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STUDIES ON BLOODSTREAM INFECTIONS AND INFECTIVE ENDOCARDITIS

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DOCTORAL DISSERTATION

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ABSTRACT

Diagnosis and treatment of bloodstream infections (BSIs) and infective endocarditis (IE) remain a challenge to treating physicians. Determining the microorganisms responsible is of major importance. New microbiological methods have emerged in the diagnosis of these entities with blood cultures remaining as a cornerstone. Changing epidemiology of IE and its risk groups pose a challenge to their treatment. BSIs and IE both contribute to a major morbidity and mortality. Hence, optimal treatment of these entities is imperative.

Study I evaluated the impact of new microbiological method, short incubation matrix assisted laser desorption/ionization time-of-flight mass spectrometry (si-MALDI-TOF), on the antimicrobial treatment of BSIs caused by *Pseudomonas aeruginosa, Enterococcus* spp. and AmpC-producing *Enterobacteriaceae* when introduced into a routine clinical setting. Patients were treated at Helsinki University Hospital (HUS) between February 2014 and March 2015. In 69 episodes si-MALDI-TOF identified the causative agent and in 55 episodes conventional identification methods were used. Identification by si-MALDI-TOF lead to 12.8% increase in episodes in which patients received appropriate antimicrobial treatment within 48 hours after the blood culture draw, which was the primary endpoint. In BSIs caused by *Enterococcus* spp. (n=62) the observed increase in appropriate antimicrobial treatment was 22.4% (87.9% vs 65.5%, P=0.038). In a subgroup of patients with immunosuppression si-MALDI-TOF method was observed to offer a significant benefit. Implementation of si-MALDI-TOF into a routine clinical setting was associated with an increased proportion of patients with appropriate antibiotic treatment within 48 hours after blood culture draw, especially in case of enterococcal BSIs.

Study II explored bacteremia in patients with complicated skin and skin structure infections (cSSSI) in a population-based study including 460 patients with cSSSI from Helsinki, Finland and Gothenburg, Sweden. Blood cultures were positive in nearly one-fourth of those 258 patients from whom they were obtained. Diabetes, symptom duration less than two days and higher CRP level were associated with more frequent blood culture sampling, whereas surgical wound infection and peripheral artery disease were associated with less frequent blood culture sampling. None of the factors associated with blood culture drawing were found to be associated with blood culture positivity. Alcohol abuse was the only distinct patient characteristic associated with blood culture positivity. Patients with bacteremia had antibiotic treatment streamlined more often compared to non-bacteremic patients, which demonstrates a clear benefit of the information acquired from positive blood cultures. Given the high percentage of bacteremia amongst those from whom blood was cultured, difficulties in predicting patients with bacteremia and benefit of the information acquired from positive blood cultures, clinicians should order blood cultures from patients with cSSSI with low threshold.

Study III evaluated the impact of pre-operative antimicrobial treatment duration on the yield of valve cultures and bacterial 16S PCR obtained during surgery for IE and the diagnostic value of PCR. The study cohort included 87 surgically treated IE patients from HUS between years 2011-2016 and from whom valve culture and PCR sample from resected endocardial material were obtained. None of the patients with preoperative antimicrobial treatment duration longer than two weeks had positive valve cultures. PCR positivity was 91% in those patients with antimicrobial treatment duration less than two weeks and 53% in those with more than two weeks. In PCR positive cases preoperative antimicrobial treatment duration was significantly shorter than in PCR negative cases. PCR sampling had a diagnostic impact in one-sixth of the cases. Pre-operative treatment duration was shown to have a negative effect on the yield of both valve cultures and PCR. PCR sampling had added diagnostic value, especially in blood culture negative cases.

Study IV was a population-based study including all adult patients diagnosed with IE in Helsinki University Hospital Area between 2013 and 2017. The objective of this study was to describe the epidemiology, the spectrum of the microorganisms responsible for IE, clinical picture and the treatment and outcome of IE in the study region. Another objective was to determine the proportions of IE episodes according to the source of infection (i.e., mode of acquisition) and to define the characteristics of these groups and compare their differences. In all, 313 episodes of IE originating from 292 patients were included in the study cohort. *Staphylococcus aureus* was the leading cause accounting for one-third of the cases, followed by viridans group streptococci and *Enterococcus* spp. Equal proportions of community-acquired IE, health care-associated IE and drug use-related IE were observed, each accounting about for one-third of the cases. Distinct features of these entities were described and also different outcomes. Health care-associated IE was associated with more underlying diseases, prosthetic valve involvement, enterococcal etiology and higher mortality than community-acquired IE. Intravenous drug use-related IE was associated with more frequent right-sided and bilateral involvement, *Staphylococcus aureus* as etiology but no difference in mortality compared to community-acquired IE. High proportion of health care-associated IE and intravenous drug use-related IE pose a challenge for treatment, but also an opportunity for selected preventive strategies. In areas with common drug use, concomitant IE may account for a substantial proportion of all IE episodes, equal to that of HAIE.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four original studies:

I Halavaara M, Nevalainen A, Martelius T, Kuusela P, Anttila VJ. Impact of short-incubation MALDI-TOF MS on empiric antibiotic therapy in bloodstream infections caused by Pseudomonas aeruginosa, Enterococcus spp., and AmpC-producing Enterobacteriaceae. Diagn Microbiol Infect Dis 2019; 94:1-6.

II Halavaara M, Jääskeläinen IH, Hagberg L, Järvinen A. Factors associated with blood culture positivity in patients with complicated skin and skin structure infection – a population- based study. Eur J Clin Microbiol Inf Dis 2019; 38:1351-1357.

III Halavaara M, Martelius T, Järvinen A, Antikainen J, Kuusela P, Salminen US, Anttila VJ. Impact of pre-operative antimicrobial treatment on microbial findings from endocardial specimens in infective endocarditis. Eur J Clin Microbiol Inf Dis 2019; 38:497-503.

IV Halavaara M, Martelius T, Anttila VJ, Järvinen A. Three separate clinical entities of infective endocarditis: a population-based study from Southern Finland 2013-2017. Open Forum Infectious Diseases, 2020; ofaa334, doi.org/10.1093/ofid/ofaa334.

The publications are referred to in the text by their roman numerals.

ABBREVIATIONS

ASP	Antimicrobial stewardship program
BSI	Bloodstream infection
CAIE	Community-acquired infective endocarditis
CCU	Cardiac care unit
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
PET-CT	^{18}F-Fluorodeoxyglucose positron emission tomography/computed tomography
ESC	European Society of Cardiology
HAIE	Health care-associated infective endocarditis
HUS	Helsinki University Hospital
HUSLAB	Helsinki University Hospital Laboratory
ICU	Intensive care unit
ID	Infectious diseases
IDSA	Infection disease society of America
IDU	Intravenous drug use
IDUIE	Intravenous drug use-related infective endocarditis
IE	Infective endocarditis
IMCU	Intermediate care unit
IQR	Interquartile range
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MRI	Magnetic resonance imaging
NVE	Native valve endocarditis
PCR	Polymerase chain reaction
PVE	Prosthetic valve endocarditis
PWID	Person who injects drugs
RDT	Rapid diagnostic testing
SI	Short incubation
SSSI	Skin and skin structure infection

1 INTRODUCTION

Bloodstream infections (BSIs) and infective endocarditis (IE) intrigue both clinicians and researchers for they are severe infections with high morbidity and mortality, and their diagnosis and treatment are a challenge. The cornerstone in the diagnosis is a blood culture and optimal antibiotic therapy is crucial in their treatment. Determining the microbiological etiology without a delay is of utmost importance (Ibrahim et al. 2000, Seifert 2009). Also, clinicians should be well informed of the current epidemiological situation and clinical profiles of these entities, especially of IE, in order to better treat and prevent them.

Matrix assisted laser ionization/desorption time of flight (MALDI-TOF) mass spectrometry has changed the field in diagnostic microbiology, especially in BSIs, for it provides earlier information on the causative microorganisms compared to traditional methods (Drancourt 2010, Patel et al. 2013). Impact of MALDI-TOF method on management and outcome of BSIs has been studied with promising results, especially when combined with antimicrobial stewardship program (ASP) intervention. However, its impact is not fully determined, particularly when it is introduced to a routine clinical setting and when short incubation technique is used.

The treating physician receives a variety of critical information from the positive blood cultures. However, blood cultures do not come without a cost and false-positive results are relatively common. When exactly should blood culture be obtained? Skin and skin structure infections (SSSIs) are traditionally considered to be seldom blood culture positive and blood culturing is only recommended in special circumstances (Stevens et al. 2014). A Finnish-Swedish study on complicated SSSIs (cSSSIs) found blood cultures to be positive at quite a high rate, challenging the traditional view (Jääskeläinen et al. 2016). Factors associated with blood culture drawing and blood culture positivity are, however, undetermined in cSSSI and possible benefit of information received from positive blood cultures is undefined in this clinical entity.

Given the severity of IE and long intravenous antimicrobial treatment required in its treatment, determining the causative agent of IE is imperative. In case of blood culture-negativity, etiology may be resolved by serological testing or by valve cultures or polymerase chain reaction (PCR) from resected endocardial material if a patient is operatively treated (Fournier et al. 2010). PCR obtained during surgery has been shown to be useful in diagnostics of IE, especially if blood cultures are negative (Miller et al. 2016). However, the impact of the duration of pre-operative effective antimicrobial treatment on the yield of PCR is undetermined.

The clinical entity of IE has changed during the past few decades (Wang et al. 2018). Health care-associated infection has emerged and it accounts at least one-fourth of the cases contemporarily. Intravenous drug use (IDU) is a major risk factor for IE and in areas where IDU is prevalent IDU-related IE is of major

importance. As a consequence of the changes in epidemiology, *Staphylococcus aureus* is now the leading pathogen responsible for IE in developed world (Moreillon and Que 2004, Fowler et al. 2005). Much of the data on IE are derived from referral centers, thus population-based studies are warranted. Studies comparing clinical profiles of IE according to the mode of acquisition are limited in number, especially studies comparing all three: community-acquired IE (CAIE), health care-associated IE (CAIE) and intravenous drug use-related IE (IDUIE). Moreover, no recent studies exist on the epidemiology and clinical entity of IE in the Capital Region in Finland.

This thesis work evaluates the clinical impact of microbiological methods: MALDI-TOF performed in BSIs and PCR applied on resected endocardial material from patients with IE. Clinical impact and utility of new microbiological methods are important to evaluate, in addition to their performance in microbiology laboratory. In this work, also the impact of duration of pre-operative antimicrobial treatment on the yield of both valve cultures and PCR is evaluated. This thesis also views blood culture positivity in patients with cSSSI. It determines factors associated with clinicians ordering blood cultures from patients with cSSSI and also factors associated with bacteremia in those patients. Finally, this thesis characterizes and compares different clinical entities of IE according to the mode of acquisition and determines their proportions of all IEs. The microbial spectrum of microorganisms responsible for IE, and the incidence, clinical characteristics and outcome of IE in the Capital Region of Finland is described.

2 REVIEW OF THE LITERATURE

2.1 BLOODSTREAM INFECTIONS

Bloodstream infections (BSIs) include infective endocarditis, catheter-related BSIs and primary BSIs or they can arise from a focal infection, e.g., from complicated skin and skin structure infection (Coburn et al. 2012).

BSIs can be acquired from community or they can be health care-associated (Friedman et al. 2002). They contribute to a major morbidity, mortality and healthcare costs (Weinstein et al. 1997, Kilgore and Brosette 2008, Pien et al. 2010, Goto and Al-Hasan 2013).

2.1.1 INCIDENCE AND BURDEN OF DISEASE

Incidence of BSIs is considered to be increasing in Finland, as was shown in a population-based registry study: the incidence increased from 147 to 168 per 100,000 person-years during the study period between 2004 and 2007 (Skogberg et al. 2012). Derived from these data, an estimated nearly 8,700 BSI episodes and nearly 1,100 deaths occurred from BSI per year in Finland (Skogberg et al. 2012, Goto and Al-Hasan 2013). An increase in incidence rate of BSIs was also reported in a population-based study from Denmark and the reported incidence rate reached 166 per 100,000 person-years in 2006 (Sogaard et al. 2011). In Denmark, one-third of BSI episodes were hospital-acquired 2002-2006 (Sogaard et al. 2011). Even higher incidence rate of 189 episodes per 100,000 person-years was reported from Olmsted County, Minnesota (Uslan et al. 2007).

In all, an estimated 1.2-1.4 million episodes of BSI occurred in year 2007 in Europe and BSI is estimated to be among top seven causes of death in many European countries and in North America (Goto and Hasan 2013). As the incidence of BSI increases with age (Uslan et al. 2007), the overall burden of BSI in developed countries is likely to increase due to aging of the population.

In a prospective evaluation of nearly a thousand cases from two tertiary centers, mortality attributable to nosocomial BSI was higher (23%) compared to community-onset (10%) and overall crude mortality was 24% (Diekema et al. 2003). A 30-day mortality in evaluation of 14,303 bacteremia episodes from Denmark 2002-2006 was 15.4% for community-acquired, 22.0% for health care-associated and 27.7% for nosocomial bacteremia (Sogaard et al. 2011). In addition, in a Finnish study including 33,473 BSI episodes, a 30-day case-fatality rate of 13% was observed, one-third of which occurred within 2 days (Skogberg et al. 2012). A finding which, for its part, highlights the importance of early identification and treatment.

2.1.2 MANAGEMENT, SOURCE OF INFECTION AND MICROBIOLOGY

The cornerstones of the management of BSIs are fast identification of the causative organism and the source of infection (Ibrahim et al. 2000, Seifert 2009). This approach enables timely administered optimal antibiotic therapy, which is a crucial factor for better outcome (Kollef 2008).

According to a prospective study from two centers in USA and including 929 episodes of BSI the two most common (culture confirmed) sources were catheter (26%) and genitourinary (16%) followed by gastrointestinal/biliary tract (12%), respiratory tract (8%) and skin or soft tissue (6%; Diekema et al. 2003). Proportions were roughly similar between community-onset BSIs and nosocomial (i.e., hospital-acquired) BSIs (Diekema et al. 2003).

In year 2017 approximately 17,000 bacteremias were recorded by National Institute of Health and Welfare in Finland (Table 1) and their number has increased steadily over the recent years (Jaakola et al. 2018). *Escherichia coli* has been the most common finding and the second has been *Staphylococcus aureus* (Table 1). Similarly, in a population-based study from Olmsted County, Minnesota *E. coli* was the most common finding, also followed by *S. aureus* (Uslan et al. 2007). The study also found clear differences between genders: *E. coli* BSI was more common in females whereas BSI caused by *S. aureus* and viridans group streptococci were more common in males.

Table 1. *Annual number of selected blood culture findings in Finland according to National Institute of Health and Welfare (Jaakola et al. 2018).*

Blood culture finding	Year 2013		Year 2017	
	Age 15-64 years	*Age ≥ 65*	*Age 15-64 years*	*Age ≥ 65*
Escherichia coli	951	2876	1174	4011
Staphylococcus aureus	641	876	846	1348
Viridans group streptococci	151	193	119	207
Enterococcus faecalis	83	301	103	395
Enterococcus faecium	97	209	80	203
Pseudomonas aeruginosa	91	230	66	288
Enterobacter spp.	90	188	98	214
Serratia spp.	32	81	39	107
Morganella morganii	18	30	16	48
All bacteremias	4189	8084	5059	11548

2.1.3 DEFINITION AND DIAGNOSIS

Universal definition of BSI does not exist, but BSI is considered in a patient with symptoms and signs consistent with an infection and with blood cultures positive with an organism associated with infection (Laupland and Church 2014). Bacteremia means presence of viable bacteria in bloodstream. It must be noted that BSI and bacteremia are not synonyms, although often used interchangeably. BSI also includes fungemia (i.e., presence of fungi in bloodstream) and another difference is that, although rarely, bacteremia may be transient and thus not representing an infection (Laupland and Church 2014).

BSI is diagnosed by performing a blood culture, which is one of the most common microbiological tests performed and has remained the first and essential diagnostic tool for the detection of BSI (Lamy et al. 2016). Blood cultures were applied in microbial diagnostics as early as late 19th century, and infective endocarditis was among the first clinical entities targeted (Allerberger and Kern 2020).

Blood for culturing is obtained from venipuncture using a single-needle technique. One blood culture (one set) contains one aerobic and one anaerobic bottle. Two blood cultures are usually recommended and they can be obtained without an interval (Dargére et al. 2014). The sensitivity of blood culturing to detect bacteria in blood is correlated with the amount of blood drawn (Li et al. 1994). Of patients with bacteremia approximately 90% will show positive results after two sets of blood cultures and over 95% after three sets (Lee et al. 2007). In addition, two blood cultures are useful in interpretation of results in case of a possible contamination, i.e., whether a growth of coagulase-negative staphylococci represents a contamination or true bacteremia (Weinstein et al. 1996). More than two cultures are seldom needed, but suspicion of endocarditis is an exception to this rule.

Antibiotic treatment hampers the utility and the sensitivity of blood culture and should be started only after the blood culture draw (Lee et al. 2007). In a study including 325 patients with severe sepsis preantimicrobial blood cultures were positive in 31.4% patients and repeated blood cultures drawn 30 to 240 minutes after initiation of antimicrobial therapy were positive in 19.4% ($P<0.001$) demonstrating the negative effect of antibiotic treatment on the yield of blood cultures (Cheng et al. 2019). Another study including septic patients demonstrated equal positivity rates in blood cultures taken within one hour before (24%) and in blood cultures taken within one hour after (23%) initiation of intravenous antibiotics and also a sharp decline in blood culture positivity after one hour of antibiotic administration (Rand et al. 2019).

2.1.4 RECOGNITION OF BACTERIA FROM POSITIVE BLOOD CULTURES, THE MALDI-TOF METHOD, AND CLINICIAN´S VIEW

In microbiology laboratory, blood culture specimens are incubated in an automated blood culture instrument, which continuously monitors the samples and signals if growth is detected. In most microbiological laboratories blood cultures are routinely incubated for 5-6 days. This incubation time is considered adequate for detecting most bacteria, including fastidious bacteria, e.g., *Haemophilus*, *Aggregatibacter, Cardiobacterium, Eikenella* and *Kingella* spp. which are referred to as HACEK-group (Baron et al. 2005, Kirn et al. 2013).

If blood culture bottles are positive (i.e., growth is detected in specimens) next step is Gram staining, which provides the first information that the clinicians receive. The gram stain results are very useful if interpreted correctly and can lead to changes in antibiotic therapy (Munson et al. 2003, Huttunen et al. 2016). In light of the Gram stain results the likely effectiveness of the empiric antibiotic treatment can preliminarily be evaluated and the possible site of infection is also hinted. Gram staining results in combination with clinical background information may shorten delay to effective antimicrobial therapy (Hautala et al. 2005).

Simultaneously with Gram staining subcultures are also performed. These allow further identification and also susceptibility testing. Identification is usually done by standard protocols using biochemical identification. In most cases recognition of the causative organism to species level may require at least 48 hours and final results with susceptibility results are at clinicians' disposal within 48 – 72 hours (Patel et al. 2013). However, it may take longer if the organism grows slow *in vitro* or is difficult to recognise.

Blood cultures have remained the most important and comprehensive method in detecting microbes causing BSIs and determining their susceptibility to antimicrobial agents, despite progress in molecular diagnostic testing (Kirn et al. 2013, Lamy et al. 2016).

Molecular methods

When blood cultures show growth it is beneficial to recognise the organisms as fast as possible. Thus, molecular rapid diagnostic testing (mRDT) has gained field in the diagnosis of BSIs in the past decade (Sullivan et al. 2019). MRDT refers to tests such as polymerase chain reaction (PCR) and MALDI-TOF. Infectious Disease Society of America (IDSA) recommends the use of RDTs in addition to conventional culture of blood samples if combined with antimicrobial stewardship program (ASP) intervention (Barlam et al. 2016). A meta-analysis on the effect of mRDTs on clinical outcomes of BSIs found an association with mRDT and decreased time to effective therapy and length of stay, and in presence with an ASP also a decrease in mortality (Timbrook et al. 2017). Other RDTs include nucleic-acid-based methods such as fluorescence *in situ* hybridization (FISH), microarrays and rapid PCR-based tests (Opota et al. 2015[a]).

The MALDI-TOF method

MALDI-TOF has revolutionized the field in microbiology diagnostics (Bizzini and Greub 2010, Dranoncourt 2010, Patel et al. 2013). It has proven to be especially useful in the diagnostics of BSIs, in which fast recognition of the causative organism is of utmost importance (Patel et al. 2013).

In MALDI-TOF method laser ionizes the clinical sample within the matrix. Then ionized molecules are funnelled through electrostatic field into a time-of-flight mass analyser. Different sized molecules have different flying times, thus creating a mass spectrum. Resulted mass spectrum is compared with spectra in a reference database resulting in identification. This process is fast and takes only few minutes. (Drancourt 2010.)

However, specimen from incubated positive blood culture bottles cannot directly be applied to mass spectrometry, but the microbes need to be purified prior to analysis. For this purpose different purification approaches are applied (Faron et al. 2017). In a labour and time-consuming MALDI-TOF technique bacteria are purified by centrifuging and lysis before the analysis (Vlek et al. 2012). This method is hereby referred to as direct MALDI-TOF method. In another less laborious method a short incubation (si) on solid media is used to isolate bacteria from the content of the blood culture bottle. This method has been found to be both accurate and useful (Idelevich et al. 2014, Verroken et al. 2015, Kohlmann et al. 2015). This method is hereby referred to as si-MALDI-TOF method.

Implementation of the MALDI-TOF method in microbiology laboratory routine has significantly shortened the time from blood culture draw to recognition of the causative organisms of BSI to species level (Idelevich et al. 2014, Vlek et. al 2012). A systematic review concluded that MALDI-TOF identification was at least 24 hours faster than conventional methods (Dixon et al. 2015). In a study of 218 patients with bacteremia direct MALDI-TOF shortened the time to organism identification by 28.8 hours compared to traditional identification (16.4 hours vs 45.2 hours; Vlek et. al 2012). Introduction of MALDI-TOF identification led to 17 hours reduction in time to identification (from 73.5 h to 56.8 h) compared to conventional identification and further reduction of 15 hours after introduction of si-MALDI-TOF (56.8 h – 41.3 h) in a study comparing these three different identification protocols (Delport et al. 2017).

The diagnostic sensitivity of MALDI-TOF is reported to be 65-99% depending on the organism (i.e., better performance for gram-negative bacteria compared to gram-positive) and it is highly specific (Faron et al. 2017). In diagnostics, polymicrobial BSIs are not accurately detected by MALDI-TOF method (Clerc et al. 2013).

The shortcoming of the currently routinely used MALDI-TOF techniques is the recognition of bacteria and yeast only to species level without any additional information on the susceptibility of the organism. For example, MALDI-TOF can identify a gram-positive coccus to be *S. aureus*, but does not provide

information on its susceptibility to methicillin. In a world of growing bacterial resistance, fast information also on possible resistance of the causative agent of BSI is increasingly important. To tackle this issue, new clinical applications of MALDI-TOF are being developed to detect antibiotic resistance and also strain type of bacteria (Oviano and Bou 2018, Idelevich and Becker 2019).

Finally, molecular methods that can be applied directly to blood samples (without prior subculturing) are being developed (Peker et al. 2018). Such methods include direct PCR methods, T2 magnetic resonance based methods (especially for *Candida* spp.) and metagenomics (Peker et al. 2018).

Although direct PCR method poses clear advantages (e.g., speed and detection of noncultivable organisms) over traditional blood cultures, it will only be supplementary to blood cultures now and in the near future (Opota et al. 2015[b]). Disadvantages of direct molecular diagnostics on blood sample include: lack of full automation and defined workflow in microbiology laboratory, too high sensitivity and incapability of reliably detect multifactorial resistance mechanisms of bacteria (Opota et al. 2015[b]).

Delays in the diagnostics of BSI and the workflow in the microbiology laboratory are demonstrated in Figure 1.

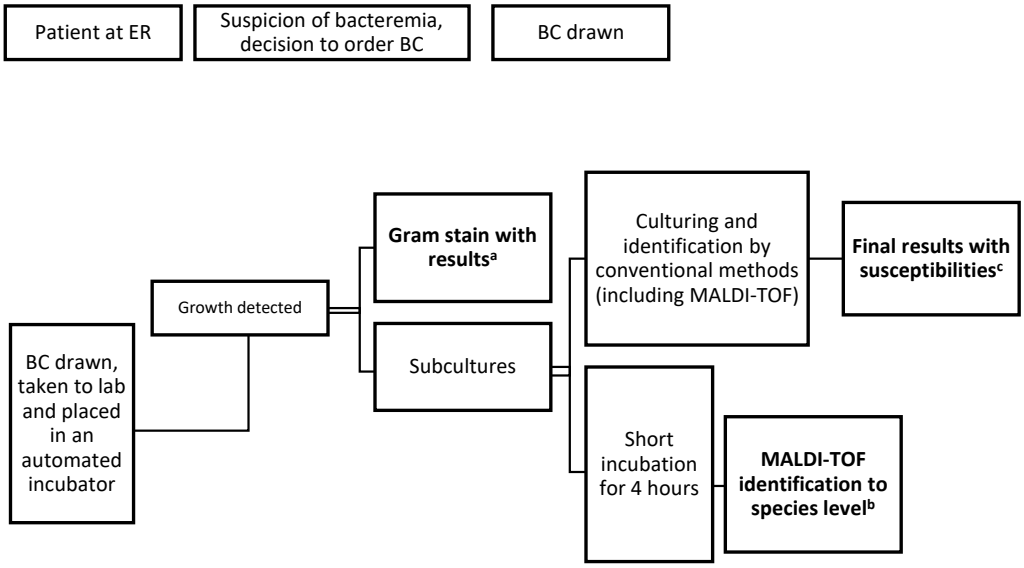

Figure 1 Work flow in the diagnosis of bloodstream infection and determining the causative agent. Upper part of the chart represents time elapsing in the ER from patient presentation to blood culture draw (usually 3 to 12 h). Lower part of the chart displays time elapsing in the microbiology laboratory. [a]This is the first information that clinician receives, 18–24 hours from blood culture draw; [b]If short incubation MALDI-TOF is done, this is the next information that clinicians receives, 24–32 hours from blood culture draw; [c]48-72 hours from the blood culture draw. Hours are approximations. In HUSLAB short incubation MALDI-TOF is done if blood culture bottle flags positive between 8 p.m. to 11 a.m. Abbreviations: ER, emergency room; BC, Blood cultures.

2.1.5 IMPORTANCE OF APPROPRIATE ANTIBIOTIC TREATMENT

Timely administered appropriate antibiotic treatment is crucial in the treatment of BSIs, for it can reduce morbidity and mortality (Leibovici et al. 1998, Ibrahim et al. 2000). Data derived from a prospective cohort study of 3414 patients hospitalized for BSI found longer hospitalization for survivors (11 vs 9 days, $P < 0.05$) and higher in-hospital mortality rate (34% vs 20%, P=0.0001) with inappropriate versus appropriate empirical antimicrobial treatment (Leibovici et al. 1998). In a multicenter study of febrile hospitalized patients (n=295) inappropriate empiric antibiotic therapy was associated with increased length of hospitalization (at least 2 days) and mortality (20% vs 12%, P=0.001; Fraser et al. 2006). Inappropriate initial therapy was an independent risk factor for increased length of hospital stay and mortality. Similarly, in patients with cSSSI appropriate empiric antibiotic treatment led to more rapid clinical stability (Jääskeläinen et al. 2017).

Moreover, time is of the essence, and the utmost importance of timely administration of effective antimicrobial therapy is shown and emphasized in septic patients (Ibrahim et al. 2000, Kumar et. al 2006). Delayed start of antibiotics was the most important individual variable associated with high mortality in a Finnish study including patients with septic shock (Varpula et al. 2007).

Of note, underlying disease is another important factor contributing to mortality, especially between one and three months after onset of infection, as was noted in a Finnish study including blood culture-positive septic patients (Rannikko et al. 2017).

The benefit of timely and effective antibiotic treatment has been shown in the treatment of BSIs caused by *Pseudomonas aeruginosa* (Kang et al. 2003, Lodise et al. 2007) and *Enterococcus* spp. (Zasowski et al. 2016). In hospital-onset enterococcal bacteremia a 3-fold increase in 30-day mortality was observed if receipt of appropriate antibiotic treatment was delayed over 48 hours (Zasowski et. al 2016) and similarly in hospital-acquired *Pseudomonas aeruginosa* BSIs delay of appropriate antibiotic treatment over approximately 48 hours increased 30-day mortality over two-fold (Lodise et. al 2007).

Enterobacter spp., *Serratia* spp. and *Morganella morganii* are Amp-C producing *Enterobacteriaceae*, which usually are nosocomial isolates (Donnerberg 2010). Due to their inducible AmpC-gene they are in most cases resistant to ampicillin and 1st generation cephalosporins and frequently also to 2nd and 3rd generation cephalosporins (Donnerberg 2010). Treatment failures occur with cephalosporin therapy, even if the isolate is susceptible *in vitro* (Harris and Ferguson 2012).

Finally, initiation of an empiric antimicrobial treatment is fine art (Kim and Gallis 1989). It should be mastered well and education helps. As 428 BSI episodes were prospectively evaluated, empirical antibiotic treatment was appropriate in 78% for the episodes treated by infectious disease (ID) specialists and 54% for the others (P<0.001; Byl et al. 1999). After blood culture results

were available corresponding percentages were 97% vs 89% (P=0.008). Additionally, ID specialists used fewer broad-spectrum antimicrobials. Similar finding of higher rate of optimal empiric therapy associated with infectious disease service was noted in another study with also a significantly higher incidence of de-escalation of antimicrobial treatment after culture results (Fluckiger et al. 2000).

2.1.6 CLINICAL IMPACT OF MALDI-TOF BASED TECHNIQUES

When a new microbiological method is adopted in microbiology laboratory, in addition to the evaluation of its performance and impact on laboratory diagnostics, also its impact on patient management and outcome along with cost-effectiveness should be examined (Doern 2014, McElvania TeKippe 2016).

Combined with ASP intervention, MALDI-TOF method decreased the time to both effective and optimal antibiotic therapy and was associated with reduced 30-day all-cause mortality (20% vs 13%, P=0.021), decreased length of intensive care unit (ICU) stay and recurrent bacteremia in univariate analysis in a pre-post study including 501 patients with bacteremia or candidemia (Huang et. al 2013). A cost-analysis performed using the data derived of patients included in the study by Huang and colleagues revealed that despite extra costs of implementing MALDI-TOF with ASP, it was cost-effective with approximately 2500 dollars saved per BSI and reduced cost was mainly due to the shortened ICU-stay (Patel et al. 2016). Cost-effectiveness of MALDI-TOF method with ASP was observed in another study including gram-negative BSIs (Perez et al. 2013).

A study including 202 episodes of gram-negative BSIs found reporting of MALDI-TOF results impact the empiric antibiotic treatment in one-third of the cases whereas reporting of the Gram stain results had an impact in one-fifth of the episodes (Clerc et al. 2013). This study included only patients with infectious disease (ID) consultation, lacked a control group and did not evaluate clinical outcome. It must be noted that independent impact of the MALDI-TOF method was not evaluated in the abovementioned studies.

Impact of MALDI-TOF without mandatory ASP intervention or ID consultation is less certain, as one study showed that MALDI-TOF combined with ASP intervention led to more favourable outcomes than MALDI-TOF alone (Berganovic et al. 2017). Similarly, in a clinical setting without ASP, MALDI-TOF had no benefit on patient management or outcomes (Jeon et al. 2018). However, a crossover study of 218 patients with BSI demonstrated that in routine clinical setting direct MALDI-TOF compared to conventional methods improved the appropriateness of antibiotic treatment within 24 hours of blood culture positivity (75% vs 64%, P=0.01; Vlek et. al 2012). Impact on other clinical outcomes was not evaluated. Another study evaluated the effect of direct MALDI-TOF when introduced to a pre-existing ASP setting and found its use to be associated with less frequent ICU admissions (23% vs 37%, P=0.02) and shorter duration of antibiotic treatment in case of contaminated blood cultures,

but had no effect on overall duration on antibiotic therapy or length of hospital stay (Osthoff et al. 2017).

Impact of short incubation MALDI-TOF technique on clinical management and outcome of patients with BSI is less studied. One study found its use to lead to a modified treatment recommendation in half of the cases, but its impact on clinical outcomes was not further assessed and the study lacked a control group (Kohlman et al. 2015). A study from London, UK including oncology and transplant patients with BSI found short incubation MALDI-TOF identification to be associated with shorter hospital stay and bacteremia associated mortality risk (Delport et al. 2017). Recent study conducted in pre-post method without a change in ASP policy with almost two thousand bacteremic patients included, found that implementation of short incubation MALDI-TOF led to a decrease in ICU admissions, shortened hospital stay and in case of gram-negative bacteremias also to decreased mortality (20% versus 16%; Zadka et al. 2019). However, as the authors note: lack of detailed data on antibiotic treatment is a major limitation of the study.

2.1.7 WHEN SHOULD A CLINICIAN ORDER BLOOD CULTURES?

First step in diagnosing a bloodstream infection is a decision by the clinician to order blood cultures. When exactly should this test be performed? Published guidelines do not clearly answer this question (Baron et al. 2013).

Blood culturing is not inexpensive and it is subject to false positive results in case of a contamination, causing quandary with the treating physicians. In hospital setting false-positive blood cultures have been found to increase the length and the cost of hospital stay (Alahmadi et al. 2011). One study found true negative blood cultures (i.e., no growth of microorganisms) to be 15 times more common than false positive blood cultures, yet much more health-care cost was related to false positive cultures (Zwang and Albert 2006).

On the other hand, BSI is a serious infection with marked mortality (Pien et al. 2010). This fact might prompt clinicians to order blood cultures liberally. One study indeed suggested that clinicians do overestimate the probability of bacteremia in their patients (Poses and Anthony 1991). In a three-center prospective study from France including 2314 adult patients with blood cultures sampled in emergency departments, 10.6% were positive for pathogens and 2.4% for contaminants (Dargére et al. 2014). In an analysis of 17,697 blood culture results collected at Helsinki University Hospital, Meilahti region during year 2012, the positivity rate was 5.4% and contamination rate less than 1% (Kalanti et al. 2013, unpublished data).

In suspicion of sepsis and septic shock, blood culturing is recommended by Surviving Sepsis Campaign (Levy et al. 2018). Blood culturing in this clinical scenario, and infective endocarditis, is feasible and is endorsed in other guidelines and reviews (Coburn et al 2012, Fabre et. 2020). In patients with severe sepsis, 31.4% of blood cultures were positive in one study (Cheng et al.

2019) and in IE blood culture positivity is over 85% (Cahill and Prendergast 2016).

Clinical scenarios with high pre-test probability of bacteremia (>50%) include: septic shock, meningitis, endovascular infections, discitis, native vertebral osteomyelitis, epidural abscess and nontraumatic native septic joints (Fabre et al. 2020). In these clinical scenarios blood culturing is always feasible. Fabre and colleagues suggest in their review that in other scenarios with lower probability of bacteremia, clinician should also take into account if culturing can be done from primary site of infection and if the results from blood cultures are likely to impact patient management when evaluating possible blood culture sampling (Fabre et al. 2020).

Fever and leukocytosis alone should not prompt blood culturing without considering the pre-test probability (Coburn et al. 2012, Linsenmeyer et al. 2016). One study including hospitalized medical patients found true positive rate of 3.6% of all blood cultures (Linsenmeyer et al 2016). The most common indications recorded by the treating physicians in the study were fever and leukocytosis, neither being highly predictive of bacteremia. A study including patients with clinical suspicion of bacteremia found CRP concentrations to be associated with bacteremia, but to have a limited role in bacteremia prediction model including white blood cell count parameters (Wyllie et al. 2015). A recent study found the sensitivity of another biomarker, procalcitonin, for bacteremia to be unacceptably low for a rule-out test (Goodlet et al. 2020).

Several clinical prediction rules to predict bacteremia have been developed. In a study of 3901 patients from whom blood cultures were drawn in emergency room, Shapiro et al. developed a clinical decision rule suggesting when to order blood culturing in a patient suspected to have an infection (Shapiro et al. 2008). This rule consists of three major or 10 minor criteria. Major criteria include: suspicion of endocarditis, temperature over 39.4 °C or indwelling catheter; minor criteria includes classical signs of severe infection, e.g., systolic blood pressure under 90 mmHg and thrombocytopenia. If one major or two minor criteria are met, blood culturing is recommended.

Systematic review found 15 validated models for predicting bacteremia and defining groups with low and high probability of bacteremia (Eliakim-Raz et al. 2015). However, these studies were heterogeneous in patient populations and variables, and with limited external validation (Eliakim-Raz et al. 2015). Moreover, none of these were found to be implemented in clinical work, including the one by Shapiro and colleagues.

A recent scoping review including 50 studies constructed an algorithm in an attempt to promote a wiser use of blood cultures, both initial and follow-up cultures (Fabre et al. 2020). This algorithm is based on pre-test probability of bacteremia in different clinical scenarios, as discussed above. As noted also in this review: clinical judgement is always a priority when considering blood culturing.

2.1.8 BLOOD CULTURE POSITIVITY IN SKIN AND SKIN STRUCTURE INFECTIONS

Skin and skin structure infections (SSSIs) are common infections encountered by physicians working in emergency departments and hospital wards (Raff et al. 2016). Beta-hemolytic streptococci and *Staphylococcus aureus* are the two most common causative agents of SSSI with former predominating in non-purulent cellulitis (Karppelin et al. 2015).

SSSI is classified as complicated (cSSSI) if it involves deep subcutaneous tissues or needs surgery (FDA 1998, Dryden 2010, Jääskeläinen et al. 2016). Thus cSSSI represent the more severe forms of SSSI. Also, presence of systemic signs of severe infection (e.g., hypotension), presence of comorbidities or a need for hospitalization can classify SSSI as complicated (Dryden 2010). The annual incidence of cSSSI in Helsinki was 9/100,000 in a population-based study between 2008 and 2011 (Jääskeläinen et al. 2016).

Gram-positive bacteria are predominating etiological agents in patients with cSSSI, as in two studies including patients with cSSSI approximately two-thirds of microbial findings were gram-positive bacteria (Jenkins et al. 2010, Garau et al. 2013). In these two studies, staphylococci accounted for 65% and 49%, streptococci 40% and 14% and gram-negative bacteria 13% and 46% of microbiological findings, respectively (Jenkins et. al 2010, Garau et al. 2013).

A population-based study from Finland and Sweden including patients with cSSSI found *S. aureus* (21%) and streptococci (16%) to be the two most commonly found pathogens in monomicrobial infections of microbiologically tested patients (Jääskeläinen et al. 2016).

A diagnosis and treatment guideline for skin and soft tissue infections by Infectious Diseases Society of America (IDSA) does not routinely recommend obtaining blood cultures in typical case of cellulitis (Stevens et al. 2014). They do recommend it in case of malignancy, severe infection or in case of unusual predisposing factors (e.g., animal bite). A study of cellulitis patients with blood culture positivity less than 5% is cited in this context as a rationale for the recommendation (Perl et al. 1999). Similar recommendation against blood culturing is stated in a study including 476 hospitalized patients with uncomplicated cellulitis with a blood culture positivity rate of 4.8% and sampling rate 53% (Bauer et al. 2016). In a recent large study from the United States including patients hospitalized for uncomplicated SSSI with low acute severity of illness and specially excluding cSSSI, found blood culture positivity rate of only 3% (and contamination rate of 2%) with blood culturing rate approximately 80%, suggesting that routine blood culturing is of low value in uncomplicated SSSI (Sutton et al. 2020).

Higher blood culture positivity rates in patients with SSSI, especially in more severe and complicated cases, have been reported, which challenge abovementioned recommendations. Bacteremia was found in 7.6% - 12% of patients subjected to blood culturing in patients with cSSSI (Jenkins et al. 2010, Garau et al. 2013). Both studies had similar blood culturing rate of 53%. In

addition, a study including patients with lower limb cellulitis reported that 19% of patients subjected to blood culture had them positive (Peralta et al. 2006). A study including hospitalized patients with SSSI found blood cultures positive in 16% of the patients from whom blood culture sample was obtained (van Daalen et al. 2017).

It must be noted that considerable variation between studies exists in blood culture positivity rates, probably due to differences is patient selection (e.g., severity of disease), amount of blood drawn and blood culture drawing rate. Indeed, the severity of SSSI affects the blood culture positivity rate, as a review found blood culture positivity rate of 4.8% in patients with erysipelas and a rate of 8% in patients with cellulitis, which involves deeper tissues than erysipelas (Gunderson and Martinello 2012).

The benefit of the information acquired from positive blood cultures has been questioned, as blood culture results seldom affected the antibiotic treatment in patients with complicated cellulitis (Paolo et al. 2013). Additionally, in patients with uncomplicated cellulitis blood cultures were exclusively due to *Streptococcal* spp. and *Staphylococcus aureus*, which were organisms usually susceptible to empiric antibiotics (co-amoxicillin) used in the study, thus bacteremia had no impact on change of empiric regimen (Bauer et al. 2016).

2.1.8.1 Microbiological findings from positive blood cultures

Streptococci were the most frequent finding in blood cultures in a study including patients with SSSI (van Daalen et al. 2017); in a review including patients with erysipelas and cellulitis (Gunderson and Moreillo 2012); in a study including patients with limb cellulitis (Peralta et al. 2006); and in a study including uncomplicated cellulitis (Bauer et al. 2016). *S. aureus* constituted 11-25% of all bacteremias in these four studies. Interestingly, proportions of *S. aureus* were equal in patients with cellulitis and erysipelas (14%) in one review, challenging the traditional view of greater role of *S. aureus* in cellulitis (Gunderson and Moreillo 2012).

In one study, even one-fourth of blood culture findings were due to gram-negative bacteria (Peralta et al. 2006). In this study, information from blood cultures led to modification of ongoing antibiotic therapy in nearly half of the cases (half of these changes were de-escalations).

In patients with cSSSI, however, *S. aureus* seems to predominate. In a large multicentre study from Europe including almost two thousand patients with cSSSI *S. aureus* was the most frequent finding in blood cultures and one-fourth of findings were gram-negative bacteria (Garau et al. 2013). In a cohort of 322 hospitalized patients with cSSSI *S. aureus* was found in eight patients and beta-hemolytic streptococci in two out of 13 blood culture positive patients (Jenkins et al. 2010).

2.1.8.2 Factors associated with blood culture sampling

A population-based study including patients with cSSSI reported that male gender and cellulitis associated with blood culture draw in univariate analysis (Jääskeläinen et al. 2016). This study included 460 patients with cSSSI from Helsinki, Finland and Gothenburg, Sweden. This is best to my knowledge the only study in this patient setting to study these factors.

In a study including patients with less severe SSSIs, acute severe clinical condition prompted blood culture sampling (van Daalen et al. 2017) and in patients with erysipelas those subject to blood culture sampling were sicker and had more often diabetes (Bläckberg et al. 2015). Higher CRP has been shown to be associated with increased blood culture sampling in patients with erysipelas (Bläckberg et al. 2015) and uncomplicated cellulitis (Bauer et al. 2016).

2.1.8.3 Factors associated with blood culture positivity

Only few studies have studied the association of patient characteristics or clinical findings (including laboratory results) with bacteremia in patients with SSSI and have not showed uniform results, as discussed in detail below. In addition, possible benefits of information gained from blood cultures are even less addressed, as noted above.

In a study including patients with limb cellulitis risk factors for bacteremia in multivariate analysis were absence of previous antibiotic treatment, two or more comorbidities, length of illness less than two days and proximal limb involvement (Peralta et al. 2006). Similarly, in a study including patients with SSSI higher blood culture positivity was observed in patients with severe co-morbidities compared to those without (van Daalen et al. 2017). Whereas, in patients with uncomplicated cellulitis alcoholism was the only distinct patient characteristic associated with bacteremia in multivariate analysis (Bauer et al. 2016).

Nonetheless, bacteremia in patients with SSSI is important to recognize because bacteremia is proposed to be associated with important outcome factors. In patients with cSSSI, when comparing bacteremic patients to the rest of the study group, bacteremia was associated with clinical failure and prolonged length of hospital stay (Jääskeläinen et al. 2016) as well as to later clinical stability (Jääskeläinen et al. 2017). Moreover, bacteremia was associated with longer duration of hospitalization in multivariate analysis also in patients with uncomplicated cellulitis (Bauer et al. 2016).

Given the incidence of SSSI and prognostic factors associated with bacteremia, Lipsky and colleagues constructed a prediction model to help clinicians to identify patients at high risk for bacteremia (Lipsky et al. 2010). They used a large database of adults who needed hospitalization for acute SSSI in USA and found 11 independent predictors for bacteremia. In their material

half of the patients had blood cultured with a positivity rate of 12%. They observed a three times higher in-hospital mortality in bacteremic patients compared to patients without bacteremia. *S. aureus* was the most common finding with more than one-half of the blood culture isolates, and with methicillin resistant *S. aureus* (MRSA) composing more than one-third of these cases (Lipsky et al. 2010). Another prediction model was constructed by Lee and colleagues based on cohort of patients from tertiary centre in Taiwan including hospitalized patients with cellulitis (Lee et al. 2016). They found four independent factors associated with bacteremia and included them in their model. These factors were: age over 65 years; involvement of non-lower extremities; liver cirrhosis; and fulfilment of systemic inflammation response syndrome criteria. Neither of these models, however, has been widely implemented in clinical work.

2.2 INFECTIVE ENDOCARDITIS

Since the days of Sir William Osler and his famous *The Gulstonian Lectures, On malignant endocarditis* (Osler 1885) where he first described the clinical entity of IE, clinicians´ battle against IE has come a long way and has had major victories (e.g., antibiotic treatment), but the adversary remains undefeated.

Despite medical progress, IE still contributes to major morbidity and mortality and the diagnosis and the treatment of IE remain a challenge (Hoen and Duval 2013, Cahill and Prendergast 2016, Wang et al. 2018). The clinical entity of IE has changed over time and it keeps on evolving (Wang et al. 2018). In the days of Sir William Osler and through most of the 20th century IE was mostly streptococcal disease of dental origin in patients with mitral stenosis caused by rheumatic fever, but in developed countries health care-associated IE with different causative agents, *Staphylococcus aureus* as the most common, has emerged along with prosthetic valve endocarditis and intravenous drug use-related IE (Moreillon and Que 2004, Murdoch et al. 2009).

Consequently, clinicians must stay alert and repeated studies on the epidemiology, etiology and clinical picture of IE are warranted (Hoen et al. 2002).

2.2.1 DIAGNOSIS AND CLINICAL PICTURE

Along with a well-informed clinician who knows when to suspect infective endocarditis, blood cultures and cardiac ultrasound are the cornerstones in the diagnosis of IE (Cahill and Prendergast 2016). Indeed, diagnosis of IE is based on demonstration of cardiac involvement and presence of causative microorganisms. Other imaging modalities, such as ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (PET-CT), echocardiogram-gated computed tomography angiography and magnetic

resonance imaging are complimentary to cardiac ultrasound. PET-CT has been shown to be especially useful in the diagnostic work-up of prosthetic valve endocarditis (de Camargo et al. 2020).

Given the perplexity of the disease, modified Duke criteria are used as a structured ground on which to base the diagnosis of IE (Li et al. 2000). Most studies concerning IE reference to these criteria. European Society of Cardiology (ESC) also adopted modified Duke criteria in their guideline on IE, but with some alterations (Habib et al. 2015). ESC includes imaging studies (i.e., CT angiography and PET-CT) as major criteria. Diagnostic criteria of IE are shown in Table 2.

Duke criteria, which were originally developed for research purposes, should not overthrow clinical judgement when considering the diagnosis of an individual patient (Cahill and Prendergast 2016, Holland et al. 2016).

Clinical presentation of IE depends mostly on the causative agent, affected valve, possible septic emboli and host factors. Classical categorisation to acute, subacute and chronic IE is outdated due to changing profile of IE (Cahill and Prendergast 2016). Classification according to the source (i.e., mode of acquisition) of IE to community-acquired IE (CAIE) or health care-associated IE (HAIE) and the type of the affected valve (native or prosthetic) is more relevant and gives tools for empirical antibiotic treatment and other treatment considerations.

Fever and elevated markers of inflammation are present in majority of cases (Murdoch et al. 2009, Selton-Suty et al. 2012). New or worsened pre-existing heart murmur is a valuable clinical sign. In some cases, first symptoms result from septic emboli (e.g., stroke). Overall, stroke as a complication of IE is found up to one-fifth of the IE patients and septic emboli to other sites in one-third (Murdoch et al. 2009, Selton-Suty et al. 2012). Immunological phenomena and other classic signs of IE, albeit classic textbook material, have become rare due to earlier presentation of the patients and better diagnostic methods (Murdoch et al. 2009).

IE should be considered especially in patients with bacteremia known to be associated with IE. In *S. aureus* BSI IE occurs in such a high proportion that routine screening for IE is warranted (Thwaites et al. 2011). In a prospective study from Finland including 430 patients with *S. aureus* bacteremia, IE was found in 17% of cases (Ruotsalainen et al. 2006). Community-acquired enterococcal BSI, in the absence of a primary focus, is a major criterion in modified Duke criteria, highlighting the importance of consideration of IE in patients with enterococcal BSI (Li et al. 2000). In a prospective study including 344 patients with *E. faecalis* bacteremia, systematic evaluation found definite IE in 26% of patients (Dahl et al. 2019). This study suggested consideration of routine screening for IE in patients with *E. faecalis* bacteremia. Risk factors for IE were: prosthetic valve, community-acquisition, persistent bacteremia, unknown portal of entry, monomicrobial bacteremia and immunosuppression (Dahl et al. 2019).

In a recent national registry study from Denmark, IE was found in 16.7% of patients with *E. faecalis* BSI, in 10.1% of patients with *S. aureus* BSI and in 7.1% of patients with *Streptococcus* spp. BSI, suggesting also routine screening for IE in bacteremias caused by these agents (Ostergaard et al. 2019).

As noted, bacteremia is a precursor for IE. In a systematic search for portal of entry in a study including 318 IE patients, it was found in 74% of patients (Delahaye et al. 2016). Most frequent portal of entry was cutaneous (40% of identified portals of entry), second being oral or dental (29%). Interestingly, dental infection focus was more often involved than dental procedure (59% and 12%, respectively). In one-fourth of the cases, portal of entry was gastrointestinal.

Table 2. *Diagnostic criteria of infective endocarditis.*

Definite IE
One pathological criterion **or**
Two major criteria **or**
One major criterion and three minor criteria **or**
Five minor criteria fulfilled
Possible IE
One major criterion and one minor criterion **or**
Three minor criteria fulfilled
Rejected IE
Criteria for possible IE not met **or** firm alternative diagnosis **or** resolution of symptoms ≤ 4 days **or** no sign of IE in pathological examination of valves (with antibiotic therapy ≤ 4 days)
Pathological criteria
Microbes demonstrated by histological examination or culture from valves or septic embolus **or**
Pathological lesion consistent with vegetation or abscess present in histological examination showing active endocarditis
Major criteria
Microbiological evidence
Blood cultures positive with typical microbe causing IE **or**
Blood cultures persistently positive with microbe consistent with IE **or**
Single positive blood culture or serology positive for *Coxiella burnetii*
Evidence of endocardial involvement
Echocardiogram findings consistent with IE (e.g., vegetation) **or**
PET-CT showing activity adjacent to prosthetic valve[a] **or**
Paravalvular lesion detected in cardiac CT[a]
Minor criteria
Predisposing heart condition or injection drug use
Fever more than 38 C
Vascular phenomena (e.g., septic emboli, intracranial hemorrhage, Janeway lesions)
Immunological phenomena (e.g., glomerulonephritis, rheumatoid factor, Osler nodes)
Microbial evidence: blood cultures positive with microbe consistent with IE, but not meeting the major criteria **or** serological evidence of active infection with microbe consistent with IE
Adapted from modified Duke criteria (Li et al. 2000) and ESC guidelines (Habib et al. 2015). Note: [a] These additional imaging studies are included only in ESC guidelines, not in modified Duke criteria.

2.2.2 DETERMINING THE ETIOLOGY

Considering the challenges in the treatment of IE, antibiotic treatment should be as optimal as possible for better outcome. Thus, determination of the causative organism is imperative. The absence of etiology has been shown to be an independent predictor of in-hospital mortality (Díez-Villanueva et al. 2016).

2.2.2.1 Blood culture

In more than 85% of cases, the etiology of IE is provided by blood cultures (Murdoch et al. 2009, Hoen and Duval 2013, Cahill and Prendergast 2016). Infective endocarditis is an infection of endocardium of the heart, mainly the valves. Thus, in IE bacteremia is continuous. Three sets of blood cultures are recommended with at least an hour interval between first and last draw (Baddour et al. 2015, Habib et al. 2015). In critically ill patients two sets are adequate because receipt of antibiotic therapy should not be delayed in this setting. An interval between the blood culture draws is not considered necessary in one review (Liesman et. al 2017). The most common reason for blood culture negativity is prior antibiotic treatment, in one study nearly two-thirds of blood culture negative IE cases had received antibiotics within seven days of culturing (Murdoch et al. 2009). Causative agents may also be fastidious, require special media in order to grow or be non-cultivable.

2.2.2.2 Serology and PCR

Blood cultures are negative in 10-15% of IE cases (Hoen and Duval 2013). In blood culture-negative endocarditis etiology may be revealed by serological testing, by PCR from blood or by PCR and culture of resected endocardial material in patients who undergo valve surgery due to IE (Fournier et al. 2010).

In case of blood culture negativity, ESC recommends, according to local epidemiology, serological testing for *Coxiella burnetii, Bartonella* spp., *Legionella* spp., *Brucella* spp., *Mycoplasma* spp. followed by specific PCR assays for *Bartonella* spp., *T. whipplei* and fungi from the blood (Habib et al. 2015). Serology for *C. burnetii,* the etiologic agent of Q-fever, is one of the major criteria of modified Duke criteria and is considered most reliable of the serologic tests (Li et al. 2000, Baddour et al. 2015). Other serological tests lack proper validation and standardization. However, serologic testing for *Bartonella* spp. is found useful in clinical work and is recommended to use with *C. burnetii* in the diagnostic work-up of blood culture-negative IE (Liesman et al. 2017). Systematic serological testing provided an etiology in 8% of IE cases in a study including 427 IE cases (Raoult et al. 2005).

In a study including 177 patients with definite, culture-negative IE from Marseille, France broad spectrum PCR from blood detected pathogens in three patients (1.7%) and specific PCR from blood in 24 patients (13.5%; Fournier et al. 2017). The two PCR methods from blood were, however, sole providers of etiology only in 14 cases and both were significantly less sensitive in detecting the causative organisms of IE than serology or culture or PCR from valves.

2.2.2.3 Microbial sampling of the resected endocardial material

Valve cultures

Valve culturing is recommended for IE patients who undergo valve surgery (Habib et al. 2015). The rationale behind this recommendation is that valve culture is a major criterion in modified Duke criteria and also culture results guide the duration of antibiotic treatment (Li et al. 2000, Habib et al. 2015). A study of 94 surgically treated IE patients did not find valve culture positivity to influence short- or long-term outcome (Dessap et al. 2009).

Valve cultures are not particularly sensitive in detecting the causative organism of IE and due to contamination they are subject to false positive results (Liesman et al. 2017). Sensitivity rates of 26%-44% have been reported (Miller et al. 2016, Peeters et al. 2017). Contamination rates between 13%-35% have been reported (Voldstedlund et al. 2008, Vondracek et al. 2011).

PCR

Broad-range PCR followed by sequencing is a sensitive method in detecting the causative organisms of IE from resected valve material (Gauduchon et al. 2003). In a study of 29 histologically confirmed IE cases, PCR was positive in 27 and contributed to the diagnosis and management in 6 cases (Gauduchon et al. 2003). Several studies have shown the usefulness of the PCR method from resected endocardial material in establishing the etiological diagnosis in IE and in particular when blood cultures are negative (Bosshard et al. 2003, Lang et al. 2004, Greub et al. 2005). In addition to those early works, in a study from Belgium including 120 surgically treated definite IE patients, the sensitivity of 16S rRNA PCR to detect the causative agent from resected valve was 87% and had an added diagnostic value in one fifth of the cases (Peeters et al. 2017). Similarly, in a study of 68 patients PCR contributed to the microbial diagnosis in one-third of the patients and contributed to a clinical decision in one-sixth (Miller et al. 2016). A prospective study from Sweden including 57 patients undergoing heart surgery for IE and a control group (n=61) without IE, observed PCR to be positive in 44 (77%) of cases and valve cultures in 13 (23%) of cases in IE group (Vondracek et al. 2011). In the control group of the Swedish study, PCR was negative in all cases, but valve culture was positive in eight (*Propionibacterium* spp. in seven and *Corynebacterium* spp. in one) and all were ruled as contaminations.

Addition of the PCR method to the diagnostic criteria of IE has been proposed (Bosshard et al. 2003, Peeters et al. 2017).

2.2.2.4 Impact of pre-operative antimicrobial treatment on the yield of valve cultures and PCR

Pre-operative antimicrobial treatment has been found to have a negative effect on valve cultures (Upton et al. 2005, Kotilainen et al. 2006, Voldstedlund et al 2008, Dessap et al. 2009). One study, however, reported no effect (Peeters et al. 2017). In a study by Upton et al. 131 episodes of streptococcal IE in which operation was performed during antimicrobial treatment were retrospectively reviewed. They found that only in one case valve culture was positive in patients with pre-operative antimicrobial treatment duration more than two weeks (Upton et al. 2005). In two studies the median (or mean) duration of pre-operative antimicrobial treatment was significantly shorter in patients with valve cultures positive compared to those with valve culture negative (Kotilainen et al. 2006, Voldstedlund et al. 2008).

In previous studies, the duration of pre-operative antimicrobial treatment had no effect on the yield of PCR from the resected valve (Rovery et al. 2005, Kotilainen et al. 2006, Voldstedlund et al. 2008, Peeters et al. 2017). In three studies the median (or mean) duration of pre-operative antibiotic treatment was similar in patients with PCR positivity and PCR negativity (Rovery et al. 2005, Kotilainen et al. 2006, Voldstedlund et al. 2008). In a study by Rovery et al. similar proportions of PCR positivity were observed in patients with pre-operative antibiotic treatment duration of 15 days or shorter and in patients with antibiotic treatment longer than 15 days (Rovery et al. 2005).

2.2.3 EPIDEMIOLOGY

Reported incidence rates of IE vary between studies (Ambrosioni et al. 2017, Cresti et al. 2017). This is due to differences between populations studied, but also due to changes in population and risk groups for IE over time. For example, injection drug use is a major risk for IE and thus changing proportion of persons who inject drugs (PWID) in population is reflected on incidence of IE. In addition, changes in antibiotic prophylaxis guidelines might have an impact on incidence (Dayer et al. 2015).

In a population-based survey from France including 497 definite cases of IE the annual incidence of IE was 3.4 per 100,000 inhabitants (Selton-Suty et al. 2012). A recent population-based registry study from Finland estimated the incidence rate of IE admissions to be 6.3/100,000 person-years (Ahtela et al. 2019).

In a recent review, the overall incidence rate of IE was concluded to have been rather stable in recent years (Ambrosioni et al. 2017). However, studies evaluating the evolution of incidence over time show somewhat conflicting results; some report an increase in incidence while others do not. For example, a study from Spain reported an increase by 2% per year during the study period between 2003 and 2014 (Olmos et al. 2017) and a report from England also showed an increase (Dayer et al. 2015), whereas a study from states of California and New York, USA did not (Toyoda et. al 2017). In summary, results of the latest studies on trends in the incidence of IE in different regions and countries show that the incidence of IE is probably increasing (Delahaye and Duclos 2017).

Increasing incidence of IE is most obvious among the elderly people (Slipczuk et al. 2013, Cresti et al. 2017, Olmos et al. 2017, Cuervo et al. 2018). Additionally, three 1-year population-based studies carried out in years 1991, 1999 and 2008 in same regions in France observed an increasing mean age of IE patients from 58 years to 62 years, whilst overall incidence of IE stayed the same (Duval et al. 2012). Similarly, an increase in age of patients with IE was noted in a study from South-western Finland between years 1980-2004 (Heiro et al. 2006).

2.2.4 RISK FACTORS

Several risk factors for IE exist. More than half of IE cases occur in patients over sixty-years of age (Hill et al. 2007, Selton-Suty et al. 2012, Habib et al. 2019). This is probably due to aging of general population and accumulation of other risk factors (e.g., valvulopathy and increase of invasive diagnostic and therapeutic procedures) with age. Male predominance is observed in most studies with male-female ratio over 2:1 (Hill et al. 2007, Selton-Suty et al. 2012). Injection drug use is a major risk factor and is discussed separately (section 2.2.7.2). Poor dental health is considered a risk factor for IE from oral streptococci (Lockhart et al. 2009). In addition, haemodialysis, diabetes, indwelling venous catheters and immunosuppression are risk factors for IE (Hoen and Duval 2013, Cahill et al. 2017). Moreover, variety of invasive non-dental procedures has been proposed to be associated with risk of development of IE (e.g., cystoscopy and colonoscopy; Janszky et al. 2018).

High cardiac risk factors include prosthetic valve, certain congenital heart diseases and previous IE, whereas rheumatic heart disease, degenerative valve disease, implantable cardiac devices (e.g., pacemaker) and hypertrophic cardiomyopathy are considered moderate risk (Cahill et al. 2017).

2.2.5 MODE OF ACQUISITION

Defining the mode of acquisition of IE is important as the microbiological etiology and outcome, and to some extent also the clinical picture, differs according to it. IE can be acquired from community or it can be health care-associated (Friedman et al. 2002). IE occurring in persons who inject drugs (PWID) is considered separately from these, since PWID are a major distinct risk group (Moss and Munt 2003). IE is considered to be community-acquired when IE develops before or within 48 hours of hospitalization in a patient not meeting the criteria for HAIE.

2.2.5.1 Health care-associated IE (HAIE)

HAIE refers to an episode of IE that (1) develops 48 hours after hospital admission, (2) is acquired in association with recent hospitalization or invasive procedure or (3) occurs in patients with recent extensive contact with health care (e.g., wound care or hemodialysis) or (4) occurs in persons who reside in nursing homes (Friedman et al. 2002, Ben-Ami et al. 2004, Fernandez-Hidalgo et al. 2008).

HAIE can be further classified to nosocomial (i.e., hospital-acquired) HAIE or non-nosocomial HAIE. Hospital-acquired IE has traditionally included IE episodes developed after 48 hours after admission or occurring 2-3 months after hospitalization with invasive procedure. In their work Ben-Ami et al. suggested broadening of this definition to include IE episodes arising within six months after hospitalization (Ben-Ami et al. 2004). Non-nosocomial HAIE includes patients with ambulatory invasive procedures done (e.g., urologic procedures, angiography) before the onset of IE (Fernandez-Hidalgo et al. 2008, Lomas et al. 2010), but can be extended to include also patients with extensive contact with health care (e.g., recent wound care or residence in a nursing home; Friedman et al. 2002). Clinical characteristics and outcome of nosocomial and non-nosocomial HIAE are similar (Benito et al. 2009).

The reported share of HAIE of all IE cases varies between studies and depends on the definition of HAIE applied, study population and selection criteria (Table 3). Overall, HAIE is estimated to account for at least one-fourth of all IE cases (Table 3). In a large population epidemiology study including New York State and California HAIE accounted for more than half of the native valve IE (Toyoda et al. 2017).

The incidence of HAIE is increasing, as was shown in Spanish study including IE patients from 1984 to 2007 (Lomas et al. 2010). The abovementioned study from USA showed an increase in HAIE likely acquired from outpatient health care and a decrease in HAIE likely acquired from hospital setting during the study period between years 1998-2013 (Toyoda et al. 2017). Reasons for the increasing incidence of HAIE are manifold: diagnostics for IE are improved, the definition of HAIE is broadened and also more and

increasingly older people are subject to invasive procedures, both diagnostic and therapeutic. Microbiological etiology of HAIE in selected studies is shown in Table 3.

HAIE has a higher mortality rate compared to CAIE (Fernandez-Hidalgo et al. 2008, Lomas et al. 2010, Selton-Suty et al. 2012). This is mostly due to higher rates of staphylococcal etiology and older patients with higher co-morbidity. HAIE is also considered to be an independent predictor of death (Fernandez-Hidalgo et al. 2008, Benito et al. 2009, Lomas et al. 2010).

Given the increasing incidence and high mortality rates of HAIE, preventive measures in health-care setting are of utmost importance (Benito et al. 2014).

Table 3. *Proportion of HAIE of all IE episodes and proportions of Staphylococcus aureus, viridans group streptococci and enterococci of all HAIE episodes in studies on IE.*

Reference (number of patients included)	**Country / Region**	**Proportion (%) of HAIE cases by etiology**			**Proportion (%) of HAIE of all IE cases**
		S. aureus	**VGS**	**enterococci**	
Fernandez-Hidalgo et al. 2008 (n=292)	Barcelona, Spain[a]	33.7	10.8	22.9	28.4
Benito et al. 2009 (n=1622)	Multinational[b]	45	8	15	34[c]
Sy and Kritharides 2010 (n=1536)	Australia[d]	33	16	16	30
Lomas et al. 2010 (n=793)	Andalusia, Spain[b]	30.7	2.4	17.3[e]	16[f]
Selton-Suty et al. 2012 (n=497)	France[d]	32.8	10.7	4.1	26.7

Abbreviations: IE, Infective endocarditis; HAIE, Health care-associated IE; VGS, viridans group streptococci
[a] Single referral center study
[b] Multicenter study
[c] Only native valve IEs with people who do not inject drugs included in the study
[d] Population-based study
[e] *Enterococcus faecalis* only
[f] Only left-sided native valve IE included in the study

2.2.5.2 Intravenous drug use-related IE (IDUEI)

Intravenous drug abuse is a major risk factor for IE and is also included as a minor criterion in modified Duke criteria (Li et al. 2000, Wang et al. 2018). Proposed factors predisposing PWID to IE, and particularly right-sided IE, include: endothelial damage to the tricuspid valve caused by substances injected, altered right-sided blood circulation circumstances (e.g., drug-induced pulmonary hypertension), injection of high load of bacteria or yeast concomitantly with drug injection (e.g., from skin or from the drug itself; Frontera et al. 2000). Also, if saliva is used to dilute the drug, oral bacteria or

yeast may be injected in vein. Methamphetamine has cardiac toxicity and negative effect on the immune system (Yu et al. 2003) and thus it may predispose to IE similarly to cocaine (Cooper et al. 2007).

Incidence of IDUIE varies greatly between studies as noted in large review (Slipczuk et al. 2013). A population-based study from Spain reported a decrease in prevalence of IDUIE during the study period from 2003 to 2014 and overall prevalence less than 5% (Olmos et al. 2017) and in France prevalence of IDUIE remained stable and under 10% in three population-based surveys 1991, 1999 and 2008 (Duval et al. 2012). Whereas a study from USA, where the opioid crisis is prevalent, report an increasing proportion of IDUIE of all IE cases: from 27% in years 1999-2000 to 40% in years 2009-2010 in Kentucky (Seratnahaei et al. 2014). Similarly in a national registry-based study from USA IDUIE increased from 15% to 29% of all IE cases between 2010 and 2015 (Rudasill et al. 2019). A study from a Finnish teaching hospital between years 1980-2004 and with 326 episodes of IE reported a significant increase of IDUIE up to 20% of cases during the last 5-year period (Heiro et al. 2006).

Clinical entity of IDUIE differs from non-IDUIE in many aspects. Patients with IDUIE are younger with less co-morbidities (Ruotsalainen et al. 2006, Leahey et al. 2019, Rudasill et al. 2019). *Staphylococcus aureus* is clearly the most common causative agent (Sousa et al. 2012, Rodger et al. 2018, Leahey et al. 2019). In most studies, PWID with IE have right side affected more often than left side, whereas right-sided IE in general is less common, comprising 5-10% of cases (Cahill and Prendergast 2016). Vast majority of right-sided IE cases occur in PWID.

In a study including 202 cases of first-episode IE cases in PWID, right-sided infection was more common (61%) and *S. aureus* caused 77% of IE cases and no cases of HAIE were observed (Rodger et. al 2018). In a Finnish study right-side IE was observed in 60% of IDUIE cases caused by *Staphylococcus aureus* (Ruotsalainen et al. 2006). A study from single-center from Boston, USA compared IDUIE (103 patients) to non-IDUIE (278 patients) and found more frequent right-sided and both-sided valve involvement, history of previous IE, *S. aureus* as etiology and interestingly also operative treatment in IDUIE compared to non-IDUIE (Leahey et al. 2019).

A reduced mortality of IDUIE compared to non-IDUIE at index hospitalization has been observed (6.8% vs 9.6%, $P<0.001$), probably due to younger age and more frequent right-side involvement (Rudasill et al. 2019). However, despite IDUIE patients having fewer comorbid diseases and younger age compared to non-IDUIE patients, no difference were observed in one-year all-cause mortality (16% vs 13%, $P=0.58$; Leahey et al. 2019). Left-sided IE in PWID is associated with worse prognosis (Thalme et al. 2007, Ortiz-Bautista et al. 2015).

In the treatment of IDUIE concomitant treatment of addiction is in a key role and is associated with reduced mortality (Rosenthal et al. 2016, Rodger et al. 2018). Multidisciplinary endocarditis teams might include also psychiatry and social services in the treatment of this complex entity (Yanagawa et al. 2018).

2.2.6 PROSTHETIC VALVE IE

In a review including only population-based observational studies, a significant increase in IE in patients with prosthetic valve was observed (Tleyjeh et al. 2007). Risk of development of prosthetic valve IE (PVE) is highest during the initial three months after surgery and remains high during the 12 months after operation (Karchmer and Longworth 2002). The risk is estimated to be 1-5% during the first post-operative year and 1% annually after that (McDonald 2009). Valve operation for IE during active infection increases the risk of developing early PVE (Piper et al. 2001). PVE is considered early if it occurs within one year after the operation and early PVE is also defined to be HAIE (Piper et al. 2001). Risk of early IE seems to be higher and risk of late IE lower for mechanical valves compared to bioprosthetic valves (Stanbridge et al. 1997, Piper et al. 2001). Causative agents of late PVE resemble those causing native valve IE, but during the first post-operative year staphylococci predominate (Stanbridge et al. 1997, Piper et al. 2001). In a recent large multicenter study 30.1% of all IE patients had PVE and 9.9% intracardiac device-related IE (Habib et al. 2019). Proportions of PVE of all IE cases in selected studies on IE are presented in Table 4.

2.2.7 CAUSATIVE MICROBES OF IE

The microbiology of IE depends on the mode of acquisition of IE and whether IE occurs in native or prosthetic valve.

The emergence of *Staphylococcus aureus* as the leading causative organism of IE at the expense of viridans group streptococci has been reported in many studies and reviews (Fowler et al. 2005, Murdoch et al. 2009, Selton-Suty et al. 2012, Slipczuk et al. 2013, Vogkou et al. 2016). However, the predominance of *S. aureus* is not supported in all studies (Cuervo et al. 2018). In population-based studies from Olmsted County, Minnesota viridans group remained as the leading cause in a study between years 1970-2000 (Tleyjeh et al. 2005), but was outnumbered by *S. aureus* in a study between 2007 and 2013 (DeSimone et al. 2015). Similarly, *S. aureus* outnumbered viridans group streptococci during the study period 1980-2004 in a Finnish study and the cause of this shift was increased proportion of IDUIE (Heiro et al. 2006).

A recent large prospective multi-center study observed a high frequency of enterococcal IE (16%; Habib et al. 2019). The percentage of enterococcal IE was, however, counted using blood culture-positive cases as denominator instead of the whole study group and blood culture-negative cases were surprisingly many (21%). A trend of increasing enterococcal IE was observed in a large review of IE studies (Slipczuk et al. 2013). Enterococcal IE is increasingly recognised as emerging and challenging clinical entity of IE (Pericás et al. 2020).

Three most common groups responsible for IE in most studies are *Staphylococcus aureus,* viridans group streptococci and enterococci (Table 4).

Coagulase negative staphylococci (CoNS) constitute approximately 10% of all IE cases (Murdoch et al. 2009). The role of CoNS as causative agents is greater in PVE, as 16% of PVE cases were due to CoNS in large multicenter study after excluding PWID (Chu et al. 2009).

The HACEK group constitutes less than 5% of all IEs (Murdoch et al. 2009). The HACEK group refers to certain gram-negative bacteria and they were classically categorized to blood culture-negative IEs because they were difficult to cultivate but this is no longer the case (see also subsection 2.1.2).

Rare of all IEs, but common causes of blood culture-negative IEs, are *Bartonella* spp., *Coxiella burnetii* and *Tropheryma whipplei*. A rigorous diagnostic approach revealed an etiology in 63% of blood culture-negative IEs in a French referral center (Fournier et al. 2010). Out of 476 cases with identified causative agent, 229 were *C. burnetii*, 85 Bartonella spp. and 12 *T. whipplei*.

Table 4. *Proportions of Staphylococcus aureus, viridans group streptococci (VGS) and enterococci as an etiology of IE of all IE cases in studies on IE. Proportion of PVE of all IE cases is also shown.*

Reference	Country / Region	Inclusion criteria and number of cases included (number)	Proportion (%) of all IE cases by etiology			Proportion of PVE of all IE cases (%)
			S. aureus	VGS	entero-cocci	
Heiro et al. 2006	Turku, Finland	Definite and possible IE (326)[a]	33	20	6[b]	16
Murdoch et al. 2008	Multi-center and -national	Definite IE (2781)	31	17	10	21
Sy and Kritharides 2010[c]	Australia	ICD-10 code (1536)	32	23[d]	9	13
Selton-Suty et al. 2012[c]	France	Definite IE (497)	26.6	18.7[e]	10.5	20.9
Jordal et al. 2018	Western Norway	All episodes (439)	31.4	22.5	13.0	31.0
Cuomo et al. 2018	Spain	Left-sided IE, PWID excluded (595)[f]	18.5	24.4	15.6	36.3

Abbreviations: IE, Infective endocarditis; PVE, Prosthetic valve endocarditis; VGS, Viridans group streptococci
[a] Proportions presented are from the last five-year period in the study (2000-2004) including 96 episodes
[b] *Enterococcus faecalis* only
[c] Population-based study
[d] All streptococci, VGS not specified in study
[e] Oral streptococci
[f] Proportions presented are from the last four-year period in the study (2012-2015) including 135 episodes

2.2.8 TREATMENT AND OUTCOME

Treatment of IE is multidisciplinary. Treatment consists of prolonged intravenous antimicrobial therapy, management of complications and surgery if necessary (Cahill and Prendergast 2016).

Antimicrobial treatment

Antimicrobial treatment of IE is intravenous and preferably bactericidal (Habib et al. 2015). Recent study, however, showed promising results with oral antibiotics after initial intravenous course in selected patients (Iversen et al. 2019). The duration and the need for possible combination therapy depend on the causative pathogen and its susceptibility (and minimum inhibitory concentration rate) and whether the valve is native or prosthetic.

In Helsinki University Hospital (HUS) pathogen specific treatment follows ESC guidelines (Habib et al. 2015, Martelius et al. 2016; Table 5). In PVE caused by staphylococci, combination therapy including cloxacillin or vancomycin (depending on the susceptibility of staphylococci), gentamycin and rifampicin is recommended, whereas non-staphylococcal PVE treatment is recommended as for NVE, but with minimum duration of six weeks (Habib et al. 2015, Martelius et al. 2016). Possible allergies and kidney function should be taken into account when considering the treatment.

Recommendations for empirical treatment differ. HUS guideline recommends, in case of NVE and late onset PVE, 2nd generation cephalosporin with addition of vancomycin in septic cases (Martelius et al. 2016), whereas ESC recommends ampicillin plus cloxacillin plus gentamycin in community-acquired NVE and late onset PVE and vancomycin plus gentamycin plus rifampicin in health care-associated NVE and early onset PVE (Habib et al. 2015).

Table 5. *Pathogen-specific treatment of the common causative agents of native valve IE according to HUS antimicrobial guideline (Martelius et al. 2016).*

Pathogen	Recommended first line regimen	Duration (weeks)
Staphylococcus aureus (MSSA)	Cloxacillin	4-6
Staphylococcus aureus (MRSA)[1]	Vancomycin	4-6
Viridans group streptococci		
• MIC ≤ 0.125	G-penicillin **or**	4
	Ceftriaxone	4
• MIC 0.25-2	G-penicillin **or** ceftriaxone	4
	+ gentamycin	2
Enterococci	Ampicillin + gentamycin **or**	4-6
	Ampicillin + ceftriaxone **or**	6
	Vancomycin[2] + gentamycin	6
Abbreviations: MSSA, methicillin susceptible *S. aureus*; MRSA, methicillin resistant *S. aureus*; MIC, minimum inhibitory concentration		
[1] also methicillin-resistant coagulase negative staphylococci [2] In case of allergy or if strain ampicillin-resistant		

Surgery

Approximately one-fourth to one-half of the patients with IE needs surgery during the acute phase of infection (Prendergast and Tornos 2010). In the treatment of IE surgery is needed in cases of heart failure due to valvular dysfunction (or hemodynamic problems), uncontrollable infection (e.g., abscess or fistula formation or persistent bacteremia) or prevention of systemic emboli (Habib et al. 2015).

In a large international, observational study, high mortality was observed in IE patients with indication for surgery, but who were treated conservatively (Habib et al. 2019). PWID have about 10 times higher hazard of death or reoperation compared to non-PWID between 3 and 6 months after operation for IE (Shrestha et al. 2015) and optimal use of surgery in PWID with IE is still somewhat unclear (Wang et al. 2018). A recent large meta-analysis found no difference in postoperative 30-day or in-hospital mortality between patients with IDUIE and non-IDUIE and who were surgically treated for IE (Hall et al. 2020).

Outcome

A large multicenter study including 2781 patients with IE (study period 2000-20005) reported an in-hospital mortality rate of 18% (Murdoch et al. 2009). Compared to a more recent similar large multicenter study of 3116 patients (study period 2016-2018) mortality was not substantially decreased, for the reported in-hospital mortality rate was 17% (Habib et al. 2019). In addition, in-hospital mortality rates of 20% and 23% were reported in population-based studies from Spain and France, respectively (Selton-Suty et al. 2012, Olmos et al. 2017) and a rate of 17% in a register study from Germany with case-fatality rate remaining constant during the study period 2005-2014 (Keller et al. 2017). In a Finnish register study a 30-day mortality after IE admission was 11.3% (Ahtela et al. 2019). Reported long-term mortality rates after IE diagnosis are higher: 22% within 6 months (Hill et al. 2006), 24% within 3 months (Toyoda et al. 2017) and 37% within one year (Toyoda et al. 2017). The observations of stable mortality rate are in keeping with other studies and clearly demonstrate a paradox: despite medical progress, mortality of IE remains high and is not decreasing. Some have even reported an increase in mortality (Cresti et al. 2017).

In-hospital mortality of IE has been shown to be associated with *S. aureus* infection (Murdoch et al. 2009). A large prospective multinational study found age, hemodialysis, hospital-acquired infection, prosthetic valve, *S. aureus* infection and IE complications, such as heart failure, persistent bacteremia and stroke to be strongly associated with 6-month mortality (Park et al. 2016). Viridans streptococcal etiology was associated with lower mortality and also, surgery during index hospitalization, but it was performed less frequently in low risk patients (Park et al. 2016). Risk of long-term major adverse events including ischemic stroke, hemorrhagic stroke, myocardial infarction, readmission for heart failure, sudden death or ventricular arrhythmia and all-cause death are increased in patients who survive an episode of IE (Shih et al. 2014). In a Swedish nationwide study long-term relative mortality risk after IE remained elevated up to five years and excluding the first year, the long-term mortality risk was 2.2 times higher compared to general population (Ternhag et al. 2013).

Repeat IE

Patients are at risk of repeat IE after the first episode of IE (Chu et al. 2005). Haemodialysis, IDU and a history of previous IE were found to be independent risk factors for repeat IE in a large multinational cohort of IE patients (Alagna et al. 2014). Indeed, PWID are at high risk of repeat IE after the first episode, as was demonstrated in a study in which 25% of PWID who survived the first IE episode developed a repeat episode after the initial IE episode with most repeat IE episodes being re-infections (86.4%) rather than relapses and occurring within one year after the initial IE episode (77.3%; Huang et al. 2018). Furthermore, all of the patients with repeat IE acknowledged a relapse of intravenous drug use (Huang et al. 2018). In another study including 212 first-

episode IE cases in PWID, 32.1% developed a repeat episode, which was significantly more frequent compared to non-PWID (32.1% vs 6.2%, P<0.001; Rodger et al. 2019). A study from Boston, USA including 102 patients with IDU-IE observed high rates of readmissions and recurrences and suboptimal addiction interventions (Rosenthal et al. 2016).

2.2.9 CHANGING CLINICAL PICTURE OF IE

Is the clinical picture of IE changing? Based on the studies reviewed above, it is safe to say: yes. But, more precise answer is more complex. Results of IE studies depend on several factors. Studies from single referral centers tend to include more severe and complex cases of IE, thus influencing the characterization of IE and probably overestimating for example surgery rates, proportion of PVE and thus might show poorer outcomes. As in a population-based study from Rochester, USA only 20% of patients from Olmsted County needed operation for IE during the study period 2007-2013 whereas 44% of non-Olmsted County residents treated at Mayo Clinic, Rochester were operated (DeSimone et al. 2015). On the other hand, population-based studies depend on the population being studied and results might not be generalizable outside the study population, as demonstrated by studies from Spain and Kentucky, USA with staggering differences in prevalence of IDUIE and even in an estimate whether incidence is increasing or not (Seratnahaei et al. 2014, Olmos et al. 2017). In addition, studies differ in selection criteria and diagnostic criteria used. For example, studies including only left-sided IE leave major proportion of PWID out. Also, definitions may vary (e.g., definition of HAIE). Major part of data on IE is derived from studies conducted in referral centers leaving population-based studies in minority (Slipczuk et al. 2013).

From the vast literature on the epidemiology of IE, few conclusions can be drawn. First, the incidence of IE is not decreasing. Second, in a population excluding PWID incidence in the elderly is increasing. Thirdly, proportion and importance of HAIE is increasing. Fourthly, *Staphylococcus aureus* as a causative agent dominates. Fifthly, repeated studies on IE are warranted to keep up with the old foe – and studies should preferably be of population-based nature.

3 AIMS OF THE STUDY

Objectives of this study were:

1. To determine the impact of short incubation MALDI-TOF MS on the antimicrobial treatment in selected BSIs when introduced into a routine clinical setting. (Study I)

2. To evaluate the blood culture positivity in patients with cSSSI: the rate of blood culture positivity; factors associated with clinicians ordering blood cultures; factors associated with blood culture positivity and the benefit of information obtained from positive blood cultures. (Study II)

3. To evaluate the impact of pre-operative antimicrobial treatment duration on the yield of valve cultures and valve PCR from resected endocardial material in patients operated for IE and to assess the diagnostic utility of the PCR method in this setting. (Study III)

4. To describe the epidemiology, spectrum of causative agents and the outcome of infective endocarditis in a population-based study in the Capital Region of Finland 2013-2017 and also to compare clinical entities of IE according to the mode of acquisition and determine their proportions of all IEs. (Study IV)

4 MATERIALS AND METHODS

4.1 STUDY I: IMPACT OF SI-MALDI-TOF ON ANTIBIOTIC TREATMENT OF BLOODSTREAM INFECTIONS

4.1.1 STUDY DESIGN AND PATIENT POPULATION

All adult patients with blood cultures positive with *Pseudomonas aeruginosa, Enterococcus* spp. and AmpC-producing *Enterobacteriaceae* (*Enterobacter* spp., *Serratia* spp., *Morganella morganiae*) who were treated in Helsinki University Hospital, Helsinki, between March 2014 and December 2015 were included in the study. Only the first BSI episode was included. Exclusion criteria were: lack of verified clinical data, patient not receiving appropriate antibiotic treatment at all (e.g., due to poor prognosis), bacteremia episode during appropriate antibiotic treatment or blood cultures ruled as a contamination.

HUS is a tertiary hospital serving population about one and a half million in the Hospital District of Helsinki and Uusimaa. HUS is the national center for solid organ transplantations and is also responsible for the majority of stem cell transplantations nationally. The prevalence of multi-drug resistant *Pseudomonas aeruginosa* (65 cases year 2015) and vancomycin resistant enterococci (under 20 cases annually) are low from clinical samples.

Study design was an observational retrospective cohort study. Patients with a BSI caused by the abovementioned bacteria were identified, and information on si-MALDI-TOF results was retrieved, from the clinical microbiology laboratory database at Helsinki University Hospital Laboratory (HUSLAB). Demographic and clinical data were collected from the electronic medical records.

4.1.2 STUDY GROUPS AND IDENTIFICATION OF BACTERIA IN HUSLAB

Blood cultures were incubated in blood culture bottles in Bact/Alert® (BioMérieux, France) instrument for a total of 5-6 days or until reported as positive.

Study groups and the si-MALDI-TOF method

The si-MALDI-TOF identification was done from all blood culture bottles, which flagged positive between 8 p.m. and 11 a.m. If this analysis yielded a result, the bacteremic episode was assigned to si-MALDI-TOF group. If blood culture bottle flagged positive between 11 a.m. and 8 p.m., the si-MALDI-TOF was not done and hence the bacteremic episode was assigned to control group.

For the si-MALDI-TOF analysis, 50-100 microliters of blood culture content was applied on chocolate agar plates. These plates were incubated for 3-4 hours at +35 C in CO_2 atmosphere. After incubation, a sample for the MALDI-TOF analysis was obtained by streaking the application area on the chocolate agar. One spot was inoculated for each sample. The analysis (Vitek MS; bioMérioux) was performed even if no growth was visible. The si-MALDI-TOF results were reported between 11 a.m. and 2 p.m. the same day.

Simultaneously with the si-MALDI-TOF analysis another sample from blood culture bottle was Gram stained and subcultured on agar plates for susceptibility testing and confirmatory identification testing. In addition, a sample from a positive blood culture bottle was used for conventional bacterial identification; in-house identification set containing 10 biochemical tests along with chromogenic plates. Also, conventional MALDI-TOF was performed in cases giving unreliable identification in conventional biochemical methods, usually the next day.

Identification by si-MALDI-TOF resulted in initial identification roughly 22-24 hours earlier compared to identification done by conventional MALDI-TOF and biochemical identification methods.

4.1.3 STUDY DEFINITIONS

Polymicrobial bacteremia was defined as the isolation of more than one bacterial species during the same bacteremia episode, except in case of a contamination. Index bacteria refer to one of the bacteria that are included in the study. If a patient had a polymicrobial bacteremia caused by two of the index bacteria, these were analysed as separate episodes.

Appropriate antibiotic therapy was defined as follows: an antibiotic regimen to which the index bacteria was susceptible *in vitro*, considered standard treatment according to HUS antimicrobial treatment guide (Martelius et al. 2016) and, with the exception of fluoroquinolones, delivered intravenously. Appropriateness of antibiotic therapy in polymicrobial infections was assessed from the perspective of the index bacteria. Time to appropriate antibiotic therapy was calculated from the first positive blood culture draw to the delivery of effective antimicrobial regimen.

4.2 STUDY II: FACTORS ASSOCIATED WITH BLOOD CULTURE POSITIVITY IN PATIENTS WITH COMPLICATED SKIN AND SKIN STRUCTURE INFECTION

4.2.1 STUDY DESIGN AND PATIENT POPULATION

All adult patients residing in Helsinki, Finland and Gothenburg, Sweden and who were hospitalized for cSSSI during the study period 2008-2011 were included in the study (Jääskeläinen et al. 2016). The study hospitals were HUS and Helsinki City Hospital in Finland and Sahlgrenska University Hospital in Gothenburg, Sweden. Patients were identified using ICD-10 codes suitable for SSSI and those who met the inclusion criteria were included in the study. In short, inclusion criteria were: (1) adult patient with hospitalization, (2) SSSI affected deeper soft tissue (e.g., cellulitis or fasciitis) or needed surgery, (3) SSSI in lower extremity in a patient with diabetes or peripheral vascular disease and (4) patient with a major abscess or an infected ulcer. Patients needed also to have local signs of cSSSI present plus one systemic sign of infection.

The study was an observational retrospective population-based cohort study. Data on demographics, clinical variables, laboratory and microbiological results were collected from medical records.

4.2.2 STUDY DEFINITIONS

Cellulitis/fasciitis was defined as SSSI without an abscess, diabetic foot or an infected ulcer. Streamlining was defined as a change of antibiotic therapy to pathogen specific one. Clinical stability was defined as improvement of systemic and local signs of infection. Treatment failure was defined as need for unplanned surgery due to infection, no improvement in clinical situation after five days of treatment or judgement of treatment failure by the treating physicians. Coagulase-negative staphylococci and other normal skin flora commensals were generally considered as contaminants and infectious disease specialist assessed each case.

4.3 STUDY III: IMPACT OF PRE-OPERATIVE ANTIBIOTIC TREATMENT ON MICROBIAL FINDINGS FROM RESECTED ENDOCARDIAL MATERIAL

4.3.1 PATIENTS AND STUDY DESIGN

Inclusion criteria of the study were: (1) valve surgery due to infective endocarditis between 2011-2016 in HUS, (2) age ≥ 18 years, (3) a sample for PCR and valve cultures were obtained during surgery and (4) IE was post-operatively classified as definite or possible according to modified Duke criteria (Li et al. 2000). The study was a retrospective single-centre study. Patients were recognized using ICD-10 codes for infective endocarditis (i.e., I33, I38 and I39) from operating room´s database. Data on demographics, clinical features, laboratory results and microbiology were collected from electronic patient records.

In all, 115 patients were identified. Twenty-two were excluded because a sample for PCR was not obtained, two were excluded due to missing data and two were excluded because IE was classified as "rejected" according to modified Duke criteria. In addition, yeast was a causative agent in two patients and they were excluded because the broad-range PCR does not recognise yeast and thus these were out of the scope of the study.

HUS is responsible for cardiac surgery in the Hospital District of Helsinki and Uusimaa and two smaller hospital districts in Southern Finland. Also, patients from the entire country are referred to HUS for cardiac surgery in special circumstances (e.g., congenital heart diseases).

Since 2010, surgeons have been advised to routinely include a sample for PCR analysis along with a sample for bacterial culture from resected endocardial material, vegetation or pus. Surgeons themselves decided the best location for sample collection during operation. Hence, this study evaluates the value of PCR analysis implemented in routine clinical setting.

4.3.2 MICROBIOLOGICAL PROCEDURES

Blood cultures were processed as described in section 4.1.2. Samples obtained during surgery were sent straight to microbiology laboratory (i.e., HUSLAB) for analysis. If necessary, samples were kept in storage for maximum of two days in +4 °C before analysis. The clinical samples were cultured by routine diagnostics at HUSLAB. Samples were cultivated on chocolate agar and fastidious anaerobe agar and incubated at 37 °C until growth was detected or for 7 days in 5% CO_2 or under anaerobic conditions. Additionally, samples were inoculated into thioglycollate broth and cultivated ad 37 °C.

For 16S rRNA analysis (i.e., PCR analysis), the clinical samples were homogenized using a Precellys bead-beater (Bertin Instruments). During the

study period two different DNA extraction instruments were used: SelectNAplus (Molzym) or Easymag (Biomérieux). Each method has been validated and no difference on positivity rate has been observed. Detailed description is provided in the original article, supplement Table 1. The ribosomal 16S DNA was amplified using primers Forward CLSI and Reverse Bosshard (Edwards et al. 2012). Also, used polymerases, PCR instruments and thermal cycling conditions are listed in supplement Table 1 of the original article. PCR-amplified fragments were separated using gel electrophoresis and visualized under ultraviolet light. Amplified fragments were then purified and sequencing was done with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) using forward Bosshard primer (Edwards et al. 2012) and an ABI Prism 3100 genetic analyser (Thermo Fisher Scientific). After these steps, analysis was performed using Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, Bethesda, MD, USA). Each sample was tested with an inhibition control (amplification of lambda DNA or *Oryza sativa* gene) and with each run a nontemplate control was tested.

4.3.3 STUDY DEFINITIONS

Antibiotic treatment was defined effective if causative agent of IE was susceptible to it *in vitro* or it was according to the ESC guideline (Habib et al. 2015). It also had to be delivered intravenously. Duration of effective antibiotic treatment was counted from the delivery of first dose of intravenous antibiotic to the day of operation.

The PCR result was considered to have a diagnostic impact if it solely provided the etiology of IE, if it confirmed positive serology results or if it resolved a discrepancy between blood and valve culture results.

In order to study the impact of the duration of the pre-operative antibiotic treatment on the yield of the PCR method two approaches were used. First, the median duration of antibiotic treatment of those PCR positive was compared to those PCR negative. Second, study population was stratified according to the length of antibiotic treatment to five categories (Table 6) to demonstrate and to allow comparison between proportions of PCR/valve culture positive patients to PCR/valve culture negative patients. In the final analysis, we chose to compare a group of patients who received pre-operative antibiotic treatment less than 2 weeks (n=46) to those who received it 2 weeks or longer (n=34). In order to control for possible confounding, independent variables associated with PCR-positivity in univariate analysis ($P<0.2$) or with clinical significance were entered into bivariate logistic regression analysis.

Table 6. *The study cohort (n=87) stratified to five categories depending on the pre-operative antibiotic treatment duration.*

Duration of pre-operative effective IV antibiotic therapy	Whole study cohort	PCR positive patients	Valve culture positive patients
One day or less	11	10 (91%)	4 (36%)
2-13 days	35	32 (91%)	15 (43%)
14-28 days	17	10 (59%)	0
More than 28 days[1]	17	8 (47%)	0
7 days or less, but previous longer course[2]	7	4 (57%)	0

[1] One patient in this group had a pre-operative IV antibiotic treatment for more than 28 days, but the exact duration could not be verified from medical records and thus this one case is left out of the time analysis comparing median durations

2 These patients received for a less than a week IV antibiotics before surgery, but had had a longer continuous course of antibiotic treatment before this (targeting other foci in most cases). Thus, this group is left out of all time analysis, because true length of effective antibiotic treatment cannot be reliably determined in relation to other cases

4.4 STUDY IV: THREE DIFFERENT CLINICAL ENTITIES OF INFECTIVE ENDOCARDITIS

4.4.1 STUDY DESIGN

The design was an observational retrospective study. All adult patients residing in the Helsinki University Hospital Area and diagnosed with IE during 2013-2017 were included in the study cohort. The study hospitals included HUS and two Helsinki City Hospitals. The catchment areas of these hospitals form the Helsinki University Hospital Area and have the only emergency departments in the area. These hospitals are responsible for the treatment of severe infections, such as IE. Thus, virtually all patients with IE are being treated in the study hospitals. This enables the population-based approach.

Patients were recognised using ICD-10 codes for IE, i.e., I33, I38 and 139. An infectious disease specialist (M.H.) collected all data from the electronic patient records. Patients meeting the criteria for possible or definite IE according to the modified Duke criteria (Li et al. 2000) were included. Those meeting the modified Duke rejected criteria and those residing outside the study area were excluded.

4.4.2 PATIENT POPULATION

The member municipalities of Helsinki University Hospital Area are Espoo, Helsinki, Kauniainen, Kerava, Kirkkonummi and Vantaa. Adult patients who resided in these municipalities during the study period were included. A total of 0.993 million adult patients (approximately one-fifth of Finnish adult population) resided in this area 2017. The population is mainly urban. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was low during the study period in study region: 5% of all *S. aureus* bacteremias in year 2017 (Haiko et al. 2018). In year 2012, in the study area there were an estimated 5,600-10,300 problem drug users (i.e., abusers of amphetamine and/or opioids), which accounts for one-third of all problem drug users in Finland (Varjonen 2015).

4.4.3 STUDY DEFINITIONS

IE episodes were categorized according to the mode of acquisition into mutually exclusive groups: community-acquired IE (CAIE), health care-associated IE (HAIE) and intravenous drug use-related IE (IDUIE).

HAIE was defined as follows: 1) onset of IE more than 48 hours after admission or within six months after discharge from hospital stay of ≥ 2 days, 2) IE developed within six months after a significant procedure performed during hospitalization or ambulatory setting, (3) IE developed within one month after extensive out-of-hospital contact with health care (e.g., dialysis, wound care, intravenous treatment) or (4) residence in a nursing home or similar facility (Friedman et al. 2002, Ben-Ami et al. 2004, Fernandez-Hidalgo et al. 2008). PVE was defined as "early onset" if it occurred within 12 months after surgery and was defined as HAIE.

Patients with a history of intravenous drug use within one month before diagnosis of IE were classified as IDUIE even if health care association was present.

Patients not meeting the definition of HAIE or IDUIE were classified as CAIE. Dental procedure was not considered a criterion for HAIE (Fernandez-Hidalgo et al. 2008).

IE was defined as definite or possible according to the modified Duke criteria (Li et al. 2000). An episode of IE occurring after the initial IE episode was considered a relapse if it occurred within six months after the end of the antimicrobial therapy of the initial IE episode and the causative agent was similar (Chu et al. 2005), in any other case it was considered a new episode (i.e., re-infection) and was included in the study as a separate episode.

Valve abnormalities (e.g., degenerative valve lesions), prosthetic valve and cardiac devices, congenital heart disease and non-mild hypertrophic cardiomyopathy were considered as predisposing heart condition to IE. Left-sided IE was defined as IE affecting only aortic and/or mitral valves, and right-

sided IE only tricuspid and/or pulmonary valves. Location of IE was determined by echocardiogram, other imaging studies, surgery or clinically. Septic emboli or deep focus was defined as a focus of infection (other than cardiac) verified by imaging studies or clinically and related to IE. Operative treatment for IE was considered in a case where a patient had valve operation with IE indication during ongoing antimicrobial treatment. Time to operation was calculated from the first day on effective antimicrobial treatment. Effective antimicrobial treatment was defined as a regimen to which the causative agent of IE was susceptible to *in vitro* or was in accordance to recommendation of ESC (Habib et al. 2015) and delivered intravenously.

4.5 STATISTICAL METHODS (STUDIES I-IV)

Descriptive statistics were applied in summarizing the variables. Categorical variables were summarized using counts and percentages. Continuous variables were summarized using means with standard deviation or medians with min and max values and interquartile range (IQR) or range, if sample was small.

For comparison of two groups, categorical variables were compared using either chi-square test or Fisher´s exact test, when appropriate, and continuous variables were compared using Mann Whitney U –test. Odds ratio (OR) was calculated with 95% confidence interval (CI).

P-value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS versions 22.0 – 25.0 (IBM Corp., Armonk, N.Y., USA).

Study I. Kaplan-Meier method and Log-Rank test were used to analyze survival type data.

Study II. Bivariate logistic regression analysis (enter method) was used to determine the independent factors associated with blood culture sampling and blood culture positivity. First, univariate analysis was performed. Variables with P-value less than 0.15, those with clinical significance and those not multicollinear were then entered into the model.

Study III. To control for possible confounding, bivariate logistic regression analysis (enter method) was used in analysis of the length of pre-operative antibiotic treatment on PCR positivity. After univariate analysis, variables with P-value less than 0.2 and those with clinical significance were included in the model.

Study IV. Percentages were counted using total number of IE episodes as the denominator, unless otherwise specified. Poisson regression was used to calculate the 95% CIs for incidence rates and to examine the trends in incidence

rates. Comparisons of CAIE to HAIE and CAIE to IDUE were done by multinomial logistic regression.

4.6 COMPLIANCE WITH ETHICAL STANDARDS

The research board at the Inflammation Center, HUS approved the study protocols for studies I-IV. Additionally, for study II, the ethical committee of Sahlgrenska University Hospital, Sweden also approved the study protocol, along with local conventional manner. For study IV, additional approve from Helsinki City was obtained. Given the retrospective nature of all studies, no ethical committee approval from HUS was needed.

Throughout this thesis work, the research was conducted in strict compliance with Guideline of the responsible conduct of research (Varantola et al. 2012).

5 RESULTS

5.1 STUDY I: IMPACT OF SI-MALDI-TOF ON ANTIBIOTIC TREATMENT OF BLOODSTREAM INFECTIONS

5.1.1 PATIENTS, STUDY GROUPS, FOCUS/SOURCE OF INFECTION AND OUTCOME OF BSIS

A total of 164 episodes of bacteremia were identified. After excluding 40 episodes, 124 bacteremia episodes originating from 120 patients were included. One patient had two separate bacteremias of different index bacteria during the study period. Three patients had a polymicrobial bacteremia caused by two of the index bacteria. Fifteen polymicrobial bacteremia episodes including one of the index bacteria were included.

A total of 124 bacteremia episodes were divided into two groups: i) si-MALDI-TOF group (n=69) in which si-MALDI-TOF was used for bacterial identification with a positive result and ii) control group (n=54) in which identification was done by means of conventional methods.

There were no significant differences between the study groups in baseline characteristics, focus of infection or outcomes (Table 7).

The acquisition site of blood cultures were as follows: emergency room 33.9% (n=42), ICU 12.9% (n=16), internal medicine ward (incl., neurology and pulmonology) 4.8% (n=6), hematology ward 13.7% (n=17), surgical ward 25.8% (n=32), gynecology ward 3.2% (n=4) and oncology ward 5.6% (n=7) of cases.

5.1.2 MICROBIOLOGY AND ANTIBIOTIC TREATMENT

Pseudomonas aeruginosa caused 16.9% (n=21), *Enterococcus* spp. 50% (n=62) and AmpC-producing *Enterobacteriaceae* 33.1% (n=41) of included bacteremia episodes. Detailed description of the causative bacteria is presented in Table 8.

Of monobacteremias (n=103), si-MALDI-TOF was used in 65 and yielded a result in 62. It did not recognise the bacteria in one *E. faecalis* and in two *E. cloacae* bacteremias. Of polymicrobial bacteremias, si-MALDI-TOF was used in 14 and yielded a result in 7 (for the index bacteria).

Early empiric antibiotic therapy

Within 12 hours after the positive blood culture draw in 75.8% (n=94) of bacteremia episodes antibiotic treatment was either started or changed, in 19.4% (n=24) no change was made to the ongoing antibiotic treatment and in

4.8% (n=6) patient did not receive any antibiotic treatment. This early empiric antibiotic treatment was appropriate only in 22.6% (n=28) cases.

Table 7. *Patient characteristics, infection focus or source and clinical outcome of the study population (n=124) and according to study groups.*

	Whole study group (n=124)	Si-MALDI-TOF Group (n=69)	Control group (n=56)	P-value
Patient characteristics				
Age, years (mean; SD)	61.3; 15.2	62.7; 15.6	59.6; 14.7	0.257
Gender, male; n (%)	74 (59.7)	42 (60.9)	32 (58.2)	0.762
Chronic heart disease	14 (11.3)	9 (13.0)	5 (9.1)	0.490
Chronic kidney disease	22 (17.7)	11 (15.9)	11 (20.0)	0.557
Dialysis	4 (3.2)	2 (2.9)	2 (3.6)	>0.99
Liver cirrhosis	5 (4.0)	4 (5.8)	1 (1.8)	0.381
Chronic lung disease	18 (14.5)	11 (15.9)	7 (12.7)	0.614
Diabetes	26 (21.0)	14 (20.3)	12 (21.8)	0.835
Blood malignancy[1]	14 (11.3)	10 (14.5)	4 (7.3)	0.207
Acute leukemia	15 (12.1)	9 (13.0)	6 (10.9)	0.717
Stem cell transplant	5 (4.0)	2 (2.9)	3 (5.5)	0.654
Solid organ malignancy	32 (25.8)	16 (23.2)	16 (29.1)	0.456
Solid organ transplant	18 (14.5)	10 (14.5)	8 (14.5)	0.993
Immunosuppressive treatment[2]	70 (56.5)	44 (63.8)	26 (47.3)	0.066
Neutropenia[3]	21 (16.9)	14 (20.3)	7 (12.7)	0.265
Infection focus or source				
Pulmonary	5 (4.0)	2 (2.9)	3 (5.5)	0.654
Urinary tract	21 (16.9)	13 (18.8)	8 (14.5)	0.526
Skin / musculoskeletal	7 (5.6)	5 (7.2)	2 (3.6)	0.461
Abdominal cavity	55 (44.4)	28 (40.6)	27 (49.1)	0.343
Central nervous system	1 (0.8)	1 (1.4)	0	>0.99
Heart	2 (1.6)	1 (1.4)	1 (1.8)	>0.99
Indwelling catheter	5 (4.0)	1 (1.4)	4 (7.3)	0.170
Unknown	28 (22.6)	18 (26.1)	10 (18.2)	0.296
Clinical outcome[4]				
7-day mortality	3 (2.4)			
30-day mortality	16 (12.9)			

[1] other than acute leukemia
[2] anti-rejection medication, cancer chemotherapy, prednisolone more than 10 mg/day and anti-rheumatic medications including biological medications
[3] absolute neutrophil cell count less than 0.5 x 10^5 neutrophils/ml
[4] no statistically significant difference between the study groups

In a subgroup of BSIs caused by *Enterococcus* spp. (n=62) early empiric antibiotic therapy was inappropriate in 83.9% (n=52) cases. An effective coverage was reached by widening the regimen with vancomycin or linezolid in two-thirds of the cases and alternatively with a beta-lactam effective against enterococci in one-third of the cases.

Table 8. *Blood culture isolates (number) in the study group (n=124) according to the index bacteria*

Index bacteria	All episodes	MALDI-TOF group	Control group	P-value
Pseudomonas aeruginosa	21	14	7	0.265
Enterococcus spp.[1]	62	33	29	0.588
AmpC-producing *Enterobacteriaceae*[2]	41	22	19	0.754
[1] *E. faecalis* n=22; *E. faecium* n=37; *E. casseliflavum* n=3; with equal distribution in the 2 study groups				
[2] *Enterobacter cloacae* n=21; *E. aerogenes* n=2; *Serratia liquefaciens* n=1; *S. marcescens* n=13; *Morganella morganii* n=4; with equal distribution in the 2 study groups				

5.1.3 IMPACT OF SI-MALDI-TOF ANALYSIS ON ANTIBIOTIC TREATMENT

In 72.6% (n=90) of all bacteremia episodes patients received appropriate antibiotic treatment within 48 hours from the first positive blood culture draw. This percentage was 78.3% (n=54/69) in si-MALDI-TOF group and 65.5% (n=36/55) in control group (P=0.112). In a Kaplan-Meier analysis similar trend was observed (log rank 0.139; Study I, Figure 1).

Median time from blood culture draw to receipt of appropriate antibiotic treatment was 33.5 hours (IQR, 19.3-49.5) in whole study group and in si-MALDI-TOF group median time was 31.9 hours (IQR, 14.9-46.8) and in control group 39.7 hours (IQR, 21.2-61.4); P=0.230.

Pseudomonas aeruginosa **group (n=21)**

In si-MALDI-TOF group 78.6% (n=11/14) received appropriate antibiotic treatment within 48 hours from the blood culture draw compared to 71.4% (n=5/7) in control group; P>0.99.

***Enterococcus* spp. group (n=62)**
In Si-MALDI-TOF group 87.9% (n=29/33) received appropriate antibiotic treatment within 48 hours from the blood culture draw compared to 65.5% (n=19/29) in control group; P=0.036.

Amp-C-producing *Enterobacteriaceae* group *(n=41)*
In si-MALDI-TOF group 63.6% (n=14/22) received appropriate antibiotic treatment within 48 hours from the blood culture draw compared to 63.2% (n=12/19) in control group; P=0.975.

Subgroup of patients with immunosuppressive medication (n=70)
In si-MALDI-TOF group 86.4% (n=38/44) received appropriate antibiotic treatment within 48 hours from the blood culture draw compared to 57.7% (n=15/26) in control group (P=0.007; Study I, Figure 2).

5.2 STUY II: FACTORS ASSOCIATED WITH BLOOD CULTURE POSITIVITY IN PATIENTS WITH COMPLICATED SKIN AND SKIN STRUCTURE INFECTION

5.2.1 STUDY COHORT, BLOOD CULTURE POSITIVITY AND RESULTS

In all, 460 patients with cSSSI were included in the study. Demographics, co-morbidities and clinical features are summarized in Table 1, Study II.

Blood cultures were obtained from 258 patients (51.6%) and they were positive in 61 patients (23.6%). The proportion of blood culture positivity was nearly equal in the two centres: 23% in Helsinki and 25% in Gothenburg.

Streptococcus pyogenes (n=19, 31%) and *Staphylococcus aureus* (n=19, 31%) were the two most common findings followed by non-A beta-hemolytic streptococci (n=12, 20%). *Streptococcus pneumoniae* was found in one patient. Notably, in only one case gram-negative bacteria was isolated. Five cases (8%) were polymicrobial and four (7%) were unknown.

5.2.2 FACTORS ASSOCIATED WITH BLOOD CULTURE SAMPLING

To determine the factors associated with blood culture draw we compared patients from whom blood cultures were drawn (n=258) to those from whom they were not drawn (n=202; Table 1, Study II). We used both univariate and multivariate analysis, which differs from the analysis done in the primary publication (Jääskeläinen et al. 2016). Results are shown in full in Table 1, Study II.

In multivariate analysis, diabetes (OR 1.9, CI 1.2-2.9), duration of symptoms shorter than 2 days (3.0, 1.8-5.2) and CRP > 150 mg/L at the time of diagnosis of cSSSI (1.8, 1.2-2.8) were associated with more frequent blood culture sampling. Whereas peripheral vascular disease (0.5, 0.3-0.8) and post-surgical wound infection (0.4, 0.2-0.8) were associated with less frequent blood culture sampling. In addition, median CRP measured at the time of the diagnosis was significantly higher in patients with blood culturing compared to those without blood cultures (181 mg/L vs 130 mg/L, P=0.003).

5.2.3 FACTORS ASSOCIATED WITH BLOOD CULTURE POSITIVITY

In order to determine the factors associated with blood culture positivity we compared patients with positive blood cultures (n=61) to those from whom blood culture sample was taken but it was negative (n=197). This approach differs from the original publication of this material where blood culture positive patients were compared to the rest of the study group (Jääskeläinen et al. 2016).

In multivariate analysis only alcohol abuse was significantly associated with blood culture positivity (OR 5.5, CI 2.3-13.2). Full results of univariate and multivariate analysis are shown in Table 2, Study II. In addition, neither the CRP measured at the time of the diagnosis of cSSSI nor the highest CRP measured during the hospitalization was associated with bacteremia (Table 2, Study II). Blood culture positivity rates among the patients from whom blood culture was obtained categorized according to CRP count are illustrated in Figure 2. No statistical difference between the groups in percentages of bacteremic patients was observed (P=0.455, Chi-square).

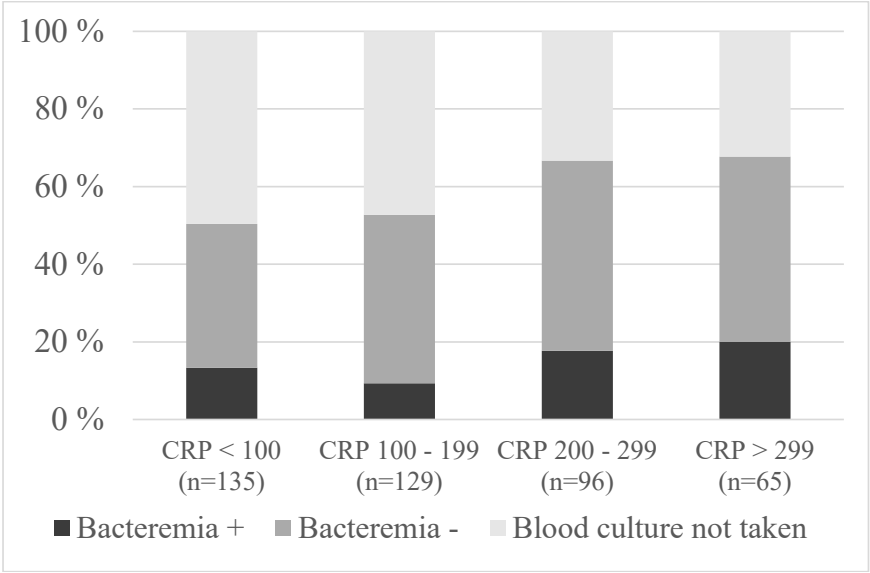

Figure 2 Blood culture positivity and negativity by categorized CRP levels.

Clinical endpoints in blood culture positivity

Patients with blood cultures positive had antibiotic treatment streamlined more often compared to blood culture negative patients (23.3% vs 6.3%, OR 4.5 [CI 2.0-10.5], P<0.001). In addition, patients with bacteremia reached clinical stability less likely in three days compared to patients without bacteremia (30% vs 52%, OR 0.4 [CI 0.2-0.8], P=0.006). Bacteremia was also associated with increased ICU admissions and longer hospital stay. However, no differences were observed in rate of surgical interventions after the diagnosis of cSSSI, length of antibiotic therapy or 30-day mortality. See Table 3, Study II for specific details.

5.3 STUDY III: IMPACT OF PRE-OPERATIVE ANTIBIOTIC TREATMENT ON MICROBIAL FINDINGS FROM ENDOCARDIAL SPECIMENS

5.3.1 PATIENT DEMOGRAPHICS, CLINICAL FEATURES AND OUTCOME

In all, 87 patients were included in the study cohort. Eighty-five were classified as having definite IE and two patients had possible IE according to the modified Duke criteria (Li et al. 2000). Mean age of the patients was 52.7 years and 71

(81.6%) of them were male. Known predisposing heart disease was observed in 24 (27.6%) patients and seven (8%) had a history of previous IE. Co-morbidities were found as follows: chronic kidney disease in four (of these three were on hemodialysis and one on peritoneal dialysis), liver cirrhosis in four and diabetes (requiring medication) in 17 (19.5%). Eleven (12.6%) patients had a history of alcohol abuse and 14 (16.1%) had intravenous substance abuse.

Most of the operated patients had NVE (n=76, 87.4%). A cardiac device was involved in two cases. A total of 105 valves were operated, 50 of these were aortic, 47 mitral, seven tricuspid and one pulmonary valve.

Septic emboli or other deep focus complicated the course in approximately half of the cases (n=48, 55.2%). Relapse of IE occurred in four cases within 12 months of the operation. 30-day all-cause mortality was observed in 8% (n=7) and 365-day all-cause mortality in 17.2% (n=15) of cases.

5.3.2 MICROBIOLOGY OF BLOOD CULTURES, VALVE CULTURES AND PCR

A total of 89 samples for PCR were obtained from 87 patients. From two patients two samples were obtained, and one in the rest of the cases. In case of 80 patients a sample for PCR was obtained from endocardial material or vegetation, and in seven cases from pus (e.g., from paravalvular abscess). A total of 101 valve cultures were obtained, two samples from 14 patients and one sample from 73 patients. All PCR and valve culture results are hereby presented as per patient.

The full results of the blood and valve cultures and PCR are presented in Table 4, Study III.

Blood cultures

Blood cultures were positive in 74 (85%) of cases. *Staphylococcus aureus* (n=25) was the most common finding followed by viridans group streptococci (n=20) and *Enterococcus faecalis (n=9)* and coagulase-negative staphylococci (n=8). Of the coagulase-negative staphylococci four were *Staphylococcus epidermidis*, three *Staphylococcus lugdunensis* and one was not named to species level. In 13 (15%) of cases blood cultures were negative, and etiology was determined in 10 of these cases by PCR from resected valve.

Valve cultures

Valve cultures were positive in 19 (22%) cases (Table 4, Study III). In two cases the valve and blood culture results differed. In one case, *Streptococcus mitis* was cultured from valve sample and also found by PCR, but blood cultures were negative. In the other case, *Staphylococcus epidermidis* were found in valve cultures, but *Streptococcus salivarius* was found in blood cultures and in valve PCR. In this case the valve culture result was regarded as a contaminant. In addition to this, in only one other case culture result was considered as a

contaminant. In this other case *Cutibacterium acnes* grew on culture (taken from pus from a mediastinal site) and blood cultures grew *Enterococcus faecalis*. Infectious disease specialists evaluated both these cases.

16S rRNA PCR and sequencing

In total of 64 (74%) of cases PCR was positive (Table 4, Study III). Of these cases 54 were also blood culture positive with matching results and in 10 cases blood cultures were negative. In one case valve culture was positive but PCR remained negative. No false-positive results were found. In one case a mixed sequence was suspected (two viridans group streptococci). In the two cases where two samples for PCR were taken they showed similar results (both positive in both cases).

5.3.3 DIAGNOSTIC IMPACT OF THE PCR RESULT

PCR was considered to contribute markedly to the etiological diagnosis in 12 patients (Table 5, Study III). In five cases PCR solely provided the etiology: *Bartonella quintana* in two cases and *Coxiella burnetii, Streptococcus gordonii* and *Streptococcus pneumoniae* each in one. In the cases of the latter two, blood cultures were negative due to prior antimicrobial treatment.

5.3.4 IMPACT OF THE DURATION OF THE PRE-OPERATIVE ANTIBIOTIC TREATMENT ON THE YIELD OF THE VALVE CULTURE AND PCR

All patients with positive valve cultures (n=19) received pre-operative antibiotic treatment less than two weeks.

The median duration of pre-operative effective antibiotic therapy in PCR-positive patients (n=60) was significantly shorter compared to PCR-negative patients (n=19 [8.5 days vs 24 days, P=0.001]). In addition, the proportion of PCR-positive patients in those who received less than two weeks of antibiotic treatment pre-operatively was significantly higher compared to the proportion of PCR-positive patients in those who received it for two weeks or longer (91% vs 53%, P<0.001), see also Table 6. Moreover, in multivariate logistic regression analysis only duration of pre-operative antibiotic treatment less than 2 weeks remained significantly associated with PCR-positivity (OR 7.2, 95% CI 2.0-26.0, P=0.003). Results of the analysis are presented in Supplement Table 2, Study III.

5.3.5 IE CAUSED BY BARTONELLA QUINTANA, COXIELLA BURNETII AND TROPHERYMA WHIPPLEI (CASES FROM STUDIES III AND IV)

A total of nine patients had IE caused by *Bartonella quintana*. Median age was 55 years (range 24-86) and eight patients were male. Aortic valve alone was affected in five, both aortic and mitral valves in one and mitral valve alone in three cases. Two patients had septic emboli and three patients had IE-associated glomerulonephritis. Five patients had a history of alcohol abuse and/or homelessness, but four did not. Six patients were surgically treated and all had valve PCR positive. One conservatively treated patient had PCR positive from pseudoaneurysm of superior mesenteric artery. Serology was tested for six patients and was positive in every case. One patient died during hospitalization, others survived. Interestingly, two patients had PVE (bioprosthesis) and these two cases were also blood culture positive (in extended culturing). In both cases microbiology laboratory was informed beforehand of the suspicion of *Bartonella quintana* IE.

Two patients had IE caused by *Tropheryma Whipplei*. Patients were: 65 year-old man and 48 year-old woman, and both were otherwise healthy but had a long history of arthralgia before diagnosis. Diagnosis was established by valve PCR in both cases.

Two patients had IE caused by *Coxiella burnetii*. Both were male, aged 66 years and 71 years. Sixty-six year old man had a predisposing heart condition and a travel history to Canary Islands, Spain. IE diagnosis was established by valve PCR and serology. The other patient had a bioprosthesis and travel history to endemic area. In this case diagnosis was based on serology.

5.4 STUDY IV: THREE DIFFERENT CLINICAL ENTITIES OF INFECTIVE ENDOCARDITIS

5.4.1 PATIENTS

In all, 313 episodes of IE occurring in 291 patients were included in the study cohort. Median age was 55 years (range, 19-98; interquartile range [IQR], 36-71). A male-to-female ratio was approximately 2:1, as 68.7% (n=215) of IE episodes occurred in men and 31.3% (n=98) in women. Of IE episodes, 78.9% (n=247) were classified as definite and 21.1% (n=66) as possible according to the modified Duke criteria (Li et al. 2000).

Of the whole study cohort, 40.9% (n=128) had known pre-existing cardiac risk factor, 14.4% (n=45) had diabetes, 2.9% (n=9) liver cirrhosis, 10.9% (n=34) chronic kidney disease, 7.0% (n=22) were on dialysis and 5.4% (n=17) had immunosuppressive treatment. A history of previous IE was documented in 15.0% (n=47) of patients.

Mode of acquisition

Community-acquired IE (CAIE) accounted for 38.0% (n=119) of IE episodes, health care-associated IE (HAIE) 31.0% (n=97) and intravenous drug use-related IE (IDUIE) 31.0% (n=97).

Median age in CAIE group was 64 years (IQR, 52-75), in HAIE group 69 years (IQR, 58-77) and in IDUIE group 35 years (IQR, 29-38). Patients in IDUIE group were significantly younger compared to CAIE group (P<0.001) and to HAIE group (P<0.001) and no statistical difference in age between CAIE and HAIE groups was observed (P=0.365). Figure 3 shows the number of IE episodes by age and mode of acquisition.

The median age in patients without a history of IDU was 66 years (IQR 53-75.8) and women were older than men (median 72 years vs median 65 years, respectively, P=0.001).

Comparison of CAIE group to HAIE and IDUIE in patient demographics and co-morbidities is shown in Table 3, Study IV. Of note, patients with HAIE had more underlying comorbidities compared to CAIE. Eventhough in IDUIE group male predominance was observed (59.8%), the proportion was significantly smaller compared to CAIE (73.1%, P=0.039).

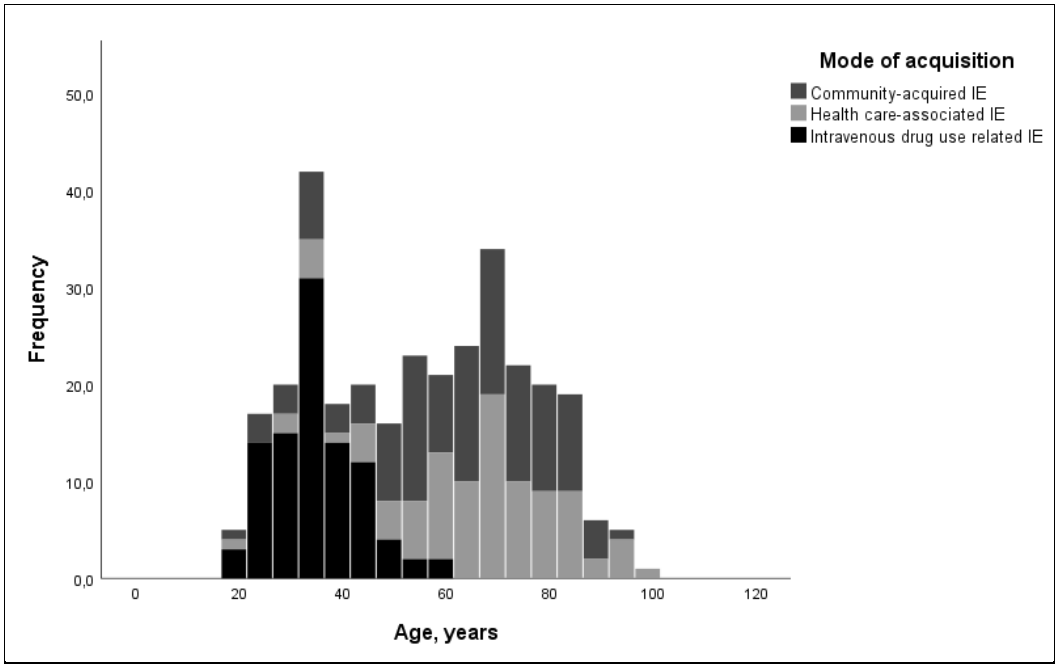

Figure 3 Number of IE episodes by age and mode of acquisition.

Incidence

The incidence was found to be 6.48/100,000 person-years (95% CI, 5.80-7.24) in adults. No significant change in incidence between the study years was observed. Annual incidence rates according to the mode of acquisition were 2.46/100,000 (95% CI, 2.06-2.95) for CAIE, 2.01/100,000 (95% CI, 1.65-2.45) for HAIE and 2.01/100,000 (95% CI, 1.65-2.45) for IDUIE. No significant change in these incidence rates during the study period by mode of acquisition was observed either: CAIE P=0.903, HAIE P=0.324, IDUIE P=0.650. In Figure 4 are presented the numbers of IE episodes by year of the diagnosis and mode of acquisition.

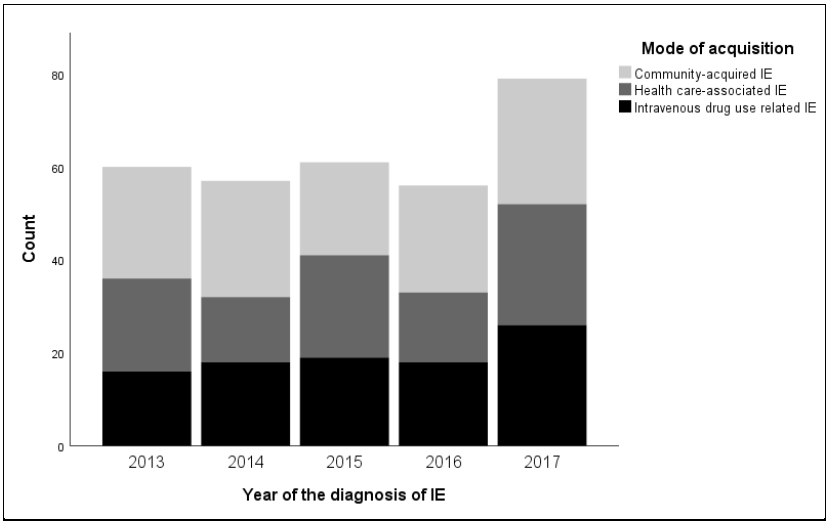

Figure 4 Number of IE episodes by year of the diagnosis of IE and mode of acquisition.

5.4.2 LOCATION OF IE

Transthoracic echocardiography was performed in every episode and transesophageal echocardiography in 57.5% (n=180) of cases. Native valve was affected in 83.7% (n=262) of cases. Majority of PVEs occurred in biovalves (n=39) following mechanical valves (n=7), mitral plasty (n=2) and transcatheter aortic valve implant (TAVI; n=3). Composite graft was involved in 1.3% (n=4) and pacemaker in 1.6% (n=5) of IE episodes. One-fifth of PVEs were early PVEs (n=11/51).

Aortic valve alone was affected in 40.3% (n=126) of cases, mitral valve alone in 31.6% (n=99), both aortic and mitral valves in 3.5% (n=11) and tricuspid valve

alone in 20.1% (n=63). Pulmonary valve was affected in four cases, of which in two cases alone and in one with mitral valve and in one with tricuspid valve. IE was left-sided in 75.4% (n=236) of cases, right-sided in 21.1% (n=66) and both-sided in 3.5% (n=11). Comparison of location of IE according to mode of acquisition is presented in Table 3, Study IV. Of note, patients with IDUIE had right-sided and both-sided infection more commonly than patients in CAIE group. Prosthetic valve was affected in one-third of HAIE cases, and over three times more frequently than in CAIE.

5.4.3 ETIOLOGY

The etiology of IE was determined in 91.4% (n=286) of episodes. Blood cultures were positive in 87.5% (n=274) of cases. Of the 39 blood culture-negative cases etiology was resolved in 30.8% (n=12) by following methods: PCR from resected valve (n=4), PCR and culture from resected valve (n=1), PCR from resected valve and serological testing (n=2), culture from other site (i.e., deep focus or septic embolus; n=3), PCR from thrombotic mass in artery and serology (n=1) and serology only (n=1). Etiology remained unknown in 8.6% (n=27) of cases.

Staphylococcus aureus was the leading causative agent. It caused 36.1% (n=113) of IE cases. Second was viridans group streptococci 21.4% (n=67) and third *Enterococcus* spp. 9.9% (n=31). Detailed microbiology results are presented in Table 2, Study IV. Comparison of microbial etiology of IE according to the mode of acquisition is presented in Table 3, Study IV. *Staphylococcus aureus* was equally common in CAIE and HAIE. Viridans group streptococci were more frequently and *Enterococcus spp*. less frequently responsible for IE in CAIE compared to HAIE. In fact, two-thirds of all enterococcal IEs belonged to HAIE group. *S. aureus* caused 74.2% of IDUIE cases.

In left-sided IE (n=236) *S. aureus* constituted 22% (n=52), viridans group streptococci 27.5% (n=65) and *Enterococcus* spp. 12.3% (n=29) of cases.

In patients with left-sided IE, no difference between PVE and NVE were observed in cases caused by *S. aureus* (17.4% vs 23.2%, respectively, P=0.397) or viridans group streptococci (21.7% vs 29.1%, P=0.317), but *Enterococcus* spp. were the etiological agent more often in PVE (21.7%) compared to NVE (10.0%, P=0.030).

5.4.4 COMPLICATION OF IE

Septic complication was observed in 51.8% (n=162) of IE episodes. Cerebral complication (e.g., radiologically verified infarct or haemorrhage) occurred in 14.4% (n=45) episodes and septic emboli other than cerebral complicated the course of IE in 46.3% (n=145) of cases (Table 1, Study IV). Besides cerebral complications, septic emboli were detected in lungs in 24.9% (n=78), in visceral

organs in 12.8% (n=40), in musculoskeletal sites in 17.6% (n=55) and in other sites in 8.0% (n=25) of IE cases.

In 9.3% (n=29) of IE episodes patients were admitted to intensive care unit (ICU) during the hospitalization (post-operative admissions not included). Of the patients who were not admitted to ICU (n=284), nearly one-third (n=94) needed treatment in intermediate care unit (IMCU) or cardiac care unit (CCU). Mechanical or non-invasive ventilation due to heart failure was needed in 14.7% (n=46).

There were no difference in cerebral complication between CAIE and HAIE or CAIE and IDUIE, but patients with IDUIE had significantly more septic emboli (or deep foci) compared to CAIE group (Table 4, Study IV).

5.4.5 TREATMENT AND OUTCOME

Median duration of effective intravenous antibiotic treatment was 35.5 days (IQR, 29-44.8) for the whole study group (data missing in 1 case). In patients with NVE median duration was 33 days (IQR, 29-43) and for PVE 43 days (IQR, 29-49).

In cases where empirical antimicrobial treatment was started after blood culture sampling (n=238), in 79 % (n=188) of cases it was considered effective against the causative organism and in 21% (n=50) ineffective. In 27 episodes the etiology remained unresolved and thus the effectiveness of empirical antibiotic therapy could not be evaluated. In 45 episodes intravenous antimicrobial treatment was initiated after preliminary blood culture results and in two episodes patients did not receive effective intravenous antibiotic at all.

In 23% (n=72) of IE episodes valve surgery was performed during ongoing antimicrobial treatment. Median time to surgery from the start of effective intravenous antimicrobial treatment was 14 days (IQR, 7.3-34.8). In NVE operation was performed in 23.3% (n=61/262) cases and median time to surgery was 11 days (IQR, 7-32.5) and in PVE operation was performed in 21.6% (n=11/51) and median time to surgery was 39 days (IQR, 19-70). No statistical difference was observed in surgery rates between NVE and PVE (P=0.790), but the median time to operation was significantly longer for patients with PVE (P=0.005). Patients with CAIE had surgery more often than IDUIE group (Table 4, Study IV).

In-hospital all-cause mortality rate was 17.3% (n=54) and one-year all-cause mortality rate was 25.9% (n=81). Of those who survived the IE episode (n=259) 3.5% (n=9) had a relapse. Comparison between mortality rates according to the mode of acquisition is presented in Table 4, Study IV. Of note, HAIE was associated with increased both in-hospital and one-year mortality compared to CAIE.

For patients with PVE in-hospital mortality was 25.5% (n=13) and with NVE 15.6% (n=41; P=0.089). One-year mortality for PVE was 43.1% (n=22) and for NVE 22.5% (n=59; P=0.002).

6 DISCUSSION

6.1 STUDY I: IMPACT OF SI-MALDI-TOF ON ANTIBIOTIC TREATMENT OF BLOODSTREAM INFECTIONS

Considering that empiric antibiotic treatment is seldom modified until more microbial or clinical evidence is obtained, which may take up to 48 hours, microbiological methods that accelerate the identification of bacteria in positive blood cultures are of utmost importance. This is especially emphasized in BSIs caused by bacteria with such natural resistance profile that the commonly used empirical antibiotic treatments may be ineffective, e.g., the bacteria included in our study. Indeed, in the present study we observed that antibiotic treatment was appropriate only in 22.6% of bacteremia episodes within 12 hours after the blood culture draw.

In this study si-MALDI-TOF was shown to increase the proportion of bacteremia episodes with appropriate antibiotic treatment by 12.8% within 48 hours from the blood culture draw (78.3% vs 65.5%, P=0.112). This is in line with a crossover study including 253 episodes of bacteremia comparing traditional identification methods to direct MALDI-TOF in routine clinical setting which showed an 11.3% increase in proportion of patients on appropriate antibiotic treatment within 24 hours after blood culture positivity (64% vs 75%, P=0.01; Vlek et al. 2012).

Identification by direct MALDI-TOF led to a treatment modification in 13% of BSIs in a prospective study including 157 BSIs (Martiny et al. 2013). However, this study lacked a control group. A study assessing the benefits of short incubation MALDI-TOF in clinical routine found information acquired from it leading to a change in empiric antibiotic therapy in one-fifth of patients with bacteremia (179 antibiotic orders analysed) and three quarters were evaluated rational by the authors (Köck et al. 2017). Contradicting our findings, Jeon and colleagues observed that use of MALDI-TOF alone did not significantly reduce the time to effective therapy and pointed out the benefit of concomitant ID assessment (Jeon et al. 2018). However, they did not use direct or short incubation technique, but a subculture of 18-24 hours, which method differs from ours.

The impact of si-MALDI-TOF was shown especially in BSIs caused by *Enterococcus* spp. in which a significantly higher percentage of cases received appropriate antibiotic therapy within 48 h after blood culture draw in si-MALDI-TOF group (87.9%) compared to control group (65.5%, P=0.036).

In a study including 202 gram-negative bacteremias, impact of enhanced MALDI-TOF reporting on empiric antibiotic therapy was maximal in case of AmpC-producing *Enterobacteriaceae* as MALDI-TOF had an impact on empiric antibiotic therapy in 35% of bacteremia episodes in whole group, but in case of AmpC-producing *Enterobacteriaceae* (n=27) in 60% (Clerc et al. 2013). In our

study we could not confirm this finding, eventhough the number of AmpC-producing *Enterobacteriaceae* in our study was higher. Possible explanation is that in a study by Clerc and colleagues only patients with ID consultation were included whereas in our study it was not mandatory. In a recent study conducted in a setting with active ASP and low resistance rates, direct MALDI-TOF had limited impact on antibiotic treatment in whole study group (n=240), but in a subgroup of patients (n=100) with BSI caused by AmpC-producing, non-fermenting organisms, *S. aureus, Streptococcus* and *Enterococcus* spp. active therapy was administered more frequently within 48 hours in MALDI-TOF group (Osthoff et al. 2017).

An interesting and important finding in our study was the impact of si-MALDI-TOF in a subgroup of patients with immunosuppressive medication (n= 70). In si-MALDI-TOF group in 86.4% (n=38/44) of bacteremic episodes patients received appropriate antibiotic treatment within 48 hours after blood culture draw whereas the corresponding percentage was 57.7% (15/26) in control group (P=0.007). The difference remains significant also when looking at only those episodes with inappropriate initial empiric antibiotic therapy (n=54, 81.8% vs 46.7%, P=0.008). Also, the proportions of different bacteria in study groups were the same. Possible explanation might be that treating physicians observe this group of patients more closely and hence the full potential of rapid identification is exploited.

Our study adds to the weight of evidence on the benefits of si-MALDI-TOF identification on antibiotic treatment of BSI. However, as a recent review noted, further studies are needed, and it emphasized that earlier information should also lead to an appropriate clinical response (Dixon et al. 2015). This remark is underlined by a study showing that MALDI-TOF method was more powerful when combined with ASP intervention than alone (Berganovic et al. 2017).

The strength of this study is that it provides real-life data on the effect of si-MALDI-TOF implementation in routine microbial diagnostics in a low resistance setting and its effect can be independently evaluated. We chose to include only BSIs caused by certain bacteria (i.e., the index bacteria) which are often health care-associated and due to their natural resistance profile are often non-susceptible to first line empiric antibiotic treatment. This approach enabled the high number of the index bacteria in our study compared to others, but may overestimate the impact of si-MALDI-TOF. Another strength is diverse patient population.

This study has limitations. It is a single center study and it is retrospective. Bacteremia episodes were not randomized into separate groups, so inevitable selection bias may exist. Appropriate de-escalation of antibiotic therapy was not evaluated. However, the natural resistance profile of the index bacteria itself necessitates use of broader-spectrum antibiotics. This study was done in a low resistance environment so these results might not be generalizable to a high resistance setting. Finally, the study was not large enough to evaluate the si-MALDI-TOF method´s impact on clinical outcomes, such as mortality or length of stay. However, time to appropriate antibiotic therapy is probably better

indicator to assess the benefits of molecular rapid detection, as true mortality benefits may be difficult to detect (Timbrook et al. 2017).

6.2 STUDY II: FACTORS ASSOCIATED WITH BLOOD CULTURE POSITIVITY IN PATIENTS WITH CSSSI

Blood cultures were positive in 23.6% of patients from whom blood was sampled. This percentage is higher than in most studies. A similar observational multi-center study from Central and Southern Europe including also patients with cSSSI observed a blood culture positivity of 12% of those who had blood cultures performed with similar blood culturing rate of 53% as in our study (Garau et al. 2013). A study including 334 hospitalized patients with skin and soft-tissue infection found bacteremia in 16% of patients from whom blood was cultured (52%; van Daalen et al. 2017).

Most common blood culture isolates were *Streptococcus pyogenes* and *Staphylococcus aureus* with equal shares (n=19, 31%). Non-A beta-hemolytic streptococci were found in 20% (n=12) and gram-negative bacteria only in one case. These findings differ from the study by van Daalen and colleagues who found more gram-negative bacteria than *Staphylococcus aureus* and Peralta and colleagues who found a gram-negative etiology in one-fourth of bacteremias (Peralta et al. 2006, van Daalen et. al 2017). Differences might be explained by differences in patient selection. In addition, in a study by Peralta et al. blood culture sampling rate was only 14% indicating probably that blood culturing were performed in more severe cases (positivity rate was 19%; Peralta et al. 2006). Similar predominance of beta-hemolytic streptococci and *S. aureus* has been observed in observational study including cSSSI (Jenkins et al. 2010). In abovementioned large study including patients with cSSSI 40% of all blood culture isolates were *S. aureus*, but only 22% were beta-hemolytic streptococci (Garau et al. 2013), whereas in our study corresponding percentages were 31% and 51%.

Factors found to be associated with increased blood culturing were diabetes, symptom duration shorter than two days and CRP measure over 150 mg/L at the time of the diagnosis. CRP over 150 mg/L was found to be associated with increased blood sampling rate in multivariate analysis and CRP measured at time of the diagnosis was significantly higher in patients from whom blood was cultured (median 181 mg/L) compared to those not subjected to blood culture sampling (median 130 mg, P=0.003). Similarly, in a study including 476 patients with uncomplicated cellulitis higher CRP was linked to more blood culturing (77 mg/L vs 119 mg/L, P<0.001; Bauer et al. 2016) and in another study including patients with erysipelas mean CRP was 91 mg/L for those who had blood cultured compared to 57 mg/L for those who did not (Bläckberg et al. 2015). Eventhough higher CRP seemed to encourage clinicians to order blood cultures, it was not associated with blood culture positivity in multivariate analysis in our study. In a study by Bauer and colleagues CRP was significantly

higher in bacteremic patients (187 mg/L) than in non-bacteremic patients (116 mg/L, P=0.02) in univariate analysis, but this result did not persist in multivariable analysis (Bauer et al. 2016). In addition, another study also found diabetes to be associated with increased blood culturing in patients with erysipelas (Bläckberg et. al 2015). Of note, Bauer and colleagues and Peralta and colleagues used multivariate analysis in their study, but Bläckberg and colleagues did not.

In multivariate analysis only alcohol abuse was found to be significantly associated with blood culture positivity, as 54% of patients with a history of alcohol abuse had positive blood cultures compared to 20% of those who did not. Similarly, alcohol abuse was the only discriminant patient characteristic linked to bacteremia in patients with uncomplicated cellulitis (Bauer et al. 2016).

Interestingly, prior antibiotic treatment was not negatively associated with blood culture positivity in our study, which is in contrast to one previous study (Peralta et al. 2006). However, prior antibiotic treatment discouraged clinicians from ordering blood cultures as patients with antibiotic treatment before the diagnosis of cSSSI had blood culture taken in 45% of cases compared to 55% who did not (P=0.004). These factors did not remain significant in multivariate analysis models. In any case, knowledge of prior or ongoing antibiotic treatment alone should not discourage clinicians from ordering blood cultures.

Patients with cellulitis/fasciitis (i.e., no abscess or ulcer present) were more likely to have blood cultured compared to other patients (66% vs 23%). This difference was statistically significant in univariate analysis (P<0.001) and a strong trend was seen in multivariate analysis (OR 1.6, P=0.052). Accordingly, the opposite clinical conditions (i.e., by definitions used to define these conditions), such as an abscess, infected ulcer or post-surgical wound made sampling less likely (in univariate analysis). Clinicians, perhaps, evaluated microbial sampling from these foci to be sufficient in determining the etiology of infection. Also, patients with cellulitis/fasciitis were likely to be bacteremic and patients with an abscess less likely in univariate analysis, but in multivariate analysis significance of this difference was not confirmed.

Patient´s severe co-morbidity (van Daalen et al. 2016) and presence of two or more co-morbid factors (Peralta et al. 2006) have been linked to bacteremia, but we could not confirm these findings.

Patients with positive blood cultures had antibiotic treatment significantly more often streamlined compared to blood culture negative patients (23.3% vs 6.3%, P<0.001). This is a clear benefit of blood culturing and challenges the finding from patients with uncomplicated cellulitis where blood culture positivity rarely affected antibiotic treatment (Paolo et al. 2013). In accordance to our results, in a study including 308 patients with lower limb cellulitis, a change in the antibiotic therapy was noted in 49% of patients after blood culture results (Peralta et al. 2006).

In our study, bacteremia was linked to later clinical stability, which without the information from blood cultures might lead to a premature change of

antibiotic treatment to more broad-spectrum. Furthermore, one-third of blood culture isolates were *S. aureus*, which is a clinically important finding for it warrants prolonged antibiotic treatment, and search for deep foci such as endocarditis (Thwaites et al. 2011). In conclusion, several benefits of information gathered from positive blood cultures could be demonstrated in this study. The importance of blood culture-directed therapy in countries with higher antibiotic resistance has also been pointed out (Eron and Lipsky 2006).

The strengths of this study derive from its population-based nature. Although, some patients may have been left unrecognized due to coding inexactness. Limitation of this study is its retrospective nature and missing data in some variables. In addition, 56% of patients were subjected to blood culturing causing selection bias. Previous analysis of this patient material showed an association between blood culture draw and higher mortality, and this probably indicates that clinicians ordered blood culturing from sicker patients (Jääskeläinen et al. 2016). Despite difference in blood culture drawing rates between the two centers, the positivity rate was similar, suggesting that in patients with cSSSI blood culturing should be done with low threshold.

6.3 STUDY III: IMPACT OF PRE-OPERATIVE ANTIBIOTIC TREATMENT ON MICROBIAL FINDINGS FROM RESECTED ENDOCARDIAL MATERIAL

In our study all valve cultures were negative in patients who received more than two weeks of effective antibiotic treatment before surgery. Similarly, mean duration of effective pre-operative antibiotic treatment of 4 days (range 2-8 days) for those who had valve cultures positive was observed (Kotilainen et al. 2006). In another study, sensitivity of valve PCR and valve culture were similar in detecting the causative organisms if pre-operative antibiotic treatment was less than 5 days, but after that sensitivity of valve culture dropped markedly but PCR did not (Voldstedlund et al. 2008). In accordance, in a study of streptococcal IE, all but one valve culture were negative after two weeks of antibiotic therapy (Upton et al. 2005). One study did not find negative effect of pre-operative antibiotic treatment duration on valve culture yield (Peeters et al. 2017). A quite high number of false-positive valve cultures have been reported in some studies raising a concern of their utility (Liesman et al. 2017). Alleviating this concern, in the present study only two valve cultures were ruled as contaminants.

In this study we demonstrated a negative impact of the duration of the pre-operative antibiotic treatment on the positivity of valve PCR. Patients with pre-operative duration of antibiotic therapy shorter than two weeks had PCR positive in 91% of cases and after two weeks the positivity dropped markedly to 53% ($P<0.001$). In addition, a significant difference in the median durations was observed between PCR positive and negative cases (8.5 days vs 24 days, $P=0.001$). Other studies have been unable to demonstrate a similar effect

(Rovery et al. 2005, Kotilainen et al. 2006, Voldstedlund et al. 2008, Peeters et al. 2017). The mean duration of preoperative antibiotic treatment was 19.6 days (range 1-58) for patients who had PCR-positivity compared to 22.6 days (range 0-60 days) who had valve PCR-negative (P=0.65) in study from South-western Finland (Kotilainen et al. 2006). Voldstedlund et al. observed no significant difference in the median duration of pre-operative antibiotic treatment in PCR-positive and PCR-negative cases (Voldstedlund et al. 2008). In a study of 126 patients operated during active IE the median duration of 19.5 days of antimicrobial treatment (range 1-150) for patients with negative PCR and 14 days (range 1-79) for patients with positive PCR (P=0.28) was observed (Rovery et al. 2005). Also, the proportion of PCR positivity in patients with antibiotic treatment for 15 days or less was 66% and for those over 15 days (55%, P=0.19).

It is noteworthy that after four weeks of effective therapy nearly half of the patients still had PCR test positive. In one patient PCR was positive after six months of initial IE episode caused by *Granulicatella adiacens*. At the time of the surgery the patient was still receiving oral antibiotic due to concomitant spondylodiscitis. After operation the antibiotic was stopped and no relapse occurred. Similarly, one patient from the patient cohort of study IV had PCR positivity (*E. faecalis*) 18 months after treatment of IE without signs of active IE and no relapse occurred eventhough antibiotic treatment was discontinued after surgery. These findings point out that bacterial DNA may persist for long period in heart valves and cannot be used as a guide for therapy. This was further demonstrated in the abovementioned study in which patients with active treatment for endocarditis had valve PCR positivity in 60% (76 out of 126) of cases whereas patients who had completed the antibiotic course for IE at time of surgery had PCR positivity in 37% (11/30) of cases, P=0.02 (Rovery et al. 2005). Median time for PCR positivity in latter group (i.e., 11/30) was 167 (range 45 – 2,920 days). Also, they found that PCR was positive significantly more often if histological signs of IE were present and also if Gram staining was positive.

In conclusion, the meaning of persistence of bacterial DNA for considerable long time after successful treatment is uncertain. Interestingly, this applies also to Gram staining, which may remain positive also for longer time and is found positive also in patients with treated IE and sterile valve cultures and negative histopathology (Morris et al. 2003).

The diagnostic value of the PCR have been shown in several studies, especially in cases of blood culture-negative IE (Vondracek et al. 2011, Miller et al. 2016). Our results add to weight of this evidence as we showed the diagnostic impact of PCR in 14% of cases. This is in agreement with a study from France in which PCR contributed to the microbiological etiology in one-fifth of IE patients (Gauduchon et al. 2003). A study from Canada of 68 IE patients showed even higher contribution of PCR method (in 31% of cases) and it made a contribution to the clinical decision in 13% of cases (Miller et al. 2016).

In the studies III and IV we describe nine cases of IE caused by *Bartonella quintana*, two cases of IE caused by *Coxiella burnetii* and two cases of IE caused by *Tropheryma whipplei*. First case of IE caused by *Bartonella quintana* in

Finland was reported from South-western Finland (Jalava et al. 1995). This case was included in their study from 2006 including a total 56 cases of operatively treated IE (Kotilainen et al. 2006). However, no other *B. quintana* IE cases were found. In studies from Sweden and Denmark including patients with IE undergoing surgery and molecular methods applied, no IE cases caused by *Bartonella* spp. were found (Voldstedlund et al. 2008, Vondracek et al. 2011). Also, in a prospective study of 334 IE cases from western Sweden, serological findings for *B. quintana* or *C. burnetii* were rare (Werner et al. 2003). In a multinational IE study, however, most cases of these two infections came from Europe (Murdoch et al. 2009). These observations highlight the geographical variation of causative agents of IE and the importance of population-based studies from different parts of the world.

In the present study, one-third of *Bartonella* IE cases had IE-associated glomerulonephritis, a finding which is suggested also in previous literature (Khalighi et al. 2014). Two cases were also blood culture positive. In both cases laboratory had prior information of the suspicion, which probably enabled the finding. This underlines the importance of collaboration between clinicians and microbiologists.

Seeing that IE is relatively rare condition and surgery is required in one-third to one-half of the cases, the 87 patients included in our study make it one of the largest of its kind. However, 22 patients were excluded because operating surgeon did not obtain a sample for PCR, so selection bias might exist. On the other hand, 12 of these occurred during the first year of study and then 1-3 cases annually, so the most likely explanation is that surgeons had yet not adopted a new protocol, rather than other systematic bias. Samples for PCR were not standardized. Operating surgeons obtained the sample from the sites most clearly infected in the visual and manual inspection of the valve.

6.4 STUDY IV: THREE DIFFERENT CLINICAL ENTITIES OF INFECTIVE ENDOCARDITIS

In the patient cohort, CAIE, HAIE and IDUIE were observed in almost equal proportions, each accounting for approximately one-third of the cases.

During the study period 2013-2017 the incidence was 6.48/100,000 person-years. No significant change in incidence between the study years was observed. The observed incidence is in line with a recent population-based registry study from entire country of Finland, which reported annual occurrence rate of IE admissions to be 6.33 per 100,000 person-years (Ahtela et al. 2019). Incidence rates in person-years according to the mode of acquisition per 100,000 were: 2.46 for CAIE, 2.01 for HAIE and 2.01 for IDUIE. No significant change in these incidence rates by mode of acquisition during the study period was observed either. Rates for these different entities have rarely been reported. A population-based study from Australia reported an annual incidence of HAIE to be 1.5/100,000 (Sy and Kritharaides 2010). However, the definition of HAIE used

in the Australian study is narrower than commonly used, thus probably underestimating the true incidence.

The present study confirms the emergence and major proportion of HAIE. In the whole study cohort one-third and in non-PWID nearly one-half of IE episodes were HAIE. In a population-based study from France one-quarter of all IE episodes were HAIE (Selton-Suty et al. 2012) and in a large multinational study excluding PWID and PVE (Benito et al. 2009) the proportion of HAIE was approximately one-third, which are smaller proportions compared to our material. In a more recent population-based study from USA (States of New York and California) one-half of IE cases were HAIE (Toyoda et al. 2017). Together with our observation, this highlights the increasing significance of HAIE.

In comparison of CAIE to HAIE the present study describes distinct features: patients with CAIE had less frequently diabetes, chronic kidney disease and immunosuppressive therapy and *Enterococcus* spp. responsible for IE and more frequently viridans group streptococci as causative agent than patients with HAIE. The observation of higher comorbidity in HAIE has also been made in a population-based study from Australia (Sy and Kiritharides 2010) and in two studies from Spain (Fernandez-Hidalgo et al. 2008, Lomas et al. 2010). These three studies have also found patients with HAIE to be older compared to CAIE, but we could not confirm this finding.

IDUIE accounted for one-third of the cases. This proportion is quite large compared to other studies (Slipczuk et al. 2013). Although, a study from Kentucky, USA, where the opioid epidemic is prevalent, reported an IDUIE proportion of 40% (Seratnahaei et al. 2014) and a study from Western Norway reported a proportion of 23.5% (Jordal et al. 2018). Both these two studies noted also an increase in IDUIE during their study periods. An increase in IDUIE was also noted in a national register study from USA (Rudasill et al. 2019). These studies highlight the growing importance of IDUIE due to increasing drug abuse in many western countries. However, population-based registry study from Spain reported proportions of less than 5% during the study period 2003-2014 with decreasing numbers (Olmos et al. 2017) and a population-based study from France reported 5.8% share of IDUIE (Selton-Suty et al 2012). These studies demonstrate the geographical variation in proportions of IDUIE of all IE cases and point out the need to be cautious when extrapolating data on IDUIE from different regions. Drug abuse predisposing to IE may be higher in our study´s region compared to many other regions. Approximately one-third of Finnish problem drug users (i.e., abusers of methamphetamine and opioids) reside in the study area and in 2012 there were an estimated 5,600-10,300 problem users in the study area (representing 0.73%-1.34% of the population aged between 15 and 65; Varjonen 2015). Also, incidence of drug abuse in Finland is increasing (Kankaanpää et al. 2016). Altogether, our finding of IDUIE accounting for one-third of the IE episodes, an equal share compared to HAIE, is one of the key findings in this study and

indicates that in areas where drug use is common concomitant IDUIE contributes to a large part of all episodes of IE.

Studies comparing features of IDUIE to other community-acquired IEs are very rare and most studies compare IDUIE to non-IDUIE in which case the non-IDUIE group also includes those with health care-association. In our material, patients in CAIE group were older with higher comorbidity and proportion of males than in IDUIE. Also, left-sided IE and viridans group streptococci as etiology were more frequent in CAIE than IDUIE. Whereas, in CAIE both-sided infection, a history of previous IE and *S. aureus* as etiology was less frequent. A recent study comparing IDUIE to non-IDUE found similar differences as we describe, although in IDUIE group itself left-sided IE occurred more frequently compared to right-sided (45% vs 35%) which differs from our material (Leahey et al. 2019). A study describing a cohort of 202 IEs in PWID described characteristics very similar to ours: median age of 34, right-sided infection in 64% and *S. aureus* being responsible for 77% of cases (Rodger et al. 2018).

Results of this study confirm the role of *S. aureus* as a leading pathogen causing IE in a population level. In whole study group *S. aureus* constituted one-third of the cases, followed by viridans group streptococci (21.4%) and enterococci (9.9%). Similar proportions are reported from other studies (Murdoch et al. 2009, Selton-Suty et al. 2012). Some studies have noted an increase in enterococcal IE and with proportions higher than ours, 13%-16% (Jordal et. al 2018, Habib et al. 2019).

Approximately in one-sixth of the IE cases cerebral complication occurred. Septic emboli to other sites occurred in half of the cases, and less frequently in CAIE compared to IDUIE. This is probably to do with more *S. aureus* IE in IDUIE.

Overall, 23% of patients needed operative treatment. This is in line with other population-based studies (Sy and Kritharides 2010, DeSimone et al. 2015, Toyoda et al. 2017). Higher surgery rates are reported from referral centre-based studies (DeSimone et al. 2015, Olmos et al. 2017). CAIE cases were operated more frequently than patients with IDUIE, probably due to left-sided dominance of location of IE in CAIE.

We report in-hospital mortality rate of 17%. Most population-based studies report similar rates (Selton-Suty et al. 2012, Keller et al. 2017). Both in-hospital mortality and one-year mortality were lower in CAIE compared to HAIE (15% vs 28% and 19% vs 43%, respectively). Similar observations of higher mortality in HAIE have been made in previous studies as well and they have found HAIE to be an independent risk factor for death (Fernandez-Hidalgo et al. 2008, Lomas et al. 2010). Lower mortality-risk in patients with IDUIE compared to non-IDUIE was observed in a large registry-base study from USA (Rudasill et al. 2019), but in our study we found no statistical difference (9% vs 15%, P=0.200). The trend toward lower mortality in IDUIE compared to CAIE is, however, observed and lack of statistical significance is probably due to low total numbers of cases. Interesting observation is the increase in mortality in IDUIE group when comparing in-hospital mortality to one-year mortality (from 9.3% to

17.5%) while similar change is not seen in CAIE (from 15% to 19%). Given the younger age, lower co-morbidity and more frequent right-sided location (i.e., all factors associated with better come) in IDUIE, the increase in mortality is therefore probably explained by some other factor. The most plausible being continuing drug abuse, which highlights the importance of concomitant addiction treatment. Addiction treatment has been noted to impact better outcome (Rodger et. 2018).

The major strength of this study is its population-based nature. Most data on IE are derived from patients treated in referral centrals (Slipczuk et al. 2013). This introduces a selection bias towards more complicated cases (Kanafani et al. 2010). We decided to categorize IE according to the mode of acquisition for following reasons: 1) to determine their proportions and incidence and 2) to describe their unique features and differences. IDUIE is a well-known distinct clinical entity of IE and IDU is a strong risk factor for IE (Moss and Munt 2003), so IDUIE was reasonable to consider as its own entity. Moreover, health care-association in IDUIE cases is rare (Asgeirsson et. al 2016, Rodger et al. 2018). Another strength of this study is complete information on microbiology and very few missing data. In addition, an infectious disease specialist (M.H.) collected and reviewed all data.

Limitations derive from the retrospective nature of the study. Data on patient history before hospitalization were retrieved from medical patient records thus the accuracy of the data rely on the notes of the treating physicians. Patients were recognized using ICD-10 codes, so some cases may have been left out due to coding inaccuracy. If a patient had moved outside the study region during the following year the follow-up data might be inaccurate. Findings of this population-based study might not be generalizable outside the study population.

6.5 FUTURE CONSIDERATIONS

The benefit of new microbial methods should be examined also from the clinical standpoint. The impact of MALDI-TOF on the treatment and outcome in patients with BSI is well studied, but further studies are needed. Our study showed that even when introduced into a routine clinical setting si-MALDI-TOF had a positive impact on the treatment of selected BSIs. However, MALDI-TOF is probably even more powerful if combined with ASP intervention, i.e., in the hands of experts who are capable of fully exploiting the provided new information. The lack of ASP probably explains why si-MALDI-TOF did not provide additional impact in case of AmpC-producing *Enterobacteriaecae* BSI. In addition to ASP intervention, electronic reminders and antibiotic guidelines might help to fully exploit the benefits of the new rapid diagnostic methods. The impact and cost-effectiveness of MALDI-TOF alone versus in combination with ASP should be further studied. Various rapid diagnostic tests, including MALDI-TOF, are being developed and studied to target also the possible resistance of the bacteria causing BSI and their clinical impact should be studied from

clinicians' perspective. However, currently and in the near future these will only be supplementary to conventional blood cultures.

Only few studies exist to study the impact of pre-operative antimicrobial treatment duration on the yield of microbial samples obtained during heart surgery in patients with IE. This should be further studied. If the yield of PCR method is confirmed to diminish with prolonged treatment that would in blood culture negative patients even advocate earlier timing of surgery. The cost-effectiveness of the PCR method routinely obtained from patients with IE is undetermined and should be examined. The data from our, and other studies as well, strongly support the usefulness of routine valve PCR in surgery performed for IE.

In case of SSSI, when should a clinician order blood culturing? Based on our study, in case of cSSSI even a routine blood culture sampling might be feasible for the following reasons: 1) the high blood culture positivity rate, 2) benefit of the information gained from positive blood cultures, and 3) practical difficulties with prediction scores. At least in alcoholics with cSSSI blood culturing routinely is feasible. Other risk groups need further defining. Additionally, factors associated with bacteremia in different entities of SSSI deserve further study. The cost benefit of blood culture sampling in patients with cSSSI remains undefined and should be studied.

Infective endocarditis is a challenge for clinicians. Given the evolving nature of IE, repeated studies on its epidemiology and microbial etiology are warranted. The ageing population in developed countries underlines this need. The modified Duke criteria have been widely used since their introduction twenty years ago. Uniform criteria are necessary in studies of IE. However, they should be modified again. ESC has proposed other imaging studies to be added along with cardiac echocardiogram to improve sensitivity and specificity. A recent large multinational study (EURO-ENDO study) used these new criteria. Also, PCR from valve samples should be added to the diagnostic criteria of IE. As HAIE is a growing and challenging entity of IE, generally accepted definition of HAIE is called for. Prevention of IE is its best treatment and this warrants further studies, given the emerging and challenging entities of HAIE and IDUIE. As HAIE is often a result of bacteremia associated with health-care procedures, especially intravascular catheter use, all effort should be made to reduce the risk of such bacteremia.

7 CONCLUSIONS

Following conclusions can be drawn from the results of the studies:

Study I
This study demonstrated that implementation of si-MALDI-TOF method into a routine clinical setting had a favourable impact on the empiric antibiotic treatment of selected BSIs. A significant benefit was demonstrated in patients with enterococcal BSIs.

Study II
A population-based study including patients with cSSSI found that none of the factors associated with blood culture sampling were associated with blood culture positivity. Alcohol abuse was the only discriminant patient characteristic associated with blood culture positivity. Given the high proportion of bacteremic patients with cSSSI and the demonstrated clear benefit of information acquired from blood cultures, we recommend that clinicians should order blood cultures in this scenario with low threshold.

Study III
In a large cohort of operatively treated patients with IE we found that pre-operative antibiotic treatment decreased the diagnostic yield of microbiological samples obtained from resected endocardial material. All valve cultures were negative in patients who had received pre-operative antimicrobial treatment for more than two weeks. PCR positivity also dropped from 91% to 60% after two weeks of antimicrobial treatment, but remained approximately in 50% after four weeks of treatment. PCR from resected endocardial material had a diagnostic impact in one-sixth of the cases in whole study cohort and it was especially useful if blood cultures were negative.

Study IV
The present population-based study was the first to explore the incidence, characteristics, microbial etiology and outcome of IE in the Helsinki University Hospital area. The study described IE incidence of 6.48 per 100,000 person-years in adults in the study area. Almost equal shares of CAIE, HAIE, and IDUIE were observed and their distinct clinical features and outcome were described. In areas where drug use is common, concomitant IDUIE seems to constitute to a major part of all IE episodes, and even with an equal share than HAIE. These two entities also differ from CAIE. Findings of this study are of benefit to physicians treating the challenging entity of IE and suggest that in future studies on IE the three entities should be considered separately.

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" Olin ehyt ja rento ja tiesin mitä hain "

- *Juice Leskinen*

Helsinki, August 2020

Mika Halavaara

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