IMPROVEMENT OF MICROBIOLOGICAL DIAGNOSTICS IN SEPSIS

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Improvement of microbiological diagnostics in sepsis

Thesis for Doctoral Degree (Ph.D.)

By

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Popular science summary of the thesis

Sepsis is a life-threatening condition characterized by organ failure triggered by infection. Despite changing definitions over the years, sepsis remains a leading cause of death worldwide. The effective treatment of sepsis relies on promptly identifying the infectious agent in order to give appropriate antibiotic therapy. Yet, our primary tool for identifying these deadly pathogens—the blood culture—relies on techniques developed over a century ago. Despite advancements, blood cultures often fail to detect bacteria in more than half of all sepsis cases.

The overall aim of this thesis is to study the current and future methods of detecting bacteria in blood in sepsis. In the first study we questioned the need to draw blood from multiple skin sites and revealed that sampling from a single site can be just as effective and might minimize contamination errors.

In the second and third project, we studied the results of blood cultures in varying conditions. The second study compared microbiological findings in patients fulfilling the new definition of sepsis introduced in 2016 versus the old definition. We found that most patients fulfilled both definitions, with blood cultures finding pathogens in around one third of patients. In the third project, we studied blood cultures in COVID-19 patients, revealing lower chance of finding bacteria in the bloodstream and an increased risk of sample contamination in this group.

The last part of the thesis highlights the potential but also the limits of non-culture based diagnostics. These new and innovative methods, which detect bacterial DNA directly, provide an exciting new frontier of microbiological diagnosis in sepsis. The fourth study reveals that many sepsis patients may harbor bacteria previously undetectable by conventional means, but that these novel analyses still suffer substantial logistical delays.

The findings presented in this thesis are meant to be used by clinicians in their daily work. For example, the change in practice to single-sampling strategy means that blood cultures are now sampled more effectively and with less discomfort to patients. We also now know more about what kind of microorganisms we can expect to find using the new definition of sepsis, and that we do not find bacteria in COVID-19 patients as often as previously thought. Lastly, we demonstrated that non-culture based tests are a valuable complement to blood cultures, but also that the logistic chain needs to be optimized to take full advantage of them.
Populärvetenskaplig sammanfattning

Sepsis är ett livshotande tillstånd som kännetecknas av organsvikt orsakad av en infektion. En tillförlitlig och snabb metod för att identifiera den orsakande mikroorganismen är en förutsättning för att kunna ge effektiv behandling. Ändå bygger vårt främsta verktyg för att identifiera dessa dödliga mikroorganismer – blododlingen – på tekniker som utvecklades för över ett sekel sedan. Trots förbättringar i blododlingsmetodiken misslyckas vi fortfarande med att odla fram bakterier i mer än hälften av alla sepsisfall.


Den fjärde och sista delen av avhandlingen undersöker värdet av nya metoder som kan detektera bakteriellt DNA direkt från blodet. Denna studie avslöjade med hjälp av dessa metoder att många sepsispatienter kan vara infekterade av bakterier som tidigare varit oidentifierbara med blododling, men att de nya metoderna fortfarande lider av fördröjningar i provhanteringen.

Fynden i denna avhandling är tänkta att användas i den kliniska vardagen. Vi tar nu blododlingar på ett sätt som är effektivare och innebär mindre obehag för patienten. Vi har sett att förekomsten av mikroorganismer är den samma efter införandet av den nya definitionen av sepsis, och att det är ovanligare att hitta bakterier i blodet vid COVID-19 än vad vi först trodde. Slutligen har vi lärt oss att nya metoder för detektion av bakteriellt DNA utgör ett nytt och viktigt komplement i diagnostiken vid sepsis, men att en effektiv logistik är av yttersta vikt för att den nya tekniken ska komma till sin fulla potential.
Abstract

Sepsis, a life-threatening condition characterized by a dysregulated host response to infection, poses significant diagnostic and therapeutic challenges, contributing to high morbidity and mortality rates worldwide. The overall objective of this thesis is to provide an insight in how to improve the microbiological diagnosis of sepsis both by optimizing current blood culture (BC) practices and by evaluating novel diagnostic methods. The thesis also explores the impact on microbiological diagnosis imposed by two major recent events; the change of clinical sepsis criteria and the COVID–19 pandemic. The thesis consists of the four following projects:

Study I was a prospective non-inferiority study, assessing the utility of implementing a single-sampling strategy (SSS) versus multi-sampling strategy (MSS) for BCs in sepsis. The study group consisted of 549 suspected BSI episodes. The outcomes were detection of pathogens and occurrence of sample contamination. We found no significant difference in pathogen detection rates, and a tendency toward less contamination using SSS, thereby suggesting a potential simplification of the BC sampling protocol without compromising diagnostic yield.

In Study II, we described microbiological findings when using different sepsis criteria (Sepsis–2 versus Sepsis–3) in 514 patients with suspected sepsis. There were 357/514 (79.5%) Sepsis–3 and 411/514 (80.0%) Sepsis–2 episodes, with two-thirds of patients fulfilling both definitions. The BC positivity rate was 130/357 (36.1%) and 145/411 (35.3%) in Sepsis–2 and Sepsis–3, respectively. The study shows that even with the improved definition, the frequency of BC positivity remains largely consistent.

Study III explored the changes in bacteremia rates during the COVID–19 pandemic, comparing BCs from patients with COVID–19 (n = 3,027) with two control groups without COVID–19, consisting of a contemporary group (n = 6,663) during the same period, as well as historical group (n = 8,175) from a year prior. Clinically relevant growth was found in 6.5% of the BC episodes in the COVID–19 group compared to 10.8% and 10.4% in the control groups respectively. Contamination was present in 8.4% in the COVID–19 group compared to 5.0% and 4.3% in the control groups. The study concluded that in COVID–19 patients, frequency of relevant growth was lower and contamination rates were higher.
Study IV retrospectively evaluated the diagnostic performance of T2Bacteria, a rapid molecular-based tool, against traditional BCs. We compared 640 T2Bacteria results to all BCs sampled within ± 72 hours of the T2Bacteria sampling. In total, 46/101 (45.5%) episodes were T2Bacteria positive/BC negative and 26/101 (25.7%) were T2Bacteria negative/BC positive. Only 29 (28.7%) episodes were positive using both methods. Total turn-around time was 27 hours and 33 minutes for T2Bacteria and 36 hours and 48 minutes for BCs (p < 0.001). We concluded that identification using molecular methods directly from blood provide a valuable complement to BCs.

Collectively, the findings from these studies adds to the understanding of microbiological diagnosis in sepsis, and highlights the need of both improving established methods, as well as the judicious implementation of novel techniques.
List of scientific papers

I. Single-sampling strategy versus multi-sampling strategy for blood cultures in sepsis: a prospective non-inferiority study

David Yu, Anna Ekwall-Larson, Åsa Parke, Christian Unge, Claes Henning, Jonas Sundén-Cullberg, Anna Somell, Kristoffer Strålin*, Volkan Özenci*

*equal contribution


II. Correlation of clinical sepsis definitions with microbiological characteristics in patients admitted through a sepsis alert system; a prospective cohort study

David Yu, David Unger, Christian Unge, Åsa Parke, Jonas Sundén-Cullberg, Kristoffer Strålin, Volkan Özenci


III. Low prevalence of bloodstream infection and high blood culture contamination rates in patients with COVID-19

David Yu, Karolina Ininbergs, Karolina Hedman, Christian G. Giske, Kristoffer Strålin, Volkan Özenci

PLoS ONE (2020) 15(11)

IV. Performance of T2Bacteria in relationship to Blood Cultures: a retrospective comparative study

David Yu, Anna Ekwall-Larson, Volkan Özenci

Submitted
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AST</td>
<td>Antimicrobial susceptibility testing</td>
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<tr>
<td>BC</td>
<td>Blood culture</td>
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<td>BSI</td>
<td>Blood stream infection</td>
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<td>CFU</td>
<td>Colony forming units</td>
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<td>CLABSI</td>
<td>Central line associated blood stream infection</td>
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<td>COVID-19</td>
<td>Coronavirus disease 2019</td>
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<td>DAMP</td>
<td>Damage associated molecular pattern</td>
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<tr>
<td>ED</td>
<td>Emergency department</td>
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<td>ESI-MS</td>
<td>Electrospray ionization mass spectrometry</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
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<td>MSS</td>
<td>Multi-sampling strategy</td>
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<td>NPV</td>
<td>Negative predictive value</td>
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<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PPR</td>
<td>Pattern recognition receptor</td>
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<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>RETTS</td>
<td>Rapid emergency triage and treatment system</td>
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<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<td>SOFA</td>
<td>Sequential organ failure assessment</td>
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<td>SSS</td>
<td>Single-sampling strategy</td>
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<tr>
<td>TAT</td>
<td>Turnaround time</td>
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<td>TTD</td>
<td>Time to detection</td>
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<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 Introduction

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (1). The healthcare burden of sepsis is substantial and a major contributor to morbidity and mortality worldwide. Incidence is difficult to estimate as varying case definitions has been used over the years. A meta-analysis estimated the incidence of sepsis to 437 cases per 100,000 person–years, however the analysis only included studies performed in high-income countries (2). Epidemiological data for low-and middle-income countries remain scarce due to missing reliable population-based data (2). Even in high-income countries, heterogeneity in relevant case definitions and outcome measures across studies is a major obstacle in obtaining robust epidemiological data. A contemporary study reported a 48.9 million cases worldwide and 11 million deaths in 2017, with a markedly higher incidence in low- and middle-income countries (3).

Outcomes have been improved throughout time, with decreasing mortality despite an increased incidence (4). Mortality is reported to be around 25–30% (5, 6), and appears correlated to the presence of multiple organ failure, age and co-morbidities, presence of immunosuppression, as well as delayed treatment (4). In 2017, sepsis was declared a “Global Health Priority” by the World Health Organization (WHO), emphasizing the need for further research to improve outcomes in sepsis. The programme states that the aim is “to improve the prevention, diagnosis and clinical management of sepsis”, and outline the present goals of obtaining correct epidemiological data based on appropriate consensus case definitions, public awareness and establishment of sepsis surveillance (7).

The cornerstones in the management of sepsis includes early recognition, early effective antibiotic therapy, and supportive measures such as hemodynamic and respiratory support (8). Achieving a microbiological diagnosis in sepsis is critical and carry two main functions. One is to establish the presence of an infectious cause in a case of suspected sepsis, and the other is to guide appropriate antimicrobial therapy. The importance of early appropriate antimicrobial therapy to reduce mortality and health care costs has been confirmed in several studies (9–12). There is utmost need for optimization of both traditional blood culture (BC) based methods and investigation of novel molecular–based methods in the diagnosis of sepsis. This thesis explores the challenges in achieving a microbiological diagnosis in sepsis.
2 Literature review

2.1 Pathophysiology

The pathophysiology of sepsis remains elusive, and it is highly likely that mechanisms of disease vary between cases due to complex differences in microbial pathogenicity, host factors, or host-microbial interactions. There is evidence of dysregulation of the immune system as a key component, with innate immune responses to microbial pathogen-associated molecular patterns (PAMPs) as one of the central mechanisms. Pattern recognition receptors (PRRs) recognize PAMPs, and intracellular signaling leads to release of cytokines such as TNF-α, IL-1, IL-2 and IL-6 (5). In addition, several bacteria produce toxins which provides other mechanisms to elicit a host immune response (13). The concentration of bacteria or other pathogens in blood has been suggested as an important factor in the resulting disease severity. The proposed mechanism is directly linked to the immunological background of sepsis; a higher pathogen count provides more antigens that are immunologically recognized, amplifying the host immune response. It has been shown, using quantitative methods, that mortality is correlated with bacterial load in blood (14). The correlation of bacterial load and disease severity is likely complex and dependent on other confounding factors, such as underlying disease predisposing to bacteremia, certain foci of infection such as endocarditis and intravascular device infection, and the pathogen involved (14). Modern BC diagnostics are not quantitative, however time to detection (TTD) of a positive BC after incubation may yield indirect information regarding bacterial load (15).

After the initial microbial insult, further damage may be caused by the host response. Damage to endothelium and other host tissues results in release of damage associated molecular patterns (DAMPs), which in turn produce further inflammation and tissue injury via recognition by PRRs and further release of cytokines and proinflammatory peptides, creating a detrimental cascade of events (5). Inflammation and perturbation of the microcirculatory milieu leads to endothelial dysfunction and subsequent organ dysfunction. Organ dysfunction occurs mainly in organs highly dependent of adequate perfusion and oxygen delivery. In a Swedish study, cerebral, renal and pulmonary dysfunction is relatively common in sepsis, occurring in 25% of cases, with increasing incidence of pulmonary dysfunction with age (16).
2.2 Sepsis definitions

The clinical definition of sepsis has varied over time (Table 1). Despite the marked clinical response to sepsis, the current understanding of sepsis is incomplete, and a recent attempt to characterize sepsis using disease phenotypes revealed significant variability in expression of biomarkers, pattern of organ dysfunction and clinical parameters (17). Acknowledging that the pathophysiology of sepsis remains complex and poorly understood, health care providers have relied on clinical definitions in the diagnosis of sepsis.

In the first definition of sepsis (Sepsis-1), systemic inflammatory response syndrome (SIRS) was a central component in identification of sepsis (18). SIRS criteria include two or more of the following: heart rate exceeding 90/min, respiratory rate over 20/min, body temperature above 38°C or below 36°C, and leukocyte count either greater than 12 x 10⁹/L or less than 4 x 10⁹/L. Additionally, two specific subcategories were defined: severe sepsis, characterized by sepsis concurrent with organ failure, and septic shock, defined as sepsis accompanied by persistent hypotension despite the administration of intravenous fluids. In 2001 a new definition, Sepsis-2, was introduced. The major change was the inclusion of further clinical parameters specifying systemic inflammation in addition to SIRS, clarifying a list of general criteria as well as hemodynamic and organ perfusion criteria to diagnose sepsis (19). However, both Sepsis-1 and Sepsis-2 have been criticized to lack specificity and called for subsequent attempts to redefine the clinical diagnosis of sepsis (20).

To address the problems with Sepsis-1 and Sepsis-2, the current definition was introduced in 2016 as Sepsis-3 (21). The central change consisted of the use of specific parameters related to organ dysfunction due to infection, in contrast to the systemic inflammatory response syndrome (SIRS) used in the previous definitions (Sepsis-2 and Sepsis-3). As a crucial part of grading organ dysfunction, Sepsis-3 relies on the Sequential Organ Failure Assessment (SOFA) score to assess and quantify affected parameters. In the Sepsis-3 definition, sepsis is considered present when infection exists in combination with an increase in SOFA score more or equal to two points from baseline. Items to be assessed with SOFA includes respiratory, circulatory, coagulation, liver, renal and central nervous system dysfunction. To aid clinicians, quick SOFA (qSOFA), consisting of three items (mental alteration, increased respiratory rate, and hypotension) was
suggested as a tool for identification of septic patients at risk for adverse outcome (22). While recommended as a tool for stratifying risk, qSOFA is only marginally better than the original SIRS at identifying high risk patients and as SIRS, lacks specificity to diagnose sepsis. Therefore, qSOFA is not intended to be used as a screening tool to assess whether sepsis is present or not (5, 22).

Table 1: Past and present sepsis definitions

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<tbody>
<tr>
<td><strong>Sepsis</strong></td>
<td>SIRS* with infection (presumed or proven).</td>
<td>Signs and symptoms associated with sepsis was defined and expanded, in addition to SIRS.</td>
<td>Life-threatening organ dysfunction, defined as an increase in SOFA score by 2. Caused by a dysregulated host response to infection.</td>
</tr>
<tr>
<td><strong>Severe sepsis</strong></td>
<td>Sepsis (as above) with evidence of acute organ dysfunction.</td>
<td>Sepsis (as above) with evidence of acute organ dysfunction.</td>
<td>Severe sepsis was removed in the Sepsis-3 definition.</td>
</tr>
<tr>
<td><strong>Septic shock</strong></td>
<td>Sepsis with persistent hypotension after fluid resuscitation.</td>
<td>Sepsis with persistent hypotension after fluid resuscitation.</td>
<td>A subset of sepsis with persistent hypotension requiring vasopressors to maintain MAP of ≥65 mm Hg and a serum lactate level of &gt;2 mmol/L despite adequate volume resuscitation.</td>
</tr>
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</table>

*Sepsis criteria adapted from (19, 21, 23) respectively.
SIRS: Systemic Inflammatory Response Syndrome. SOFA: Sequential Organ Failure Assessment.

A common and persistent challenge to all published sepsis definitions is the lack of a golden standard against which sepsis criteria is compared and measured. Measures have included correlation with clinicians’ adjudication of the final diagnosis (20), International Classification of Diseases (ICD) codes (24), as well as microbiological outcomes such as the presence of blood stream infection (BSI) (16). This diversity in validation approaches illustrate the challenges in establishing an universally applicable clinical definition of sepsis.
2.3 Blood culture diagnostics

The practice of sampling blood for culture was first described in 1869 (25) and initially involved rudimentary aseptic techniques and blood collection methods including the use of leeches. The evolution of BCs experienced several landmarks during the beginning of the 20th century including the development of culture broths and media as well as improved collection techniques. Later, the introduction of automated systems to culture and detect bacterial growth led to the standardization of BC pre-processing and integration in the clinical workflow (26).

At the time of writing this thesis, BC is still the gold standard in diagnosis of blood stream infections (BSIs). The contemporary BC procedure consists of collecting an amount, usually 30–40 ml blood into bottles pre-filled with broth for analysis in the microbiology laboratory. The broths used today are complex and usually have proprietary formulas, usually consisting of a base of soybean casein digest, and various supplements aimed at providing optimal growth conditions (27).

As soon as possible after sampling, the BC bottles is incubated in continuous-monitoring automated systems with the purpose to rapidly increasing bacterial concentration. Most systems in use today monitors the metabolic activity of growing bacteria by analysing CO2 levels using colorimetric methods and will provide an alert when reaching a threshold value.

The subsequent analysis is usually performed by sub-culturing of blood from the positive BC bottle onto agar plates, and analysis of growth on different culture media. The identification of bacteria is aided by direct visualization on gram-stained preparations of the positive BC bottles, using adjuncts such as matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and in some cases, with molecular based tests. Antibiotic susceptibility testing (AST) is performed in parallel with the identification process, and additional tests is often needed to provide an accurate AST after the identification process. The entire process is time consuming, and even though most positive BC bottles is signalling growth within 24 hours (15), the complete assessment including definitive identification and AST often takes up to 2–3 days.

BC diagnostics can be viewed using a “bundle” approach, divided into preanalytical, analytical, and postanalytical phases (28). This approach takes in
account all the steps from recognizing the need for BCs followed by optimal sampling and transportation (preanalytical phase), pre-processing and analysis of the sample (analytical phase) and finally delivery of results to the clinician and translation into clinical use (postanalytical phase). In this bundle approach, the aim is to maximize diagnostic accuracy of BCs, while minimizing impact from contamination. The pre-analytical phase is an important determinant of the successful outcome of BCs (29). In this phase, the goal is to optimize sampling of cultures so that microbes can be isolated even in cases with a low bacterial load. Challenges include collecting an appropriate amount of blood, minimizing contamination by skin flora, using an optimal sampling method as well as maintaining optimized logistic chains for transportation of the sample (30).

The sampled BC volume is highly correlated to the ability to detect microorganisms within the sample (29, 31, 32). Most international guidelines advocate the collection of 4–6 bottles per sampling, each bottle containing 8–10 mL blood (30). Single-set BCs, also called solitary BCs, should be avoided due to an inferior detection rate compared with the recommended strategy (33). However in clinical practice, inadequate BC volume is common and solitary BCs occur in 10–33% (30). Problems include difficulties obtaining intravenous access, decreased venous filling in patients with shock, time constraints due to resuscitation efforts and a lack of continuous education regarding optimal BC sampling.

Contamination rates must be minimized, and guidelines recommend that laboratories keep contamination rates lower than 3% of all bottles received (34). Contamination commonly results from bacteria from the skin entering the sample, which erroneously might lead the clinician to assume that the subsequent growth represents blood stream infection. The most frequent contaminant microorganisms are coagulase-negative staphylococci, which is the isolated microorganism in 75–88% of contaminated samples (34). Strategies to minimize contamination include avoiding sampling from existing peripheral intravenous catheters, meticulous education of phlebotomists and implementation of optimal disinfection techniques, and optimal sampling methods (30, 34).
The sampling method, also sampling strategy (Figure 1), determines if the blood is drawn from a single or from two or more venipuncture sites. In the multi-sampling strategy (MSS), the sampled blood is collected from two different venipuncture sites. MSS is the dominating approach in most countries including Sweden due to the assumption that repeated venipunctures would increase the volume of blood collected, and allow clinicians to discriminate between suspected contaminations from separate venipuncture sites (30). In contrast, in the single-sampling strategy (SSS), the blood is collected from a single venipuncture site. The use of SSS is simpler and facilitates earlier completion of BC sampling which may save valuable time in the management of septic patients. In SSS, another benefit is introduced as second venipuncture is avoided, and thus eliminates one of two skin breaches which are the source of most BC contaminations (34).

Dargère et al. reported a study on BCs where two BC sets were sampled from a single venipuncture site, and a third BC set was sampled through a second venipuncture set within 24 hours after the first two draws (35). The authors suggested that SSS outperformed MSS both in terms of microorganisms detected and contamination rates. In the study by Dargère et al., interval between the samplings and low sample size imply that the SSS should be investigated further. Potential downsides of SSS are that isolated skin flora may pose a diagnostic difficulty as results from another venipuncture site, which may enable differentiating contamination from clinically relevant growth, is lacking. Moreover, there are certain scenarios where MSS is preferable to SSS. Examples include the
presence of indwelling catheters, as the diagnosis of central line associated blood stream infection (CLABSI) is aided by the difference in time to detection in samples collected from the central line and a peripheral vein (30).

BCs in sepsis are frequently negative. Even with optimal BC collection techniques, isolation of pathogens is only possible in around 30% of sepsis cases (36–39). There are several hypothetical possible mechanisms for negative BCs in sepsis. One is that there are no bacteria present in the blood stream in some cases of sepsis. Localized infectious and non-infectious processes may trigger systemic inflammatory responses and organ dysfunction, even in the absence of the migration of pathogens into the blood stream. As both current and previous sepsis definitions are entirely based on clinical parameters, it is reasonable to assume that fulfilment of sepsis criteria does not accurately predict the presence of pathogens in the blood stream in all cases.

On the other hand, if pathogens are assumed to be present in the blood stream, other explanations can be suspected to be the cause of a negative BC. Bacteremia may be present only intermittently, which can interfere with retrieval of pathogens if BC collection is not performed at the time of bacteremia. This suggested mechanism has led to the common practice of collecting BCs at several points in time rather than at a single occasion. However, the practice of collecting BCs intermittently, i.e., when there is a fever spike, has not been showed to increase the diagnostic performance (40).

Lastly, if assumed that BCs are indeed collected when bacteremia is ongoing, there are still mechanisms that can explain a negative BC. In BSIs, the concentration of microbes ranges from 0.01 to 10 colony forming units (CFU) per mL blood to $1 \times 10^3$ and $1 \times 10^4$ CFU/mL (30, 41). Several studies have showed that about 50% of BSI episodes have a bacteria concentration that ranges between 0.01 – 1 CFU/mL (30). However, the minimum in-vivo concentration of bacteria that can be detected by conventional BC is not known, and there is likely a lower threshold that prevents detection even in perfect pre-analytical and analytical circumstances.

To overcome the inherent limitations of BCs, there is a growing interest in culture-independent methods to provide the microbiological diagnosis in sepsis.
2.4 Molecular diagnostics

Molecular based methods including PCR-based diagnostics have the potential to improve the clinical workflow (Figure 2). As the culturing process remain the rate-limiting factor, molecular diagnostics offer the possibility to circumvent several steps, by either providing the diagnosis from a bacterial colony grown on an agar plate, a positive BC bottle, or from whole blood, i.e. freshly sampled blood from a patient. Currently, the only molecular based method that is firmly established in the clinical BC workflow is MALDI-TOF (29).

MALDI-TOF is typically performed after obtaining a colony from a cultured agar plate, after which the ribosomal mass to charge ratio (m/z) is analyzed using a time-of-flight method. The resulting spectrometry profile is then compared to a library, yielding a preliminary species identification. In addition to the established use of MALDI-TOF on a microbial colony grown on agar plate, the use of MALDI-TOF directly from a BC bottle is a theoretically promising approach (42). This method reportedly achieves successful identification of bacterial species in 80% of samples tested, with the potential to deliver results within 20 to 40 minutes following the detection of a colorimetric signal from an incubated BC bottle (28). However, the use of MALDI-TOF directly on positive BC bottles has several limitations which prevents routine use, including the requirement for pre-processing of the BC, need for sample batching, and suboptimal detection of fungemia and polymicrobial bacteremia (29, 42).

Polymerase chain reaction (PCR)-based tests target and amplify DNA in a sample, synthesising a high number of copies of a specific gene to be detected using a fluorescent reporter technique. This technique is labelled quantitative PCR, or real-time PCR, and allows for detection when the amount of fluorescence reach a pre-defined threshold. PCR-based techniques on whole blood offers several potential advantages in the microbiological diagnostics of BSI. First, administration of antibiotic therapy does not affect results as unlike BCs, PCR is not dependant on the presence of living microorganisms. Second, quantitative PCR have the potential ability to indicate the microbial burden in the patient, theoretically allowing for monitoring of microbial quantity in the blood and therefore efficacy of treatment. Lastly, PCR can detect important resistance genes causing phenotypic resistance in common microorganisms (43).
Major technical challenges in PCR-based diagnosis of BSI include extraction of bacterial DNA and reducing interaction with host DNA. The high quantity of host DNA in blood leads to unspecific detection, and methods to degrade and remove human DNA is needed to provide a suitable sample for analysis. Furthermore, natural PCR inhibitors in blood such as haemoglobin, immunoglobulins and lactoferrin must be neutralized using different reagents (43). Strategies used in overcoming these challenges include combining PCR with sequencing (44) and electrospray ionization mass spectrometry (PCR/ESI-MS) to provide species identification, and in some cases also relevant resistant genes (45, 46).
There are also some important limitations to PCR based diagnostics. Analogous with the problem encountered using traditional BC diagnostics, low bacterial load limits the sensitivity in PCR based diagnostics as well. Inherently, PCR is more sensitive than BC. However, in contrast to BCs in which optimally 30–40 ml of blood is sampled (30), current extraction techniques only allow for analysis of 1–5 ml blood in PCR-based methods (43). To overcome the problem with low bacterial concentration in blood, it is possible to first collect BCs to enrich the bacteria population in an incubator, after which PCR is used to identify bacteria. Several multiplex techniques have been utilized in this manner (47–49). As a positive signal may be detected only after several hours of incubation, the time to result is longer when compared to analysis directly on blood. Nonetheless, PCR on positive BC bottles may prove a useful adjunct to standard diagnostics.

Only a handful of methods utilizing whole blood has been used clinically (43). PCR/ESI–MS was a promising method utilizing mass spectrometry to identify up to 780 microorganisms as well as numerous resistance genes. The method had been validated in a series of prospective studies with demonstrated clinical benefit when used in adjunct with BCs (50), and was used in clinical routine in Karolinska University Hospital among other centres. Unfortunately, due to financial and regulatory issues, the method was discontinued despite promising results (51).

Recently, a novel method employing superparamagnetic particles and T2 magnetic resonance to detect microbial DNA, T2MR, (52–55) was described, and recently received clearance by the US Food and Drug Administration (FDA) as well as the CE mark in Europe (56). Initially commercialized as the T2Candida assay designed for Candida spp. detection (57), T2MR was further developed to include a bacterial assay, T2Bacteria. Pathogens included in the T2Bacteria panel is *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, and *Acinetobacter baumannii* (55), constituting a group of bacteria that is frequently associated with a high prevalence of antimicrobial resistance. The time to result has in prospective studies been shown to be around 4–7 hours, however only including the time for the assay. It shows high sensitivity (about 90%) and specificity (96%–98%) for in-panel pathogens (55), outperforming BCs in certain scenarios. As a diagnostic method on whole blood with promising performance metrics, both T2Candida and T2Bacteria show potential in rapid pathogen identification that may improve infection management and patient outcomes.
A key limitation of PCR-based diagnostics for BSI is the risk of false positive results. This occurs because bacterial DNA might be detected in blood even when viable bacteria are not present. Consequently, interpreting a positive result from a PCR-based method alongside a negative BC result can sometimes be challenging. This issue is a common problem when evaluating new BSI diagnostic methods against BCs, as there is no absolute method to confirm the presence of bacteria in blood samples in clinical settings.

To assess the diagnostic performance of new tests, current studies often use a composite reference standard of clinical adjudication, BC results, and other microbiological findings to establish a reference standard for comparison (49, 55, 58-60). However, it’s crucial to understand that none of these criteria alone can definitively indicate a true BSI. Indeed, it is well known that composite reference standards inherently introduce bias, termed imperfect reference standard bias (61, 62). Therefore, all performance metrics in a given study on a molecular based method, such as sensitivity, specificity, PPV, and NPV, relies on the chosen definition of the reference standard. Currently, there is no composite reference standard that is consistently used, which remains an obstacle for comparing results from different studies (28, 63).

2.5 The role of blood stream infection in COVID-19

Exposure to pandemics is increasingly recognized as a major risk factor for sepsis (5). Bacterial infections secondary to viral infections are well described. In influenza virus infection, viral replication affects the agents of the innate immune system including neutrophils and macrophages, decreasing the resistance to bacterial co-infection (64). Commonly, Staphylococcus aureus infections complicating seasonal influenza is known to carry considerable morbidity and mortality (65). The coronavirus SARS-CoV-2, which is the etiologic agent responsible for coronavirus disease 2019 (COVID-19) caused a major outbreak leading to a pandemic in 2020. This provided a unique opportunity to study features of bacterial co-infection present together with a novel viral infection.

The clinical experience from the early management of SARS-COV-2 infection was that the initial manifestation was non-specific, with major challenges in differentiating the hyperinflammation of COVID-19 and sepsis related SIRS. Indeed,
common pathophysiological pathways have been elucidated, including dysregulation of innate immune responses and elevated levels of proinflammatory cytokines (66).

The clinical similarities between sepsis and COVID-19 often lead to ordering BCs in both diseases before the etiology is known. In a study by Sepulveda et al, the surge of COVID-19 led to an increase of 34.8% in BC ordering (67). The prevalence of bacterial infection in COVID-19 therefore has major implications for the initial management in severe COVID-19, especially considering the need for early broad-spectrum antibiotic therapy. If the prevalence is high, antibiotic therapy may be a cornerstone in treatment. In contrast, if prevalence is low, unnecessary administration of antibiotic therapy may lead to adverse effects such as side-effects and/or development of antibiotic resistance. In addition, bacterial involvement in COVID-19 warrants additional considerations before administering immunosuppressant therapy for COVID-19 such as glucocorticoids and interleukin-6 inhibitor therapy, as there is a risk for uncontrolled bacterial infection in an immunosuppressed state (68). At the onset of the pandemic, the prevalence of concomitant BSI infection in COVID-19 was not known. Significant efforts, of which some are presented in this thesis, was important to provide data on bacterial infections in COVID-19 needed to guide appropriate antibiotic utilization.

2.6 Current gaps in knowledge

Currently, the main areas for improving microbiological diagnostics in sepsis include finding optimal ways to refine sampling methods to enhance diagnostic accuracy, to improve clinical identification of patients at high risk of BSIs, and to advance laboratory analytical techniques. A comprehensive approach combining all these elements is essential for optimizing the microbiological diagnosis in sepsis.

Key questions that are the principal targets in this doctoral project include: (i) How can the BC sampling process by optimized? (ii) How does the clinical diagnosis of sepsis correlate with microbiological findings? (iii) What is the impact of novel viral infections such as COVID-19 on sepsis and concurrent BSI? (iv) What is the role of novel non-culture based methods in the diagnosis of sepsis?
3 Research aims

The overall research aim of the present doctoral project is to provide an understanding for the determining factors of successful microbiological diagnosis in human sepsis.

I. Study I aims to compare the two described sampling methods, MSS and SSS. Specifically, the aim is to investigate whether SSS is non-inferior to MSS in terms of ability to detect pathogens in the blood stream in sepsis patients.

II. Study II aims to describe differences in BC findings between cases fulfilling the old (Sepsis-2) the new (Sepsis-3) definition, as well as investigate clinical differences.

III. Study III aims to explore the frequencies of positive BCs and contamination rates during the COVID-19 pandemic, in comparison to control groups.

IV. Study IV aims to determine the additional diagnostic value of a novel molecular-based BSI diagnosis tool (T2Bacteria), compared to BCs taken during the same BSI episode.
4 Materials and methods

4.1 Ethical considerations

Study I and II was approved by the Swedish Ethical Review Authority (reference number 2017/1358-31).

4.1.1 Study I

When the clinical protocol associated with a sepsis alert system was introduced in the emergency department in Karolinska University Hospital Huddinge, the method of drawing six BC bottles at once was introduced (the standard is to draw four). This enabled us to study the performance of sampling strategies using a prospective design. There were two main ethical considerations regarding this change in clinical routine. One was that more blood was to be taken from the patient, that had not been taken otherwise. The act of sampling 20 mL of additional blood for culture was, however, part of the routine that was introduced in a clinical context and thus did not require specific patient consent for research purposes. This distinction was clearly stated in the research ethical permit. From the clinical viewpoint, when introducing the sepsis alert system, we reasoned that the extra blood taken for the third BC (16–20 mL) is too low to make a clinical difference in adult patients. In addition, the third BC was to be taken through an existing venipuncture, therefore avoiding adding further discomfort to the patient.

The other ethical consideration was the collection of personal data. The data was pseudonymized to ensure patient integrity and stored securely. The data handling and analysis aligned with the principles of Good Clinical Practice.

4.1.2 Study II

The same ethical permit used in study I (reference number 2017/1358-31) was applied to this study. For Study II, patients’ clinical data was used in combination with microbiological data to investigate microbiological profiles in sepsis. The clinical data was pseudonymized, and care was taken to have sensitive data stored safely. No specific research intervention was performed, however, the sepsis alert itself could be viewed as an intervention. However, the sepsis alert was part of an improvement project that was mainly motivated clinically, with the purpose to improve care for patients.
4.1.3 Study III

In Study III, we exported sample data from the laboratory system, which did not contain sensitive personal information. The analysis was performed on the level of isolate data, and not on the patient level. As the data did not contain sensitive personal information, and the study was done on the information from the laboratory system and not the samples directly, this study did not require an ethical permit.

4.1.4 Study IV

As in Study III, data was retrospectively analyzed from the laboratory data system in Study IV. As before, no sensitive personal information was included, and no work was performed on the samples themselves. Therefore, no ethical permit was needed for study IV.

4.2 Data sources

4.2.1 The sepsis alert system

A cohort from a newly introduced sepsis alert was the subject of Study I and II. The sepsis alert system was implemented at Karolinska University Hospital Huddinge Emergency Department in September 2017 and consisted of a triage tool and a set of mandated tasks, with similarities to other published sepsis alert systems (69–72). In the present sepsis alert system, used as the study setting for Study I and II, the Rapid Emergency Triage and Treatment System (RETTS) (73, 74) is used to prioritize patients upon arrival at the emergency department and is used nationwide in Sweden. Within this framework, patients presenting with suspected infections were evaluated for sepsis alert inclusion based on their RETTS triage priority. Only patients classified as priority one (the highest urgency level) with a suspected infection, and those at priority levels two or three with a measured lactate level exceeding 3.2 mmol/L, were considered for mandated care according to the sepsis alert system (Figure 3). Conversely, patients categorized as priority four, indicative of the lowest urgency, were excluded from the sepsis alert system.
Sepsis alert in the ED

Figure 3: Sepsis alert activation criteria. Adapted from the Swedish version. ED: emergency department. Triage priority denotes Rapid Emergency Triage and Treatment (RETTS) priority.

Upon identification of an eligible patient, the sepsis alert protocol required that triage nurses would promptly transfer the patient to a designated resuscitation area. Additionally, immediate evaluation of the patient was done by an emergency medicine physician. A crucial part of the protocol, shown in Figure 4, was the involvement of a collaborative, multi-disciplinary response: an infectious diseases specialist and an intensive care physician were promptly notified and joined the emergency medicine physician. This team approach ensured a comprehensive and rapid assessment of the patient, involving the necessary specialties early in patient management.
In line with the Swedish guidelines in effect during the study period, we collected two sets of BCs using MSS from two separate peripheral venipuncture sites. Skin preparation was conducted using a 0.5 mg/ml chlorhexidine disinfectant prior to venipuncture. Additionally, we emulated SSS by collecting an additional BC set from the first venipuncture location.

For identification and tracking purposes, we pre-labeled the BC bottles from one to six (Figure 5). From the first venipuncture site, we collected and labeled bottles 1, 2, 3, and 4, with bottles 1 and 3 designated for aerobic cultures and bottles 2 and 4 for anaerobic cultures. Correspondingly, bottles 5 (aerobic) and 6 (anaerobic)
were obtained from the second site. This dual-sampling approach, representing both MSS and SSS for each patient, later permitted a direct comparison between the two methods.

**Figure 5:** Labeling of blood culture bottles 1-6 in the sepsis alert cohort.

### 4.2.2 Laboratory data system

The microbiological laboratory database in QlikView (Qlik, King of Prussia, PA, USA), was the main source of data for studies III and IV. The database includes pertinent information about microbial isolates from BCs, including bacterial and fungal microorganisms. Additionally, results from other cultures and results from non-culture based methods such as T2Bacteria were present in the database. Each isolate’s data were recorded at the sample level, allowing for a detailed analysis. In addition, the database included fundamental patient demographics such as age and gender, as well as the clinic where sampling was performed.

The database also tracked the detailed timing of key laboratory processes, from specimen collection to result delivery, which was critical for assessing the
diagnostic performance in Study IV. To maintain data integrity, we followed validation protocols and conducted regular quality checks.

4.3 Methods

4.3.1 Study I

Study I was a prospective, non-inferiority trial that compared pathogen detection in BCs using SSS versus MSS at Karolinska University Hospital Huddinge in Stockholm, Sweden. The study period was September 2017 to February 2019.

Participants: Adult patients that triggered the sepsis alert system were eligible for the study. If a patient had multiple episodes, each could be included in the study provided they were separated by a minimum of 72 hours. Episodes were excluded if fewer than six BC bottles were collected, or if collection of blood was not performed in accordance with the study protocol. The microbiological analysis followed the hospital's standard practices.

BC collection: Patients were their own control, as MSS and SSS were collected simultaneously in each patient. BC bottles were pre-labelled 1 to 6. MSS consisted of bottles 1, 2, 3 and 4, and SSS consisted of bottles 1, 2, 5 and 6. Bottles 1 through 4 were sampled from the same venipuncture site. Bottles 5 and 6 were collected from the other site. Bottles 1, 3, and 5 were BactAlert FA Plus bottles, and bottles 2, 4, and 6 were BactAlert FN Plus bottles. BC bottles were sampled consecutively in the labeled order. In cases of indwelling intravascular catheters, bottles 1–4 were sampled from the catheter. When comparing MSS to SSS, microbiological data from all six BC bottles were used as reference.

Outcomes: The primary outcome was the difference in rate of detection of pathogenic organisms in BCs between MSS and SSS, and the secondary outcome was the rate of contamination growth. Results were categorized as either clinically relevant growth or contaminants, using a standardized set of criteria.

Statistical Analysis: Non-inferiority of SSS to MSS was assessed using a predefined 5% margin for the difference in detection rates. We predicted a 30% positivity rate based on clinical experience and earlier studies. To ensure 80% power at a 0.05 alpha level, 520 episodes were required to establish non-inferiority for the 5% margin. Confidence intervals was calculated using the Wald
method. Data were analyzed using frequencies, percentages, means and standard deviations. McNemar’s test was used for categorical paired comparisons between MSS and SSS.

### 4.3.2 Study II

Study II was a prospective descriptive study that examined the differences in clinical and microbial findings between patients that fulfilled Sepsis-2 and Sepsis-3, respectively. The cohort studied was the same cohort as in Study I. In addition, comprehensive clinical data was collected from patient charts.

**Participants:** Patients were included in the study if they activated the sepsis alert due to suspected sepsis. As a result, the study population also consisted of patients who were ultimately diagnosed with conditions other than sepsis and/or infection. Similar to study I, an "episode" refers to an instance where a patient activated the sepsis alert system. To ensure the reliability of BC outcomes, episodes were excluded if less than six BC bottles were collected. Furthermore, in contrast to study I, only the first episode for a patient was included to prevent bias from repeated events.

**Definitions of sepsis:** Clinical and microbiological information was gathered from the hospital’s electronic health record system and the electronic laboratory information system, respectively. The presence of Sepsis-3 was determined based on two specific criteria: A) The clinical suspicion of infection at the time of admission to the Emergency Department (ED); and B) The presence of organ dysfunction, indicated by an increase in the SOFA score of 2 or more points from the baseline. The baseline SOFA score was established using the most recent physiological data points from the 3 months preceding the study enrollment. If baseline data were missing, the respective baseline SOFA score was set to zero. Clinical suspicion of infection (criterion A) was deemed to be met if broad-spectrum antibiotics were started within 48 hours of BC sampling and continued for at least 4 days, consistent with definitions used in prior epidemiological studies (75).

Sepsis-2 was identified if two different criteria were met: (A) as defined previously, and (C), the presence of two or more Systemic Inflammatory Response Syndrome (SIRS) criteria.
Statistical analysis: Categorical variables were summarized using frequencies and percentages, whereas numerical variables were described using means and standard deviations, as well as medians and interquartile ranges.

4.3.3 Study III

Study III was a retrospective cohort study comparing the BC outcomes of patients with COVID-19 to two control groups. The study took place from March 1, 2020, to April 30, 2020, at Karolinska University Hospital. BCs were collected and analyzed at the Karolinska University Laboratory, which receives specimens from six tertiary care hospitals across Stockholm and its neighboring cities and suburbs.

Participants: The study group was comprised by all patients that tested positive for SARS-CoV-2 RNA through reverse transcriptase PCR in respiratory secretions during the study period. BCs collected during the study timeframe from the same patients were also analyzed. The study group is subsequently referred to as the “COVID-19 group.”

Two distinct control groups were included. The first was a historical control group, consisting of patients in whom BC results were recorded between March 1 and April 30, 2019, and is henceforth known as “control group-2019.” The second was a contemporary control group, consisting of individuals with BC results from the same period in 2020 as the COVID-19 group but who did not test positive for SARS-CoV-2 via PCR. This group is referred to as “control group-2020.” Initially, during the pandemic’s onset, testing was limited to patients exhibiting symptoms indicative of COVID-19, not encompassing all admitted patients. Consequently, the control group-2020 included a mix of patients who tested negative for SARS-CoV-2 and those not tested at all. Nonetheless, since patients presenting with COVID-19-like symptoms were likely to be tested, it can be reasonably inferred that those not tested did not exhibit clinical signs of COVID-19.

Outcomes: The main outcome was the difference in proportions of BC positivity between patients from the COVID-19 group and the two control groups. The main outcome was further divided to examine difference in clinically relevant growth, and contaminant growth, respectively.
**Statistical analysis:** BC results in patients with COVID-19 was pairwise compared to control group-2020 and control group-2019 using the Pearson’s chi-square test.

### 4.3.4 Study IV

Study IV was a retrospective cohort study comparing the performance between T2Bacteria and BCs. The study was performed at Karolinska University Laboratory, Huddinge.

**Participants:** T2Bacteria samples collected from patients between May 18, 2022, to November 21, 2023, were included. The time period within ± 72 hours was defined as an “episode” in the context of Study IV. Then, BCs sampled within episode time frame from same patients were included and assigned to the corresponding episode. Other non-BC cultures were also included and assigned similarly to the corresponding episodes. T2Bacteria samples were excluded under the following conditions: if a patient had a previous T2Bacteria sample taken within the preceding 7 days, or if the assay results of the T2Bacteria samples were invalid. Furthermore, episodes that did not include any BCs were also excluded, as these were not applicable for the comparative analysis between T2Bacteria and BCs.

**Data analysis:** For the comparative analysis between T2Bacteria samples and BCs, all BCs taken during each patient episode were considered. The evaluation of BC performance was based on the set of microorganisms contributed to from all BCs collected within the same timeframe belonging the T2Bacteria sample. Additionally, data from non-BC cultures were incorporated into the sensitivity and specificity analyses for each episode. For the main analysis, only bacteria that were part of the T2Bacteria panel were considered. To estimate the effect of out-of-panel bacteria on test performance, a separate analysis was performed.

**Outcome measures:** The primary outcome was the proportion of positive results obtained with T2Bacteria in comparison to BCs. As a secondary outcome, the study evaluated the turnaround time (TAT) for both diagnostic methods.

**Statistical analysis:** Paired comparison for agreement between proportions of T2 and BC positivity was performed using McNemars Chi-2 test, and comparison of TAT was performed using the Mann–Whitney U test.
Table 2: Summary of the methodology used in the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aim</strong></td>
<td>To compare SSS vs. MSS in patients with suspected sepsis.</td>
<td>To analyze clinical and microbiological characteristics in relationship to Sepsis-3 and Sepsis-2 definitions.</td>
<td>To analyze BC data in COVID-19 patients compared to patients without COVID-19</td>
<td>To compare diagnostic performance of T2Bacteria to standard BCs</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td>Prospective non-inferiority diagnostic study</td>
<td>Prospective descriptive cohort study</td>
<td>Retrospective cohort study</td>
<td>Retrospective cohort study</td>
</tr>
<tr>
<td><strong>Cohort</strong></td>
<td>Consecutive adult patients admitted through a sepsis alert system</td>
<td>Study group: SARS-CoV-2 positive patients Control group 1: Historical cohort Control group 2: Contemporary cohort (during the same period as the study group)</td>
<td>Consecutive patients with T2Bacteria samples and concomitantly sampled BCs.</td>
<td></td>
</tr>
<tr>
<td><strong>Study setting</strong></td>
<td>Emergency department, Karolinska University Hospital Huddinge</td>
<td>Karolinska University Laboratory in Huddinge, analyzing microbiological samples from six tertiary care hospitals in the greater Stockholm area as well as surrounding cities and suburbs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Study period</strong></td>
<td>Sept 2017 - Febr 2019</td>
<td>1 March 2020 - 30 April 2020</td>
<td>May 18 2022 - Nov 21 2023</td>
<td></td>
</tr>
<tr>
<td><strong>No. of patients</strong></td>
<td>514 patients with 549 episodes</td>
<td>549 patients</td>
<td>Study group: 2240 patients Control group 1: 6841 patients Control group 2: 6022 patients</td>
<td>544 patients with 640 episodes</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td><strong>Primary outcome:</strong> Difference between detection rates in SSS and MSS.</td>
<td><strong>Descriptive outcomes:</strong> BC results and clinical characteristics in patients fulfilling sepsis-2 criteria, sepsis-3 criteria, neither or both criteria.</td>
<td><strong>Primary outcome:</strong> Difference in BC positivity rate in the study group compared to the control groups</td>
<td><strong>Primary outcome:</strong> Proportion of positive findings with T2Bacteria compared to the BCs. <strong>Secondary outcome:</strong> Difference in turnaround time between T2Bacteria and BC.</td>
</tr>
</tbody>
</table>

5 Results

5.1 Study I:
The final analysis included 514 patients with 549 suspected episodes in total. The study inclusion flow chart is shown in Figure 6.

Out of the total 549 suspected sepsis episodes, positive BCs was observed in 209 episodes (38.1%) across 200 unique patients, using all six BC bottles implemented in the study. Of these episodes, clinically relevant microorganisms were isolated in 170 episodes (31.0%), with 11 of these also presenting concurrent contaminant growth. In contrast, 39 episodes (7.1%) had exclusively contaminant growth. Within the 170 episodes with clinically significant microbial growth, monomicrobial growth was noted in 140 cases (82.4%), while polymicrobial growth was observed in 30 cases (17.6%). Clinical data for the episodes is shown in Table 3.
Table 3: Clinical characteristics of the episodes in study I. Total N = 549

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex – n (%)</td>
<td>338 (61.6)</td>
</tr>
<tr>
<td>Age – yr*</td>
<td>69.5 ± 16.7</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>95 (17.3)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>166 (30.2)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>71 (12.9)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>51 (9.3)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>107 (19.5)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>130 (23.7)</td>
</tr>
<tr>
<td>Chronic kidney failure (GFR &lt;60)</td>
<td>135 (24.6)</td>
</tr>
<tr>
<td>Chronic liver failure</td>
<td>17 (3.1)</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>89 (16.2)</td>
</tr>
<tr>
<td>Source of infection, n (%)**</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract†</td>
<td>174 (37.2)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>102 (21.8)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>39 (8.3)</td>
</tr>
<tr>
<td>Soft tissue/skin/skeletal/joint</td>
<td>37 (7.9)</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>114 (24.4)</td>
</tr>
<tr>
<td>Disease severity/outcomes</td>
<td></td>
</tr>
<tr>
<td>SOFA score at admission††</td>
<td>3 (2 – 5)</td>
</tr>
<tr>
<td>Subgroup category after record review</td>
<td></td>
</tr>
<tr>
<td>- Sepsis according to Sepsis-3, n (%)‡</td>
<td>387 (70.5)</td>
</tr>
<tr>
<td>- Infection without sepsis‡</td>
<td>81 (14.8)</td>
</tr>
<tr>
<td>- No infection</td>
<td>80 (14.6)</td>
</tr>
<tr>
<td>Admission to intensive care unit during hospital stay, n (%)</td>
<td>46 (8.4)</td>
</tr>
<tr>
<td>28-day mortality, n (%)</td>
<td>73 (13.3)</td>
</tr>
</tbody>
</table>

*Denotes mean ± standard deviation.
** Source of infection was only analyzed for patients with infection (n=468). Two or more different sources of infection was found in 9 episodes, therefore total percentage exceed 100%.
† Includes lower and upper respiratory tract infections.
‡ Denotes median (interquartile range).
‡‡ Sepsis was determined as present if there was an increase in SOFA score by 2 compared to baseline, as well as evidence of infection. Infection was in this study was defined as present if the patient was administered intravenous antibiotic therapy within 48 hours from admission and during at least four days.

5.1.1 Comparison of sampling strategies

Figure 7 shows the comparative detection between MSS and SSS. Comparing MSS and SSS, clinically relevant isolates were identified in 162/549 episodes (29.5%) using MSS and in 160/549 episodes (29.1%) using SSS. The observed difference in detection rates between the two methods was 0.36%, with a 95% confidence
interval (CI) ranging from -1.33% to 2.06%. This difference did not surpass the pre-established non-inferiority margin of 5%.

Contaminant growth was identified in 29/549 episodes using MSS and 40/549 episodes using SSS.

Figure 7: Comparative detection for clinically relevant growth (A) and contamination (B).
MSS: Multi-sampling strategy. SSS: Single sampling strategy. Statistical comparison was performed with McNemar’s $\chi^2$ test. Total N = 549 episodes.

In episodes of monomicrobial growth, where only one type of microorganism was present, MSS and SSS showed similar performance, detecting microorganisms in 132 (24.0%) and 130 (23.7%) episodes, respectively. However, when considering all clinically relevant isolates in polymicrobial episodes, MSS detected all microorganisms in 93% (28 out of 30) of the episodes compared to 73% (22 out of 30) for SSS. ($p = 0.08$). The discordant polymicrobial episodes are further detailed in Table 4.
Table 4: Discordant results between MSS and SSS* in episodes with polymicrobial growth. Site of infection and isolated microorganisms are presented.

<table>
<thead>
<tr>
<th>Site of infection</th>
<th>Isolates detected by both methods</th>
<th>Isolates detected by MSS only</th>
<th>Isolates detected by SSS only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen</td>
<td><em>Escherichia coli</em></td>
<td><em>Enterococcus faecium</em></td>
<td>-</td>
</tr>
<tr>
<td>Abdomen</td>
<td><em>Klebsiella pneumoniae,</em></td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>-</td>
</tr>
<tr>
<td>Abdomen</td>
<td><em>Enterococcus faecalis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental</td>
<td><em>Streptococcus anginosus</em></td>
<td><em>Parvimonas micra</em></td>
<td>-</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td><em>E. coli</em></td>
<td>-</td>
<td><em>K. pneumoniae</em></td>
</tr>
<tr>
<td>Soft tissue</td>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td>Soft tissue</td>
<td><em>Streptococcus pyogenes</em></td>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
</tr>
<tr>
<td>Urinary tract</td>
<td><em>E. coli, S. aureus</em></td>
<td><em>E. faecalis</em></td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td><em>Citrobacter freundii,</em></td>
<td><em>E. faecalis</em></td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td><em>Helcococcus</em></td>
<td><em>Globicatella</em></td>
<td><em>E. faecalis</em></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td><em>Alcaligenes faecalis</em></td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td><em>E. coli</em></td>
<td><em>Candida parapsilosis,</em></td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td><em>Lactobacillus</em></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td><em>Actinomyces</em></td>
<td><em>Streptococcus mitis</em></td>
<td>-</td>
</tr>
</tbody>
</table>

5.2 Study II

During the study period, 652 sepsis alert episodes were considered for analysis. This initial cohort was the same as in Study I. The study flow chart and criteria for exclusion are illustrated in Figure 8. Following exclusions, 514 episodes were eligible for final analysis. The average age of patients was 69.3 years (standard deviation ±17.0), with males constituting 60.5% of the study population. A total of 1542 BCs, corresponding to 3084 individual BC bottles, were analyzed.

Out of the 514 episodes analyzed, 357 (69.5%) met Sepsis-3 criteria (referred to as Sepsis-3 positive episodes), and 411 (80.0%) met Sepsis-2 criteria (termed Sepsis-2 positive episodes). A total of 341 episodes (66.3%) satisfied both Sepsis-3 and Sepsis-2 criteria. Figure 9 illustrates the distribution of episodes that fulfilled either, both, or neither of the sepsis criteria.

Figure 8: Inclusion flow chart for study II. Here, bottles denote BC bottles.
Figure 9: Clinical classification of suspected sepsis episodes and blood culture results. Numbers inside the horizontal bar denote episodes fulfilling criteria for clinical classification of Sepsis-3, Sepsis-2, both, or none. Percentages above the bar denotes the cumulative percentage of all included episodes. Numbers inside circles denote episodes with clinically relevant growth, and percentages in parenthesis denote percentage of all episodes in the associated clinical classification.

The clinical characteristics of the episodes, categorized by their fulfillment of none, one, or both sepsis definitions, are detailed in Table 1. The median SOFA score was 3 (interquartile range [IQR]: 3–5) for both Sepsis-3 and Sepsis-2 positive groups. The 28-day mortality rates were 15.4% (55/357) in the Sepsis-3 group and 14.4% (59/411) in the Sepsis-2 group.
Table 5: Clinical characteristics of the episodes (N = 514 episodes).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sepsis-3 positive*/Sepsis-2 positive (n=341)</th>
<th>Sepsis-3 negative/Sepsis-2 positive (n=70)</th>
<th>Sepsis-3 positive/Sepsis-2 negative (n=16)</th>
<th>Sepsis-3 negative/Sepsis-2 negative (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male – n (%)</td>
<td>209 (61.3)</td>
<td>42 (60.0)</td>
<td>10 (62.5)</td>
<td>50 (57.5)</td>
</tr>
<tr>
<td>Age – years **</td>
<td>73.2 (14.0)</td>
<td>60.2 (19.5)</td>
<td>71.6 (15.8)</td>
<td>60.8 (20.3)</td>
</tr>
<tr>
<td>28-day mortality n (%)</td>
<td>54 (15.8)</td>
<td>5 (7.1)</td>
<td>1 (6.3)</td>
<td>8 (9.2)</td>
</tr>
<tr>
<td>Admission to intensive care unit during hospital stay, n (%)</td>
<td>38 (11.1)</td>
<td>1 (1.4)</td>
<td>3 (18.8)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>SOFA score†</td>
<td>3 (3–5)</td>
<td>1 (1–2)</td>
<td>3.5 (2–6)</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>Co-morbidities, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>66 (19.4)</td>
<td>8 (11.4)</td>
<td>4 (25.0)</td>
<td>9 (10.3)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>101 (29.6)</td>
<td>15 (21.4)</td>
<td>4 (25.0)</td>
<td>32 (36.8)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>50 (14.7)</td>
<td>5 (7.1)</td>
<td>2 (12.5)</td>
<td>7 (8.0)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>35 (10.3)</td>
<td>4 (5.7)</td>
<td>0 (0.0)</td>
<td>5 (5.7)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>74 (21.7)</td>
<td>9 (12.9)</td>
<td>3 (18.8)</td>
<td>12 (13.8)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>86 (25.2)</td>
<td>9 (12.9)</td>
<td>5 (31.3)</td>
<td>14 (16.1)</td>
</tr>
<tr>
<td>Chronic kidney failure (GFR &lt;60)</td>
<td>98 (28.7)</td>
<td>8 (11.4)</td>
<td>4 (25.0)</td>
<td>16 (18.4)</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>52 (15.2)</td>
<td>18 (25.7)</td>
<td>2 (12.5)</td>
<td>10 (11.5)</td>
</tr>
<tr>
<td>Chronic liver failure</td>
<td>9 (2.6)</td>
<td>2 (2.9)</td>
<td>1 (6.3)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Source of infection††</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>127 (37.2)</td>
<td>25 (35.7)</td>
<td>6 (37.5)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>80 (23.5)</td>
<td>15 (21.4)</td>
<td>4 (25.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>27 (7.9)</td>
<td>5 (7.1)</td>
<td>0 (0.0)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Soft tissue/skin/skeletal/joint</td>
<td>26 (7.6)</td>
<td>10 (14.3)</td>
<td>2 (12.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>2 (0.6)</td>
<td>0 (0.0)</td>
<td>1 (6.3)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>5 (1.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>81 (23.8)</td>
<td>15 (21.4)</td>
<td>3 (18.8)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>No suspected infection</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>81 (93.1)</td>
</tr>
</tbody>
</table>

SOFA: Sequential Organ Failure Assessment. GFR: Glomerular filtration rate.
* Sepsis-3 positive episodes were defined as present if there was an increase in SOFA score by 2 compared to baseline, as well as evidence of infection. Infection in this study was defined as present if the patient was administered intravenous antibiotic therapy within 48 hours from admission and during at least 4 days. Sepsis-2 positive episodes were defined as present if there were 2 or more SIRS criteria in combination with infection.
** Denotes mean ± standard deviation.
† Denotes median (interquartile range).
†† Two or more sources of infection were found in 8 episodes, so total percentage exceeds 100.
BC growth was observed in 193 out of 514 episodes (37.5%), with clinically relevant growth identified in 158 episodes (30.7%). Of these, 9 episodes also had contaminant growth. There were 35 episodes (6.8%) with only contaminant growth. Among the 158 episodes with clinically relevant growth, 132 (83.5%) had monomicrobial growth, while 26 (16.5%) had polymicrobial growth. Detailed microbiological findings, in relation to the fulfillment of sepsis criteria, are presented in Table 6.

In the Sepsis-3 positive category, 130 out of 357 episodes (36.4%) had clinically relevant BC growth, compared to 145 out of 411 episodes (35.3%) in the Sepsis-2 positive group. Contamination occurred in 30 Sepsis-3 positive episodes (8.4%) and 35 Sepsis-2 positive episodes (8.5%). Among episodes not meeting any sepsis definitions, clinically relevant growth was present in 7 out of 87 (8.0%), and contaminant growth in 8 (9.2%).

Table 6: Microbiological characteristics of the episodes (N = 514 episodes).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sepsis-3 positive*/Sepsis-2 negative (n=341)</th>
<th>Sepsis-3 positive/Sepsis-2 negative (n=70)</th>
<th>Sepsis-3 positive/Sepsis-2 negative (n=16)</th>
<th>Sepsis-3 negative/Sepsis-2 negative (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiological findings, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSI episodes with clinically relevant growth</td>
<td>124 (36.4)</td>
<td>21 (30.0)</td>
<td>6 (37.5)</td>
<td>7 (8.0)</td>
</tr>
<tr>
<td>Contaminated episodes</td>
<td>29 (8.5)</td>
<td>6 (8.6)</td>
<td>1 (6.3)</td>
<td>8 (9.2)</td>
</tr>
<tr>
<td>Episodes with G+ isolates</td>
<td>57 (16.7)</td>
<td>7 (10.0)</td>
<td>4 (25.0)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Episodes with G- isolates</td>
<td>79 (23.2)</td>
<td>14 (20.0)</td>
<td>2 (12.5)</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>Episodes with fungal isolates</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Episodes with anaerobic isolates</td>
<td>7 (2.1)</td>
<td>3 (4.3)</td>
<td>0 (0.0)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Time to Detection (h)**</td>
<td>16.5 (11.6)</td>
<td>25.5 (22.4)</td>
<td>13.9 (6.0)</td>
<td>26.2 (13.5)</td>
</tr>
</tbody>
</table>

BSI: blood stream infection
*Sepsis-3 positive episodes were defined as present if there was an increase in SOFA score by 2 compared to baseline, as well as evidence of infection. Infection in this study was defined as present if the patient was administered intravenous antibiotic therapy within 48 hours from admission and during at least 4 days. Sepsis-2 positive episodes were defined as present if there were 2 or more SIRS criteria in combination with infection.

**Denotes mean ± standard deviation.
5.3 Study III

Study III included 58,704 BC bottles, encompassing 17,865 episodes among 15,103 patients. A flow chart of the included episodes is shown in Figure 10. The total number of patients undergoing BC sampling (n = 8,262) in the 2020 study period increased with 1,421 patients from 6,841 in the 2019 study group, corresponding to an increase of 20%.

![Figure 10: Study flow chart for study III.](image)

Demographically, the COVID-19 group included 2,240 patients, of whom 790/2,240 (35.3%) were female, with a mean age (± SD) of 64 (± 18) years. Control group-2020 had 2,789/6,022 (46.3%) female with mean (± SD) age 57 (± 26) years, and control group-2019 consisted of 3,322/6,841 (48.6%) females with mean (± SD) age 60 (± 26) years.

BC results for included episodes are shown in Table 7. Clinically significant growth was observed in 197/3,027 episodes (6.5%) in the COVID-19 cohort, in contrast to
717/6,663 episodes (10.8%) in the control group–2020 (p<0.0001), and 851/8,175 episodes (10.4%) in the control group–2019 (p<0.0001) (Figure 11, panel A).

Figure 11: Blood culture episodes with clinically relevant growth (Panel A) and with contaminant growth (Panel B). Total number of episodes included for analysis were COVID–19 group: 3,027, Control group–2020: 6,663, Control group–2019: 8,175.

BC contamination was identified in 255/3,027 episodes (8.4%) among episodes with COVID–19, as opposed to 330/6,663 episodes (4.95%) in the control group–2020 (p<0.0001), and 354/8,175 episodes (4.33%) in the control group–2019 (p<0.0001) (Figure 11, Panel B). The incidence of contaminant growth in both control groups was comparable, the difference in proportions being non–significant.

Table 7: Bloodstream infection episode data for patients with COVID–19 and both control groups.

<table>
<thead>
<tr>
<th>Episode type</th>
<th>COVID–19</th>
<th>Control group–2020</th>
<th>Control group–2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Included episodes, N</td>
<td>3,027</td>
<td>6,663</td>
<td>8,175</td>
</tr>
<tr>
<td>Episodes with growth, n (%)</td>
<td>433 (14.3)</td>
<td>1,015 (15.2)</td>
<td>1,153 (14.1)</td>
</tr>
<tr>
<td>Episodes with clinically relevant growth, n (%)</td>
<td>197 (6.5)</td>
<td>717 (10.8)</td>
<td>851 (10.4)</td>
</tr>
<tr>
<td>Gram positive*</td>
<td>116 (3.8)</td>
<td>344 (5.2)</td>
<td>420 (5.1)</td>
</tr>
<tr>
<td>Gram negative*</td>
<td>64 (1.7)</td>
<td>306 (4.6)</td>
<td>351 (4.3)</td>
</tr>
<tr>
<td>Yeast*</td>
<td>2 (0.07)</td>
<td>11 (0.17)</td>
<td>8 (0.10)</td>
</tr>
<tr>
<td>Polymicrobial episodes**</td>
<td>27 (0.89)</td>
<td>56 (0.84)</td>
<td>72 (0.88)</td>
</tr>
<tr>
<td>Episodes with contaminant growth, n (%)</td>
<td>255 (8.4)</td>
<td>330 (5.0)</td>
<td>354 (4.3)</td>
</tr>
<tr>
<td>Only contaminant growth</td>
<td>236 (7.8)</td>
<td>298 (4.5)</td>
<td>302 (3.7)</td>
</tr>
<tr>
<td>Both contaminant and clinically relevant growth</td>
<td>19 (0.63)</td>
<td>32 (0.48)</td>
<td>52 (0.64)</td>
</tr>
</tbody>
</table>

*Monomicrobial episodes
**Polymicrobial episode is defined as an episode with occurrence of more than one clinically relevant isolate.
In an analysis of the incubation periods for 5,568 BC bottles, it was noted that a predominant majority (93–96%) signaled positive within 48 hours. An additional 4–5% of the bottles signaled positive by the fourth day. The remaining BCs signaling positive on the fifth day accounted for 2% in the COVID-19 group and 1% in each of the control groups.

<p>| Table 8: Distribution of microorganisms isolated from blood cultures in patients with COVID-19 and both control groups. |
|--------------------------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>All isolates, N</th>
<th>COVID-19</th>
<th>Control group-2020</th>
<th>Control group-2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive bacteria* n (%)</td>
<td>150 (66)</td>
<td>385 (49)</td>
<td>487 (52)</td>
</tr>
<tr>
<td>Coagulase negative staphylococci**</td>
<td>49 (22)</td>
<td>55 (7.1)</td>
<td>58 (6.2)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>44 (19)</td>
<td>124 (16)</td>
<td>162 (17)</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>23 (10)</td>
<td>46 (5.9)</td>
<td>75 (8.0)</td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td>22 (9.7)</td>
<td>64 (8.2)</td>
<td>63 (6.7)</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>6 (2.7)</td>
<td>47 (6.0)</td>
<td>50 (5.3)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3 (1.3)</td>
<td>21 (1.4)</td>
<td>51 (1.6)</td>
</tr>
<tr>
<td>Other Gram-positive bacteria†</td>
<td>3 (1.3)</td>
<td>28 (3.6)</td>
<td>28 (3.0)</td>
</tr>
<tr>
<td>Gram-negative bacteria* n (%)</td>
<td>66 (29)</td>
<td>352 (45)</td>
<td>397 (42)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>34 (15)</td>
<td>213 (27)</td>
<td>242 (26)</td>
</tr>
<tr>
<td>Other Enterobacterales</td>
<td>24 (11)</td>
<td>111 (14)</td>
<td>117 (13)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>7 (3.1)</td>
<td>13 (1.7)</td>
<td>14 (1.5)</td>
</tr>
<tr>
<td>Other Gram-negative bacteria†</td>
<td>1 (0.4)</td>
<td>15 (1.9)</td>
<td>24 (2.6)</td>
</tr>
<tr>
<td>Anaerobic bacteria n (%)†</td>
<td>7 (3.1)</td>
<td>32 (4.1)</td>
<td>40 (4.3)</td>
</tr>
<tr>
<td>Yeast n (%)†</td>
<td>3 (1.3)</td>
<td>11 (1.4)</td>
<td>15 (1.6)</td>
</tr>
</tbody>
</table>

Significant differences were observed in the types of microorganisms identified from BCs across the three groups under study (Table 8). Gram-positive bacteria were more prevalent in patients with COVID-19, where 150/226 (66%) isolates were Gram-positive, compared to 385/781 (49%) isolates in control group-2020 and 487/940 (52%) isolates in control group-2019 (p<0.0001 for both comparisons). The occurrence of Gram-positive growth was similar between the two control groups (not statistically significant). Conversely, the frequency of Gram-negative isolates was significantly lower in the COVID-19 group, with 66/226 (29%) isolates, as opposed to 353/781 (45%) isolates in control group-2020 and 398/940 (42%) isolates in control group-2019 (p<0.0001 and p<0.001 respectively). The numbers of Gram-negative isolates were comparable between the two control groups (not statistically significant).
5.4 Study IV

In total, 843 T2Bacteria samples were considered for inclusion. The study inclusion flow is depicted in Figure 12.

![Study inclusion flow chart for Study IV. BC: blood culture](image)

In total, 640 episodes involving 544 patients was included, consisting of 640 T2 samples and 2,117 BCs. Out of the included patients, 329/544 (60.5%) were male and the mean age (SD) was 57.3 (19.8) years. Among the 640 episodes examined, 129 episodes (20.2%) were found to be positive by T2, BC, or both, when all potential microorganisms were considered. When the analysis was restricted to bacteria included in the T2Bacteria panel, the number of positive episodes was 101 out of 640 (15.8%). Polymicrobial detections were observed in four episodes using T2, and one episode demonstrated in-panel polymicrobial growth in BCs. Additionally, when species not covered by the T2Bacteria panel were included,
polymicrobial growth in BCs was observed in 12 episodes. The overlap of positive episodes is shown in Figure 13.

![Comparison of Diagnostic Methods: T2 vs BC](image)

Figure 13: Overlap of episodes. BC: blood culture. T2: T2Bacteria. Percentages denote % of total positives of in-panel positive episodes (n = 101). Both positive episodes (n = 29) include 27 episodes in which isolate/isolates detected by T2 matched the isolate/isolates in BCs completely, while two episodes had partial matches.

Out of 29 episodes that tested positive by both T2 and BC, 27 episodes showed complete concordance between T2 and BC results, with respect to bacteria included in the T2 panel. Among the remaining two episodes that were positive with both methods, one episode was positive for E. faecium and K. pneumoniae in T2, but BC only detected E. faecium. In the other episode, T2 was positive for E. faecium, while BC identified both E. faecium and S. aureus. A significant difference in proportions was observed when comparing the 46/640 T2 positive/BC negative episodes to the 26/640 T2 negative/BC positive episodes (p = 0.018). The impact
on these proportions, resulting from changes in the time frame of episodes during which BCs were deemed relevant to the T2 sample, is depicted in Figure 14.

Figure 14: Numbers and proportions of concordant and discordant episodes as a function of the time frame used in the analyses. T2: T2Bacteria. BC: Blood culture.

In the secondary analysis, which included all detected microorganisms in the BCs, the impact of the T2Bacteria panel’s limited repertoire was assessed. Considering all microorganisms, there were 42 episodes that were T2 positive/BC negative, and 54 episodes that were T2 negative/BC positive. In 6 episodes, the bacteria identified by T2 were a subset of those found in the BCs, and in 4 episodes, T2 and BC identified non-matching bacteria. Additionally, in one episode, BC detected a subset of the bacteria found with T2.

The difference in TAT is shown in Figure 15. The mean overall TAT from sampling to preliminary report for BCs was 36 hours and 48 minutes, compared to 27 hours and 33 minutes for T2 (p < 0.001). The time from sampling to arrival to the laboratory was 1 hour and 30 minutes for BC and 15 hours for T2 (p < 0.001). The
interval from the sample’s arrival to the first result was 35 hours and 17 minutes for BC and 12 hours and 32 minutes for T2 (p < 0.001).

Figure 15: Turn-around time for T2Bacteria and blood culture samples. T2: T2Bacteria. BC: Blood culture. Only T2 and BC samples positive for bacteria included in the T2Bacteria assay panel are included (n = 75 for T2, n = 120 for BC). Total turn-around time is defined as the total time from sampling to preliminary report. Outliers are not shown.
6 Discussion

6.1 Overview

Following the results of the studies, I aim to provide an understanding to the key factors contributing to successful microbiological diagnosis in sepsis.

In Study I, we refined the pre-analytical phase of BC diagnostics by showing that we could streamline and simplify the sampling process while preserving the diagnostic capability. Studies II and III expanded our understanding of the microbiological profiles in sepsis, particularly in response to two significant developments: the adoption of new sepsis definitions and the emergence of the COVID-19 pandemic. In the last study, we focused on the evaluation of a novel diagnostic method and the challenges inherent in its adoption into clinical practice.

In the following sections, specific discussion regarding each of the studies in the present thesis will be followed by general considerations.

6.2 Discussion of the included studies

6.2.1 Study I

Study I demonstrated that SSS is non-inferior to MSS in identifying pathogenic microorganisms in BCs, with both methods positive in around 30% of cases. These findings not only validate previous theoretical models (76) but also align with empirical data, (35) supporting SSS as a routine method for BC in emergency departments. While preserving diagnostic capabilities, SSS offers the dual advantage of being less harmful to patients and less labor-intensive for staff.

In Study I, only patients with complete MSS and SSS BC sets were included. In real clinical scenarios, it is not uncommon that only solitary BCs (one BC set consisting of two BC bottles) are sampled. The rate of solitary sets varies in different settings, reaching 10–33% in most cases (30, 77, 78). In another study performed by our group, a change from MSS to SSS was associated with a decrease in solitary BCs from 28,7% to 4,1% (78). The potential for a dramatically decreased rate of solitary BCs with SSS could contribute substantially to increased performance of BCs, as the sampled volume have repeatedly been shown to influence BC sensitivity (30).
Contamination in BC diagnostics is a critical issue, with a reduction in contaminants being one of the proposed benefits of SSS (30). The study achieved an overall contamination rate below the accepted 3% upper limit (34), with SSS showing a trend towards fewer contaminants than MSS, though not statistically significant. It has been previously argued that MSS could more accurately differentiate between true pathogens and contaminants, especially when a common skin contaminant is isolated from only one of the multiple sites. Nevertheless, the practice of two venipunctures inherently doubles the risk of contamination from skin. It is also important to note that the isolation of a common skin contaminant from only one site does not conclusively indicate that it is a true contaminant. Moreover, when using a single venipuncture, the concentration of contaminants is exposed to decay with each successive bottle by an unknown factor. Despite the limited sample sizes in Study I in the present thesis and the other work previously performed by Dargère et al. (35), there was a noted reduction in the rate of contaminants in the second set of samples from the same venipuncture, though this reduction did not quite reach the threshold of statistical significance in either study. Considering both theoretical considerations and existing empirical evidence, it appears likely that SSS can reduce contamination rates with minimal impact on the interpretability of results.

The high rate of polymicrobial growth in BCs, representing 17.6% of all relevant growth in Study I, might be attributed to a higher rate of comorbid illnesses and a higher proportion of true sepsis in the cohort used in this study. Notably, MSS was more effective than SSS in detecting all microorganisms in polymicrobial episodes, although the study did not have enough statistical power for non-inferiority regarding polymicrobial growth. Further research, particularly with a larger sample of polymicrobial sepsis cases, is necessary.

This study had some limitations. The exclusion of nearly 100 sepsis alert episodes due to an insufficient number of BC bottles could have influenced the patient cohort’s etiologies or severity. Nevertheless, as patients served as their own controls, such an influence likely had minimal impact on the results. Another limitation was the small sample size, which was insufficient to definitively conclude whether SSS could surpass MSS in reducing contamination rates. Lastly, due to logistical challenges, BC bottle volumes were not measured, making it unclear if there were significant differences in blood volumes among individual bottles.
However, the similarity in growth rates between parallel bottles suggests minimal variance in blood volumes.

The strengths of this study lie in its methodology. The use of a well-defined sepsis alert system led to high positive BC rates and low contamination rates—crucial factors for assessing sampling method performance. Simultaneous implementation of both MSS and SSS in the Emergency Department, using pre-labeled BC bottles, minimized the risk of varying preconditions, such as the initiation of antibiotic therapy.

### 6.2.2 Study II

This prospective study evaluated BC positivity in patients triggering the sepsis alert in an emergency department, examining the performance of Sepsis-3 and Sepsis-2 criteria. The study found that most patients met both criteria sets, with similar BC positivity rates for both. Specifically, episodes meeting Sepsis-3 criteria had a 36.4% bacteremia rate, while those meeting Sepsis-2 criteria had a slightly lower rate of 35.3%. This is in contrast to previous studies, where bacteremia rates ranged from 22% to 48.4% for Sepsis-3 patients (16, 79), the latter potentially skewed by focusing solely on ICU admissions. The higher bacteremia rate could be attributed to differences in study design, such as prospective patient inclusion in the ED without prior intravenous antibiotic treatment and the use of six BC bottles, which might have enhanced sensitivity due to increased sample volume.

Furthermore, we employed a method to categorize clinically relevant growth and contaminants, whereas previous studies often lacked clarity on whether contaminants were excluded in their BC positivity analyses (10, 39).

Interestingly, microbiological findings were similar in Sepsis-3 and Sepsis-2 positive episodes, both in terms of pathogens and contaminants. This similarity could be due to the considerable overlap between the two groups in this study. Focusing on the episodes with discrepant sepsis categorizations, the Sepsis-3 positive group showed more episodes with clinically relevant growth and fewer contaminations compared to the Sepsis-2 group, though the small sample size precluded a definitive conclusion.

One important finding on its own is the significant overlap between patients meeting Sepsis-3 and Sepsis-2 criteria, contrasting with previous large-scale
studies that reported lower concurrence. This discrepancy may stem from the study's patient selection criteria and the use of a sepsis alert system in the ED, which increased the likelihood of patients meeting both criteria.

The clinical sepsis definitions are of unexpected importance to the microbiological diagnosis of sepsis. In determining the success of microbiological diagnosis, the performance is often thought to be synonymous to the proportion of sepsis cases that becomes positive for bacteria in blood. However, this proportion must depend on the definition of sepsis used. In Study II of the present thesis, the change to the sepsis definition did not lead to a significant change in microbiological characteristics. It is possible that there is not much more to gain in correlation between a clinical diagnosis of sepsis and presence of bacteremia, solely by adjusting clinical criteria for sepsis.

The study's strength lies in its prospective design and use of a clinical screening tool, allowing for the inclusion of patients with a high likelihood of sepsis at presentation. This approach ensured a real-world representation of patients in the emergency setting, where final diagnoses are often undetermined. The limitations of the study consist of its single-center design and predominant focus on community-acquired sepsis, though the criteria for Sepsis-3 and Sepsis-2 are applicable to both community and hospital-acquired sepsis. Additionally, the use of clinical triage criteria and lactate measurements for inclusion may have missed some sepsis cases, given the poor predictive values of existing sepsis screening tools. Despite this, the study aimed to include patients with a high probability of sepsis, reflecting the clinical realities encountered in emergency care.

6.2.3 Study III

Study III was designed in response to the COVID-19 pandemic, during a period when not much data was present regarding the prevalence of BC positivity in this patient group. This study showed that the overall BC positivity rates were comparable across the three groups: patients with COVID-19 and the two control groups. However, the proportion of episodes with clinically relevant growth was significantly lower, and contamination rates were higher, in COVID-19 patients compared to both control groups.
The lower rate of clinically significant growth can have several causes. The clinical picture in COVID-19 resembles bacterial sepsis, with a significant proportion of admitted patients during the first period of the pandemic showing signs of organ dysfunction without having concomitant bacteremia. Indeed, the immunological cascade in COVID-19 shares common pathways with bacterial sepsis, with similar immunological processes such as activation of Toll-like receptors and downstream cytokine signaling (66, 80). Differentiating between the viral and potential bacterial components in hospitalized COVID-19 patients is challenging, given the similarity in clinical and laboratory manifestations. However, the low incidence of significant bacteremia, as reported in Study III, suggests a predominance of the viral aspect in COVID-19 cases.

From the other viewpoint, contamination rates were higher in patients with COVID-19. Contamination rate in itself is not related to the prevalence of bacteremia, but to the risk of contaminating the BC bottle with bacteria from the skin. In our center, BCs typically demonstrate low contamination rates, as evidenced by the data from the two control groups. However, in the COVID-19 group, contamination rates surpassed the <3% benchmark recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines. This increase in contamination could potentially lead to unwarranted antibiotic administration and extended hospital stays for these patients if results are incorrectly interpreted. The specific causes for the elevated contamination rates in the COVID-19 cohort remain unclear. Our study noted that these rates were particularly higher in the emergency departments and ICUs, compared to other hospital units, within the COVID-19 group. Previous research has established a link between high-stress environments and increased BC contamination rates (81, 82). The combination of high pace of work in emergency departments and ICUs, coupled with the heightened risk of exposure to SARS-CoV-2 suggests that the demanding conditions in these units could be a critical factor influencing the rate of BC contamination.

At the time of publishing, Study III was one of the first reports of BC data in patients with COVID-19. The strengths of the study included a large sample size, with over 15,000 patients from six tertiary care hospitals. Furthermore, clinically relevant growth and contaminant growth were separated which allowed correct analysis of the otherwise similar positivity rates between groups.
Limitations of the study included the lack of a control group with a different viral respiratory infection. To include, for example, patients with influenza during the same study period would provide a relevant comparison group. In the case of the studied period, the influenza prevalence was too low to include such a group. Furthermore, as study III was a pure laboratory study, we had limited baseline clinical data. Assessment of the effects of differences in patient characteristics on the BC results could therefore not be analyzed. Lastly, clinically relevant growth in the context of isolating a common skin contaminant was defined as the presence of three or more bottles with that isolate. In cases where only one or two bottles were sampled, around 25–30% of episodes in all groups, common skin contaminants were finally adjudicated as contaminant growth due to the limited number of BCs. Therefore, even if the majority of patients across all groups had four BC bottles sampled, the limited number of bottles in some cases could potentially have led to misclassification bias.

6.2.4 Study IV

In this study, we evaluated the diagnostic capabilities of the T2Bacteria assay for identifying bacterial pathogens in BSI, compared to BCs. The combined detection rate for in-panel bacteria by both methods was 15.8%, which is higher than the bacteremia rates reported previously by Nguyen et al (55) but similar to the rate described by de Angelis et al. (52). A significant finding was the high discordance rate between the two tests, with nearly three-quarters of positive detections showing discordant results between T2Bacteria and BC.

The high discordance by the methods suggests complementary roles of BCs and non-culture based BSI diagnostics. Nearly half of the episodes in our study were detected exclusively by the T2Bacteria assay, with no corresponding positives in BCs within a 72-hour period surrounding the T2 testing. This aligns with prior evidence indicating limitations in identifying microorganisms with BCs compared to molecular methods, possibly due to the impact of recent antibiotic treatment, low bacterial load, and sub-optimal sampling. A significant challenge, as highlighted in earlier research, is establishing a reliable "gold standard" for confirming true positive results in cases where T2 is positive but BCs are negative. Our methodology which was adapted from previous efforts, was based on validating the presence of bacteria through other microbiological samples beyond BCs to
indicate true infection. Out of 46 T2 positive/BC negative episodes, only eight had matching microorganisms in other samples. This approach, however, presents reliability concerns, as even episodes positive by both T2 and BC for the same bacteria—indicative of actual infection—frequently lacked matching organisms in other samples. Another approach commonly used is the addition of clinical adjudication. However, the clinical adjudication by clinicians is not independent, but frequently influenced by the diagnostic tests themselves, potentially leading to false positive classifications. The pathogens detected by T2 are commonly associated with clinically significant infections, suggesting that a positive T2 result, even in the absence of confirmatory BCs or matching samples from other sites, could indeed represent a true infection. This is especially important in scenarios where BCs are sampled during antibiotic treatment, or similar circumstances when the sensitivity of BCs is limited.

Conversely, a quarter of episodes were T2 negative but BC positive. This stands in contrast to data reported in previous studies (52, 55, 60), which report a low frequency of T2 negative/BC positive cases. The reason for this discrepancy potentially stems from the different method of including BCs in Study IV compared to the other described studies that only considered BCs sampled at the very same occasion as T2. In our study, a higher number of BCs during a wider time interval were included, indicating a higher detection capacity for BCs when considering all sampled BCs within a 72-hour period surrounding T2 testing. Furthermore, expanding the scope to pathogens not included in the T2Bacteria panel, we found that BCs detected pathogens in 32 additional episodes, corresponding to an increase from 8.6% to 13.6% of analyzed cases. This clearly illustrates the limitations of a narrow panel repertoire which must be considered when developing a novel diagnostic method for BSI.

Regarding diagnostic performance, the sensitivity and positive predictive value (PPV) of the T2Bacteria assay were generally low, while specificity and negative predictive value (NPV) remained high. This contrasts with prior studies reporting higher sensitivity for T2, which may be attributed to our inclusion of a larger number of BCs sampled over a broader time frame, cumulatively enhancing the diagnostic power of the BCs. The optimal time frame in which to include comparators for a novel method is currently unclear. To assess the influence of different time frames, we examined the effect of changing time frames on the proportions of positivity between the two methods (Figure 14). Notably, the most
consistent agreement between tests was observed within intervals ranging from 48 hours to five days, indicating that the chosen timeframe for our analysis was appropriate.

TAT serves as an important metric for evaluating the effectiveness of diagnostic methods, incorporating both the pre-analytical phases, such as sample collection and transport, and the analytical phase consisting of the actual testing process. As expected, the TAT for BCs was found to be significantly longer than the TAT for T2Bacteria, primarily due to the inherent time required for the culture step. This duration is critical from a clinical standpoint as it is directly linked to the time to targeted antimicrobial therapy. The reduction in TAT with T2Bacteria could potentially lead to quicker clinical decisions and interventions. However, in Study IV this advantage was mitigated to some extent by logistical delays experienced with the T2Bacteria assay, with a mean delay from sampling to arrival in the microbiological laboratory reaching 15 hours. The corresponding delay for BCs was much shorter, with samples typically reaching the microbiological laboratory within one and a half hour. Previous studies reported a much shorter TAT for T2 of around 3–7 hours, however only accounting for the sample processing time, and not the pre-analytical delays. The extended pre-analytical phase for T2 in our study can be attributed to its novel status in many clinical settings, coupled with the restriction of its availability to standard work hours. This limitation not only prolongs the time before testing can commence but also illustrates a broader issue with the implementation of new diagnostic technologies in healthcare facilities. The operational constraints, such as limited work hours, highlight an important area for improvement in laboratory management and test availability. Continuous availability would eliminate delays associated with non-working hours, aligning the TAT of T2Bacteria more closely with its theoretical potential.

Study IV had some limitations. First of all, it was a retrospective study, including only patients that underwent T2Bacteria sampling. This might potentially introduce bias in patient selection as well as a limited selection of studied infections. It is possible that the decision to use T2Bacteria was influenced by several factors such as ongoing antibiotic treatment, failure to provide a microbiological diagnosis and need for rapid diagnosis associated with critical illness. Regardless, this patient group is highly representative for a population in whom a non-culture based
method adds additional diagnostic value. The study’s reliance on an imperfect reference standard for confirming true BSI presence is acknowledged, however, we used a similar approach as previous studies to assess the likelihood of true infection by including additional microbiological samples in our assessment. The laboratory was a single center, limiting the generalizability of the study. However, the samples were collected from six tertiary health care centers across Stockholm with a broad representation of patient categories.

Strengths of this research lie in its large sample size, high bacteremia rate, and comprehensive analysis of BCs within a 72-hour timeframe of T2 testing. This methodology minimized the impact of intermittent bacteremia and provided a more accurate comparison. Additionally, this is the first study to report TAT of T2Bacteria using an in-depth analysis, offering insights into the real-world performance of the method.

6.3 General considerations

6.3.1 Challenges in choosing a reference standard

Several recurring issues regarding the core concepts of bacteremia arises when interpreting the results of the studies included in the present thesis and warrants further discussion. Study I and IV compares two methods to a chosen reference standard, which in Study I consists of all six BC bottles and in Study IV of the combined findings of BC and other microbiological samples except T2Bacteria. Implicitly, these reference standards were chosen to represent the dichotomous condition of bacterial presence in the blood stream. This problem is not unique to the study of bacteremia: it arises in all fields in medicine where there is not a single diagnostic test that represent a true positive.

In clinical studies of bacteremia, commonly used reference standards are clinical adjudication (83), other microbiological samples (52), International Classification of Diseases (ICD) coding, or a composite of the above (58). While providing a reference, the usage of these is not optimal. Other microbiological samples provide only a weak reference, as the presence of microorganisms in, for example urine, do not necessarily imply the presence in blood. Clinical adjudication and ICD coding also confer its own weaknesses, as in clinical practice microbiological results, particularly from blood, have a large impact on the final clinical diagnosis which can
result in bias. In addition, BCs has been used as the reference standard in other comparisons (84), which precludes the assessment of diagnostic superiority, meaning when the new method is positive and BC is negative, the result will be interpreted as a false positive.

Furthermore, it has been shown that the sensitivity reported for a novel diagnostic test varies significantly with prevalence when using composite reference standards (61). Historically, BSI has been studied in the general setting, and the prevalence of positive BCs have been around 5-13% (30, 32). Newer studies including study II included in the present thesis, suggests that prevalence of positive BCs is around 30% when selecting a group fulfilling sepsis criteria. Of interest, the prevalence of BC positivity in study IV only reached 8.6% which may have deflated the sensitivity of the T2Bacteria test.

In light of these considerations, the intersection between sepsis definitions, clinical presentations, and microbiological diagnostics becomes a complex matrix. The reliance on clinical criteria to both prognosticate sepsis and infer bacteremia may not always align with the microbiological findings, which illustrate a limitation in our current diagnostic algorithms.

6.3.2 Interpretation of BC-negative sepsis

The current sepsis definition, a clinical diagnosis primarily guided by the SOFA score, raises critical questions about the efficacy and precision of diagnosing true bacteremia within this framework. Approximately 30% of patients diagnosed with sepsis under these criteria are confirmed to have bacteremia through BC diagnostics. However, this statistic carries uncertainties regarding the remaining 70%. One critical question is whether this significant proportion of patients, who are clinically diagnosed with sepsis but lack BC-confirmed bacteremia (Figure 16), actually harbor undetected bacterial pathogens, or do they have sepsis triggered by non-bacterial agents or other non-infectious etiologies? While the present sepsis criteria are effective in flagging patients with severe clinical presentations, they might not be as precise in confirming or excluding bacteremia. This imprecision could lead to overtreatment or undertreatment, with either scenario having profound clinical implications. Over-reliance on clinical criteria without
confirmatory microbiological evidence could contribute to unnecessary antibiotic use without clinical benefits.

Furthermore, the reliability of BCs, though a cornerstone in the microbiological confirmation of bacteremia, is fraught with limitations. False negatives can occur due to various reasons, including prior antibiotic administration, the timing of culture collection relative to the infection’s course, or the intrinsic characteristics of the bacteria, such as slow-growing or fastidious organisms.

Figure 16: Proposed explanation for negative blood cultures in sepsis. The true size proportions of the three sections, representing prevalence in negative blood cultures, are largely unknown.

Therefore, the major uncertainties in the field covered by the present thesis stem from both the frequency of bacteremia in sepsis, as well as frequency of detectable bacteria in bacteremia. To further complicate our understanding, the clinical significance of bacteremia is not a trivial question. In the thought experiment of a perfect detection method for bacteremia, it is theoretically plausible that the lowest ranges of bacterial concentrations represent bacteremia without clinical significance, possible transient bacteremia or even constitutional bacteremia. Therefore, with evolving methods of detecting successively lower concentration of bacteria in blood, the aspect of significance is important to
acknowledge. Distinguishing between DNA-emia, transient, likely contaminant bacteremia and clinically significant bacteremia will be vital in the era of newer molecular diagnostics, as treatment decisions and resource allocation will be even more influenced by the interpretation of microbiological results from blood. The differentiation is especially crucial in the context of increasing healthcare costs and the need for antibiotic stewardship.

7 Conclusions

In Study I, we showed that it was possible to simplify the sampling process without loss of diagnostic accuracy. We also challenged the prior theory that MSS was superior in terms of discriminating contaminant growth, by showing that contamination was in fact less common using SSS. In a wider context, we concluded that the preanalytical phase of BCs still warrants refinement.

In both Study II and Study III, we were interested in how bacteremia rates depended on initial clinical conditions. The most important finding in Study II was that the old (Sepsis-2) and new (Sepsis-3) definitions of sepsis overlapped to a large extent and was associated with similar BC positivity rates. In contrast, in Study III, the prevalence of COVID-19 had a large impact on the diagnostic yield of BCs. The significant change in proportions of clinically relevant and contaminant growth further reinforces that the clinical conditions must be considered when interpreting BCs. In an even wider scope, the results of Study III implies that future responses to viral pandemics must include an early assessment of the prevalence of bacterial superinfection including BSI, to ensure appropriate use of empiric therapy and reinforced efforts to reduce contamination.

The last study in the present thesis uses T2Bacteria as a model to explore the utility of novel diagnostic methods for BSI. In Study IV, we concluded that both newer diagnostic methods and BCs provide complementary diagnostic power in the diagnosis of BSI. The study also highlights many of the current limitations of non-culture based methods, such as a limited detection panel, prolonged TAT related to preanalytical delays and the absence of phenotypic AST.

The problem relating to lack of a golden standard arises frequently when comparing microbiological diagnostic methods in sepsis. In a larger perspective, it
connects to the fundamental question in sepsis of whether the disease is driven directly by the microorganisms, or mainly by the immunological and inflammatory phenomena associated with infection. In BC-negative cases, Study IV concludes that at least a subset of these cases has molecular evidence of bacterial presence in the blood stream. The remainder, however, is one of the major unexplored frontiers in bacteriology.

8 Points of perspective

8.1 Clinical implications

From the vantage point of the clinician, the critical challenges consist of optimizing BC based diagnostics, and to introduce and validate new molecular based methods.

Study I provide further evidence that SSS is a safe alternative to MSS. With increasing number of performed medical interventions and procedures, especially in the emergency department, improving feasibility and time consumption of existing procedures is important. Additionally, if no additional diagnostic gain is made by MSS, there is also an ethical motivation to use SSS instead of MSS.

Study I, together with prior evidence (35) and subsequent efforts (78), was a pivotal study in determining the recent change in Swedish national guidelines (75). However, it is important to mention that there are certain scenarios where MSS can be valuable, such as repeat BCs in a patient with previous BC results having suspected skin contamination, where MSS theoretically can be of value to discriminate clinically relevant from contaminant growth. Furthermore, MSS is the preferred sampling strategy when CLABSI is suspected, given that the difference in TTD between central and peripheral sampling is a crucial factor in this context.

Study II and Study III collectively illustrate that BC performance is closely related to clinical disease definitions and pre–test conditions. The results of Study II indicate that previous knowledge of microbiological characteristics gained from the Sepsis–2 era remain relevant and applicable within the Sepsis–3 framework. Given that a substantial portion of the microbiological diagnostic data in sepsis is derived from the Sepsis–2 and Sepsis–1 definitions, the findings of Study II enable the application of earlier data to current clinical practice. In Study III, the high degree of
contamination and the lower incidence of true bacteremia in COVID-19 is important metrics to consider when designing protocol driven care during pandemics, especially when forming recommendations concerning antibiotic treatment.

Study IV provides practical insights into implementing a molecular based method complementing BCs. The clinical challenges are illustrated by the high frequency of T2Bacteria positive/BC negative results, and the need to develop a framework to interpret the discordant results in clinical practice. Additionally, it is shown that the preanalytical phase must be optimized to fully utilize a rapid turn-around diagnostic method.

8.2 Future studies

Future studies are warranted to address important questions left by this thesis, of which some are suggested here.

1. What further optimization can be done to the sampling process in clinical practice, in terms of ensuring adequate disinfection procedures, optimization of sampling volumes and other measures known to affect BC outcomes?
2. What proportion of BC negative sepsis patients have bacterial DNAemia?
3. Are there variations in bacteremia rates among different sepsis phenotypes (17), and if so, what are the implications?
4. What is the significance of bacterial DNAemia on patient outcomes?
5. How can an effective preanalytical workflow that operates 24/7 for molecular diagnostic methods be established?
6. Can a standardized composite reference be implemented to harmonize outcomes in future studies?
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10 References


