood cultures and TTP was provided by the Department of clinical microbiology. The shortest TTP among the blood culture bottles obtained the first 24 hours was used in all analyses. We acquired clinical data through manual review of medical records and classified infectious foci, infective endocarditis cases and Charlson comorbidity index according to the description under the headline Review of medical records. We collected data on risk factors for infective endocarditis, known from previous studies (45, 86, 229, 230). The records were followed up to at least 180 days after the index blood culture. An episode started with blood cultures with monomicrobial growth of *E. faecalis* and ended after at least seven days of effective antimicrobial treatment or otherwise after 30 days. Multiple episodes per patient could be included. An episode was considered nosocomial if the index blood culture was obtained > 48 hours after admission to hospital, and otherwise as community acquired. We excluded episodes with no access to medical records (the patient was not admitted to public healthcare in the Skåne region), and episodes where the patient had obtained antimicrobial treatment within seven days preceding the index blood culture. The clinical outcomes were 30- and 90-day mortality, relapse bacteraemia within 180 days and infective endocarditis.

In statistical analyses, patient age was categorised into three groups to give similar sizes, 18 – 70, 71 – 80 and ≥ 81 years. Charlson comorbidity index was categorised into three groups, 0 - 2, 3 – 4 and ≥ 5 . TTP was analysed both as a continuous variable and dichotomised to ≤ 12.0 or > 12.0 based on its median value. Continuous TTP was compared between categorical variables using the Mann-Whitney test and the Kruskal-Wallis test. Dichotomised TTP was analysed using the Chi-square test. A logistic regression analysis was conducted with $TTP \leq 12.0$ as the outcome, including the covariates sex, age, Charlson comorbidity index, nosocomial vs. community-acquired acquisition and focus of infection. A receiver operating characteristics (ROC) curve was made with IE as the outcome and TTP as the exposure.

Paper IV

In this retrospective study, we used a large dataset of all blood cultures in Skåne from 2015 to 2023 to first study the association between TTP and TTI and to calculate correction factors, and thereafter, as a proof of concept, to test whether corrected TTP was more strongly associated than crude TTP with 7-day mortality.

We obtained blood culture data from the two LIS used during the period, and from Epicenter. Reliable timestamps for sampling time and the time for incubation start were important for calculating TTI. The sampling time was originally written on paper request forms and was entered by laboratory staff into the LIS, and could thus be absent, incomplete (date only) or incorrect. Data on 7-day mortality and comorbidity was obtained from healthcare databases.

We decided *a priori* to make separate analyses for aerobic and anaerobic bottles, and to exclude paediatric bottles as these were much fewer, have a more variable target sample volume, and were thought to contain significantly more contaminant growth. We grouped all species into 13 microbial groups, with the most common species *E. coli* and *S. aureus* having their own groups. We conducted separate analyses for all positive bottles irrespective of the species, and for individual microbial groups. The association between TTP and TTI was modelled in two different ways: We primarily sought simple linear associations that would be easy to use in clinical practice. The equation for these models was

$$TTP = \beta_0 + \beta_1 * TTI$$

where β_1 is the slope of the regression line and thus the potential correction factor, while β_0 , the y-axis intercept, represents TTP as if it were unaffected by TTI. Thus, the corrected TTP (TTP_{corr}) can be calculated as follows:

$$TTP_{corr} = TTP - \beta_1 * TTI$$

Secondly, we used restricted cubic splines with four knots to investigate potentially non-linear associations between TTI and TTP. Linear and non-linear models were compared using analysis of variance (ANOVA), and they were also compared to a so-called null-model where there was no association between TTI and TTP.

Starting from the whole dataset, we excluded observations where the bottle was paediatric, a Mycosis-bottle or of unknown type. We then excluded bottle sets with more than one species, as our analyses were stratified by microbial group, bottles with missing TTP and bottles with missing or incorrect sampling time or time for incubation start. The resulting dataset was used for the derivation of correction factors. We thereafter excluded potential contaminants, based on a list of common contaminant species (including CoNS, *Bacillus* species and *Corynebacterium* species), if the species was only growing in one blood culture set, irrespective of whether there was only one collected set or several ones. All additional blood cultures obtained within 30 days after the first positive bottle were removed from the data, as well as all bottles but the bottle with the shortest TTP. Finally, patients with missing data on 7-day mortality were excluded, resulting in the final dataset where the association between TTP and 7-day mortality was studied.

The association between crude TTP, corrected TTP and 7-day mortality was studied using restricted cubic splines as per Ljungquist *et al.* (190). Both species-independent and species-specific correction factors were tested.

Research ethics

The research followed the declaration of Helsinki. For Study I, ethical permission was not necessary according to Swedish law, as all data was anonymised when we received it. Study II was the only study in which patients were recruited prospectively. The patients received oral and written information about the study and its procedures, and a signed consent to participation was mandatory for inclusion. The procedures of Study II were permitted by the regional ethical committee in Lund (Dnr 2016/219). Study III and IV were completely retrospective and individual consent from patients was waived by the regional ethical committee in Lund (Dnr 2018/898) in the case of Study III, and by the Swedish Ethical Review Authority (Dnr 2023-03076-01) for Study IV.

Results

Paper I

Out of 149 746 sampling events that were reviewed for inclusion, 117 142 sampling events remained in the main dataset after the exclusion of 1268 single aerobic or anaerobic bottles and sampling events containing Mycosis bottles, and 31 336 sampling events from the Helsingborg hospital. The proportion of sampling events in which at least four bottles were collected, increased from 87.6% before the routine change to 92.3% after ($p < 0.001$). This change was not observed in the Helsingborg emergency department, where SSS had already been implemented (Figure 4). The proportion of sampling events with growth of relevant species remained unchanged at 13.6%, while the proportion with growth of contaminant species decreased from 5.8% to 5.5% ($p = 0.056$). Most of the contaminant species were CoNS, and the proportion of sampling events with CoNS changed from 5.2% to 4.8% ($p = 0.039$).

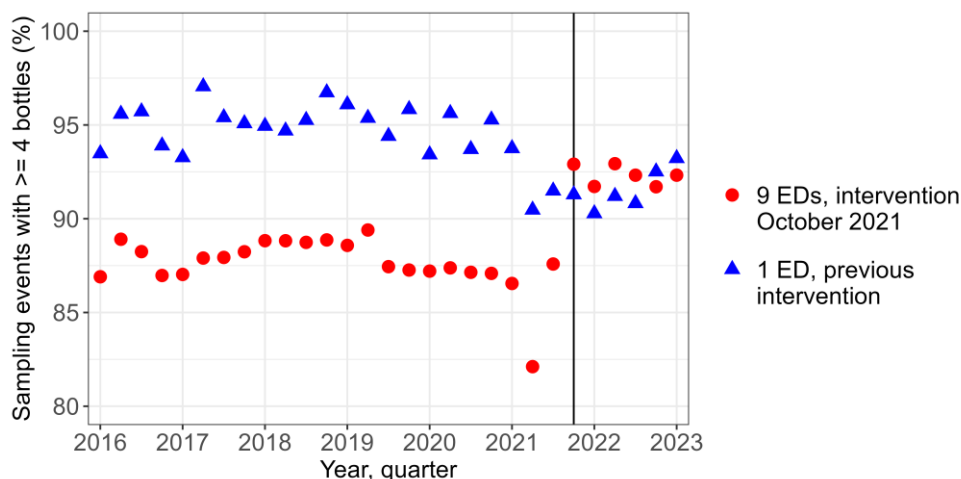


Figure 4. The proportion of sampling events with at least four bottles, represented by quarter. In nine emergency departments (EDs) single-sampling strategy was implemented in October 2021, marked by the vertical black line. In one ED, SSS was implemented before 2016. The y-axis is cut at 80%. Figure adapted from Paper I.

The dataset CoNS A was constructed from the complete dataset, after exclusion of polymicrobial sampling events and sampling events with less or more than four collected bottles and consisted of 100 064 sampling events. The CoNS B dataset was constructed from the complete dataset by exclusion of sampling events prior to March 2021 and sampling events with less or more than four collected bottles, resulting in 32 538 sampling events. In the CoNS A dataset, the proportion of sampling events with CoNS in 1-2 out of 4 bottles decreased from 4.0% to 3.3% ($p < 0.001$), while the proportion with CoNS in 3-4 out of 4 bottles increased from 0.39% to 0.49% ($p = 0.029$). The progression of these two proportions over time are visualised in Figure 5. In the CoNS B dataset, the proportion of sampling events with CoNS in 1-2 out of 4 bottles decreased from 5.2% to 3.8% ($p < 0.001$), and the proportion with CoNS in 3-4 out of 4 bottles increased from 0.62% to 1.0% ($p = 0.0016$). The multivariable analyses that included adjustment for patient age, waiting time in the emergency department and COVID-19 admission rate, did not change these results.

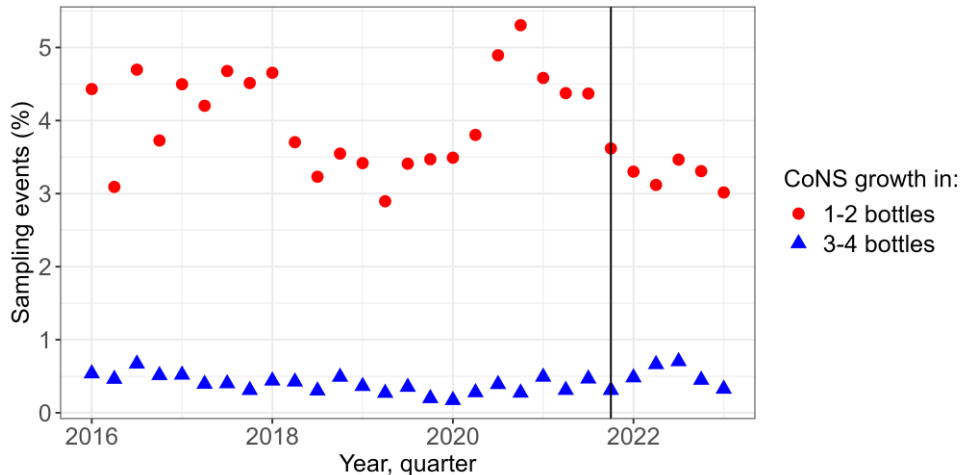


Figure 5. The proportion of sampling events with growth of coagulase-negative staphylococci (CoNS) in 1-2 and 3-4 out of 4 bottles, presented per quarter. Data from the CoNS A dataset. The implementation of single-sampling strategy and initial specimen diversion in October 2021 is marked by the vertical black line. Figure adapted from Paper I.

Paper II

The investigators were informed of a total 142 episodes of monomicrobial *E. faecalis* bacteraemia during the study period. In 31 episodes the ward was not contacted due to a lack of time on the side of the investigator. In 22 episodes, the attending physician did not have time for including the patient or considered the patient otherwise inappropriate. In 21 episodes the patient was not able give informed consent, often due to dementia, temporarily decreased cognitive function or, less commonly, unconsciousness. In 9 cases the patient was not admitted to a hospital in Skåne. In three cases the patient declined participation, and in two cases the patient had died before inclusion was possible. This left 54 episodes in 49 patients, but repeated blood cultures were only collected in 50 episodes in 47 patients. In 42 of these episodes, the new cultures were sampled in the pre-defined interval of 48 – 72 hours after initiation of active antimicrobial treatment, whereas it was sampled earlier in 3 episodes and later in 5 episodes. The following presentation contains all the episodes irrespective of the time for repeated sampling.

In 5 of the 50 episodes (10%), there was persistent growth in the repeated blood cultures. In 4 of these 5 episodes, the patient had a complicated infection according to our definition: Two had infective endocarditis, one had cholangitis that had not been drained, and one had an infected subcutaneous venous port that was not removed. The fifth had an infection with an unknown focus, a negative TOE, and no relapse within 180 days after 15 days of treatment. In the episodes without persistent bacteraemia, there were 5 episodes with infective endocarditis and 1 with aortic graft infection. The proportion of complicated infections was thus 4 / 5 (80%) in episodes with persistent growth and 6 / 45 (13%) in episodes without persistent growth ($p = 0.004$).

The median initial TTP in episodes with persistent growth was 9.4 hours, and median TTP in repeated cultures was 14.5 hours ($p = 0.13$).

Table 7. Clinical features of episodes with positive and negative repeated cultures.

Baseline characteristics	Positive repeated culture (n = 5)	Negative repeated culture (n = 45)
Age (years, median, IQR ¹)	78 (72 - 83)	82 (71 - 87)
Female	3 (60%)	12 (27%)
Charlson comorbidity index	2 (1.5 - 5.5)	2.5 (1 - 4)
Microbiology		
≥ 2 Positive cultures initially	5 (100%)	34 (76%)
TTP ² (median and IQR)	9.4 (6.6 - 16)	11 (9.7 - 13)
Site of acquisition		
Community	0	12 (27%)
Healthcare associated	5 (100%)	25 (56%)
Nosocomial	0	8 (18%)
Focus of infection		
Urinary tract	0 (0%)	18 (40%)
Biliary or abdominal	1 (20%)	2 (4%)
Infective endocarditis	2 (40%)	5 (11%)
Other ³	1 (20%)	2 (4%)
Unknown	1 (20%)	18 (40%)
Complicated infection	4 (80%)	6 (13%)

¹Interquartile range. ²The shortest blood culture time to positivity (TTP) in the initial cultures. ³One with subcutaneous venous port infection, one with endometritis and one with aortic graft infection. Table adapted from Paper II.

Paper III

Out of 359 patients that were reviewed for inclusion, 23 were excluded due to antibiotic treatment within one week from the blood culture and 13 were excluded due to unavailable medical records or TTP. The number of included patients were 323, contributing with 367 episodes. TTE plus TOE was performed in 90 (24.5%) of the episodes and only TTE in a further 70 (19.1%) episodes.

Median TTP was 11.6 hours (IQR 9.9 – 14.1). When analysing the distribution of TTP in different variables, TTP was significantly shorter in episodes with infective endocarditis, and in episodes that were community acquired (Table 8). When dichotomised TTP (≤ 12.0 and >12.0 hours) was the outcome in a multivariable analysis adjusted for sex, age, Charlson comorbidity index, site of infection acquisition, and focus of infection, only infective endocarditis was associated with a short TTP with statistical significance (OR 13.0, 95% CI 4.4 – 38).

Variables that were hypothesised to be associated with infective endocarditis were thereafter analysed, and previous *E. faecalis* bacteraemia, community acquisition of infection, duration of symptoms for at least seven days, embolisation, at least two positive blood cultures, unknown origin of infection, valve disease, auscultation of murmur and TTP ≤ 12.0 hours were associated with infective endocarditis.

Table 8. Distribution of time to positivity (TTP) within different variables. Adapted from Paper III.

	TTP median, h (IQR) ¹	p
Sex		
Female (104, 28%)	11.6 (6.9-13.0)	0.34
Male (263, 72%)	11.6 (8.1-14.3)	
Age		
18-70 (112, 31%)	11.7 (7.6-14.5)	0.54
71-80 (135, 37%)	11.7 (8.2-13.8)	
81-96 (120, 33%)	11.2 (8.0-13.8)	
Charlson score		
0-2 (209, 60%)	11.6 (10.0-14.1)	0.15
3-4 (93, 25%)	11.5 (9.4-13.3)	
>5 (65, 18%)	12.3 (10.2-15.6)	
Site of acquisition		
Community acquired (308, 84%)	11.5 (9.6-13.9)	0.04
Nosocomial (59, 16%)	12.3 (10.7-14.2)	
Focus of infection		
IE (55, 15%)	9.4 (6.4-10.6)	<0.01
Urinary tract (136, 37%)	12.1 (10.6-14.4)	
GI and biliary (29, %)	12.5 (11.1-14.4)	
Skin and soft tissue (27, 7%)	12.4 (10.5-16.1)	
Skeletal and joint (15, 4%)	13.6 (11.4-22.1)	
Other known ² (10, 3%)	12.7 (9.7-15.4)	
Unknown (94, 26%)	11.7 (10.1-14.1)	

¹ Interquartile range. ² Pneumonia n = 7, other airway infections n = 3.

In the ROC analysis for TTP vs. infective endocarditis, the area under the curve (AUC) was 0.80 (95% CI 0.74 – 0.86). The sensitivity for IE at the cutoff ≤ 12.0 hours was 93% (95% CI 83 – 97%) and the specificity was 51% (95% CI 45 – 56%). We constructed a new risk stratification score, DETOVA, where the variable “at least two positive blood cultures” in DENOVA was substituted by TTP ≤ 12.0 hours. We compared the ROC-curves for the DENOVA and DETOVA scores in their prediction of infective endocarditis (Figure 6). The AUC of both DENOVA and DETOVA was 0.98 (95% CI 0.97 – 0.99), the difference was not statistically significant ($p = 0.70$). When considering > 2 points as positive, the sensitivity of DENOVA was 100% (95% CI 94 – 100%) and the specificity 89% (95% CI 85 – 92%), while the sensitivity for DETOVA was 98% (95 % CI 90 – 100%) and the specificity 90% (95% CI 86 – 93%).

The 30-day all-cause mortality was 11% and there was no difference in TTP between deceased patients and survivors (median TTP 11.0 and 11.7 hours, respectively, $p = 0.35$). 180-day mortality was 24%, with no significant difference in TTP between diseased patients and survivors. Relapse of *E. faecalis* bacteraemia within 180 days was detected after 44 episodes, and there was no statistically significant difference between the TTP in episodes that were followed by a relapse and those that were not.

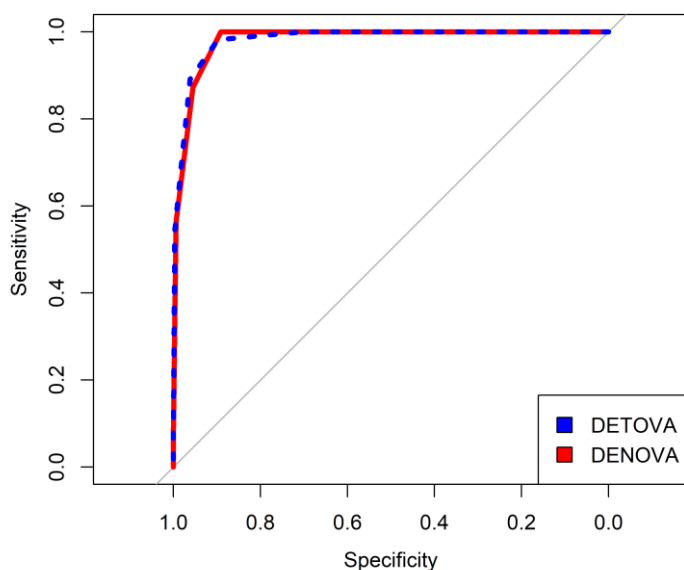


Figure 6. Receiver operating characteristics curve comparing the DENOVA and DETOVA scores.

Paper IV

Out of the total dataset of 136 969 positive blood culture bottles, 100 829 bottles were included in the dataset for derivation of correction factors, and 31 723 episodes were included in the analysis of outcome. We plotted the linear and non-linear models of TTP vs. TTI with no stratification based on microbial group (see Figure 2 in Paper IV) and found that the predictions of the non-linear models were similar to those of the linear models. The species-independent correction factor for aerobic bottles was -0.27, meaning a decrease in TTP by 16 minutes for each hour of delayed loading of the bottles, and -0.32 for anaerobic bottles, corresponding to a decrease of TTP by 19 minutes. In species-specific analyses, we found statistically significant linear associations between TTI and TTP in all microbial groups except for *Pseudomonas* species in anaerobic bottles, fungi in aerobic bottles, and the two heterogenic groups “Other anaerobic” and “Other species”. We considered the linear models to be sufficient or more realistic for our purpose of correcting TTP in all cases, although there were instances where the non-linear models resulted in a better fit to the data.

As a first simple test of the effect of the correction, the median TTP of blood cultures obtained at hospitals in Skåne with different median TTI was compared. The blood cultures obtained at hospitals with a longer median TTI had a shorter median crude

TTP, but after calculation of corrected TTP, the median corrected TTP of all hospitals was similar.

In the main analysis with 7-day mortality as the outcome, all 31 723 episodes were included, with correction of TTP based on species-independent correction factors. For TTP values under approximately 10 hours, the association with mortality was stronger for corrected TTP compared to crude TTP *i.e.*, the predicted mortality rate was higher for a given TTP (see Figure 4 in paper IV). In hospitals without on-site incubators there was no significant association between crude TTP and 7-day mortality, but the association was restored after correction.

We further compared species-independent and species-specific correction of TTP, using 7-day mortality as the outcome, in the subset of episodes where species-specific linear correction factors could be established ($n = 28\,499$). The difference between the two methods for correction was small. Finally, we studied the association between corrected and crude TTP and 7-day mortality within the individual microbial groups. There was an obvious association between TTP and 7-day mortality in *E. coli*, *S. aureus*, beta-haemolytic streptococci and *S. pneumoniae*. There were trends for associations, but with wider confidence intervals, for Other Enterobacterales, *Enterococcus* species, *Pseudomonas* species and *Bacteroides* species. In contrast, there were no discernible associations between mortality and TTP for CoNS, alpha-haemolytic streptococci and fungi, regardless of correction.

Discussion

Sampling methods

In Study I we found that after the introduction of SSS and ISDT, the proportion of sampling events with at least four bottles increased, and the proportion of sampling events with growth of relevant pathogens stayed unchanged. Overall, there was a small decrease in the proportion of sampling events with CoNS. The proportion of sampling events with growth of CoNS in 1-2 out of 4 bottles decreased, but there was an opposite, although smaller in absolute numbers, increase in the proportion with growth of CoNS in 3-4 out of 4 bottles.

An increase in the proportion of sampling events consisting of at least four bottles after introduction of SSS has also been demonstrated by Ekwall-Larsson *et al.* (129), who reported an increase by 35% from 71% to 96%. In our study, that increase started from a higher level, 87.6% to 92.3%, corresponding to an increase by 5.4%. The mechanism behind the increase has not been studied, but it is reasonable to assume that when the MSS is used, there are many possible reasons for not obtaining the second culture *e.g.*, lack of time, failure of finding a second vein for sampling, interference with other procedures around the patient *etc.* Moreover, there may be a conflict of interest between spending time on multiple sampling and avoiding delays in administration of antimicrobial treatment. When SSS is used, and if there is no problem with the venous access, many of these reasons become irrelevant and all four (or six) bottles can be collected from one venipuncture, with a significant saving of time compared to MSS (131). Ekwall-Larsson *et al.* additionally found an increased proportion of patients with relevant findings in their blood cultures when using SSS, which was explained by the increase in the proportion of patients that were sampled with at least four bottles (129). In contrast, we saw no such increase, possibly because of the proportionally lower increase of sampling events with an adequate number of bottles in our study. It is likely that hospitals with a high proportion of “solitary blood culture sets” will achieve larger benefits on the sensitivity of blood cultures when SSS is introduced.

We observed a small decrease in the overall proportion of sampling events with growth of CoNS after the intervention, irrespective of the number of positive bottles. Given the simultaneous introduction of SSS and ISDT, it is not possible to evaluate their relative contributions to this effect, and our study must be seen as an evaluation of the combined implementation. Despite a theoretical advantage of SSS over MSS

regarding contamination rate (126), only one study has so far reported a statistically significant decreased contamination rate after introducing SSS (130). Using an ISDT of the same type as that in our study has been reported to reduce the contamination rate with 30% to 66% (121-123) – significantly more than the overall reduction in CoNS of 8% that we observed. However, we made no attempts to separate true CoNS bacteraemia from contamination, as most of the previous studies did, which makes this comparison difficult. If contamination were to be defined by growth of CoNS in only 1-2 out of 4 bottles, the decrease was 18% or 27%, depending on whether the dataset CoNS A or B was studied. One possibility for why we saw a smaller decrease than previous studies, could be that the ISDT was simply not used, or used incorrectly, in a large proportion of the sampling events after the intervention.

The evidence for using the number of positive bottles with growth of CoNS as a criterion for contamination or true infection is not unanimous, with some studies advocating its use (133-135, 138) and others recommending against it (136, 137). Still, the number of positive bottles is more often than not incorporated into the definition of contamination in studies on blood culture contamination, and it is frequently used in clinical practice. The increase in the proportion of sampling events with CoNS in 3-4 out of 4 bottles, that we observed after the introduction of SSS and ISDT, has not been reported before and it could have two different causes. Firstly, the increase may have been due to an increased number of patients presenting at emergency departments with CoNS infections. The observed increase was at least 26%, and we are not aware of a change in the management of patients in our region, that could explain such a large increase in patients with CoNS infections at emergency departments. Secondly, the increase may have been caused by an increase in sampling contaminations that affected multiple bottles. It can be imagined that if SSS is used with poor technique *e.g.*, inadequate skin antisepsis, there is an increased risk of contaminating all bottles. As I see it, this risk is one of the reasons for recommending MSS, although it is seldom clearly stated. If there was an increase in the number of patients that were erroneously believed to have true CoNS bacteraemia, it could have led to an increased morbidity of those patients, as well as increased healthcare costs (114-117).

The strengths of Study I is a dataset that is significantly larger than any previous study on SSS, and our attempts at adjusting the outcomes for confounding factors, *i.e.* other important changes that occurred over time. Another strength is that we studied the number of bottles with growth of CoNS without *a priori* assumptions about what was to be considered a contamination or a relevant finding. The largest weakness is the retrospective design, which makes it impossible to establish a causal relationship between intervention and observed outcome with certainty. Because of the lower number of observations, it is especially difficult to know whether the increase in the proportion of sampling events with CoNS in 3-4 out of 4 bottles is an effect of the intervention, or an unusually pronounced fluctuation for other

reasons. Although we tried to adjust the outcomes for concomitant changes in the workload of emergency departments and in COVID-19 incidence levels, the variables that were available for doing so were very crude. The absence of data on whether individual samplings were conducted using SSS or MSS, with or without ISDT, and using peripheral venipunctures or central venous catheters, is also a major limitation. I believe that the rapid increase of sampling events with at least four bottles to previously unseen levels directly after the intervention (Figure 4), is a trustworthy indication that SSS was adopted quickly. It is more difficult to assess the frequency of ISDT use, and there is a risk that it was used less often. A manual review of the medical records of patients with CoNS might have given us a better understanding of whether they had contaminated cultures or true bacteraemia. However, such a review was out of the scope of this study, and we did not have access to the patient IDs.

Despite the many limitations, I think that Study I is a relevant description of what one can expect when simultaneously introducing SSS and ISDT in a large healthcare organisation with limited accompanying education. Some things improve, such as the proportion of sampling events with an adequate number of bottles, and the proportion of sampling events with CoNS in 1-2 out of 4 bottles, while there may be an insidious increase in contaminations affecting all bottles. It is likely that the positive aspects would be larger, and the negative aspects less pronounced, if more education had been given at the introduction of the new sampling routines.

Repeated cultures

We found persistent bacteraemia in 10% of episodes of *E. faecalis* bacteraemia. Persistent bacteraemia was more common when the patient had a complicated focus of infection, but most patients with a complicated focus of infection did not have persistent bacteraemia.

The overall prevalence of persistent bacteraemia of 10% in our study was lower than what has been reported for *S. aureus*, with proportions ranging from 17% to 49% in different studies that aimed to include all patients without bias for more or less complicated infections (40, 41, 43, 206, 207). In a study by Wiggers *et al.*, 20% of patients with enterococcal bacteraemia had positive repeated blood cultures, but these data also include *E. faecium*, which may behave differently, and it is also likely that many repeated cultures were collected before active antimicrobial therapy was started. Moreover, in that retrospective study, only 48% of patients with enterococcal bacteraemia were selected for repeated cultures, on unknown grounds, which likely means that the proportion of complicated infections was higher in that group (211). Thus, the result in our less biased cohort may be comparable. Wiggers

et al. reported an even lower prevalence of persistent bacteraemia in viridans-group streptococci (2/42, 5%) and beta-haemolytic streptococci (0/23, 0%) (211).

After our study, there has been many new results reported on persistent bacteraemia in *Enterococcus* species. In a retrospective study by Bussini *et al.* (231), repeated blood cultures were obtained in 32% of patients with monomicrobial bacteraemia. Persistent bacteraemia, defined as growth in cultures obtained ≥ 72 hours after initiation of active antimicrobial treatment, was found in 10% of patients with *E. faecalis* and 18% of patients with *E. faecium* bacteraemia. Out of 27 patients with infective endocarditis, 8 (30%) had persistent bacteraemia while 19 did not. Persistent bacteraemia was also associated with haematologic malignancy and treatment with daptomycin (231). A retrospective American study on enterococcal bacteraemia with mixed species, but dominated by *E. faecalis*, found persistent bacteraemia (at least 5 days) in 12% of cases (232). In both of these studies, there is no information on how patients were selected for sampling with repeated blood cultures, and it is likely that patients with infective endocarditis and other complicated infections were enriched (231, 232). Freling *et al.* performed a retrospective study where patients with infective endocarditis and controls with *E. faecalis* bacteraemia for other reasons were studied (113). Persistently positive blood cultures for at least 2 days resulted in a positive likelihood ratio of 6.32 (95% CI 3.39 – 11.79) for infective endocarditis. In a large Spanish study specifically on patients with enterococcal infective endocarditis (233), 13.0% of cases with *E. faecalis* and 16.7% with *E. faecium* infective endocarditis had positive repeated cultures after more than 7 days of effective antimicrobial treatment. Persistent bacteraemia was a risk factor for relapse of bacteraemia within six months, but not for 1-year mortality (233). Despite the differences in definitions of persistent bacteraemia, these results are well in line with our study, suggesting a prevalence of persistent bacteraemia of approximately 10% in *E. faecalis* bacteraemia with mixed infectious foci, and approximately 30% in patients with infective endocarditis. Notably, some studies have used persistent bacteraemia at a late timepoint as a marker for treatment failure (233, 234), rather than as a marker for complicated infection, as we intended.

Is it motivated to repeat blood cultures for risk-stratification of patients with *E. faecalis* bacteraemia? The results of our study and those conducted by others, suggest that there is a potential for using it. On the other hand, the sensitivity for infective endocarditis and complicated infections is only around 30% *i.e.*, absence of growth in repeated cultures does not exclude a complicated infection. Another problem with repeated cultures in enterococcal bacteraemia, is that it easily becomes a lengthy business: In our region, the empirical treatment for patients with an infection of unknown focus is often a cephalosporin, which has no effect against enterococci. Thus, it may take one or two days from the initial culture before the aetiology becomes known, and the antibiotic treatment is changed. Thereafter, another two to three days of waiting ensues before the repeated cultures are

obtained. Since the time to positivity might be lengthened during antimicrobial treatment (197, 198), the positive result of a repeated culture may be further delayed. To somewhat shorten the total time, it is possible that repeated cultures can be collected 24 hours after initiation of effective treatment, as suggested by Kuehl *et al.* in *S. aureus* bacteraemia (207). The relatively low proportion of persistent bacteraemia with enterococci compared to *S. aureus* might also support the appropriateness of a shortened waiting time before cultures are repeated.

In hospitals where repeated blood cultures are routinely collected in *E. faecalis* bacteraemia, persistent growth after initiation of effective treatment should be considered a marker for increased risk for a complicated infection. We do not have enough data, however, to recommend repeated cultures where this is not already a routine. Repeated blood cultures have a cost in money and staff resources, and there is also a risk of obtaining contaminated cultures or irrelevant findings that can lead to even more analyses being necessary. If further studies are made, they should ideally be without bias as to why repeated cultures were obtained. They should also evaluate whether persistent bacteraemia adds any information of importance, that is not already contained in the DENOVA score for infective endocarditis (45), in TTP, or that is easily found on clinical examination or when taking patient history.

The strength of Study II is that it is the only one, to my knowledge, that has included patients prospectively, which reduces the risk for selection bias. A major limitation are the logistical problems, which led to difficulties in asking all eligible patients for inclusion, and thus a small sample size. A pre-defined structure utilising *e.g.*, research nurses at the different hospitals would have been preferable. Furthermore, we could not include patients who were unconscious or who had an acute or permanently impaired cognitive function. The prevalence of complicated infection might have been different in these populations, potentially leading to incorrect estimates of sensitivity and specificity of repeated cultures in a mixed population. In addition, the clinicians in care of the patients were not blinded to the results of the repeated blood cultures. It might thus be that persistent bacteraemia led to further investigations, and absence of persistent bacteraemia led to reduced ambitions in finding complicated infections, but this was not something that was expressed explicitly in the medical records.

Time to positivity

In Study III we used a retrospective study design with manual review of medical records to demonstrate for the first time that TTP is of potential use as a diagnostic tool in *E. faecalis* bacteraemia – a shorter TTP means a higher probability for infective endocarditis. In the retrospective Study IV, we used a large dataset to develop a method for correcting TTP for a major disturbing factor – time to

incubation start (TTI), and we found that corrected TTP was more strongly associated with 7-day mortality than crude TTP. This is an additional proof of the increased risk of death in bacteraemia with short TTP, but most importantly, it is a proof-of-concept. Correction of TTP may facilitate a more efficient use of TTP as a predictive and diagnostic test in future studies and in clinical practice.

The observation that TTP in *E. faecalis* infective endocarditis is generally shorter than TTP in the setting of other foci of infection, is similar to what has been described for *S. aureus*. In several publications, the majority of cases of infective endocarditis have had a TTP in the lower half of the total distribution (40, 82, 170-172). In the studies by Kahn *et al.* and Strömdahl *et al.*, ROC-analyses resulted in AUCs of 0.83 and 0.72 for the performance of TTP alone as a diagnostic test for infective endocarditis (40, 82) and this can be compared with 0.80 in our study. In other words, the TTP in *E. faecalis* bacteraemia seems to contain as much information of interest for the diagnosis of infective endocarditis, as it does in *S. aureus* bacteraemia. To add a different reference to this number, the AUC of the fungal biomarker β -D-glucan as a diagnostic test for *Candida* blood stream infection is 0.81 (95% CI 0.76 – 0.86) with the assay that is currently used in Skåne (235). However, continuous variables like TTP must in some way be categorised by cutoffs into either a binary positive / negative, or multiple levels (*e.g.*, negative, borderline, low positive, high positive *etc.*), if they are to be used by clinicians. Following the practice of other authors, we decided on a cutoff at ≤ 12.0 hours, based on its proximity to the median. The sensitivity and specificity for infective endocarditis using this cutoff was 93% and 51%, corresponding to a negative predictive value (NPV) of 98% for TTP > 12.0 . Strömdahl *et al.* suggested > 13 hours as a cutoff and this resulted in a NPV of 96% for infective endocarditis. These authors were criticised by Tonizzo *et al.* and Santos-Patarroyo *et al.*, who found 13 hours to be a significantly less sensitive cutoff in their datasets (236, 237).

Since Paper III was published, other research groups have published articles on the association between TTP and infective endocarditis with *E. faecalis* as well. Michelson *et al.* (238) studied a dataset including 54 patients with *E. faecalis* bacteraemia and found an association between shorter TTP and cardiovascular infection (mainly infective endocarditis), with an ROC AUC of 0.75. With a cutoff of ≤ 10.50 hours, the sensitivity for intravascular infection was 82% and the specificity 67%. It can be noted that the median TTP for *E. faecalis* was shorter than in our study, 9.2 hours with an IQR of 3.6 to 13.4 hours. There is no description of the blood culture logistics or TTI, but with a distribution that is so shifted to lower values, it is likely that the TTP is heavily influenced by TTI. Pérez-Rodríguez *et al.* (89) studied 121 patients with *E. faecalis* bacteraemia, of whom 39 had infective endocarditis. The authors observed a shorter median TTP in patients with infective endocarditis, but it was not statistically significant (10 hours, IQR 8.0 – 13.0 vs. 11 hours, IQR 8.6 – 13.7, $p = 0.175$). A complicating factor that is mentioned, is that 13% of the controls had catheter-related bacteraemia and therefore a significantly

shorter TTP. The authors added TTP in a dichotomised form to the DENOVA score but found no improvements of the diagnostic performance of the score. In a retrospective study of 27 patients with definite *E. faecalis* infective endocarditis and 87 with other or unknown foci of infection, Robson *et al.* (239) did not find a significantly shorter TTP in the infective endocarditis group, 11.3 hours, compared to all others, 10.6 hours ($p = 0.07$). When 15 patients that were treated as infective endocarditis but did not fulfil the 2023 Duke-ISCVID criteria were moved to the endocarditis group, the TTP was shortened, but there was still no statistically significant difference compared to the controls. The median TTI in this study was 3.5 hours (IQR 1.9 – 5.4) (239). Finally, a non-peer reviewed publication by Deas *et al.* (240) retrospectively studied a dataset with 109 *E. faecalis* bacteraemia cases, of which 20 had infective endocarditis. The median TTP was 11 hours (IQR 9 – 14) and the authors found no statistically significant association between TTP and infective endocarditis, although such an association was found for *S. aureus* in the same study. In short, our results of a strong inverse correlation between TTP and probability of infective endocarditis in *E. faecalis* bacteraemia, have not been reproduced. The reasons for this are hard to comprehend, but smaller sample sizes in the later studies, perhaps in combination with a somehow less robust association than that seen between TTP and infective endocarditis in *S. aureus* bacteraemia, could be an explanation. Only one of the studies reported on TTI (239), and their reported value was higher than what we observed in hospitals with blood culture incubators in Study IV. The fact that TTE or TOE was only used in 44% of the episodes in our study, might lead to a higher rate of infective endocarditis cases that were not detected in the control group. However, that should have resulted in a weaker association with TTP, unless the undetected cases of infectious endocarditis systematically had longer TTPs.

Three important points can be discussed based on the published studies: Firstly, TTP should probably be combined with other clinical data when it is used as a diagnostic test. In none of the articles do the authors recommend clinicians to use TTP without regard of other information, it is rather suggested to be “one of many pieces in the diagnostic puzzle” (241). In my experience, clinical decisions are often based on multiple variables, but it can be hard to interpret certain test results, especially if it is a test that one has little experience of. Another approach is thus to validate an integration of TTP with other clinical information. Kahn *et al.* (40) included TTP in their POSITIVE score for *S. aureus* bacteraemia, which was a better diagnostic tool than TTP by itself. In an analysis that was not included in the published Paper III, we found that the diagnostic performance of the DENOVA score remained practically unaltered when the variable “number of positive blood cultures ≥ 2 ” (N) was replaced by $TTP \leq 12.0$ hours (T), to give a DETOVA score. Pérez-Rodríguez *et al.* (89) added TTP on top of the DENOVA score, but this makes little sense, as the “N-” and “T-variables” are likely highly collinear. As mentioned in the introduction on blood cultures in this thesis, the number of positive blood cultures or bottles, is a rough measure of the concentration of bacteria in blood, just as TTP

is. If a bacterial species like *E. faecalis*, that can physiologically grow in all bottles, is only growing in one or two bottles, it is likely that the original concentration in blood was low (105). In the case of *E. faecalis* bacteraemia, it seems that growth in at least two cultures and TTP below the median, carries similar information. The reasons why we did not include the “DETOVA score” in the final publication were that it was a *post-hoc* analysis, and that we could not verify it in a separate cohort, as the authors did with the DENOVA and POSITIVE scores (40, 45).

A second point is that information is lost when TTP is handled as a binary variable. It might be that more information could have been gathered from TTP than from the number of positive cultures, if the continuous variable had been divided into several intervals or not categorised at all. In the POSITIVE score, Kahn *et al.* (40) divided TTP into four different categories with no point added if TTP is ≥ 13 hours, and 2, 3 and 5 points added for sequentially shorter TTP categories. It thus allows for a potentially exponential increase in the probability for infective endocarditis with a shortening TTP, theoretically well in line with the exponential nature of bacterial growth in blood culture bottles. This multi-level categorisation makes it a bit more complicated to remember the score, but it is still possible to calculate the result easily. It is also possible to include TTP in prediction tools as a continuous variable, in a similar manner to how Ljungquist *et al.* (190) utilised the full spectrum of possible values of TTP in modelling the risk for mortality in bloodstream infection with non-linear methods. If that method is used unaltered in prediction models, however, the score must be calculated using software or a nomogram. It would be interesting to further study TTP as a predictor of infective endocarditis in *E. faecalis* bacteraemia without dichotomisation or statistical methods that assume linear associations.

A third important point is that however well investigators (*e.g.*, Kahn *et al.* and Strömdahl *et al.*) validate their TTP cutoffs, many centres will find that those cutoffs do not work in their own settings (236, 237). Reasons for this may be the composition of the study populations, and whether patients were sufficiently examined and thereby correctly classified to endocarditis or not endocarditis in these retrospective studies (40, 82). As mentioned in the introductory chapter on blood cultures, however, there are also technical issues that may cause systematically different TTP, mainly different blood culture systems and media, and TTI. Blood culture system is a known factor that is easy to control for, but TTI is more difficult to handle. When blood culture bottles are transported to a laboratory at another location with a certain number of scheduled transports per day, often with no transports during the night and fewer on weekends, the resulting TTI of individual bottles is extremely variable. Even if TTI is reported, such as by Robson *et al.* (239), it is hard for the reader of the article to understand just how it could have affected the results. Besides making TTP cutoffs invalid, variable TTI can lead to obscuration of associations between TTP and an outcome, as the separation in TTP

between the groups with and without the outcome, is smudged out (185, 190). This leads us to Study IV.

When studying the effect of TTI on TTP, the reduction of TTP by 16 minutes (aerobic bottles) and 19 minutes (anaerobic bottles) per hour of TTI is within the range of what has been reported in previous experimental studies, although these only reported TTP after a few, pre-defined TTIs (141, 156, 157, 191). This consistency strengthens the credibility of our method for correcting TTP. In addition, a recent study by Deas *et al.* (242) that mainly investigated the risk for a decreased blood culture yield with increasing TTI, also found an approximately linear correlation between TTI and TTP. The authors reported a reduction of TTP by 10 minutes for each hour of TTI, when all species were merged. When data for separate species are presented, the correlation coefficient is around 0.3 hours (18 minutes) for several common pathogens, such as Enterobacterales and beta-haemolytic streptococci, which is similar to our results, but their correlation coefficients for *S. aureus* and enterococci were closer to zero than ours. The high proportion of cultures with CoNS (40%) in the dataset of the study by Deas *et al.* can perhaps have led to the smaller mean decrease of TTP per hour of TTI, as we found CoNS to have a species-specific linear correlation coefficient of -0.15, corresponding to 9 minutes per hour of TTI. The proportion of CoNS in the dataset we used for derivation of species-independent correction factors was 18%.

There are no previous studies that have successfully corrected TTP and obtained better associations with an outcome of interest. Two studies with less than 100 patients each, have used crude methods for correcting TTP. Haimi-Cohen *et al.* (164) used TTP to differentiate between true CoNS bacteraemia and contamination in paediatric patients, and Turkeltaub *et al.* (157) used TTP to categorise patients into three groups of blood bacterial concentration, <100 CFU/ml, 100 – 1000 CFU/ml and >1000 CFU/ml, which in turn were compared against clinical outcomes. It is likely that the small sample sizes and the use of TTP in a categorised form was the reason for not finding any statistically significant differences between predictive performance of crude and corrected TTP in these studies.

The species-independent correction factors that we calculated naturally became dominated by the species that were most common in the dataset, *E. coli*, CoNS and *S. aureus*. When we studied whether species-specific correction resulted in stronger associations with mortality than species-independent correction, we found no such differences in the whole dataset, nor in analyses of the individual microbial groups. In certain groups such as *S. pneumoniae* and *Enterococcus* species however, the resulting confidence intervals were wide. We thus generally recommend the species-independent correction factors, but it cannot be excluded that species-specific correction is more appropriate in certain instances *e.g.*, for *S. pneumoniae* and CoNS, which seemed to be growing more slowly than other species at room temperature, or for more uncommon and slowly growing species that were placed in the groups Other and Other anaerobic. To calculate separate correction factors for

uncommon species was beyond the scope of this study, and despite the large dataset, the number of observations for such species would probably be too low. It could be more feasible in a dataset where a larger proportion of samples have a long TTI than in our dataset.

I see Study IV mainly as a proof-of-concept. It clearly demonstrates that using a method for correcting TTP for TTI improves the potential of TTP as a prognostic or diagnostic test, especially in settings where blood cultures are transported or where incubation cannot be started during nights. It also makes it possible to compare TTP more directly between different settings, and to validate cutoffs that can be used irrespective of the local blood culture logistics. Hopefully, our correction method can be used by others, but a separate validation is recommended.

There are many ways that TTP may be used in clinical practice. A short TTP might be used as an indication that the patient has a potentially more severe infection and may be benefitted by a higher level of monitoring, or urgent diagnostic or therapeutic procedures. A laboratory might use a short TTP as a criterion for expensive reflex testing, including *e.g.*, rapid identification of species and genotypic AST, if there is a need to prioritise such procedures to those in highest need. Furthermore, it is possible that instant and automatic reporting of TTP from positive blood cultures, before a Gram stain is reported, can give a timely prompt for re-evaluating the level of monitoring of the patient, or initiate antimicrobial therapy, if that has not yet been done. In these proposed uses, it is possible that other clinical information such as vital signs, patient history or other laboratory tests, have already correctly classified the severity of the patients' condition in many cases. Therefore, the additional value of TTP should be evaluated in studies designed for that purpose. If TTP is reported before a Gram-stain, the impact of false positive bottles must also be considered.

Corrected TTP should also be studied as a diagnostic test for infective endocarditis. The correction might lead to a better separation between the TTP distributions of patients with infective endocarditis and those with *e.g.*, urinary tract infections or osteoarticular infections. Thereby, the diagnostic performance of the POSITIVE score might be improved, and it would be interesting to test a new risk stratification score for *E. faecalis* bacteraemia that includes a non-dichotomised TTP, corrected for TTI.

The strengths of Study III is a comparatively large dataset, where review of medical records allowed us to retrieve details on the symptoms of the patients. On the other hand, it is a weakness that certain information can be recorded with a bias – it is *e.g.*, more likely that a physician finds and documents the auscultation of a heart murmur if infective endocarditis is suspected. Another weakness inherent to the retrospective design is that the correct diagnosis might have been missed in many cases, including cases of endocarditis. If echocardiography had been performed more frequently, it is likely that more cases would have been found (83). We

attempted to avoid the disturbing effect of TTI, by not including cases from hospitals without on-site blood culture incubators, but there might be cases with long TTI at the other hospitals as well. However, this should likely have led us to underestimate the association between TTP and infective endocarditis. We did not measure blood volume in the bottles, but as mentioned in the introduction on blood cultures, the importance on blood volume for TTP can be questioned (195, 196). In retrospect, it would have been better to investigate the association between TTP and infective endocarditis with other statistical methods, that do not necessitate dichotomisation or assume linearity.

The strengths of Study IV are mainly the large dataset that allowed us to investigate the association between TTI and TTP for different microbial groups, although most of the variation in TTP is caused by other factors. We did not make assumptions about the nature of the association and included non-linear models. By adding a separate analysis where mortality (as an external reference) was the outcome, we could prove that the correction method performed well. Some of the caveats when interpreting our results have already been mentioned *e.g.*, the species-independent correction factors may not be representative for all species, and the number of observations was relatively low for some microbial groups. We excluded polymicrobial infections, as we could not use them when deriving species-specific correction factors. However, the TTP of polymicrobial bottles is probably dominated by rapidly growing species that may be present, and we see no reason why the association between TTI and TTP should be significantly different from that of monomicrobial bottles. On the other hand, since the mechanism that links a high bacterial concentration in blood with a higher mortality is unknown, there might be a different association between the TTP and risk of death in polymicrobial infections, compared to in monomicrobial infections with typical pathogens such as *E. coli*, *S. aureus* and *S. pneumoniae*. If that is the case, however, it does not change our conclusion that corrected TTP is a better predictor of mortality than crude TTP. Another weakness is the difficulty in correctly classifying contaminations. We used the common, but very crude, method of considering common contaminant species growing in only one blood culture set (two paired bottles) as a contaminant, but as a relevant species if it was growing in more than one set. As already discussed, using the term blood culture set to define two bottles, makes little sense when SSS is used. SSS was the recommended sampling method during the last two years of the study, and it might have been better to analyse the number of bottles instead. It is furthermore probable that contaminant species growing in more than one blood culture set, were contaminants anyway in many cases. Inadvertent inclusion of contaminants, probably often with long TTPs (163, 164) and low 7-day mortality rate, could have contributed to the overall inverse association between TTP and mortality rate. However, the obvious associations between TTP and mortality in separate microbial groups, where contaminants are completely excluded, indicate that the potential effect of contaminants should have been minimal. Finally, we did not include paediatric blood culture bottles in the analysis, due to the significantly

lower number, the more variable blood volume that they are filled with, and the fact that contaminants are more common in blood cultures obtained from children in our region. These factors would have made correction factors difficult to calculate for most microbial groups, and it could be hard to compare the resulting corrected TTPs with mortality rate.

Conclusions

Introduction of SSS led to an increase in the proportion of blood culture sampling events with an adequate number of bottles, and the blood culture sensitivity was not negatively affected, both of which are known from previous studies. In my opinion, there is sufficient evidence to widely recommend SSS in blood culture collection. Furthermore, diagnostic criteria for infective endocarditis should not include demands for several venipunctures or spacing of blood cultures in time, as the evidence for these procedures are weak. Our results indicate that there may be an increased risk for contamination of more than two bottles when using SSS, but the strength of the evidence is low. If true, this can have an unfavourable impact on patients, as a high number of positive bottles is often used as an indicator for true infection. Further investigations to test whether this result can be reproduced are recommended.

We saw no obvious negative effects of combining ISDT with SSS. Using an ISDT in blood culture collection has proven benefits on the contamination rate. If an ordinary vacuum blood collection tube, that is later sent for other analyses, is used as the diversion device, it is also without an extra cost. My recommendation for other national guidelines is to adopt the use of an ISDT.

The prevalence of persistent *E. faecalis* bacteraemia is around 10% in a mixed population, which is lower than the prevalence of persistent *S. aureus* bacteraemia. There is an increased risk for infective endocarditis and other complicated infections in patients with persistent *E. faecalis* bacteraemia, but the sensitivity is low. There is currently not enough data to recommend repeated blood cultures in these patients.

We have reported compelling evidence that TTP can be useful in the diagnosis of *E. faecalis* infective endocarditis, with shorter values increasing the probability. However, other studies have failed to reproduce our results. We furthermore prove that TTP is dependent on TTI in an approximately linear fashion. When using the linear regression coefficients as correction factors for TTP, the association between TTP and short-term mortality is strengthened, and correction factors that are common to all species is likely sufficient in most cases. This means that TTP can be more easily compared between studies, and that less biased TTP cutoffs can be established for different purposes.

Future perspectives

Single-sampling strategy (SSS) is likely still used in a minority of all blood culture samplings worldwide. It seems that fear of increased difficulties in distinguishing contaminations from true infections is an important argument against SSS. Therefore, it is important to continue studying the effect of SSS on blood culture sensitivity and contamination risk. These studies should be large enough to detect increases in the proportion of sampling events with contaminations in multiple bottles and should include review of patient records to classify contaminations with higher certainty. Furthermore, there is only one study on SSS that has investigated how the number of positive blood culture bottles can be used to determine the significance of growth of CoNS (138), and more studies would be valuable, given the importance of the subject.

In future studies on the diagnostic performance of repeated blood cultures in *E. faecalis* bacteraemia, I recommend that blood cultures are repeated earlier *e.g.*, 24 hours after the start of active antimicrobials. This might increase the sensitivity for complicated infection. However, repeated blood cultures is a rather slow method for detecting cases where further diagnostic or therapeutic actions are needed, and it is likely that the type of antimicrobial and the time elapsed from the latest administration of it, have a large impact on the positivity rate. Testing of bacterial DNA concentration in blood might be more robust against such disturbing factors, is faster than waiting several days for a blood culture result, and has the benefit of returning a quantified output, rather than a binary one. Bacterial DNA is probably continuously released from intravascular foci of infection during antimicrobial treatment, although the bacteria cannot grow in blood cultures. This might be especially relevant for bacteria that seldom grow in blood cultures after administration of antibiotics *e.g.*, viridans group streptococci (211). Thus, I think that the potential of bacterial DNA measurements in blood has a larger potential than repeated blood cultures, although the cost might be higher and the availability lower.

Our study on the correction of TTP for TTI should be validated in another centre before it is used widely. For laboratories that use the BACT/ALERT VIRTUO blood culture system, which measures the blood volume in individual bottles, the impact of blood volume on TTP can be studied simultaneously. It would be a good thing to use TTP corrected for TTI in all studies on TTP, even when the TTI is generally

short. It would permit comparisons across studies and validations of cutoffs, that are currently not possible.

The association between a short TTP and a higher mortality rate is well established. In future studies on the prognostic value of TTP, the question of whether TTP has a value in addition to vital signs and other biomarkers, should be addressed. Since TTP becomes available several hours after the collection of blood culture bottles, the importance of the temporal aspects must also be studied.

I think that TTP has its largest potential in the diagnosis of infective endocarditis. TTP should probably be integrated in risk stratification scores as one of several variables, and it should be handled in a multilevel or continuous fashion, rather than as a binary variable. Although studies have not found a clear association between TTP and infective endocarditis in bacteraemia with non-beta-haemolytic streptococci (176), it might be fruitful to analyse the different species complexes separately, as they have different median TTP (176, 240). In addition, risk stratification scores for CoNS should be developed, as CoNS are some of the most common aetiologies of infective endocarditis in high-income countries.

Finally, do the risk stratification scores developed for infective endocarditis in *S. aureus* and *E. faecalis* bacteraemia have appropriate outcomes? All scores published to date have the crude aim of detecting infective endocarditis yes / no, and often with little consideration given to the temporal aspects. It should be said that the PREDICT score, however, has both an early and a late timepoint for evaluation (41) and the POSITIVE score only includes data that is known at the time of the first positive blood culture (40). In my opinion, it would be more appropriate to divide the question that the risk stratification scores are designed to answer, into two separate ones: Firstly, is the patient under risk of acute life-threatening or permanently debilitating complications of infective endocarditis, which can be prevented by urgent surgery *e.g.*, cardiogenic shock due to structural damage, or symptomatic embolism? This should be evaluated directly when the first blood culture becomes positive, and the score can contain variables that are available at that time, such as TTP, vital signs, common blood biomarkers *etc.* If positive, echocardiography is to be performed without delay. The second question is whether the patient has infective endocarditis and thereby needs special antimicrobial treatment and – possibly – less urgent surgery to reduce the risk for relapses or late complications. For this part of the score, it is assumed that patients with a high risk for acute complications have already been detected, and there is thus time to wait for results from repeated blood cultures or study the dynamics of bacterial DNA concentration in blood, before the questions of imaging of the heart or treatment length are decided on.

Populärvetenskaplig sammanfattning

Denna avhandling handlar om att på olika sätt förbättra användningen av blododlingar – en vanlig metod för att diagnosticera allvarliga infektionssjukdomar. Vid infektioner såsom njurbäckeninflammation, lunginflammation, sårinfektioner med mera, uppstår ibland sepsis. Sepsis innebär att kroppens immunförsvar reagerar kraftigt och obalanserat, och därmed orsakar rubbningar i livsviktiga system som andning eller blodcirkulation. I Sverige vårdas ca 700 – 800 personer per 100 000 invånare och år på sjukhus för sepsis, och av dem dör runt 16% inom trettio dagar. När en person har sepsis finns det ofta, men inte alltid, levande bakterier i blodet och det är dem man försöker hitta med blododlingar, så att behandlingen kan riktas in mot dem. Endokardit är en annan infektionssjukdom som är mycket ovanligare, men som är dödlig utan behandling. Vid endokardit växer bakterier (eller mer sällan svamp) på hjärtats klaffar, där det bildas så kallade vegetationer – klumpar av bakterier och koagulerat blod. Vegetationerna kan lossna och stoppa blodflödet i blodkärl där de fastnar, vilket kan orsaka stroke eller hjärtinfarkt. Dessutom blir hjärtklaffarna ofta skadade så att hjärtats förmåga att pumpa blod försämras – den drabbade får hjärtsvikt. Endokardit upptäcks oftast genom att man hittar bakterier i blododlingar som har tagits på grund av att patienten har feber, och sedan använder man ultraljud av hjärtat för att se om det finns vegetationer eller skador på klaffarna. För en läkare kan det vara svårt att veta vilka patienter som behöver ultraljud av hjärtat ifall man hittar bakterier i blodet, då risken för endokardit varierar mycket. Det finns därför behov av fler tester som kan hjälpa till att bedöma den risken, gärna med hjälp av information som redan finns. Där kan detaljer från blododlingar vara en viktig pusselbit.

Blododling görs genom att blod tappas till särskilda blododlingsflaskor, där bakterier och svampar av olika arter kan växa. Flaskorna sätts in i ett blododlingsskåp, där de långsamt vaggas vid kroppstemperatur. När en mikroorganism växer bildas koldioxid, och när det börjar bli en tydlig ökning av denna gas i flaskan, reagerar blododlingsskåpet och flaskan blir positiv. Genom mikroskopi och andra analyser på innehållet i flaskan kan man sedan avgöra vilken bakterie- eller svampart som finns i patientens blod, och man kan testa om de är resistent mot antibiotika. När man sticker för att ta blodprovet är det viktigt att rengöra huden ordentligt med sprit för att minska risken för att få med hudbakterier i flaskorna. Det kan nämligen vara svårt att veta om hudbakterier som växer i blododlingar är en orsak till patientens infektion, eller om de bara är en förorening.

I Studie I i denna avhandling undersökte vi vad som hände efter att sjukvården i Skåne hösten 2021 gick över från att använda två separata stick för att ta blodprov för blododling, till att endast använda ett stick, tillsammans med ett inledande provtagningsrör för att skölja bort förorenande bakterier ur provtagningsnålen. Vi visade att antalet provtagningar där man använde det rekommenderade antalet flaskor (fyra) ökade från 87,6% till 92,3% och lika många sjukdomsframkallande organismer hittades efter som före förändringen, vilket är bra. De vanligaste hudbakterierna som förorenar blododlingar kallas koagulas-negativa stafylokocker. Andelen provtagningar där det växte sådana bakterier i en eller två av totalt fyra flaskor minskade från 4,0% till 3,3%, men samtidigt ökade andelen provtagningar där de växte i tre eller fyra av fyra flaskor från 0,39% till 0,49%. Detta kan tyda på att det finns risk för att det blir vanligare att många flaskor i samma provtagning förorenas med hudbakterier, när man använder ett i stället för två stick för provtagning. Provtagning med ett stick är bättre på många sätt, men eftersom antalet positiva flaskor ofta används för att värdera sannolikheten för sann infektion med koagulas-negativa stafylokocker, kan detta eventuellt innebära fler svårtolkade fall.

Studie II handlar om *Enterococcus faecalis*, den tredje vanligaste orsaken till endokardit i höginkomstländer. Vi undersökte om det går att använda upprepade blododlingar för att bedöma risken för att en patient med växt av *E. faecalis* i blododlingar har endokardit eller en annan svårbehandlad typ av infektion, jämfört med mer lättbehandlade infektionstyper. Upprepade blododlingar används redan på det sättet när man hittar bakterien *Staphylococcus aureus* i blodet, och fortsatt växt i nya odlingar, som tas efter att man hittat bakterier i de första odlingarna, ökar då risken för komplicerad infektion. Vi tog nya blododlingar från patienter i 50 fall. Bland de fem fall som hade fortsatt växt i de nya blododlingarna, hade två endokardit och två andra svårbehandlade infektioner. Å andra sidan var det endast fyra av tio fall med endokardit eller annan svårbehandlad infektion som hade positiva upprepade blododlingar. Fortsatt växt av *E. faecalis* i upprepade blododlingar innebär alltså ökad risk för en svårbehandlad infektion, men eftersom så få av fallen hittas, går det inte att rutinmässigt rekommendera nya blododlingar.

I Studie III undersökte vi om den tid som går från att en blododlingsflaska sätts in i ett blododlingsskåp till att flaskan larmar positivt – tid till positiv blododling – går att använda för att bedöma risken för endokardit med *E. faecalis*. En kortare sådan tid kan innebära att det fanns mer bakterier i blodet när blodprovet togs, och det finns indikationer på att det ofta finns en högre koncentration av bakterier i blodet vid endokardit. Vi kunde visa att patienter med endokardit hade en kortare tid till positiv blododling (median 9,4 timmar) än vad den studerade gruppen som helhet hade (median 11,6 timmar), ett samband som också har setts för den vanligaste orsaken till endokardit – *S. aureus*. Även om tid till positiv blododling har potential att utvecklas vidare som diagnostiskt test, var det inte ett lika bra test som andra sätt att värdera risken för endokardit som redan används idag.

Ett stort problem med måttet tid till positiv blododling är att det påverkas mycket av tiden som går från provtagning till insättning av flaskan i blododlingsskåpet: Om flaskorna förvaras några timmar i rumstemperatur så börjar bakterierna i dem att dela sig, fast långsamt, och när de sedan sätts in i blododlingsskåpet blir tid till positiv blododling kortare, eftersom de redan har hunnit växa till sig i antal. Då blododlingsflaskornas transporttid till blododlingsskåp kan variera mycket, såväl inom ett sjukhus som mellan olika sjukhus, innebär det en risk för att skillnader i tid till positiv blododling mellan grupper, exempelvis de med och utan endokardit, missas. I Studie IV utvecklade vi därför en metod för att korrigera måttet tid till positiv blododling för tiden till insättning av flaskan i blododlingsskåp. Genom att analysera data från ca 100 000 positiva blododlingsflaskor i Skåne år 2015 till 2023, kunde vi dra slutsatsen att för varje timme som tiden till insättning i blododlingsskåp ökade, minskade tid till positiv blododling med 15 till 20 minuter. Med hjälp av denna minskningstakt kunde vi för varje blododlingsflaska sedan räkna fram en korrigerad tid till positiv blododling, dvs vad denna tid skulle ha varit om flaskan hade satts in i blododlingsskåpet direkt. När vi sedan jämförde sambanden mellan korrigerad och icke-korrigerad tid till positiv blododling med andel patienter som dog inom 7 dagar från att blododlingen togs, hade det korrigerade måttet starkast koppling till risk för död. Detta tyder på att vår metod för korrigering fungerar, och sannolikt kan användas i andra studier för att göra tid till positiv blododling till ett bättre test för exempelvis endokardit.

Sammantaget har dessa studier varit små bidrag till delvis otillräckligt utforskade ämnen. Provtagning med ett stick är på frammarsch i världen, men är fortfarande ovanligt ur ett globalt perspektiv, och fler studier som beskriver för- och nackdelar med metoden behövs. Tid till positiv blododling är ett intressant mått som så att säga kommer på köpet vid blododling. Fortsatta studier behövs för att undersöka om vår korrigering för tid till insättning av flaskan i blododlingsskåp fungerar som tänkt, och för att integrera tid till positiv blododling i system eller algoritmer som gör det lättare att identifiera personer som har hög risk för endokardit, eller som löper hög risk att dö i sin infektion.

Tack

Många förtjänar ett särskilt tack då de på olika sätt har varit oumbärliga i min process att genomgå forskarutbildningen och färdigställa denna avhandling.

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References

1. Mann T. *Der Zauberberg*. Translation: The Magic Mountain: Fisher Verlag. Translation: The Modern Library, New York; Original: 1924. Translation: 1927.
2. MeSH [29-10-2025]. Available from: <https://www.ncbi.nlm.nih.gov/mesh?Db=mesh&Cmd=DetailsSearch&Term=%22Bacteremia%22%5BMeSH+Terms%5D>.
3. Holmes CL, Albin OR, Mobley HLT, Bachman MA. Bloodstream infections: mechanisms of pathogenesis and opportunities for intervention. *Nat Rev Microbiol*. 2025;23(4):210-24.
4. Danielsen AS, Gu Q, Fostervold A, Eyre DW, Bjørnholt JV. 'Bloodstream infection': A valuable concept we should keep in our toolbox. *Journal of Infection*. 2024;89(4).
5. Martins CC, Lockhart PB, Firmino RT, Kilmartin C, Cahill TJ, Dayer M, et al. Bacteremia following different oral procedures: Systematic review and meta-analysis. *Oral Dis*. 2024;30(3):846-54.
6. Yildirim I, Okur E, Ciragil P, Aral M, Kilic MA, Gul M. Bacteraemia during tonsillectomy. *The Journal of Laryngology & Otology*. 2003;117(8):619-23.
7. Musher DM, Elisarraras FX. Is it time to retire the term 'bloodstream infection'? (Submitted as Letter to the Editor). *Journal of Infection*. 2024;89(1).
8. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-10.
9. Nakamori Y, Park EJ, Shimaoka M. Immune Deregulation in Sepsis and Septic Shock: Reversing Immune Paralysis by Targeting PD-1/PD-L1 Pathway. *Front Immunol*. 2020;11:624279.
10. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. 1996;22(7):707-10.
11. Mellhammar L, Kahn F, Whitlow C, Kander T, Christensson B, Linder A. Bacteremic sepsis leads to higher mortality when adjusting for confounders with propensity score matching. *Scientific Reports*. 2021;11(1):6972.

12. Lê-Bury P, Echenique-Rivera H, Pizarro-Cerdá J, Dussurget O. Determinants of bacterial survival and proliferation in blood. *FEMS Microbiol Rev.* 2024;48(3).
13. Coburn B, Morris AM, Tomlinson G, Detsky AS. Does this adult patient with suspected bacteremia require blood cultures? *Jama.* 2012;308(5):502-11.
14. Kontula KSK, Skogberg K, Ollgren J, Jarvinen A, Lyytikainen O. Population-Based Study of Bloodstream Infection Incidence and Mortality Rates, Finland, 2004-2018. *Emerg Infect Dis.* 2021;27(10):2560-9.
15. Ljungquist O, Blomstergren A, Merkel A, Sunnerhagen T, Holm K, Torisson G. Incidence, aetiology and temporal trend of bloodstream infections in southern Sweden from 2006 to 2019: a population-based study. *Euro Surveill.* 2023;28(10).
16. Verway M, Brown KA, Marchand-Austin A, Diong C, Lee S, Langford B, et al. Prevalence and Mortality Associated with Bloodstream Organisms: a Population-Wide Retrospective Cohort Study. *J Clin Microbiol.* 2022;60(4):e0242921.
17. Leligdowicz A, Dodek PM, Norena M, Wong H, Kumar A, Kumar A. Association between source of infection and hospital mortality in patients who have septic shock. *Am J Respir Crit Care Med.* 2014;189(10):1204-13.
18. Nannan Panday RS, Lammers EMJ, Alam N, Nanayakkara PWB. An overview of positive cultures and clinical outcomes in septic patients: a sub-analysis of the Prehospital Antibiotics Against Sepsis (PHANTASi) trial. *Crit Care.* 2019;23(1):182.
19. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet.* 2020;395(10219):200-11.
20. Laupland KB. Defining the epidemiology of bloodstream infections: the 'gold standard' of population-based assessment. *Epidemiol Infect.* 2013;141(10):2149-57.
21. Filice GA, Van Etta LL, Darby CP, Fraser DW. Bacteremia in Charleston County, South Carolina. *Am J Epidemiol.* 1986;123(1):128-36.
22. Madsen KM, Schønheyder HC, Kristensen B, Sørensen HT. Secular trends in incidence and mortality of bacteraemia in a Danish county 1981-1994. *Apmis.* 1999;107(3):346-52.
23. Uslan DZ, Crane SJ, Steckelberg JM, Cockerill FR, 3rd, St Sauver JL, Wilson WR, et al. Age- and sex-associated trends in bloodstream infection: a population-based study in Olmsted County, Minnesota. *Arch Intern Med.* 2007;167(8):834-9.
24. Skogberg K, Lyytikainen O, Ruutu P, Ollgren J, Nuorti JP. Increase in bloodstream infections in Finland, 1995-2002. *Epidemiol Infect.* 2008;136(1):108-14.
25. Nielsen SL, Pedersen C, Jensen TG, Gradel KO, Kolmos HJ, Lassen AT. Decreasing incidence rates of bacteremia: a 9-year population-based study. *J Infect.* 2014;69(1):51-9.
26. Laupland KB, Gregson DB, Flemons WW, Hawkins D, Ross T, Church DL. Burden of community-onset bloodstream infection: a population-based assessment. *Epidemiol Infect.* 2007;135(6):1037-42.

27. Sogaard M, Engebjerg MC, Lundbye-Christensen S, Schonheyder HC. Changes in blood culture methodology have an impact on time trends of bacteraemia: a 26-year regional study. *Epidemiol Infect.* 2011;139(5):772-6.
28. Laupland KB, Niven DJ, Pasquill K, Parfitt EC, Steele L. Culturing rate and the surveillance of bloodstream infections: a population-based assessment. *Clinical Microbiology and Infection.* 2018;24(8):910.e1-e4.
29. Torisson G, Rosenqvist M, Melander O, Resman F. Hospitalisations with infectious disease diagnoses in somatic healthcare between 1998 and 2019: A nationwide, register-based study in Swedish adults. *The Lancet Regional Health – Europe.* 2022;16.
30. Mehl A, Asvold BO, Lydersen S, Paulsen J, Solligard E, Damas JK, et al. Burden of bloodstream infection in an area of Mid-Norway 2002-2013: a prospective population-based observational study. *BMC Infect Dis.* 2017;17(1):205.
31. Holmbom M, Giske CG, Fredrikson M, Ostholm Balkhed A, Claesson C, Nilsson LE, et al. 14-Year Survey in a Swedish County Reveals a Pronounced Increase in Bloodstream Infections (BSI). Comorbidity - An Independent Risk Factor for Both BSI and Mortality. *PLoS One.* 2016;11(11):e0166527.
32. Fleischmann-Struzek C, Rudd K. Challenges of assessing the burden of sepsis. *Med Klin Intensivmed Notfmed.* 2023;118(Suppl 2):68-74.
33. Fleischmann-Struzek C, Mellhammar L, Rose N, Cassini A, Rudd KE, Schlattmann P, et al. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive Care Medicine.* 2020;46(8):1552-62.
34. Mellhammar L, Wullt S, Lindberg Å, Lanbeck P, Christensson B, Linder A. Sepsis Incidence: A Population-Based Study. *Open Forum Infect Dis.* 2016;3(4):ofw207.
35. Mellhammar L, Wollter E, Dahlberg J, Donovan B, Olséen CJ, Wiking PO, et al. Estimating Sepsis Incidence Using Administrative Data and Clinical Medical Record Review. *JAMA Netw Open.* 2023;6(8):e2331168.
36. Holland TL, Baddour LM, Bayer AS, Hoen B, Miro JM, Fowler VG, Jr. Infective endocarditis. *Nat Rev Dis Primers.* 2016;2:16059.
37. Cahill TJ, Prendergast BD. Infective endocarditis. *Lancet.* 2016;387(10021):882-93.
38. Habib G, Erba PA, Iung B, Donal E, Cosyns B, Laroche C, et al. Clinical presentation, aetiology and outcome of infective endocarditis. Results of the ESC-EORP EURO-ENDO (European infective endocarditis) registry: a prospective cohort study. *European Heart Journal.* 2019;40(39):3222-32.
39. Delgado V, Ajmone Marsan N, de Waha S, Bonaros N, Brida M, Burri H, et al. 2023 ESC Guidelines for the management of endocarditis. *Eur Heart J.* 2023;44(39):3948-4042.
40. Kahn F, Resman F, Bergmark S, Filiptsev P, Nilson B, Gilje P, et al. Time to blood culture positivity in *Staphylococcus aureus* bacteraemia to determine risk of infective endocarditis. *Clin Microbiol Infect.* 2021;27(9):1345 e7- e12.
41. Palraj BR, Baddour LM, Hess EP, Steckelberg JM, Wilson WR, Lahr BD, et al. Predicting Risk of Endocarditis Using a Clinical Tool (PREDICT): Scoring

- System to Guide Use of Echocardiography in the Management of *Staphylococcus aureus* Bacteremia. *Clin Infect Dis*. 2015;61(1):18-28.
42. Papadimitriou-Olivgeris M, Monney P, Mueller L, Senn L, Guery B. The LAUSanne STAPHylococcus aureus ENdocarditis (LAUSTAPHEN) score: A prediction score to estimate initial risk for infective endocarditis in patients with *S. aureus* bacteremia. *Front Cardiovasc Med*. 2022;9:961579.
 43. Tubiana S, Duval X, Alla F, Selton-Suty C, Tattevin P, Delahaye F, et al. The VIRSTA score, a prediction score to estimate risk of infective endocarditis and determine priority for echocardiography in patients with *Staphylococcus aureus* bacteremia. *J Infect*. 2016;72(5):544-53.
 44. Sunnerhagen T, Törnell A, Vikbrant M, Nilson B, Rasmussen M. HANDOC: A Handy Score to Determine the Need for Echocardiography in Non- β -Hemolytic Streptococcal Bacteremia. *Clin Infect Dis*. 2018;66(5):693-8.
 45. Berge A, Krantz A, Ostlund H, Naucier P, Rasmussen M. The DENOVA score efficiently identifies patients with monomicrobial *Enterococcus faecalis* bacteremia where echocardiography is not necessary. *Infection*. 2019;47(1):45-50.
 46. Talha KM, Baddour LM, Thornhill MH, Arshad V, Tariq W, Tleyjeh IM, et al. Escalating incidence of infective endocarditis in Europe in the 21st century. *Open Heart*. 2021;8(2).
 47. Ambrosioni J, Hernandez-Meneses M, Téllez A, Pericàs J, Falces C, Tolosana JM, et al. The Changing Epidemiology of Infective Endocarditis in the Twenty-First Century. *Curr Infect Dis Rep*. 2017;19(5):21.
 48. Snygg-Martin U. Endokarditregistret - år 2024. 2025.
 49. Wang A, Athan E, Pappas PA, Fowler VG, Jr., Olaison L, Paré C, et al. Contemporary clinical profile and outcome of prosthetic valve endocarditis. *Jama*. 2007;297(12):1354-61.
 50. Berisha B, Ragnarsson S, Olaison L, Rasmussen M. Microbiological etiology in prosthetic valve endocarditis: A nationwide registry study. *J Intern Med*. 2022;292(3):428-37.
 51. Bläckberg A, Falk L, Oldberg K, Olaison L, Rasmussen M. Infective Endocarditis Due to *Corynebacterium* Species: Clinical Features and Antibiotic Resistance. *Open Forum Infect Dis*. 2021;8(3):ofab055.
 52. Blomström-Lundqvist C, Traykov V, Erba PA, Burri H, Nielsen JC, Bongiorni MG, et al. European Heart Rhythm Association (EHRA) international consensus document on how to prevent, diagnose, and treat cardiac implantable electronic device infections-endorsed by the Heart Rhythm Society (HRS), the Asia Pacific Heart Rhythm Society (APHRS), the Latin American Heart Rhythm Society (LAHRS), International Society for Cardiovascular Infectious Diseases (ISCVID) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *Europace*. 2020;22(4):515-49.
 53. del Val D, Panagides V, Mestres CA, Miró JM, Rodés-Cabau J. Infective Endocarditis After Transcatheter Aortic Valve Replacement: JACC State-of-the-Art Review. *Journal of the American College of Cardiology*. 2023;81(4):394-412.

54. Thompson GR, 3rd, Jenks JD, Baddley JW, Lewis JS, 2nd, Egger M, Schwartz IS, et al. Fungal Endocarditis: Pathophysiology, Epidemiology, Clinical Presentation, Diagnosis, and Management. *Clin Microbiol Rev*. 2023;36(3):e0001923.
55. Dähler R, Brugger SD, Frank M, Greutmann M, Sromicki J, Marques-Maggio E, et al. A retrospective analysis of blood culture-negative endocarditis at a tertiary care centre in Switzerland. *Swiss Med Wkly*. 2022;152:40012.
56. Østergaard L, Voldstedlund M, Bruun NE, Bundgaard H, Iversen K, Køber N, et al. Temporal Changes, Patient Characteristics, and Mortality, According to Microbiological Cause of Infective Endocarditis: A Nationwide Study. *J Am Heart Assoc*. 2022;11(16):e025801.
57. DeSimone DC, Garrigos ZE, Marx GE, Tattevin P, Hasse B, McCormick DW, et al. Blood Culture-Negative Endocarditis: A Scientific Statement From the American Heart Association: Endorsed by the International Society for Cardiovascular Infectious Diseases. *J Am Heart Assoc*. 2025;14(8):e040218.
58. Ahmed O, King NE, Qureshi MA, Choudhry AA, Osama M, Zehner C, et al. Non-bacterial thrombotic endocarditis: a clinical and pathophysiological reappraisal. *Eur Heart J*. 2025;46(3):236-49.
59. Li M, Kim JB, Sastry BKS, Chen M. Infective endocarditis. *Lancet*. 2024;404(10450):377-92.
60. Kitagata Y, Tsuneyoshi H, Ito K, Yamanaka K, Hirano M, Hirose K, et al. Surgical treatment of infective endocarditis with massive vegetations in a patient with a ventricular septal defect and atopic dermatitis: a case report. *Gen Thorac Cardiovasc Surg Cases*. 2023;2(1):43.
61. Honarbakhsh S, Chowdhury M, Farooqi F, Deaner A. Syncope secondary to left ventricular outflow tract obstruction, an interesting presentation of infective endocarditis. *BMJ Case Rep*. 2015;2015.
62. Snygg-Martin U, Gustafsson L, Rosengren L, Alsiö Å, Ackerholm P, Andersson R, et al. Cerebrovascular Complications in Patients with Left-Sided Infective Endocarditis Are Common: A Prospective Study Using Magnetic Resonance Imaging and Neurochemical Brain Damage Markers. *Clinical Infectious Diseases*. 2008;47(1):23-30.
63. Santos-Patarroyo SD, Quintero-Martinez JA, Lahr BD, Chesdachai S, DeSimone DC, Villarraga HR, et al. Comprehensive Assessment of the Risk of Symptomatic Embolism in Patients With Infective Endocarditis. *J Am Heart Assoc*. 2025;14(3):e036648.
64. Baddour LM, Wilson WR, Bayer AS, Fowler VG, Jr., Tleyjeh IM, Rybak MJ, et al. Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications: A Scientific Statement for Healthcare Professionals From the American Heart Association. *Circulation*. 2015;132(15):1435-86.
65. Martí-Carvajal AJ, Dayer M, Conterno LO, Gonzalez Garay AG, Martí-Amarista CE. A comparison of different antibiotic regimens for the treatment of infective endocarditis. *Cochrane Database Syst Rev*. 2020;5(5):Cd009880.

66. Iversen K, Ihlemann N, Gill SU, Madsen T, Elming H, Jensen KT, et al. Partial Oral versus Intravenous Antibiotic Treatment of Endocarditis. *N Engl J Med*. 2019;380(5):415-24.
67. Kang DH, Kim YJ, Kim SH, Sun BJ, Kim DH, Yun SC, et al. Early surgery versus conventional treatment for infective endocarditis. *N Engl J Med*. 2012;366(26):2466-73.
68. Brown AJ. The history of blood cultures: From the research laboratory to the bedside. *Cleve Clin J Med*. 2024;91(11):661-3.
69. Durack DT, Lukes AS, Bright DK. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. *Am J Med*. 1994;96(3):200-9.
70. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Jr., Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis*. 2000;30(4):633-8.
71. Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F, et al. 2015 ESC Guidelines for the management of infective endocarditis: The Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). *Eur Heart J*. 2015;36(44):3075-128.
72. Fowler VG, Durack DT, Selton-Suty C, Athan E, Bayer AS, Chamis AL, et al. The 2023 Duke-International Society for Cardiovascular Infectious Diseases Criteria for Infective Endocarditis: Updating the Modified Duke Criteria. *Clin Infect Dis*. 2023;77(4):518-26.
73. Bai AD, Steinberg M, Showler A, Burry L, Bhatia RS, Tomlinson GA, et al. Diagnostic Accuracy of Transthoracic Echocardiography for Infective Endocarditis Findings Using Transesophageal Echocardiography as the Reference Standard: A Meta-Analysis. *Journal of the American Society of Echocardiography*. 2017;30(7):639-46.e8.
74. Hilberath JN, Oakes DA, Shernan SK, Bulwer BE, D'Ambra MN, Eltzhig HK. Safety of Transesophageal Echocardiography. *Journal of the American Society of Echocardiography*. 2010;23(11):1115-27.
75. Oliveira M, Guittet L, Hamon M, Hamon M. Comparative Value of Cardiac CT and Transesophageal Echocardiography in Infective Endocarditis: A Systematic Review and Meta-Analysis. *Radiol Cardiothorac Imaging*. 2020;2(3):e190189.
76. Jain V, Wang TKM, Bansal A, Farwati M, Gad M, Montane B, et al. Diagnostic performance of cardiac computed tomography versus transesophageal echocardiography in infective endocarditis: A contemporary comparative meta-analysis. *Journal of Cardiovascular Computed Tomography*. 2021;15(4):313-21.
77. Wang TKM, Sánchez-Nadales A, Igbinomwanhia E, Cremer P, Griffin B, Xu B. Diagnosis of Infective Endocarditis by Subtype Using (18)F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography: A Contemporary Meta-Analysis. *Circ Cardiovasc Imaging*. 2020;13(6):e010600.

78. Rosengren K, Gilje P, Rasmussen M. Clinical suspicion and empirical treatment of infective endocarditis on hospital admission - a population-based cohort study. *Infect Dis (Lond)*. 2025;57(1):27-34.
79. Nilson B, Olaison L, Rasmussen M. Clinical presentation of infective endocarditis caused by different groups of non-beta haemolytic streptococci. *Eur J Clin Microbiol Infect Dis*. 2016;35(2):215-8.
80. Chamat-Hedemand S, Dahl A, Østergaard L, Arpi M, Fosbøl E, Boel J, et al. Prevalence of Infective Endocarditis in Streptococcal Bloodstream Infections Is Dependent on Streptococcal Species. *Circulation*. 2020;142(8):720-30.
81. Østergaard L, Bruun NE, Voldstedlund M, Arpi M, Andersen CØ, Schønheyder HC, et al. Prevalence of infective endocarditis in patients with positive blood cultures: a Danish nationwide study. *European Heart Journal*. 2019;40(39):3237-44.
82. Stromdahl M, Hagman K, Hedman K, Westman A, Hedenstierna M, Ursing J. Time to Staphylococcus aureus Blood Culture Positivity as a Risk Marker of Infective Endocarditis: A Retrospective Cohort Study. *Clin Infect Dis*. 2025;80(4):727-34.
83. Dahl A, Iversen K, Tonder N, Hoest N, Arpi M, Dalsgaard M, et al. Prevalence of Infective Endocarditis in Enterococcus faecalis Bacteremia. *Journal of the American College of Cardiology*. 2019;74(2):193-201.
84. Lai CK, Leung E, He Y, Ching-Chun C, Oliver MOY, Qinze Y, et al. A Machine Learning-Based Risk Score for Prediction of Infective Endocarditis Among Patients With Staphylococcus aureus Bacteremia-The SABIER Score. *J Infect Dis*. 2024;230(3):606-13.
85. Chamat-Hedemand S, Bruun NE, Østergaard L, Arpi M, Fosbøl E, Boel J, et al. Proposal for the use of echocardiography in bloodstream infections due to different streptococcal species. *BMC Infect Dis*. 2021;21(1):689.
86. Bouza E, Kestler M, Beca T, Mariscal G, Rodriguez-Creixems M, Bermejo J, et al. The NOVA score: a proposal to reduce the need for transesophageal echocardiography in patients with enterococcal bacteremia. *Clin Infect Dis*. 2015;60(4):528-35.
87. Lai CKC, Leung E, Lee ALH, Yu Q, He Y, Li Y, et al. The MEFIER Score-A Risk Score to Stratify Infective Endocarditis in Patients With Enterococcus faecalis Bacteremia Based on an 11-Year Territory-Wide Cohort. *Open Forum Infect Dis*. 2025;12(6):ofaf287.
88. Danneels P, Chabrun F, Picard L, Martinet P, Rezig S, Lorleac'h A, et al. Enterococcus faecalis endocarditis risk assessment in patients with bacteremia: External validation of the DENOVA score. *Journal of Infection*. 2023;87(6):571-3.
89. Perez-Rodriguez MT, Lima O, Garrido M, Rincon A, Martinez-Lamas L, Perez-Gonzalez A, et al. The role of time to positive blood cultures in enhancing the predictive capability of DENOVA score for diagnosing infective endocarditis in patients with Enterococcus faecalis bacteremia. *Eur J Clin Microbiol Infect Dis*. 2024;43(7):1481-6.
90. Muir RR, James. *Manual of Bacteriology*: Edinburgh : Young J. Pentland; 1899.

91. Crookshank EM. A text-book of bacteriology, including the etiology and prevention of infective diseases: London, H. K. Lewis; 1896.
92. Hansen GT. Laboratory Blood Cultures: Past, Present, and Future. *Clinical Microbiology Newsletter*. 2016;38(15):119-28.
93. Castaneda MR. A practical method for routine blood cultures in brucellosis. *Proc Soc Exp Biol Med*. 1947;64(1):114.
94. Mc Elvania TeKippe E, Pence MA. Lysis-Centrifugation Methods of Blood Culture. In: Dunne WM, Burnham C-AD, editors. *The dark art of blood cultures*. Washington, DC: ASM Press; 2018. p. 39-57.
95. Wilson ML. Development of new methods for detecting bloodstream pathogens. *Clinical Microbiology and Infection*. 2020;26(3):319-24.
96. Dunne WM. Historical Perspectives on the Art and Science of Blood Culture. In: Dunne WM, Burnham C-AD, editors. *The dark art of blood cultures*. Washington, DC: ASM Press; 2018. p. 1-19.
97. S12 - Sepsis and systemic or disseminated infections: Standards Unit, Specialised Microbiology and Laboratories, UKHSA; 2023 [updated 31.01.2023. Available from: <https://www.rcpath.org/static/3f51b8e5-1ebe-469d-a79f3a3323bfaec9/7e38a3d1-fdc7-4c6e-aadc7e55b698ef26/uk-smi-s-12i1-sepsis-and-systemic-or-disseminated-infection-january-2023-pdf.pdf>.
98. Li J, Plorde JJ, Carlson LG. Effects of volume and periodicity on blood cultures. *J Clin Microbiol*. 1994;32(11):2829-31.
99. Henning C, Jonsson B, Monsen T, Wallgren K, Akerlund A, Ozenci V, et al. Diagnostik av infektioner i blod orsakade av bakterier och svamp ver 2.0 [Internet]. Föreningen för klinisk mikrobiologi; 2024 [updated 2024 Mar 11; cited 2024 Jun 13]. Available from: https://www.mikrobiologi.net/organization/document/Diagnostik_av_infektioner_i_blod_orsakade_av_bakterier_och_svamp_v_2_0b_20240311.pdf.
100. Wilson ML. Principles and procedures for blood cultures. 2. ed. [S.l.]: Clinical and laboratory standard institute; 2022.
101. Riedel S, Bourbeau P, Swartz B, Brecher S, Carroll KC, Stamper PD, et al. Timing of specimen collection for blood cultures from febrile patients with bacteremia. *J Clin Microbiol*. 2008;46(4):1381-5.
102. Werner AS, Cobbs CG, Kaye D, Hook EW. Studies on the bacteremia of bacterial endocarditis. *Jama*. 1967;202(3):199-203.
103. Lamy B, Dargere S, Arendrup MC, Parienti JJ, Tattevin P. How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infections? A State-of-the Art. *Front Microbiol*. 2016;7:697.
104. Kiehn TE, Wong B, Edwards FF, Armstrong D. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. *J Clin Microbiol*. 1983;18(2):300-4.
105. Jonsson B, Nyberg A, Henning C. Theoretical aspects of detection of bacteraemia as a function of the volume of blood cultured. *Apmis*. 1993;101(8):595-601.
106. Cockerill FR, 3rd, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis*. 2004;38(12):1724-30.

107. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? *J Clin Microbiol.* 2007;45(11):3546-8.
108. Bouza E, Sousa D, Rodriguez-Creixems M, Lechuz JG, Munoz P. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? *J Clin Microbiol.* 2007;45(9):2765-9.
109. Patel R, Vetter EA, Harmsen WS, Schleck CD, Fadel HJ, Cockerill FR, 3rd. Optimized pathogen detection with 30- compared to 20-milliliter blood culture draws. *J Clin Microbiol.* 2011;49(12):4047-51.
110. Henning C, Aygul N, Dinnetz P, Wallgren K, Ozenci V. Detailed Analysis of the Characteristics of Sample Volume in Blood Culture Bottles. *J Clin Microbiol.* 2019;57(8).
111. Blodgett R. BAM Appendix 2: Most Probable Number from Serial Dilutions: U.S. Food and Drug Administration; 2023 [updated 09/14/2023. Available from: <https://www.fda.gov/food/laboratory-methods-food/bam-appendix-2-most-probable-number-serial-dilutions>.
112. Berge A, Morenius C, Petropoulos A, Nilson B, Rasmussen M. Epidemiology, bacteriology, and clinical characteristics of HACEK bacteremia and endocarditis: a population-based retrospective study. *European Journal of Clinical Microbiology & Infectious Diseases.* 2021;40(3):525-34.
113. Freling SR, Richie I, Norwitz D, Canamar CP, Banerjee J, Davar K, et al. Interpreting Blood Culture Results as Early Guidance for Infective Endocarditis. *JAMA Netw Open.* 2025;8(5):e258079.
114. Klucher JM, Davis K, Lakkad M, Painter JT, Dare RK. Risk factors and clinical outcomes associated with blood culture contamination. *Infect Control Hosp Epidemiol.* 2022;43(3):291-7.
115. Dargère S, Cormier H, Verdon R. Contaminants in blood cultures: importance, implications, interpretation and prevention. *Clinical Microbiology and Infection.* 2018;24(9):964-9.
116. Schinkel M, Boerman A, Carroll K, Cosgrove SE, Hsu YJ, Klein E, et al. Impact of Blood Culture Contamination on Antibiotic Use, Resource Utilization, and Clinical Outcomes: A Retrospective Cohort Study in Dutch and US Hospitals. *Open Forum Infect Dis.* 2024;11(2):ofad644.
117. Doern GV, Carroll KC, Diekema DJ, Garey KW, Rupp ME, Weinstein MP, et al. Practical Guidance for Clinical Microbiology Laboratories: A Comprehensive Update on the Problem of Blood Culture Contamination and a Discussion of Methods for Addressing the Problem. *Clin Microbiol Rev.* 2019;33(1).
118. Garcia RA, Spitzer ED, Beaudry J, Beck C, Diblasi R, Gillean-Blabac M, et al. Multidisciplinary team review of best practices for collection and handling of blood cultures to determine effective interventions for increasing the yield of true-positive bacteremias, reducing contamination, and eliminating false-positive central line-associated bloodstream infections. *Am J Infect Control.* 2015;43(11):1222-37.
119. Sautter RL, Parrott JS, Nachamkin I, Diel C, Tom RJ, Bobenchik AM, et al. American Society for Microbiology evidence-based laboratory medicine practice

- guidelines to reduce blood culture contamination rates: a systematic review and meta-analysis. *Clin Microbiol Rev.* 2024;37(4):e0008724.
120. Patton RG, Schmitt T. Innovation for reducing blood culture contamination: initial specimen diversion technique. *J Clin Microbiol.* 2010;48(12):4501-3.
 121. Lalezari A, Cohen MJ, Svinik O, Tel-Zur O, Sinvani S, Al-Dayem YA, et al. A simplified blood culture sampling protocol for reducing contamination and costs: a randomized controlled trial. *Clin Microbiol Infect.* 2020;26(4):470-4.
 122. Binkhamis K, Forward K. Effect of the initial specimen diversion technique on blood culture contamination rates. *J Clin Microbiol.* 2014;52(3):980-1.
 123. Syed S, Liss DT, Costas CO, Atkinson JM. Diversion Principle Reduces Skin Flora Contamination Rates in a Community Hospital. *Arch Pathol Lab Med.* 2020;144(2):215-20.
 124. Rupp ME, Cavalieri RJ, Marolf C, Lyden E. Reduction in Blood Culture Contamination Through Use of Initial Specimen Diversion Device. *Clin Infect Dis.* 2017;65(2):201-5.
 125. Arenas M, Boseman GM, Coppin JD, Lukey J, Jinadatha C, Navarathna DH. Asynchronous Testing of 2 Specimen-Diversion Devices to Reduce Blood Culture Contamination: A Single-Site Product Supply Quality Improvement Project. *J Emerg Nurs.* 2021;47(2):256-64 e6.
 126. Lamy B, Roy P, Carret G, Flandrois JP, Delignette-Muller ML. What is the relevance of obtaining multiple blood samples for culture? A comprehensive model to optimize the strategy for diagnosing bacteremia. *Clin Infect Dis.* 2002;35(7):842-50.
 127. Dargere S, Parienti JJ, Roupie E, Gancel PE, Wiel E, Smaiti N, et al. Unique blood culture for diagnosis of bloodstream infections in emergency departments: a prospective multicentre study. *Clin Microbiol Infect.* 2014;20(11):O920-7.
 128. Yu D, Larsson A, Parke A, Unge C, Henning C, Sunden-Cullberg J, et al. Single-Sampling Strategy vs. Multi-Sampling Strategy for Blood Cultures in Sepsis: A Prospective Non-inferiority Study. *Front Microbiol.* 2020;11:1639.
 129. Ekwall-Larson A, Yu D, Dinnézt P, Nordqvist H, Özenci V. Single-Site Sampling versus Multisite Sampling for Blood Cultures: a Retrospective Clinical Study. *J Clin Microbiol.* 2022;60(2):e0193521.
 130. Andersson Norlen E, Widerstrom M, Lindam A, Olsson J, Ryding U. Blood cultures with one venipuncture instead of two: a prospective clinical comparative single-center study including patients in the ICU, haematology, and infectious diseases departments. *Infect Dis (Lond).* 2023;55(9):591-8.
 131. Goehringer F, Soudant M, Alauzet C, Selton-Suty C, Agrinier N, Virion JM, et al. Single- Versus Multiple-sampling Strategy for Blood Cultures in the Diagnosis of Infective Endocarditis: The Prospective Multicenter UniEndo Study. *Clin Infect Dis.* 2025;81(4):756-65.
 132. Senneby E, Göransson L, Weiber S, Rasmussen M. A population-based study of aerococcal bacteraemia in the MALDI-TOF MS-era. *Eur J Clin Microbiol Infect Dis.* 2016;35(5):755-62.

133. Herwaldt LA, Geiss M, Kao C, Pfaller MA. The positive predictive value of isolating coagulase-negative staphylococci from blood cultures. *Clin Infect Dis*. 1996;22(1):14-20.
134. Kitaura T, Chikumi H, Fujiwara H, Okada K, Hayabuchi T, Nakamoto M, et al. Positive Predictive Value of True Bacteremia according to the Number of Positive Culture Sets in Adult Patients. *Yonago Acta Med*. 2014;57(4):159-65.
135. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, et al. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin Infect Dis*. 1997;24(4):584-602.
136. Mirrett S, Weinstein MP, Reimer LG, Wilson ML, Reller LB. Relevance of the number of positive bottles in determining clinical significance of coagulase-negative staphylococci in blood cultures. *J Clin Microbiol*. 2001;39(9):3279-81.
137. Peacock SJ, Bowler IC, Crook DW. Positive predictive value of blood cultures growing coagulase-negative staphylococci. *Lancet*. 1995;346(8968):191-2.
138. Leyssene D, Gardes S, Vilquin P, Flandrois JP, Carret G, Lamy B. Species-driven interpretation guidelines in case of a single-sampling strategy for blood culture. *Eur J Clin Microbiol Infect Dis*. 2011;30(12):1537-41.
139. Wilms MC, Stanzel S, Reinert RR, Burckhardt I. Effects of preincubation temperature on the detection of fastidious organisms in delayed-entry samples in the BacT/ALERT 3D blood culture system. *J Microbiol Methods*. 2009;79(2):194-8.
140. Sautter RL, Bills AR, Lang DL, Ruschell G, Heiter BJ, Bourbeau PP. Effects of delayed-entry conditions on the recovery and detection of microorganisms from BacT/ALERT and BACTEC blood culture bottles. *J Clin Microbiol*. 2006;44(4):1245-9.
141. Ronnberg C, Mildh M, Ullberg M, Ozenci V. Transport time for blood culture bottles: underlying factors and its consequences. *Diagn Microbiol Infect Dis*. 2013;76(3):286-90.
142. Lamy B, Sundqvist M, Idelevich EA, Escmid Study Group for Bloodstream Infections E, Sepsis. Bloodstream infections - Standard and progress in pathogen diagnostics. *Clin Microbiol Infect*. 2020;26(2):142-50.
143. Åkerlund A, Petropoulos A, Malmros K, Tängdén T, Giske CG. Blood culture diagnostics: a Nordic multicentre survey comparison of practices in clinical microbiology laboratories. *Clinical Microbiology and Infection*. 2022;28(5):731.e1-.e7.
144. Bruins MJ, Egbers MJ, Israel TM, Diepeveen SHA, Wolfhagen MJHM. Reduced length of hospital stay through a point of care placed automated blood culture instrument. *European Journal of Clinical Microbiology & Infectious Diseases*. 2017;36(4):619-23.
145. Schaumburg F, Schuler F, Fobker M, Esser J. Integrated decentralized blood culture incubation: A step towards a 24/7 microbiology service? *J Microbiol Methods*. 2024;223:106973.
146. Patel R. Matrix-assisted laser desorption ionization-time of flight mass spectrometry in clinical microbiology. *Clin Infect Dis*. 2013;57(4):564-72.

147. Schubert S, Weinert K, Wagner C, Gunzl B, Wieser A, Maier T, et al. Novel, Improved Sample Preparation for Rapid, Direct Identification from Positive Blood Cultures Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry. *The Journal of Molecular Diagnostics*. 2011;13(6):701-6.
148. Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K. Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. *Clin Microbiol Infect*. 2014;20(10):1001-6.
149. Vyas S, Patel D, Bandali A, Giordano P, Roland R, Kessler J. Impact of BioFire® Blood Culture Identification (BCID) panels on antibiotic management of bacteremia due to select organisms. *Diagn Microbiol Infect Dis*. 2024;110(1):116384.
150. Matuschek E, Brown DF, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin Microbiol Infect*. 2014;20(4):O255-66.
151. Messiaen AS, Vandendriessche S, De Muynck E, Strubbe G, De Bus L, Schelstraete P, et al. Impact of reporting rapid susceptibility results in Gram negative bloodstream infections: a real world prospective study. *Eur J Clin Microbiol Infect Dis*. 2025;44(4):847-53.
152. Åkerlund A, Jonasson E, Matuschek E, Serrander L, Sundqvist M, Kahlmeter G. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories. *J Antimicrob Chemother*. 2020;75(11):3230-8.
153. Menchinelli G, Liotti FM, Fiori B, De Angelis G, D'Inzeo T, Giordano L, et al. In vitro Evaluation of BACT/ALERT(R) VIRTUO(R), BACT/ALERT 3D(R), and BACTEC FX Automated Blood Culture Systems for Detection of Microbial Pathogens Using Simulated Human Blood Samples. *Front Microbiol*. 2019;10:221.
154. Laupland KB, Edwards F, Dettrick Z, Harris PNA. Time to positivity is a risk factor for death among patients with bloodstream infections: a population-based cohort. *Clin Microbiol Infect*. 2024;30(7):899-904.
155. Blot F, Schmidt E, Nitenberg G, Tancrède C, Leclercq B, Laplanche A, et al. Earlier positivity of central-venous- versus peripheral-blood cultures is highly predictive of catheter-related sepsis. *J Clin Microbiol*. 1998;36(1):105-9.
156. Haimi-Cohen Y, Vellozzi EM, Rubin LG. Initial concentration of *Staphylococcus epidermidis* in simulated pediatric blood cultures correlates with time to positive results with the automated, continuously monitored BACTEC blood culture system. *J Clin Microbiol*. 2002;40(3):898-901.
157. Turkeltaub L, Kashat L, Assous MV, Adler K, Bar-Meir M. Estimating bacterial load in *S. aureus* and *E. coli* bacteremia using bacterial growth graph from the continuous monitoring blood culture system. *Eur J Clin Microbiol Infect Dis*. 2024;43(10):1931-8.
158. Kitagawa H, Kojima M, Tadera K, Kogasaki S, Omori K, Nomura T, et al. Clinical diagnostic performance of droplet digital PCR for pathogen detection in

- patients with *Escherichia coli* bloodstream infection: a prospective observational study. *BMC Infect Dis.* 2025;25(1):22.
159. Gutierrez J, Guimaraes AO, Lewin-Koh N, Berhanu A, Xu M, Cao Y, et al. Sustained Circulating Bacterial Deoxyribonucleic Acid Is Associated With Complicated *Staphylococcus aureus* Bacteremia. *Open Forum Infect Dis.* 2019;6(4):ofz090.
 160. Reshes G, Vanounou S, Fishov I, Feingold M. Timing the start of division in *E. coli*: a single-cell study. *Phys Biol.* 2008;5(4):046001.
 161. Belay N, Rasooly A. *Staphylococcus aureus* growth and enterotoxin A production in an anaerobic environment. *J Food Prot.* 2002;65(1):199-204.
 162. Falconer K, Hammond R, Parcell BJ, Gillespie SH. Investigating the time to blood culture positivity: why does it take so long? *J Med Microbiol.* 2025;74(1).
 163. Kassis C, Rangaraj G, Jiang Y, Hachem RY, Raad I. Differentiating culture samples representing coagulase-negative staphylococcal bacteremia from those representing contamination by use of time-to-positivity and quantitative blood culture methods. *J Clin Microbiol.* 2009;47(10):3255-60.
 164. Haimi-Cohen Y, Shafinoori S, Tucci V, Rubin LG. Use of incubation time to detection in BACTEC 9240 to distinguish coagulase-negative staphylococcal contamination from infection in pediatric blood cultures. *The Pediatric Infectious Disease Journal.* 2003;22(11).
 165. Chatzinikolaou I, Hanna H, Darouiche R, Samonis G, Tarrand J, Raad I. Prospective study of the value of quantitative culture of organisms from blood collected through central venous catheters in differentiating between contamination and bloodstream infection. *J Clin Microbiol.* 2006;44(5):1834-5.
 166. St Geme JW, 3rd, Bell LM, Baumgart S, D'Angio CT, Harris MC. Distinguishing sepsis from blood culture contamination in young infants with blood cultures growing coagulase-negative staphylococci. *Pediatrics.* 1990;86(2):157-62.
 167. Blot F, Nitenberg G, Chachaty E, Raynard B, Germann N, Antoun S, et al. Diagnosis of catheter-related bacteraemia: a prospective comparison of the time to positivity of hub-blood versus peripheral-blood cultures. *Lancet.* 1999;354(9184):1071-7.
 168. Orihuela-Martín J, Rodríguez-Núñez O, Morata L, Cardozo C, Puerta-Alcalde P, Hernández-Meneses M, et al. Performance of differential time to positivity as a routine diagnostic test for catheter-related bloodstream infections: a single-centre experience. *Clin Microbiol Infect.* 2020;26(3):383.e1-e7.
 169. Dhaliwal M, Daneman N. Utility of Differential Time to Positivity in Diagnosing Central Line-Associated Bloodstream Infections: A Systematic Review and Meta-Analysis. *Clin Infect Dis.* 2023;77(3):428-37.
 170. Khatib R, Riederer K, Saeed S, Johnson LB, Fakhri MG, Sharma M, et al. Time to positivity in *Staphylococcus aureus* bacteremia: possible correlation with the source and outcome of infection. *Clin Infect Dis.* 2005;41(5):594-8.
 171. Marra AR, Edmond MB, Forbes BA, Wenzel RP, Bearman GM. Time to blood culture positivity as a predictor of clinical outcome of *Staphylococcus aureus* bloodstream infection. *J Clin Microbiol.* 2006;44(4):1342-6.

172. Kim J, Gregson DB, Ross T, Laupland KB. Time to blood culture positivity in *Staphylococcus aureus* bacteremia: association with 30-day mortality. *J Infect.* 2010;61(3):197-204.
173. Simeon S, Le Moing V, Tubiana S, Duval X, Fournier D, Lavigne JP, et al. Time to blood culture positivity: An independent predictor of infective endocarditis and mortality in patients with *Staphylococcus aureus* bacteraemia. *Clin Microbiol Infect.* 2019;25(4):481-8.
174. Santos-Patarroyo SD, Quintero-Martinez JA, Lahr BD, Chesdachai S, Abu Saleh O, Michelena HI, et al. Correlation Between Blood Culture Time to Positivity and Vegetation Size in *Staphylococcus aureus* Infective Endocarditis. *Antibiotics (Basel).* 2025;14(5).
175. Huang L, Sun L, Yan Y. Time to positivity of blood culture is predictive for nosocomial infection and infectious endocarditis instead of other clinical characteristics and prognosis in *Acinetobacter baumannii* bloodstream infection. *J Infect.* 2014;68(2):198-200.
176. Krus D, Kahn F, Nilson B, Sunnerhagen T, Rasmussen M. Blood culture time to positivity in non-beta-hemolytic streptococcal bacteremia as a predictor of infective endocarditis-a retrospective cohort study. *Eur J Clin Microbiol Infect Dis.* 2022;41(2):325-9.
177. García-Ceberino PM, Anguita-Santos F, Llenas-García J, Montero-Alonso M, Chueca-Porcuna N, Borrajo E, et al. External validation of the HANDOC score in a Spanish cohort-Analysis of blood culture scoring and time to positivity. *Enferm Infecc Microbiol Clin (Engl Ed).* 2025;43(9):555-62.
178. Hsieh YC, Chen HL, Lin SY, Chen TC, Lu PL. Short time to positivity of blood culture predicts mortality and septic shock in bacteremic patients: a systematic review and meta-analysis. *BMC Infect Dis.* 2022;22(1):142.
179. Yagupsky P, Nolte FS. Quantitative aspects of septicemia. *Clin Microbiol Rev.* 1990;3(3):269-79.
180. Sowden D, Anstey C, Faddy M. Blood culture time to positivity as a predictor of mortality in community acquired methicillin-susceptible *Staphylococcus aureus* bacteremia. *J Infect.* 2008;56(4):295-6.
181. Cillóniz C, Ceccato A, de la Calle C, Gabarrús A, Garcia-Vidal C, Almela M, et al. Time to blood culture positivity as a predictor of clinical outcomes and severity in adults with bacteremic pneumococcal pneumonia. *PLoS One.* 2017;12(8):e0182436.
182. Peralta G, Roiz MP, Sánchez MB, Garrido JC, Ceballos B, Rodríguez-Lera MJ, et al. Time-to-positivity in patients with *Escherichia coli* bacteraemia. *Clin Microbiol Infect.* 2007;13(11):1077-82.
183. Liao CH, Lai CC, Hsu MS, Huang YT, Chu FY, Hsu HS, et al. Correlation between time to positivity of blood cultures with clinical presentation and outcomes in patients with *Klebsiella pneumoniae* bacteraemia: prospective cohort study. *Clin Microbiol Infect.* 2009;15(12):1119-25.
184. Chen SY, Weng TH, Tseng WP, Fu CM, Lin HW, Liao CH, et al. Value of blood culture time to positivity in identifying complicated nontyphoidal *Salmonella* bacteremia. *Diagn Microbiol Infect Dis.* 2018;91(3):210-6.

185. Bläckberg A, Svedevall S, Lundberg K, Nilson B, Kahn F, Rasmussen M. Time to Blood Culture Positivity: An Independent Predictor of Mortality in *Streptococcus Pyogenes* Bacteremia. *Open Forum Infect Dis.* 2022;9(6):ofac163.
186. Nunes CZ, Marra AR, Edmond MB, da Silva Victor E, Pereira CA. Time to blood culture positivity as a predictor of clinical outcome in patients with *Candida albicans* bloodstream infection. *BMC Infect Dis.* 2013;13:486.
187. Keighley C, Pope AL, Marriott D, Chen SC, Slavin MA, Australia, et al. Time-to-positivity in bloodstream infection for *Candida* species as a prognostic marker for mortality. *Med Mycol.* 2023;61(4).
188. Church D, Somayaji R, Viczko J, Gregson D, Naugler C. A longitudinal population-based study of predictors of mortality from bloodstream infections in Calgary, Alberta, Canada. *Future Microbiol.* 2022;17:17-25.
189. Hamilton F, Evans R, Ghazal P, MacGowan A. Time to positivity in bloodstream infection is not a prognostic marker for mortality: analysis of a prospective multicentre randomized control trial. *Clin Microbiol Infect.* 2022;28(1):136 e7-e13.
190. Ljungquist O, Tverring J, Oldberg K, Sunnerhagen T, Torisson G. Association of time to positivity with disease severity in bloodstream infections-a population-based cohort study. *Clin Microbiol Infect.* 2025;31(9):1532-8.
191. Adamik M, Hutchins A, Mangilit J, Katzin B, Totty H, Deol P. Effect of delayed entry on performance of the BACT/ALERT FAN PLUS bottles in the BACT/ALERT VIRTUO blood culture system. *Eur J Clin Microbiol Infect Dis.* 2021;40(4):699-705.
192. Bläckberg A, Lundberg K, Svedevall S, Nilson B, Rasmussen M. Time to positivity of blood cultures in bloodstream infections with *Streptococcus dysgalactiae* and association with outcome. *Infect Dis (Lond).* 2023;55(5):333-9.
193. Lamy B. Blood culture time-to-positivity: making use of the hidden information. *Clin Microbiol Infect.* 2019;25(3):268-71.
194. Maffezzoli P, Kestler M, Burillo A, Corcione S, De Rosa FG, Muñoz P, et al. Diagnostic and prognostic value of time to positivity in blood cultures. An opinion paper. *Rev Esp Quimioter.* 2025;38(1):8-20.
195. Weinstein MP, Mirrett S, Wilson ML, Reimer LG, Reller LB. Controlled evaluation of 5 versus 10 milliliters of blood cultured in aerobic BacT/Alert blood culture bottles. *J Clin Microbiol.* 1994;32(9):2103-6.
196. Kim SC, Kim S, Lee DH, Choi SR, Kim JS. Effect of blood volume in standard anaerobic blood culture bottles of the BacT/ALERT 3D system used for the detection of pathogens and time to detection. *PLoS One.* 2015;10(2):e0116728.
197. Zaghi I, Cricca M, Roberts JA, Brandolini M, Colosimo C, Montanari S, et al. Development of blood culture time-to-positivity as a pharmacodynamic indicator for monitoring antibiotic therapy against MDR Gram-negative bacteria. *J Antimicrob Chemother.* 2025;80(10):2705-13.
198. Yuan S, Yang J, Deng N, Peng J, Ma L, Du S. Comparative study of antibiotic adsorption capacity of three brands of blood culture bottles. *Front Cell Infect Microbiol.* 2025;15:1571264.

199. Martínez JA, Pozo L, Almela M, Marco F, Soriano A, López F, et al. Microbial and clinical determinants of time-to-positivity in patients with bacteraemia. *Clin Microbiol Infect.* 2007;13(7):709-16.
200. Edwards F, Waller M, Laupland KB. Determinants of time to positivity in bloodstream infections: an analysis of a population-based cohort in Queensland, Australia. *Eur J Clin Microbiol Infect Dis.* 2025;44(5):1197-204.
201. Wiggers JB, Daneman N. The culture of follow-up blood cultures. *Clinical Microbiology and Infection.* 2020;26(7):811-3.
202. Kok W. Is It Useful to Repeat Blood Cultures in Endocarditis Patients? A Critical Appraisal. *Diagnostics (Basel).* 2024;14(14).
203. Berge A, Ekdahl C, Ekspong L, Kurland S, Olaison L, Rasmussen G, et al. Vårdprogram infektiös endokardit, rev 2023: Svenska infektionsläkarföreningen; 2023 [Available from: https://infektion.net/wp-content/uploads/2023/10/vardprogram_infektios_endokardit_rev_2023.pdf].
204. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.
205. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. *Clin Microbiol Infect.* 2012;18 Suppl 7:19-37.
206. Fowler VG, Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Intern Med.* 2003;163(17):2066-72.
207. Kuehl R, Morata L, Boeing C, Subirana I, Seifert H, Rieg S, et al. Defining persistent *Staphylococcus aureus* bacteraemia: secondary analysis of a prospective cohort study. *Lancet Infect Dis.* 2020;20(12):1409-17.
208. Maskarinec SA, Park LP, Ruffin F, Turner NA, Patel N, Eichenberger EM, et al. Positive follow-up blood cultures identify high mortality risk among patients with Gram-negative bacteraemia. *Clin Microbiol Infect.* 2020;26(7):904-10.
209. Thaden JT, Cantrell S, Dagher M, Tao Y, Ruffin F, Maskarinec SA, et al. Association of Follow-up Blood Cultures With Mortality in Patients With Gram-Negative Bloodstream Infections: A Systematic Review and Meta-analysis. *JAMA Netw Open.* 2022;5(9):e2232576.
210. Shinohara J, Hanai S, Jung J, Song KH, Iwata M, Terasawa T. Association of Repeated Blood Cultures With Mortality in Adult Patients With Gram-Negative Bacilli Bacteremia: A Systematic Review and Meta-analysis. *Open Forum Infect Dis.* 2022;9(12):ofac568.
211. Wiggers JB, Xiong W, Daneman N. Sending repeat cultures: is there a role in the management of bacteremic episodes? (SCRIBE study). *BMC Infect Dis.* 2016;16:286.
212. Park SY, Chang EJ, Ledebøer N, Messacar K, Lindner MS, Venkatasubrahmanyam S, et al. Plasma Microbial Cell-Free DNA Sequencing from over 15,000 Patients Identified a Broad Spectrum of Pathogens. *J Clin Microbiol.* 2023;61(8):e0185522.

213. Esse J, Träger J, Steininger P, Bihlmaier K, Fürst J, Bardonicsek-Depnering Z, et al. Metagenomic analysis of microbial cell-free DNA from plasma of patients with suspected infections: performance and therapeutic impact in clinical routine. *Clin Microbiol Infect.* 2025;31(6):1018-25.
214. Peker N, Couto N, Sinha B, Rossen JW. Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. *Clinical Microbiology and Infection.* 2018;24(9):944-55.
215. Ziegler I, Cajander S, Rasmussen G, Ennefors T, Mölling P, Strålin K. High nuc DNA load in whole blood is associated with sepsis, mortality and immune dysregulation in *Staphylococcus aureus* bacteraemia. *Infect Dis (Lond).* 2019;51(3):216-26.
216. Rapszky GA, Do To UN, Kiss VE, Kóti T, Walter A, Gergő D, et al. Rapid molecular assays versus blood culture for bloodstream infections: a systematic review and meta-analysis. *eClinicalMedicine.* 2025;79.
217. Fida M, Wolf MJ, Hamdi A, Vijayvargiya P, Esquer Garrigos Z, Khalil S, et al. Detection of Pathogenic Bacteria From Septic Patients Using 16S Ribosomal RNA Gene-Targeted Metagenomic Sequencing. *Clin Infect Dis.* 2021;73(7):1165-72.
218. Lieu A, Harrison LB, Harel J, Lawandi A, Cheng MP, Domingo MC. The microbiological outcomes of culture-negative blood specimens using 16S rRNA broad-range PCR sequencing: a retrospective study in a Canadian province from 2018 to 2022. *J Clin Microbiol.* 2024;62(3):e0151823.
219. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat Microbiol.* 2019;4(4):663-74.
220. Nielsen ME, Søgaard KK, Karst SM, Krarup AL, Albertsen M, Nielsen HL. Application of rapid Nanopore metagenomic cell-free DNA sequencing to diagnose bloodstream infections: a prospective observational study. *Microbiol Spectr.* 2025;13(5):e0329524.
221. Nestor D, Peker N, Couto N, Hiss AN, Herbig A, Krause J, et al. Performance of shotgun metagenomics on whole blood from patients with suspected bloodstream infection: Challenges remain. *J Microbiol Methods.* 2025;237:107231.
222. Guimaraes AO, Gutierrez J, Maskarinec SA, Cao Y, Hong K, Ruffin F, et al. Prognostic Power of Pathogen Cell-Free DNA in *Staphylococcus aureus* Bacteremia. *Open Forum Infect Dis.* 2019;6(4):ofz126.
223. Ziegler I, Lindström S, Källgren M, Strålin K, Mölling P. 16S rDNA droplet digital PCR for monitoring bacterial DNAemia in bloodstream infections. *PLoS One.* 2019;14(11):e0224656.
224. Ziegler I, Josefson P, Olcén P, Mölling P, Strålin K. Quantitative data from the SeptiFast real-time PCR is associated with disease severity in patients with sepsis. *BMC Infect Dis.* 2014;14:155.
225. Rello J, Lisboa T, Lujan M, Gallego M, Kee C, Kay I, et al. Severity of pneumococcal pneumonia associated with genomic bacterial load. *Chest.* 2009;136(3):832-40.

226. Population per month by region, age and sex. Year 2000M01 - 2024M12: Statistics Sweden; 2026 [Available from: https://www.statistikdatabasen.scb.se/pxweb/en/ssd/START_BE_BE0101_BE0101A/BefolkManad/].
227. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *Journal of Chronic Diseases*. 1987;40(5):373-83.
228. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health Care–Associated Bloodstream Infections in Adults: A Reason To Change the Accepted Definition of Community-Acquired Infections. *Annals of Internal Medicine*. 2002;137(10):791-7.
229. Anderson DJ, Murdoch DR, Sexton DJ, Reller LB, Stout JE, Cabell CH, et al. Risk Factors for Infective Endocarditis in Patients with Enterococcal Bacteremia: A Case-Control Study. *Infection*. 2004;32(2):72-7.
230. Dahl A, Lauridsen TK, Arpi M, Sørensen LL, Østergaard C, Sogaard P, et al. Risk Factors of Endocarditis in Patients With Enterococcus faecalis Bacteremia: External Validation of the NOVA Score. *Clin Infect Dis*. 2016;63(6):771-5.
231. Bussini L, Rosselli Del Turco E, Pasquini Z, Scolz K, Amedeo A, Beci G, et al. Risk factors for persistent enterococcal bacteraemia: a multicentre retrospective study. *J Glob Antimicrob Resist*. 2022;29:386-9.
232. Shephard EA, Mondy K, Reveles KR, Jaso T, Rose DT. A Comparison of Outcomes With and Without Infectious Diseases Consultation for Enterococcal Bacteraemia in a Multicenter Healthcare System. *Int J Antimicrob Agents*. 2022;60(5-6):106665.
233. Pericàs JM, Llopis J, Muñoz P, Gálvez-Acebal J, Kestler M, Valerio M, et al. A Contemporary Picture of Enterococcal Endocarditis. *J Am Coll Cardiol*. 2020;75(5):482-94.
234. Shah NH, Shutt KA, Doi Y. Ampicillin-Ceftriaxone vs Ampicillin-Gentamicin for Definitive Therapy of Enterococcus faecalis Infective Endocarditis: A Propensity Score-Matched, Retrospective Cohort Analysis. *Open Forum Infect Dis*. 2021;8(4):ofab102.
235. Oldberg K, Stenmark J, Hammarström H. β -D-Glucan Testing in Candidemia: Determinants of Positivity and Association With Mortality. *Mycoses*. 2025;68(5):e70067.
236. Tonizzo A, Muñoz P, Burillo A, Adán I, Alcalá L, Bouza E. Is Time to Positivity in Staphylococcus aureus Bacteremia a Harbinger of Endocarditis? *Clin Infect Dis*. 2025.
237. Santos-Patarroyo SD, Abu Saleh O, Chesdachai S, Lahr BD, Michelena HI, Quintero-Martinez JA, et al. Blood Culture Time to Positivity and Risk of Infective Endocarditis. *Clinical Infectious Diseases*. 2025:ciaf359.
238. Michelson K, Löffler B, Höring S. Time to positivity as a prognostic factor in bloodstream infections with Enterococcus spp. *Diagn Microbiol Infect Dis*. 2021;101(3):115396.

239. Robson C, Khan H, Junckerstorff RK, Graham M, Stuart RL, Nicholls SJ, et al. The relationship between blood culture time-to-positivity and *Enterococcus faecalis* infective endocarditis. *Pathology*. 2025;57(7):932-5.
240. Deas G, Shuttleworth J, Carson A, Knapper F, North P, Williams P, et al. Blood culture time-to-positivity can identify *S aureus*, but not Enterococcal or Streptococcal endocarditis. *NIHR Open Research*. 2025;5:74.
241. Strömdahl M, Ursing J. Time to Positivity in *Staphylococcus aureus* Bacteremia—One of Many Pieces in the Diagnostic Puzzle. *Clinical Infectious Diseases*. 2025:ciaf360.
242. Deas G, Hamilton F, Williams P. Less haste, more speed: Does delayed blood culture transport time lead to adverse incubation times or yield? *Journal of Infection*. 2025;91(1):106520.

About the author

Although blood cultures have been used for over a hundred years in the diagnosis of infectious diseases, it is still possible to make small improvements in their use. This thesis explores the significance of the sampling procedure, and the value of repeated blood cultures and of time to positivity.



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