

JUHA RANNIKKO

Patients with Bacteraemia in Emergency Department

Underlying diseases, cfDNA, PCSK9 and outcome

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ACADEMIC DISSERTATION

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ABSTRACT

Patients with bacteraemia have viable bacteria in the blood. A part of the patients with bacteraemia have sepsis, but bacteraemia is not mandatory in sepsis by definition. The day 7 and day 28 all-cause mortality in patients with these infections is high. However, this all-cause mortality does not express how lethal the infection itself is. It has been known that underlying diseases have a role in the deaths of patients with bacteraemia and sepsis, but the magnitude of this role has hardly been studied at all.

Studies on biomarkers are conducted to get new tools for diagnostics or indicators for prognosis, for example. Studies on biomarkers also elucidate the pathogenesis of diseases. Some studies have shown cell-free DNA (cfDNA) as a prognostic biomarker, but it has not yet been studied against, or in combination with, a prognostic score qSOFA (quick sequential organ failure assessment). Evidence has also accumulated that cfDNA is a major factor in the pathogenesis of disseminated intravascular coagulation (DIC).

The proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme is known for its role in cholesterol metabolism. There are two antibodies, alirocumab and evolocumab, that are in use to lower cholesterol. Recent studies have indicated that PCSK9 also regulates the clearance of lipopolysaccharides (Gram-negative bacteria) and lipoteichoic acid (Gram-positive bacteria), and hence researchers are already recruiting patients to trials with the antibodies. However, studies on the level of this enzyme in infections are still scarce.

Against this background, the aim of this study was to elucidate the role of underlying diseases in deaths after a bacteraemia (Study I), to determine the role of plasma cfDNA in predicting prognosis in bacteraemia (Study II), and to assess the level of PCSK9 in bacteraemic patients and to determine the level in patients with severe disease or in relation to day 7, day 28, and day 90 deaths (Study III).

This cohort study involved 497 cases (484 patients) with bacteraemia gathered from the emergency department of Tampere University hospital during the years 2012-2014. The clinical data and the blood samples were gathered prospectively. The

biomarker analysis was done from 481 cases. Day 7, day 28, and day 90 mortality was 9%, 14%, and 20%, respectively.

In Study I, a new method was developed to categorise the patients that died by day 90 into three groups. By clinical judgement, the deaths of Group 1 patients were bacteraemia-related, and the patients did not have a rapidly fatal underlying disease. The deaths of Group 2 patients were also bacteraemia-related, but the patients had a rapidly fatal underlying disease. The deaths of Group 3 patients were only underlying disease related. The result was 16 patients (16% of all deaths) in Group 1, 45 patients (46%) in Group 2 and 37 patients (38%) in Group 3. The most common underlying disease in Groups 2 and 3 was solid cancer with metastasis. It was also found that death by day 7 does not equal that the death is directly related to bacteraemia as 66% of deaths by day 7 belonged to Group 2 and 3.

In Study II, the median level of cfDNA on day 0 was 1.38 µg/ml. The median level was significantly higher on day 0 in those who died by day 7 compared to those who survived ($p<0.001$). CfDNA had an area under curve (AUC) of 0.77 (95% Confidence Interval (CI) 0.69 to 0.85) in predicting death by day 7. QSOFA having AUC 0.77 (95% CI 0.70 to 0.85) was comparable with cfDNA, but C-reactive protein (CRP) had an AUC of only 0.52 (95% CI 0.43 to 0.60). Patients with cfDNA level of ≥ 1.69 µg/ml combined with qSOFA score ≥ 2 had an odds ratio of 20 of death by day 7. Amongst the five cases with the highest cfDNA levels, there were three patients with severe DIC.

In Study III, the median level of PCSK9 on day 0 was 376 ng/ml, which is a significantly higher level than in a control group of healed bacteraemia patients ($p<0.001$). Patients that died by day 7 and day 28 had a significantly lower level of day 0 PCSK9 ($p=0.022$ and $p=0.001$, respectively) than those who survived. There was no statistically significant difference in the level of PCSK9 between Gram-positive or Gram-negative bacteria ($p=0.499$). PCSK9 level had a significant positive correlation with CRP ($p<0.001$).

In conclusion, the role of the underlying diseases in deaths of patients with bacteraemia was found to be significant, cfDNA is, in contrast with CRP, a prognostic biomarker, and the level of PCSK9 is elevated in patients with bacteraemia, but less elevated in those that died by day 7 and day 28.

TIIVISTELMÄ

Bakteremiassa potilaalta löydetään eläviä bakteereita verenkierrasta. Osalla bakteremiapotilaista on myös sepsis, mutta sepsiksessä ei välttämättä tarvitse olla bakteremiaa. Molemmissa näistä päivän 7 ja päivän 28 kuolleisuus on kirjallisuuden mukaan korkea. Kuolleisuutta koskevissa tutkimuksissa ei kuitenkaan ole huomioitu erikseen pitkäaikaissairauksien vaikutusta. Pitkäaikaissairauksien tiedetään vaikuttavan infektiokuolleisuuteen, mutta pitkäaikaissairauksien ja infektioiden keskinäistä suhdetta on kuitenkin tutkittu vähän.

Sepsiksen diagnostiikan ja ennusteen arvioinnin apukeinoina on tutkittu erilaisia verenkierrasta mitattavia biologisia merkkiaineita. Biologisten merkkiaineiden tutkimus on lisännyt myös ymmärrystä sairauksien patogeneesistä. Muutamat tutkimukset ovat osoittaneet soluvapaalla DNA:lla (cell-free DNA, cfDNA) olevan kuolleisuutta ennustavaa arvoa, mutta tutkimuksia, joissa sitä olisi verrattu sepsiksen ennustetta mittaavaan pisteytykseen, qSOFA:n (quick Sequential Organ Failure Assessment), ei ole aiemmin tehty. Viimeisimpien tutkimusten mukaan cfDNA on myös merkittävä tekijä yleistyneen hyytymishäiriön (DIC:n) patogeneesissä.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) –entsyymillä on rooli kolesteroliaineenvaihdunnassa, ja kaksi siihen vaikuttavaa vasta-ainetta, alirokumabi ja evolokumabi, ovat käytössä kolesterolilääkkeinä. Viimeaikaiset tutkimukset ovat osoittaneet saman entsyymin säätelevän myös gram-negatiivisten bakteereiden hajoamisessa syntyvän lipopolysakkaridin ja gram-positiivisten bakteereiden lipoteikkohapon poistamista verenkierrasta. Tästä syystä molemmilla yllä mainituilla lääkkeillä on jo aloitettu tutkimukset sepsispotilailla, vaikka tutkimuksia tämän entsyymin veripitoisuuksista infektiopotilailla on vasta vähän.

Tätä taustaa vasten tutkimuksen tavoitteena oli selvittää pitkäaikaissairauksien osuutta bakteremian jälkeisessä kuolleisuudessa (Tutkimus I), tutkia bakteremiapotilailla cfDNA:n ennustearvoa (Tutkimus II) ja selvittää PCSK9-entsyymin pitoisuuksia verenkierrassa bakteremiapotilailla sekä niiden korrelaatiota bakteremian vaikeusasteeseen ja potilaan ennusteeseen (Tutkimus III).

Kyseessä oli kohorttitutkimus, jonka aineisto muodostui 497:stä bakteremiatapauksesta 484:llä eri potilaalla. Bakteremiapotilaat olivat olleet Tampereen yliopistollisen sairaalan päivystyspoliklinikalla vuosina 2012-2014.

Kliininen aineisto sekä verinäytteet kerättiin prospektiivisesti. Biomarkkereita analysoitiin 481:ltä tapaukselta. Kuolleisuus oli päivään 7 mennessä 9%, päivään 28 mennessä 14% ja päivään 90 mennessä 20%.

Tutkimuksessa I kehitettiin metodi, jossa infektiolääkärit jaottelivat 90 päivän sisällä bakteremiasta kuolleet potilaat kolmeen ryhmään. Ensimmäiseen ryhmään jaoteltiin ne potilaat, joiden kuoleman he katsoivat liittyvän bakteremiaan ja joilla olisi ollut yli puolen vuoden elinaikaennuste ilman bakteremiaan sairastumista. Ryhmään kaksi jaoteltiin ne potilaat, joiden kuolema liittyi bakteremiaan, mutta joiden ennuste olisi joka tapauksessa ollut alle puoli vuotta. Kolmanteen ryhmään jäivät ne bakteremiapotilaat, joiden kuolema katsottiin liittyvän vain pitkäaikaissairauksiin. Ryhmään yksi jaoteltiin 16 potilasta (16% kaikista kuolleista), ryhmään kaksi 45 potilasta (46%) ja ryhmään kolme 37 potilasta (38%). Yleisin pitkäaikaissairaus ryhmissä 2 ja 3 oli etäpesäkkeinen pahanlaatuinen kasvain. Tutkimuksessa todettiin myös, ettei edes päivään 7 mennessä tapahtuvia kuolemia voida pitää pelkästään bakteremiasta johtuvana vaan tässäkin ryhmät 2 ja 3 muodostivat enemmistön (66%).

Tutkimuksessa II bakteremiapotilaiden päivystyspoliklinikalla otettujen (päivä 0) cfDNA:n arvojen mediaani oli 1,38 µg/ml. Mediaani oli tilastollisesti merkittävästi korkeampi päivään 7 mennessä menehtyneillä kuin henkiin jääneillä ($p < 0,001$). CfDNA:n AUC-arvo (area under curve) päivään 7 mennessä tapahtuvan menehtymisen ennustamisessa oli 0,77 (95% luottamusväli (LV) 0,69-0,85). QSOFA:lla oli samankaltainen ennustearvo (AUC 0,77, 95% LV 0,70-0,85), mutta C-reaktiivisella proteiinilla (CRP:llä) AUC oli vain 0,52 (95% LV 0,43-0,60). Jos potilaan cfDNA oli yli 1,69 µg/ml ja samanaikaisesti qSOFA-pisteytys 2 tai yli, potilaan vetosuhde (odds ratio) menehtymisestä päivään 7 mennessä oli 20-kertainen verrattuna niihin, joilla sekä cfDNA-taso että qSOFA-pisteytys olivat matalampia. Viiden korkeimman cfDNA-arvon saaneiden joukossa oli kolme vaikean DIC-komplikaation saanutta potilasta.

Tutkimuksessa III bakteremiapotilaiden päivystyspoliklinikalla otettujen (päivä 0) PCSK9:n arvojen mediaani oli 376 ng/ml, mikä on tilastollisesti merkitsevästi korkeampi kuin bakteremiasta parantuneilla potilailla. Sekä päivään 7 että päivään 28 mennessä menehtyneillä oli muita bakteremiapotilaita tilastollisesti merkitsevästi alhaisempi PCSK9:n taso ($p = 0,022$ ja $p = 0,001$, tässä järjestyksessä). Gram-positiivisten ja gram-negatiivisten bakteremioiden välillä ei ollut tilastollisesti merkitsevää eroa PCSK9:n pitoisuuksissa ($p = 0,499$). PCSK9:n pitoisuuksilla oli merkitsevä positiivinen korrelaatio CRP:n pitoisuuksien kanssa. ($p < 0,001$).

Yhteenvedona voidaan sanoa pitkäaikaissairauksilla olevan merkittävä rooli bakteremian jälkeisessä kuolleisuudessa. Lisäksi cfDNA:n kyky ennustaa kuolleisuutta bakteremiapotilailla osoittautui hyväksi, toisin kuin CRP:llä. PCSK9 nousi bakteremian yhteydessä, mutta sen pitoisuudet olivat matalammat päivään 7 ja 28 mennessä menehtyneillä kuin eloonjääneillä.

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ABBREVIATIONS

APACHE II	Acute Physiology, Age, Chronic Health Evaluation II
apt	activated partial thromboplastin time
AUC	area under curve
CI	confidence interval
cfDNA	cell-free deoxyribonucleic acid
ctDNA	cell-free circulating tumour DNA
DAMP	damage-associated molecular pattern
DIC	disseminated intravascular coagulation
DM2	diabetes mellitus, type 2
ED	emergency department
ELISA	enzyme linked immunosorbent assay
ESBL	extended-spectrum beta-lactamase
F	female
GCS	Glasgow Coma Scale
GOF	gain-of-function
HDU	high dependency unit
ICU	intensive care unit
IQR	interquartile range
INR	international normalized ratio
LDL-R	low-density lipoprotein receptor
LOF	loss-of-function
LPS	lipopolysaccharide
M	male
MOF	multiple organ failure
MODS	multiple organ dysfunction syndrome
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
NETs	neutrophil extracellular traps
NEWS	National Early Warning Score
PAMP	pathogen-associated molecular pattern
PBS	Pitt Bacteraemia Score

PCSK9	proprotein convertase subtilisin/kexin type 9
PCT	procalcitonin
qSOFA	quick sequential organ failure assessment
RNA	ribonucleic acid
ROC	receiver operating characteristic
SIRS	systemic inflammatory response syndrome
SNP	single nucleotide polymorphism
SOFA	sequential organ failure assessment
suPAR	soluble urokinase-type plasminogen activator receptor
TAUH	Tampere University Hospital

ORIGINAL PUBLICATIONS

This dissertation is based on the following papers which are referred to in the text by their Roman numerals. Some additional data has also been included. The original publications are reproduced under Creative Commons licence or with the kind permission of the copyright holders.

- I **Rannikko, J**, Syrjänen, J, Seiskari, T, Aittoniemi, J and Huttunen, R (2017): Sepsis-related mortality in 497 cases with blood culture-positive sepsis in an emergency department. *Int J Infect Dis* 58: 52-7.
- II **Rannikko, J**, Seiskari, T, Huttunen, R, Tarkiainen, I, Jylhävä, J, Hurme, M, Syrjänen, J and Aittoniemi, J (2018): Plasma cell-free DNA and qSOFA score predict 7-day mortality in 481 emergency department bacteraemia patients. *J Intern Med* 284: 418-26.
- III **Rannikko, J**, Jacome Sanz, D, Ortutay, Z, Seiskari, T, Aittoniemi, J, Huttunen, R, Syrjänen, J and Pesu, M (2019): Reduced plasma PCSK9 response in patients with bacteremia is associated with mortality. *J Intern Med*: Epub ahead of print

1 INTRODUCTION

Bacteraemia is the presence of viable bacteria in the blood (Bone et al. 1992). Patients with bacteraemia have sepsis if the bacteraemia has evoked a life-threatening response from the immune system, but bacteraemia is not mandatory in the definition of sepsis (Singer et al. 2016). Moreover, those patients that fulfil the current definition of sepsis are on average in a slightly worse shape than bacteraemia patients, and may require care in high dependency units (HDUs) or intensive care units (ICUs) (Rhodes et al. 2017). This is due to the fact that the current definition of sepsis include patients that have an organ failure as did the old definition of severe sepsis (Singer et al. 2016). Nevertheless, most patients with bacteraemia have an infection for which the care, at least at the start, is reasonable to be given in hospitals.

There is medical literature about bacteraemia, but sepsis is far more abundantly covered in the literature, especially when the topic is the treatment of infections. In studies conducted mainly in ICUs, mortality in severe sepsis is reported to be up to 50% (Bernard et al. 2001, Peake et al. 2014, Yealy et al. 2014, Freund et al. 2017). These figures originate often from randomised controlled trials in which the in-hospital or day 28 mortality of the control group would be the mortality to sepsis. These figures represent often all-cause mortality, and the role of sepsis itself vs. the role of the underlying diseases in the cause of death is seldom estimated.

Plasma cell-free DNA (cfDNA) derives from cellular necrosis, apoptosis, and lysis (Rhodes and Cecconi 2012, Hou et al. 2016). There is also a secreted form, neutrophil extracellular traps (NETs), that has been shown to have a crucial role in thrombus initiation and progression (Fuchs et al. 2010, Martinod and Wagner 2014, Duplessis et al. 2018). Plasma cfDNA level is low in healthy individuals, but the level has been shown to rise in various conditions such as trauma, cancer, stroke, myocardial infarction, and sepsis (Wijeratne et al. 2004, Spindler et al. 2015, Ahmed et al. 2016, Clementi et al. 2016). As the level rises in various conditions, studies on the role of cfDNA in discriminative diagnostics are few, but several studies have concentrated on its use as a prognostic biomarker. CfDNA may have value in predicting the mortality of septic ICU patients (Zeerleder et al. 2003, Rhodes et al. 2006, Saukkonen et al. 2008, Dwivedi et al. 2012), but the value in prognosis of

patients with infection outside ICUs has been studied less (Moreira et al. 2010, Huttunen et al. 2011a).

The Proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme is known for its role in cholesterol metabolism (Seidah and Prat 2012, Spolitu et al. 2019). Two PCSK9 targeting antibodies, alirocumab and evolocumab, are currently used to treat statin-resistant or statin-intolerant hypercholesterolemia patients (Robinson et al. 2015, Sabatine et al. 2017). However, PCSK9 does not only regulate cholesterol metabolism, but regulates also the clearance of lipopolysaccharides (Gram-negative bacteria) and lipoteichoic acid (Gram-positive bacteria) (Grin et al. 2018). Before the current study, the level of PCSK9 in patients with infection has been evaluated in two studies (Walley et al. 2014, Boyd et al. 2016). These studies have indicated that the level would be higher than average in patients with more severe disease. Two clinical trials are ongoing to evaluate whether PCSK9 inhibiting antibodies could be used to treat patients with sepsis (clinicaltrials.gov). So far, no other therapeutic interventions on endotoxins have succeeded in meeting their objectives (Angus et al. 2000, Ankawi et al. 2018, Dellinger et al. 2018).

In the present study, the role of sepsis and the role of underlying diseases in deaths after a bacteraemia were evaluated. The value of cfDNA level in the prognosis of patients with bacteraemia, as well as the level of PCSK9 to the severity of the infection were also assessed.

2 REVIEW OF THE LITERATURE

2.1 Bacteraemia and sepsis

2.1.1 Definitions

Bacteraemia is the presence of alive and reproducible bacteria in the circulation (Bone et al. 1992). Other almost identical terms are bloodstream infection and septicaemia, but these also include the presence of yeasts in the blood (also known as fungemia). Contaminants, i.e. bacteria deriving from the skin puncture site, are excluded from bacteraemia studies. Before 2016, the definition of sepsis was the systemic inflammatory response syndrome (SIRS) in response to an infection (Bone et al. 1992, Levy et al. 2003) (Table 1). SIRS was the presence of two or more of the following: abnormal heart rate, body temperature, respiratory rate, and white blood cell count. At that time, patients with sepsis were categorized having severe sepsis if they had an organ dysfunction and septic shock if they had a persistent low blood pressure despite adequate administration of fluids.

The new definitions of sepsis and septic shock were published in 2016 (Singer et al. 2016). This SEPSIS-3 criterion abandoned SIRS and the definition of sepsis changed to “life-threatening organ dysfunction caused by a dysregulated host response to infection”. The term severe sepsis was also abandoned and serum lactate level was added to the definition of septic shock (Shankar-Hari et al. 2016). To fulfil the criteria of organ dysfunction, patients need to have an acute change in total Sequential (Sepsis-related) Organ Failure Assessment (SOFA) score ≥ 2 points consequent to the infection (Vincent et al. 1996, Singer et al. 2016). Furthermore, a new score, qSOFA, was introduced as a quick way to find patients who are in risk of dying. In developing this score, patients that had an alteration in mental status, systolic blood pressure ≤ 100 mm Hg, or respiratory rate ≥ 22 /min were found to have a higher in-hospital mortality (Seymour et al. 2016). If a patient fulfils two or three of these criteria (positive qSOFA score), the patient has a risk of death that is equivalent to the raise of SOFA score of 2 points or more (Freund et al. 2017). Nevertheless, in a research setting, positive qSOFA in patient with infection does

not necessitate that the patient has sepsis. Only those patients with infection that fulfil the SOFA criteria have sepsis by definition even though SOFA is rarely used in emergency departments (EDs) and wards (Singer et al. 2016, Keeley et al. 2017).

Neither the old nor the new definition of sepsis necessitate that sepsis patients should have a bacteraemia or that all patients with bacteraemia would have sepsis. Nevertheless, before Sepsis-3 definition, in practise all patients with bacteraemia had also sepsis (Jones and Lowes 1996, Coburn et al. 2012, Vincent et al. 2016a). The new definition of sepsis is based on organ dysfunction which all patients with bacteraemia don't have (Singer et al. 2016, Ljungstrom et al. 2017).

Table 1. The old and the new definition of sepsis

	SEPSIS	SEVERE SEPSIS	SEPTIC SHOCK
The old definition (Sepsis-2)	≥2 SIRS criteria met and (suspected) infection Criteria: Tachycardia Tachypnea Fever/hypothermia Leucopenia/leucocytosis	Sepsis and organ failure	Persistent hypotension
The new definition (Sepsis-3)	(Suspected) infection and organ failure (Increase of ≥2 SOFA points)	Not applicable	Persistent hypotension and hyperlactatemia

2.1.2 Incidence

According to the report of the Finnish National Institute for Health and Welfare, there were more than 17 000 episodes of bacteraemia in the year 2017 in Finland, being 1.7 times more than the annual number of episodes in the year 2008 (Jaakola et al. 2018). In year 2018 there was 1542 patients with positive blood culture in Tampere University Hospital (data from SAI-registry). In the United States, there are approximately 200 000 episodes of bacteraemia each year, with an incidence of about 10 per 1000 hospital admissions (Coburn et al. 2012). The incidence of sepsis in all patients of the Finnish EDs is not known. However, between 2007 and 2012, the incidence of severe sepsis in patients transferred to ICU has risen from 38 per 100 000 adult population to 60 per 100 000 (Karlsson et al. 2007, Poukkanen et al. 2013). The incidence of sepsis in the United States and other parts of the western world has also increased (Angus et al. 2001, Martin et al. 2003).

There are no studies giving definite answers as to why these incidences have changed, but at least in Finland the taking of blood cultures as a part of clinical evaluation of patients has increased (Skogberg et al. 2008). Furthermore, the aged (>65 years) account for the majority of all episodes of both bacteraemia and sepsis (Blanco et al. 2008, Esper and Martin 2009, Kaukonen et al. 2014, Jaakola et al. 2018) and thus the aging of the population might have led to the change. Other explanations could be immunosuppression and better awareness of the possibility of sepsis (Rintala and Karlsson 2017).

The incidence of positive blood cultures among patients with sepsis is dependent on the severity of the infection. In one study, a positive culture was found in 17% of patients with a SIRS-positive sepsis, in 25% with severe sepsis, and in 69% of patients with septic shock (Rangel-Frausto et al. 1995). In different studies, the proportion of community-acquired vs. hospital-acquired bloodstream infections has been around 50% (Diekema et al. 2003, Shorr et al. 2006). These two differ in causative organisms, patient groups and prognosis. (Diekema et al. 2003, Gaynes and Edwards 2005).

2.1.3 Pathophysiology

Bacterial structures are triggers for host response. Macrophages are the major cells responsible for innate immunity by recognizing and binding to microbial components (i.e. pathogen associated molecular patterns, PAMPs). They release proinflammatory cytokines inviting additional inflammatory cells, for example leucocytes. Anti-inflammatory cytokines have a role of limiting the response. Host response is capable of clearing a limited number of bacteria, and sepsis occurs when the response is both generalized and excessive. (Aird 2003, Cinel and Dellinger 2007, Chen and Nunez 2010)

The precise mechanism of cellular injury in sepsis is not known. Tissue ischemia, cytopathic injury and an altered rate of apoptosis have been proposed to be at least part of the explanation (Angus and van der Poll 2013). This cellular injury causes the release of danger-associated molecular patterns (DAMPs) that, in addition to PAMPs, are recognised by the receptors on the surface of immune cells. (Hotchkiss et al. 2001, Janeway and Medzhitov 2002, Chen and Nunez 2010, Zhang et al. 2010).

The complement system is another important factor in innate immunity. It has a role in defending against pyogenic bacterial infection, disposing of the products of inflammatory injury and immune complexes, and bridging adaptive and innate

immunity (Walport 2001). Inhibition of complement cascade has decreased sepsis mortality in several animal models (Riedemann et al. 2002, Liu et al. 2007), but this has not been adapted into clinical use.

2.1.4 Blood culture findings

Among adult patients in Finland, Gram-negative bacteria are causative microorganism in ca. 50% of all bacteraemias and Gram-positives in ca. 45%. Anaerobes constitute ca. 4% of cases (Jaakola et al. 2018). The vast majority (71 %) of positive blood cultures are detected in aged persons. The most common microorganism in Finland is *Escherichia coli* (23% of all bacteraemia) followed by *Staphylococcus aureus* (13%) and *Klebsiella* (6%) (Jaakola et al. 2018). These figures are approximately the same elsewhere in the developed world (Cisterna et al. 2001, Ortega et al. 2007).

2.1.5 Risk factors

Some of the known risk factors for bacteraemia and sepsis are age, i.e. being infant or aged, trauma, and hospitalization. Other risk factors include various diseases and treatments such as AIDS, diabetes, asplenia, malignancies and treatments for malignancies (Williams et al. 2004, Martin et al. 2006, Wang et al. 2012, Torres et al. 2015, Novosad et al. 2016). These risk factors weaken the body's natural borders such as the skin or the intestine or they weaken the immune system or both.

Furthermore, there are numerous genetic variations (mostly single nucleotide polymorphisms, SNPs) that are associated with susceptibility to infection (Kenney et al. 2017). These SNPs are in genes that encode various cell surface receptors, cytokines and enzymes of the immune defence. However, in clinical practice, genetic sequencing of a patient with infection is uncommon.

2.1.6 Clinical manifestations

The majority of bacteraemic patients have fever, but this also reflects the fact that fever is a common reason why clinicians have originally decided to take the blood cultures (Coburn et al. 2012). The presence of shaking chills (rigors) has been shown

to be independently associated with positive blood culture (Lee et al. 2012). Absence of fever has been associated with increased mortality (Yahav et al. 2016).

The current definition of sepsis is based on a score that measures organ dysfunction (Singer et al. 2016). Hence the clinical manifestations of sepsis patients represent more severe types of infection than patients with sepsis by the old definition used to have (Abraham 2016). The classical signs and symptoms of sepsis are fever, increased heart rate, tachypnea and hypotension. Lactatemia, metabolic acidosis, oliguria and skin manifestations are possible (Gotts and Matthay 2016). Patients with bacteraemia may have all of the aforementioned signs and symptoms or only some of them. Especially the aged and other patients with vasculopathy of the brain commonly have an altered mentation. The aged may lack fever and the only sign of septic infection could be poor condition. (Angus and van der Poll 2013, Yahav et al. 2016, Rintala and Karlsson 2017).

Sepsis affects a number of laboratory values. Leucocytosis or leucopenia, thrombocytopenia, creatinine increase, elevated C-reactive protein (CRP) or procalcitonin, hyperbilirubinemia, hyperlactatemia, acidosis, and elevation of INR or aPTT are all possible, but none of these is specific to infection (Angus and van der Poll 2013, Dellinger et al. 2013).

When the generalized and excessive response to infection has started, all organs are in risk of dysfunction. Vasodilatation and other factors lead to hypotension which in turn leads to tissue hypoperfusion and ischemia. Cardiopathy, renal failure, liver failure and haematological disturbances are all common in the most severe types of sepsis. If a patient has a progressive disturbance of homeostasis, the entity is called multiple organ dysfunction syndrome (MODS) or multiple organ failure (MOF) having a high mortality (Marshall et al. 1995).

2.1.7 Treatment

The more severe the infection, the more important the timing of the antibiotic is (Kumar et al. 2006, Seymour et al. 2017). Bacteraemic pyelonephritis may be treated with oral fluoroquinolones (Peterson et al. 2008, Sandberg et al. 2012), but patients with sepsis are initially treated with intra-venous antibiotic (Gotts and Matthay 2016, Rintala and Karlsson 2017). The choice of antibiotic is based on multiple different factors such as the focus, underlying diseases, the state of immunosuppression and carriage of resistant microbes. Local antibiotic guidelines should be followed. Empirical treatment of sepsis with unknown focus should cover the possibility of

both Gram-negatives and Gram-positives (Savage et al. 2016, Rhodes et al. 2017). Follow-up blood cultures should be taken in certain circumstances, such as in *Staphylococcus aureus* bacteraemia (Holland et al. 2014, Canzoneri et al. 2017).

A patient with life-threatening infection needs fluid resuscitation (balanced salt solutions) (Sethi et al. 2018). In general, mean arterial pressure should be kept over 65 mmHg (Rivers et al. 2001, Vincent et al. 2016b). Glucocorticoid therapy should not be given routinely, but current guidelines recommend its use in septic shock if the patient is not responding to vasopressor treatment (Rhodes et al. 2017). An ongoing glucocorticoid treatment should not be stopped, particularly for those with longstanding high doses (van der Goes et al. 2014).

2.2 Prognosis in bacteraemia and sepsis

2.2.1 Overview of the prognosis and the problems with the estimates

The prognosis in sepsis depends on the multitude and severity of organ dysfunction. Even though there has been improvement in managing the condition, the all-cause in-hospital mortality for severe sepsis both in Finland and the rest of the western world is 20% to 30% (Angus et al. 2001, Kumar et al. 2011, Poukkanen et al. 2013, Kaukonen et al. 2014, Kaukonen et al. 2015). After discharge, these patients still carry an increased risk of death (Perl et al. 1995, Nessler et al. 2013, Linder et al. 2016). In a Finnish study on severe sepsis patients treated in ICU, in-hospital mortality was 28%, blood cultures were taken from 68% of patients and cultures were positive in 40% (Karlsson et al. 2007). In some studies, in-hospital and day 30 mortality to bacteraemia have been approximately the same or slightly less than mortality to severe sepsis (Alberti et al. 2003, Coburn et al. 2012, Kethireddy et al. 2018, Meier et al. 2018). In one study, cases with sepsis that had a positive culture had higher day 28 mortality than cases with culture-negative sepsis (Nannan Panday et al. 2019).

The mortality of sepsis varies depending on the study material. In one study from the United States, mortality based on administrative claims data was 15% to 140% higher (range 168 000-381 000 deaths per year) than annual estimates generated using death certificate data (range 146 000-159 000 deaths per year) (Epstein et al. 2016). Both figures are still rough estimates as both of the data have numerous limitations. Sepsis is a consensus definition with no confirmatory diagnostic test

(Chen et al. 2019a). In clinical practise, the diagnosis is not based on scores, but on evidence of infection and clinical judgement (Lever and Mackenzie 2007). Moreover, death certificates have both the underlying and the immediate cause of death (CDC 2016). There is no consensus on whether sepsis mortality should be equal with sepsis in the death certificate as both of these causes, or either one of them is enough. In addition, in clinical trials the mortality depends on the inclusion and exclusion criteria, and hence the figures differ.

2.2.2 Virulence and other factors influencing the outcome

The clinical manifestations of bacteraemia are varied. Transient bacteraemia are possible after dental procedures (Wilson et al. 2007, Horliana et al. 2014) or other operations affecting mucous membranes. Most of these bacteraemia are probably undetected as they do not have clinical relevance. The other end of clinical manifestation is a patient with septic shock and high mortality. This difference in clinical manifestation is multi-factorial and explanations lie both on patient characteristics and bacterial factors, such as bacterial virulence (Diard and Hardt 2017). As an example, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Capnocytophaga canimorsus*, *Neisseria meningitis* and *Staphylococcus aureus* are more virulent than bacteria such as *Enterococcus faecium* (Gao et al. 2018). Bacteraemias caused by methicillin-resistant *Staphylococcus aureus* have worse prognosis over methicillin-sensitive *S. aureus* infection, and polymicrobial infections over monomicrobial infection (Sogaard et al. 2011, Labelle et al. 2012, Jokinen et al. 2017)

Comorbidities and the type of infection are known determinants of outcome. Comorbidities associated with poor prognosis include diseases such as liver disease, alcoholism, immunosuppression and cancer (Williams et al. 2004, Wang et al. 2012, Torres et al. 2015). The prognosis is worse for nosocomial infection than community acquired infections (Shorr et al. 2006). Moreover, the site of infection plays a role. As an example, sepsis originating from the urinary tract has a low mortality, but the mortality exceeds 75% in sepsis associated with ischemic bowel (Labelle et al. 2012).

2.2.3 Statistical methods to find the sepsis-caused deaths

In sepsis studies, there are two different statistical methods that could be of help to get closer to the attributable fraction of sepsis-caused deaths. The first is to do a multi-variable logistic regression model, and include variables such as patient age,

length of stay, re-admission to hospital, and number of diagnoses. One example of this is an Irish study that calculated the attributable mortality of hospital-acquired bloodstream infections (Brady et al. 2017). They found the highest attributable mortality for *E. faecium* and the lowest attributable mortality for *E. coli*. The other is propensity-matched groups, and it is also used to find attributable mortality (Shankar-Hari et al. 2018, Kadri et al. 2019). In both of these methods the results are dependent on the variables and their ability to standardise the groups.

2.2.4 The use of scores and indexes in the prognosis

The new definition of sepsis is based on calculations on the prognosis among patients with infection (Seymour et al. 2016). Especially in the developing of the new score qSOFA, the task force tried to find as simple a calculator as possible that has high predictive validity for in-hospital mortality among patients with suspected infection outside ICU. In their patient cohort, they calculated an area under curve (AUC) of 0.81 (95% CI 0.80-0.82). However, in a recent meta-analysis of qSOFA, the median AUC for in-hospital mortality was not that good (0.68, 95% CI 0.65-0.71) (Lo et al. 2019). The prognostic accuracy of qSOFA has also been questioned in geriatric patients (Bastoni et al. 2019).

There have already been numerous different scores to predict the mortality of both sepsis patients and ED/ICU patients in general. As an example, National Early Warning Score (NEWS) has had better predictive validity than qSOFA (Churpek et al. 2017a, Churpek et al. 2017b, Brink et al. 2019). However, none of these scores developed so far are as easy to calculate as qSOFA.

The importance of the underlying diseases in sepsis mortality has been long known. The McCabe classification was created already in the 1960s to categorise bacteraemia patients by their prognosis (McCabe and Jackson 1962). Patients are categorized whether they have rapidly fatal, ultimately fatal or non-fatal underlying disease. This old classification has been shown to be a better predictor of mortality in patients suspected of Gram-negative sepsis than the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (Perl et al. 1995). This classification also has a variation designed for ICU patients, the Sabadell score (Fernandez et al. 2006).

Scoring systems like Charlson Comorbidity Index (Charlson et al. 1987) or Chronic Disease Score (Von Korff et al. 1992) have tried to adjust the underlying diseases and conditions to get a better evaluation of the mortality. The problem with

these scores is that they should be validated regularly as the prognosis of the different underlying diseases changes over time. For example, the prognosis of an HIV patient has changed dramatically since 1987. There are variations of the original Charlson Index, but regular validation has not happened. Furthermore, there are other scores that have been shown to be better predictors of mortality than the Charlson Index. For example, Pitt Bacteraemia Score (Korvick et al. 1991) has been shown to have better sensitivity and specificity than the Charlson Index (or APACHE II) (Rhee et al. 2009).

2.2.5 The use of biomarkers to assess prognosis

A working group at the National Institute of Health has defined biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (NIH 2001). The most widely used infection marker in Finland is CRP, a short pentraxin described already in 1930s (Tillett and Francis 1930). Nevertheless, high level of CRP is not prognostic. Among patients with infection, death is as possible in all levels of CRP (Zhao et al. 2013, Magrini et al. 2014, Reichsoellner et al. 2014, Tziolos et al. 2015).

It has been suggested that procalcitonin (PCT) should be used instead of CRP as it is more rapid to rise and it would be more specific (Riedel 2012, Wacker et al. 2013). Although there are several studies concerning PCT-guided antibiotic discontinuation, death has been the primary outcome in only one study, and all the studies have had a high risk of bias (Pepper et al. 2019). This one study with death as primary endpoint did not show benefit in survival (Bloos et al. 2016). In another study, the failure of PCT level to fall by day 4 predicted day 28 mortality (Schuetz et al. 2017), and in a meta-analysis on survival data there was a statistically significant difference in the level of PCT between survivors and non-survivors (Arora et al. 2015).

Leukocytosis of more than $12 \times 10^9/l$, leukopenia of less than $4 \times 10^9/l$ or white-cell count with more than 10% of immature forms are examples of the inflammatory variables used in the suspicion of sepsis (Levy et al. 2003). Leukopenia as well as thrombocytopenia in sepsis has been associated with poor prognosis (Kreger et al. 1980, Thierry-Antier et al. 2016). In one study, persistent lymphopenia on day 4 following the diagnosis of bacteraemic sepsis predicted mortality (Drewry et al. 2014).

Serum lactate is commonly elevated in patients with severe sepsis, and the measurement of lactate is included in the diagnosis of septic shock (Shankar-Hari et al. 2016). Elevated lactate is associated with poor prognosis (Mikkelsen et al. 2009, Casserly et al. 2015, Haas et al. 2016), and the kinetics of lactate have value as well (Chertoff et al. 2015). A recent study concluded that those bacteraemic patients with sepsis who are using metformin have a higher mean level of lactate affecting the prognostic accuracy (Chen et al. 2019b).

There are around 200 other sepsis biomarkers (Sandquist and Wong 2014). One of the most studied ones is soluble urokinase plasminogen activator receptor (suPAR). This biomarker does not have much diagnostic value (Eugen-Olsen 2011), but has had an AUC between 0.79 and 0.84 in predicting death by day 30 (Kofoed et al. 2008, Huttunen et al. 2011b, Uusitalo-Seppala et al. 2012). Other examples of these biomarkers are pro-adrenomedullin, pentraxin-3, presepsin, CD-64, interleukin-6, bactericidal/permeability-increasing protein, phospholipase A₂ group IIA, and cell-free DNA.

2.3 Cell-free DNA

2.3.1 Overview of cell-free DNA

Cell-free DNA (cfDNA) consists of short-lived fragments of DNA that are released because of apoptosis and cell necrosis (Sandquist and Wong 2014). It can be detected in plasma, urine, sputum, saliva, stool, and cerebrospinal fluid (Weerakoon and McManus 2016). Apart from cellular death, it can be of bacterial origin and it is also released from activated host cells such as macrophages and neutrophils (Bhagirath et al. 2015). Neutrophils release cfDNA in structures known as neutrophil extracellular traps (NETs) (Fuchs et al. 2010, Engelmann and Massberg 2013, Liaw et al. 2016). The half-life of cfDNA in blood is ca. 16 min (Lo et al. 1999).

Cell necrosis is not sepsis-specific and hence there are numerous conditions and diseases that have been shown to increase the total circulating cfDNA. They include pregnancy, stroke, myocardial infarction, trauma and cancer (Lo et al. 2000, Lau et al. 2002, Wu et al. 2002, Chang et al. 2003, Rainer et al. 2003). A prognostic value has been shown in acute stroke and acute myocardial infarction (Chang et al. 2003, Rainer et al. 2003).

A very low level of cfDNA (ca. 0.02 µg/ml) is present in healthy populations (Wu et al. 2002). In recent years, the origins of this cfDNA have been under investigation. By studying the methylation patterns, one study concluded that the majority of cfDNA in healthy persons originates from white blood cells (55%) and erythrocyte progenitors (30%), and to a lesser extent from vascular endothelial cells (10%) and hepatocytes (1%) (Moss et al. 2018). Furthermore, the evaluation of mutated DNA in circulation can be used to detect, genotype and monitor cancer (Wan et al. 2017), and foetal cfDNA in maternal blood is a non-invasive test of foetal chromosomal abnormality (Fan et al. 2012).

2.3.2 The role in coagulation and fibrinolysis

CfDNA is one of the damage-associated molecular patterns (DAMPs). It has a pathogenic role in sepsis as the NET-form of cfDNA activates coagulation (O'Brien et al. 2017). Moreover, increased levels of cfDNA in sepsis impair fibrinolysis by inhibiting plasmin-mediated fibrin degradation (Gould et al. 2015). In summary, cfDNA acts as a link between innate immunity and coagulation. Expectedly, cfDNA has been shown to be elevated in patients with deep vein thrombosis (Fuchs et al. 2012). Evidence has also accumulated that cfDNA is a major factor in the pathogenesis of disseminated intravascular coagulation (DIC), but this has not yet been established in clinical studies (Liaw et al. 2016).

2.3.3 Cell-free DNA in infections

Plasma cfDNA is elevated in various conditions and therefore the total cfDNA cannot be used as a diagnostic tool. Despite this, one prospective study found that, with clinical judgement as a golden standard, within febrile patients it would have an extremely high AUC of 0.99 to diagnose an infection (Moreira et al. 2010). Nevertheless, it has value in prognosis of patients with bacteraemia and severe sepsis (Rhodes et al. 2006, Huttunen et al. 2011a, Dwivedi et al. 2012, Avriel et al. 2014, Forsblom et al. 2014, Garnacho-Montero et al. 2014). In these studies, non-survivors have had statistically significantly higher values compared to survivors. In patients with severe sepsis, AUC in prediction of ICU mortality has been as high as 0.97 (Dwivedi et al. 2012). In predicting prognosis, it has been reported to be better than APACHE II score and biomarkers such as PCT and IL-6 (Dwivedi et al. 2012, Avriel et al. 2014).

In recent years, the origin of cfDNA in sepsis has been under investigation (Moss et al. 2018). In fourteen sepsis patients, the majority of cfDNA originated from granulocytes, but if the patient had an elevation of alanine aminotransferase, biomarker of hepatocyte damage, more than half of the origin of cfDNA was from hepatocytes (Moss et al. 2018). In another study, the identification of circulating microbial cfDNA with next-generation sequencing test yielded a 94% agreement with blood-culture (Blauwkamp et al. 2019).

2.4 PCSK9

2.4.1 Overview of PCSK9 and the role in cholesterol clearance

The proprotein convertase subtilisin/kexin enzyme 9 (PCSK9) is a liver-produced enzyme that binds to the low density lipoprotein receptor (LDL-R) on the surface of hepatocytes (Seidah and Prat 2012, Seidah et al. 2018). Binding to the receptor leads to the degradation of the LDL-R and higher plasma LDL-cholesterol levels. Patients with a PCSK9 loss-of-function (LOF) mutation of the PCSK9 gene have a reduced cholesterol level (Cohen et al. 2006, Benn et al. 2010), and the same vice versa, patients with the gain-of-function (GOF) mutations have an increased level of cholesterol (Leren 2004). If the binding to the receptor is inhibited with antibodies, plasma cholesterol level is reduced (Robinson et al. 2015, Sabatine et al. 2017). Alirocumab and evolocumab are the first antibodies that are in clinical use to lower the cholesterol level, especially among patients with familial hypercholesterolemia. The results of a meta-analysis on the effects of the antibodies show that they reduce both cardiovascular mortality and all-cause mortality (Navarese et al. 2015).

Meta-analysis of 15 cholesterol studies concluded that the normal level of plasma PCSK9 in healthy subjects is ca. 200 ng/ml (95% confidence interval (CI) 170-220) (Boyd et al. 2016). There is a correlation between plasma PCSK9 level and LDL-cholesterol level, and the level of PCSK9 is increased in those who use statins. (Nozue 2017).

2.4.2 PCSK9 in bacteraemia and sepsis

Low level of LDL-cholesterol has been associated with mortality in sepsis (Wilson et al. 2003, Chiarla et al. 2004, Vermont et al. 2005). A study published in 2014 by a Canadian group was the first to show that the same receptor that binds to LDL-cholesterol also binds to lipopolysaccharides (LPSs, endotoxins of Gram-positive bacteria) (Walley et al. 2014). Later on, another study concluded that also the uptake of Gram-positive lipoteichoic acid (LTA) occurs through LDL-R (Grin et al. 2018). Therefore, PCSK9 blockade should clear these pathogenic lipids as the blockade prevents the LDL-R from degrading. This new therapeutic strategy has not yet been proven in clinical trials, but investigators are starting to recruit septic patients into two randomised controlled trials with both alirocumab and evolocumab (NCT03634293 and NCT03869073, respectively).

There are studies that support this strategy. In the 2014 study LOF genetic variants were associated with improved survival in septic shock (Walley et al. 2014). In another study by the same Canadian group, the presence of multiple (but not single) PCSK9 LOF alleles decreased the risk of 1-year death in sepsis survivors (Genga et al. 2018). A third study by the same group concluded that the level of PCSK9 enzyme would be increased in patients with sepsis (Boyd et al. 2016). In this study, the level among septic patients would be higher in those that have cardiovascular or respiratory failure and lower in those without any organ failure. Statistical analysis on mortality could not be made in their study as only ten of their patients died by day 28. Lastly, a murine model by another group has also supported this theory (Dwivedi et al. 2016).

This theory of the beneficial effect of the blockade on bacterial infections has had setbacks. In cholesterol studies, there has been no beneficial effect on infections, although infections have probably not been under special surveillance. On the contrary, some elevation of the incidence of upper respiratory tract infections was seen in the alirocumab study (Robinson et al. 2015, Khademi et al. 2018). In the 2014 work, PCSK9-blocking antibody significantly increased survival of septic mice (Walley et al. 2014), but in a later work with mice, the inhibition of PCSK9 did not have the same effect on LPS-induced mortality (Berger et al. 2017). Furthermore, in a cohort study done with black people living in the United States, analysis of loss-of-function PCSK9 genetic variants revealed that they were not in increased risk of hospitalization for a serious infection (Mitchell et al. 2019).

3 AIMS OF THE STUDY

The aims of this study were:

1. To study the characteristics and outcome of blood-culture positive infections
2. To study the importance of the underlying diseases in deaths associated with bacteraemia and the role of the underlying diseases in relation to day 7, day 28, and day 90 deaths
3. To assess the level of cfDNA in bacteraemic patients, and to determine the role of plasma cfDNA in predicting prognosis in bacteraemia
4. To assess the level of PCSK9 in bacteraemic patients and to determine the level in patients with severe disease or in relation to day 7, day 28, and day 90 deaths

4 MATERIALS AND METHODS

4.1 The setting

Tampere University Hospital (TAUH) is the tertiary hospital of the Pirkanmaa County with a catchment population of 524 700. At the time of patient collection, the hospital ED divided the patients into basic and specialised emergency care patients. In specialised care, the vast majority of the patients were internal medicine and surgical patients. The hospital has a 24-bed ICU and four different HDUs (pulmonary, surgical, cardiology, and internal medicine), all taking care of patients with infection.

4.2 Patients

4.2.1 The selection of the cases

The study cohort was gathered prospectively. In the ED, blood cultures are taken routinely from patients with suspected infection. The population of the study comprises all the blood culture-positive adult patients that were treated in the hospital ED specialised care between March 1, 2012 and February 28, 2014. The culture-positive cases that were considered as contaminants were excluded. These included cases with coagulase-negative *Staphylococcus*, *Bacillus*, *Micrococcus*, *Cutibacterium*, and *Corynebacterium* without clinical relevance and with detection in a single blood culture bottle. Due to technical reasons, some of the culture-positive cases were missed. These included cases where the culture became positive later than 72 hours after the admission to the ED. At that point the blood sample of the admission day needed for the biomarker analysis was missed.

4.2.2 Clinical data

The clinical data such as underlying diseases, medication, and clinical parameters at the time of the arrival to the ED was gathered from the medical records (paper and digital) by the principal investigator. (Data collection form added as an appendix.) The site of infection and the main underlying disease associated with death were decided by the investigator based on medical records and autopsy records, if available. Diagnoses of sepsis, severe sepsis and septic shock were defined according to Sepsis-2 criteria (Levy et al. 2003). The definition of sepsis changed at the time of the writing of Study I. Hence the naming of the patient cohort changed from sepsis patients to bacteraemia patients in Studies II and III. The definition of quick Sequential Organ Failure Assessment (qSOFA) was made according to Sepsis-3 criteria (Seymour et al. 2016) in cases where the necessary data was available. The qSOFA score was positive if at least two of the following criteria were fulfilled: respiratory rate ≥ 22 /min, systolic blood pressure ≤ 100 mmHg or the verbal section of the Glasgow Coma Scale was altered. The Pitt Bacteraemia Score was calculated as presented in the original study (Korvick et al. 1991). Bacteraemia was estimated to be healthcare-associated if the symptoms started more than 48 h after admission to a healthcare institution, or the bacteraemia was related to a surgical operation in the preceding 30 days.

4.2.3 The categorization of the cause of death

All patients that died before day 90 were categorized into three different groups based on their cause of death. If the death of the patient was bacteraemia-related and the patient had a life expectancy of more than six months, the patient was categorized into Group 1. If the death was bacteraemia-related, but the life expectancy was less than six months, the patient belonged to Group 2. If the death was unrelated to bacteraemia, the patient belonged to Group 3. The term bacteraemia-related refers to culture-positive cases where the immediate cause of death was the bacteraemia, or the bacteraemia was a factor in the chain of events leading to death. This categorization was done by two infectious diseases clinicians independently using medical records and autopsy records, if available. If the categorization differed between the two, a meeting was held together with a third clinician and the final decision was made. This categorization was based on the severity of the patient's underlying disease(s), the patient's pre-performance, severity of the bacteraemia, and recovery after the bacteraemia. This categorization was

developed by the authors of Study I by partly combining the McCabe classification (McCabe and Jackson 1962) and the classification commonly used in death certificates (immediate cause of death and underlying cause of death).

4.3 Microbiological methods and blood sample collection

Blood cultures were collected in BacT/Alert Aerobic (FA Plus) and Anaerobic (FN Plus) blood culture bottles. These bottles were placed in the automated microbial detection system BacT/Alert 3D (bioMérieux, France). When a blood culture that fulfilled the above-mentioned criteria turned out positive, the clinical microbiologist started to collect the blood samples for study analytes. The blood samples were collected from patients alongside treatment-based routine laboratory testing. The day 0 blood sample was collected mostly within the first hours after the patient was admitted to the ED, but samples collected within up to 24 hours were included. The plasma was extracted from the leftover blood samples and frozen.

4.4 Cell-free DNA

The quantification of plasma cfDNA was done by Qubit dsDNA HS Assay Kit and Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). The manufacturer's instructions were followed at each step. The analysis was done twice, and the final result was the average of the two samples. The intra-assay coefficients of variation from control samples were between 3.6% and 5.7%.

4.5 PCSK9

Plasma PCSK9 was quantitated from plasma samples using an ELISA assay (R&D Systems, cat#DPC900) and the manufacturer's recommended protocol. The reproducibility of the ELISA was validated by measuring part of the samples twice. The intra-array and between-array variations of the samples were on average 3.5% of the defined final concentration.

There were also control samples from another bacteraemia cohort taken one year after surviving a bacteraemia (n=17). The method of the collection of these samples is described elsewhere (Huttunen et al. 2010). This control group had a median level

of 188 ng/ml (inter quartile range (IQR) 139-264) which is in line with the normal human range presented in a meta-analysis of non-septic patients (170-220 ng/ml) (Boyd et al. 2016).

4.6 Statistical methods

The IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) was used for most of the statistical analysis excluding some of the analysis in Study III that were done with R (version 3.4., www.r-project.org). Nonparametric data were analysed with Mann-Whitney U-test or Kruskal–Wallis test when appropriate. A P-value of <0.05 was considered significant. Categorical data were analyzed by Chi-square test. AUC/receiver operating characteristic (ROC) statistic was used for the predictive performance, and the Youden index was used to select optimal cut-off value. A logistic regression model was used to study the independent effect of a biomarker adjusted to potential confounders. Kaplan–Meier method was used for the calculation of the survival curve, and survival differences between groups were compared by a log-rank test.

4.7 Ethical considerations

The study was approved by the Ethics Committee of Tampere University Hospital, Finland, and the National Supervisory Authority for Welfare and Health. The need for informed consent was waived as no additional blood sampling was needed and routine patient care was not modified.

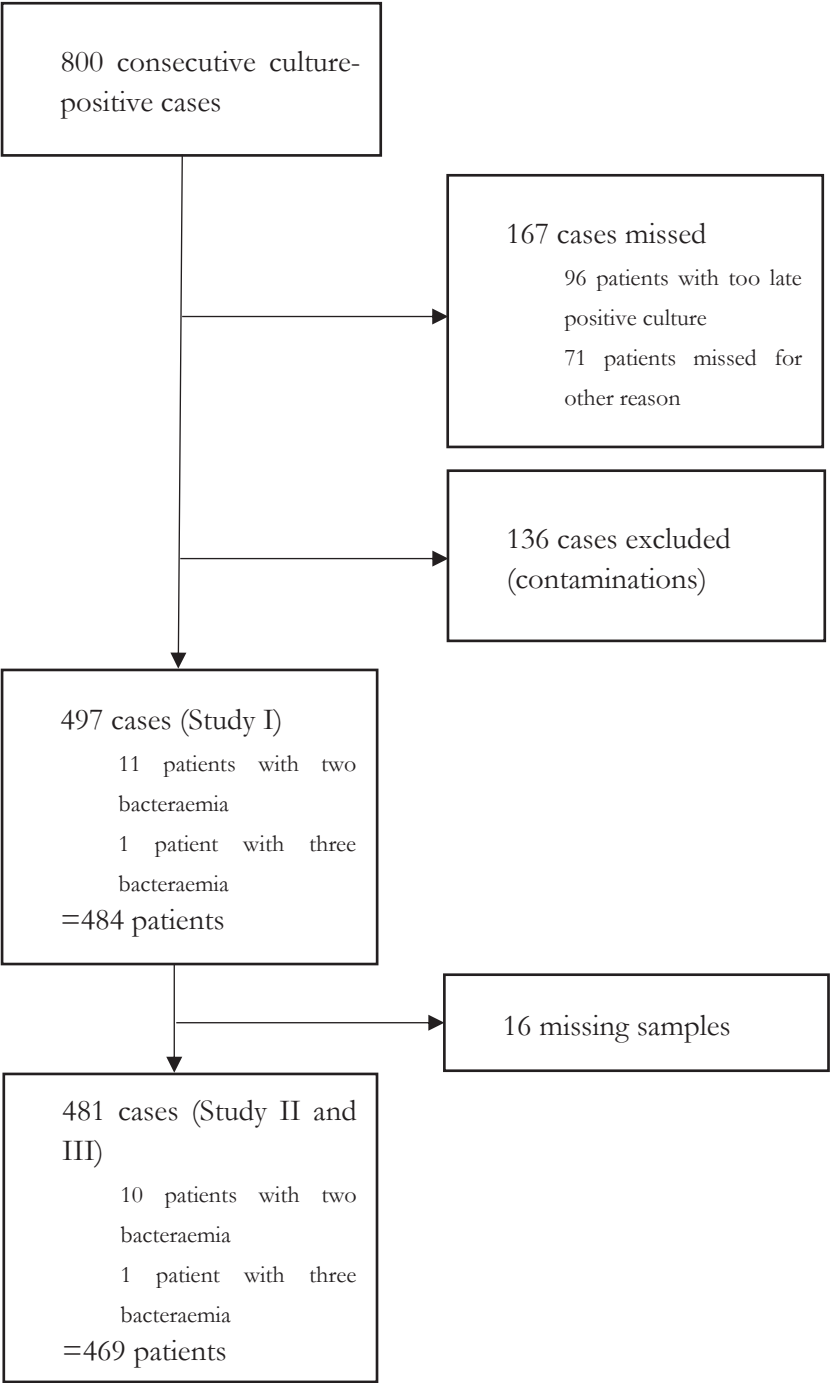
5 RESULTS

5.1 Characteristics of the study patients (Study I and some additional data)

5.1.1 The final cohort

During the study period, there were 800 positive blood cultures in patients over 16 years of age (Figure 1). Contaminants (136) were excluded and 167 were excluded for technical reasons. Eleven patients had bacteraemia twice on different admissions and one patient had bacteraemia three times. Thus, 497 cases of positive blood culture among 484 patients were included in Study I. One box containing 16 samples went later missing, hence the total number of 481 cases in Studies II and III. The final number of the plasma samples were: day 0: 481, day 1: 446, day 2: 389, day 3: 300, day 4: 137. The declination of the plasma samples was caused by various reasons, for example the patient was discharged, the patient died, or routine laboratory tests were not taken on that day.

Figure 1. Patient inclusion in studies I-III



5.1.2 Characteristics

Demographic data, chronic medical conditions and previous medications are presented in Table 2. Of the total study population, median age was 68 years and 53% were male. Majority of the culture-positive cases had some chronic disease, only 11% did not have any chronic medical condition. Furthermore, 19% of cases were on oral corticosteroids.

Table 2. Characteristics of the 497 cases with bacteraemia admitted to the Emergency Department

Characteristics	Data
Demographic	
Cases/Patients	497/484
Men, n (% of cases)	262 (53)
Women, n (% of cases)	235 (47)
Median age, y (range)	68 (16-95)
Chronic medical condition	n (% of cases)
Heart disease	176 (35)
Diabetes mellitus (any type)	139 (28)
Neurological disease	112 (23)
Pulmonary disease	100 (20)
Kidney disease	68 (14)
Rheumatological disease	52 (11)
Alcohol abuse ¹	52 (11)
Substance abuse ²	10 (2)
Solid tumor with metastasis	57 (12)
Haematological malignancy	45 (9)
MRSA carrier	16 (3)
ESBL carrier	13 (3)
Any of the above	426 (86)
Previous medication	n (%)
Oral corticosteroids	94 (19)
Antibiotic treatment on admission	58 (12)
Cancer chemotherapy	56 (11)
Disease-modifying antirheumatic drugs	21 (4)
Biological therapy	10 (2)
Antirejection medication	8 (2)

¹ Social or medical problems of alcohol abuse in the past 12 months

² Social or medical problems of substance abuse in the past 12 months

5.1.3 Causative organisms of blood culture positivity

Table 3 shows the causative organisms of blood culture positivity. Gram-positive and Gram-negative cases were equally common. MRSA and ESBL cases were rare. There were no ESBL-*Klebsiella* cases and only one *Candida* species. Patients who had a polymicrobial infection were mostly gastro-surgical cases.

Table 3. Blood culture findings (n=497)

Organisms	n (%)
Gram positive	225 (45)
<i>Staphylococcus aureus</i>	74 (15)
MRSA	3 (1)
Coagulase-negative Staph.	11 (2)
<i>Streptococcus pneumonia</i>	52 (11)
β -hemolytic streptococci	45 (9)
Viridans streptococci	21 (4)
Enterococci	17 (3)
Other gram positive	5 (1)
Gram negative	223 (45)
<i>E. coli</i>	159 (32)
ESBL- <i>E.coli</i>	9 (2)
<i>Klebsiella</i> sp.	21 (4)
<i>Pseudomonas aeruginosa</i>	18 (4)
Other gram negative	25 (5)
Others	49 (10)
Anaerobes	15 (3)
Fungi	1 (0.2)
Polymicrobial	33 (7)

5.1.4 Site of infection and initial treatment

As presented in Table 4, the most common focus was the urinary tract (26%). The bacteraemia was associated with healthcare in 81 culture-positive cases (16%). Median time from admission to ED to the start of the antibiotic was 174 min (range

from -283 min to 1269 min, data available in 446 cases). The range started from negative minutes as in some cases the antibiotic was started already at the referral site. The median time was 83 minutes in cases that were transferred to ICU. Empiric antibiotic was 2nd generation cephalosporin in 57% of the cases. Nineteen cases (4%) were treated with sepsis corticosteroids and an additional 6% got corticosteroids due to some other indication or the previous corticosteroid dose was adjusted due to the infection.

Table 4. Site of infection and the choice of antimicrobial (n=497)

Variable	n (%)
Site of infection ³	
Unknown	118 (22)
Urinary tract infection	138 (26)
Intra-abdominal	84 (16)
Skin, soft tissue and bones	72 (14)
Lower respiratory tract	54 (10)
Surgical site or foreign body	36 (7)
Others	24 (5)
Antibiotic started on the day of admission	
None	36 (7)
Monotherapy	385 (77)
2 nd generation cephalosporin	241 (48)
3 rd generation cephalosporin	60 (12)
Betalactam and betalactamase-inhibitor combination	34 (7)
Fluoroquinolone	20 (4)
Carbapenem	11 (2)
Other betalactam	9 (2)
Other	10 (2)
Combination therapy	76 (15)
Betalactam and fluoroquinolone	30 (6)
Betalactam and metronidazole	30 (6)
Other combination	16 (3)

5.1.5 Severity of bacteraemia

The clear majority of the culture-positive cases (76%) were treated in general wards and 10% of the cases were transferred to ICU. Furthermore, 76% of the cases that were qSOFA positive were treated in general wards. Forty-four patients (9%) died

³29 cases with two different foci

by day 7, 70 (14%) by day 28, 98 (20%) by day 90, and 143 (30%) within one year. Table 5 shows the severity of the bacteraemia, essential laboratory values and the unit where the case was transferred from ED. All cases had sepsis according to the Sepsis-2 definition. The definition of severe sepsis is different from the definition of positive qSOFA. Nevertheless, in the material 62% of the cases fulfilled both of the definitions, 22% fulfilled neither of them and in 16% there was a discrepancy between the definitions (data for the gathering of qSOFA was available on 473 cases). High (≥ 4) points in Pitt Bacteraemia Score (PBS) was predictive of death. In this study, 39% of patients with high points died by day 7 compared to 26% with positive qSOFA. However, the sensitivity of high PBS points was poor as 47% of deaths by day 7 were missed. The results regarding the predictive value of qSOFA are elaborated in Chapter 5.3.

Table 5. Severity of the bacteraemia, laboratory values, and the unit where the patient was transferred from the ED (n=497)

Variable	n (%)
Severity of the bacteraemia	
qSOFA score $\geq 2^4$	136 (29)
Severe sepsis (≥ 1 organ failure)	152 (31)
Septic shock	37 (7)
Need of vasopressor	51 (10)
Need of mechanical ventilation	26 (5)
Pitt Bacteraemia Score $\geq 4^5$	61 (12)
Laboratory values	
Median day 0 CRP, mg/l (IQR) ⁶	113 (46-218)
Median day 0 leucocyte count, $10^9/l$ (IQR) ⁷	11.7 (7.9-15.8)
Thrombocytes lower than $100 \times 10^9/l$ on days 0-4, n (%)	122 (25)
Case was transferred from ED to	
Intensive care unit	48 (10)
High dependency unit	52 (11)
Other wards	375 (76)
Home ⁸	16 (3)
Patient died in the ED	6 (1)

⁴ Data available on 473 cases

⁵ Data available on 490 cases

⁶ Data available on 494 cases

⁷ Data available on 494 cases

⁸All called the following days, and all but one came back to the hospital. No health deteriorations even though patients were discharged on day 0.

5.2 The role of the underlying diseases in deaths after the bacteraemia (Study I)

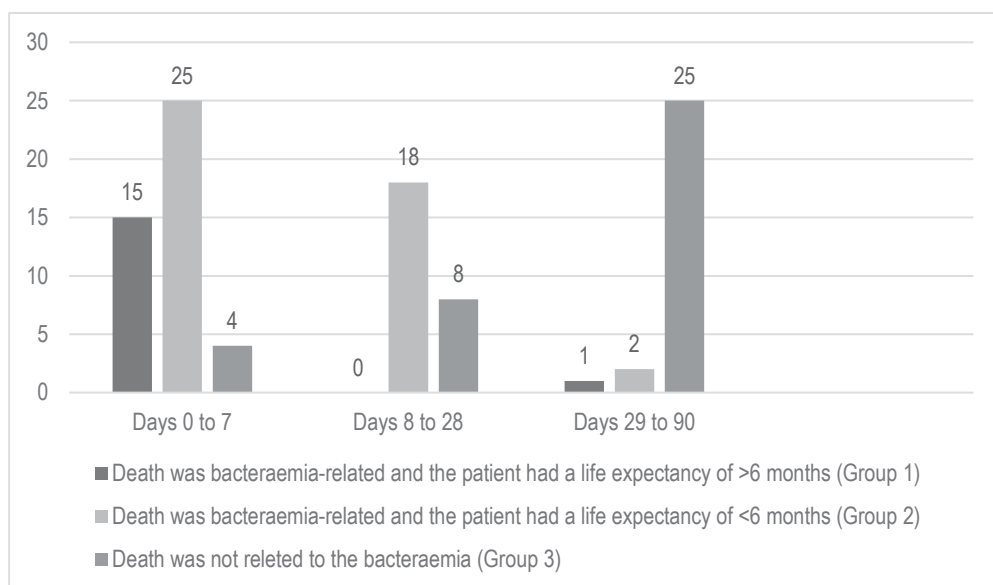
5.2.1 The categorization of the cause of death

As detailed in the methods, 98 patients who died by day 90 after the bacteraemia were categorised into three different groups. Sixteen patients (16%) did not have any rapidly fatal underlying disease and the death was bacteraemia-related (Group 1). For 45 patients, the death was related to the bacteraemia in that it weakened the patient leading to the death, which was in any case expected as the patient had a life expectancy of less than 6 months (Group 2). For 37 patients, death was not related to the bacteraemia as the immediate cause of death was not the bacteraemia nor was the bacteraemia a factor in the chain of events leading to death (Group 3). The median age in Groups 1, 2 and 3 were 77, 78 and 70, respectively. Of those cases that were transferred to ICU (n=48), 21 patients died by day 90. Of these succumbed, 11 patients belonged to Group 1.

5.2.2 The day of death in Groups 1, 2 and 3

As illustrated in Figure 2, the vast majority of the deaths in Groups 1 and 2 (the deaths related to the bacteraemia) happened before day 28 after admittance to the ED. Only three patients (3%) in these groups died on days 29 to 90. Furthermore, all but one patient in Group 1 died within 7 days after admittance to the ED. Nevertheless, the deaths of Group 1 were still a minority of all the deaths by day 7 as 66% of these deaths belonged to Group 2 and 3.

Figure 2. Number of patients (n=98) in Groups 1, 2 and 3 in relation to the day of death



5.2.3 Detailed analysis of patients in Group 1 (additional data)

Sixteen patients were categorised to Group 1. The life expectancy of these patients was evaluated as being more than 6 months without the bacteraemia. Nevertheless, only one patient in this group did not have any underlying disease. Compared to Groups 2 and 3, patients in Group 1 had more frequently both positive qSOFA (81%) and septic shock (56%) and they were the most frequently transferred to ICU (69%). The description of the course of events is detailed in Table 6.

Table 6. The description of the 16 patients whose death was bacteraemia-related and who had a life expectancy of more than 6 months

Age and gender	Day of death	Microbe	Underlying diseases and description on the course of events
51, M	7	<i>Capnocytophaga canimorsus</i>	Alcoholism. Sepsis after a dog bite. 6 days in ICU because of DIC, renal failure, seizure, and hypotension. Transferred to normal ward, found dead the first night. Forensic autopsy records not available.
55, M	0	<i>E. coli</i>	Alcoholism, liver fibrosis, schizophrenia. Found lying in bed with low GCS points. MODS diagnosed, dies in ICU the same day.
55, M	0	<i>Str. pneumoniae</i>	Alcoholism, liver fibrosis, neurocognitive impairment, renal disease. Found lying in bed with low GCS points. Diagnosed in ED with pneumonia and sepsis, dies in ICU the same day.
60, M	0	<i>Staph. aureus</i>	Alcoholism. Cortisone started to symptoms resembling polymyalgia rheumatica. Then vomiting, feeling ill and arrived to ED with renal failure, condition deteriorated and MODS diagnosed, died in ICU the next day.
63, F	39	<i>Str. pneumoniae</i>	Coeliac disease. 39 days in ICU because of persistent MODS and multiple amputations due to severe DIC. Died in ICU.
73, F	0	<i>E. coli</i>	DM2, mentally retarded. Arrived with a difficult dyspnoea and dies in ED. Autopsy revealed severe pulmonary embolism and pyelonephritis. Embolism categorized being secondary to infection.
74, F	0	<i>Bacteroides uniformis</i>	DM2. Carcinoma of the sigmoid and metastasis in regional lymph nodes operated half a year before. Died in operation room due to peritonitis caused by adhesive occlusion of intestine.
76, M	0	<i>E. coli</i>	DM2, nephropathy, metabolic syndrome and colostomy due to operated tubular adenoma of colon years ago. Arrived to ED because of abdominal pain, diagnosed with intestinal perforation, condition deteriorates in operation room, operation ceased and dies shortly after.
78, M	0	<i>E. coli</i>	DM2, heart failure, Alzheimer. Arrived to ED with abdominal pain, diagnosed with perforation of the sigma. Operated, but dies because of renal and cardiac failure in ICU.
78, F	3	<i>Staph. aureus</i>	No underlying diseases. Pain in neck for a week, no fever. Seeks help from acute primary care. Condition deteriorates and gets cardiac arrest. Resuscitated, return of spontaneous circulation in 24 minutes. Transferred through ED to ICU. Diagnosed with anoxic brain damage and dies in ICU. Forensic autopsy records not available.
79, M	0	<i>E. coli</i>	Chronic obstructive pulmonary disease, aortic stenosis and regurgitation. Arrived to regional hospital because of pelvic pain. Diagnosed with urethral stone. Condition deteriorates and

			transferred through ED to ICU. Diagnosed with urosepsis and MODS. Restrained from mechanical ventilation and dialysis due to prior low pulmonary capacity and dies in ICU the same day.
80, M	0	<i>Klebsiella oxytoca</i> , <i>E. coli</i>	Asthma. Arrives to ED with hypotension and MODS. Dies in ED. Clinical suspicion in ED and autopsy records conclude that patient died to cholecystitis and sepsis.
82, F	0	<i>Klebsiella pneumoniae</i>	DM2, previous stroke. Diagnosed in ED with both sepsis and a new cerebral infarct. Dies in ICU. <i>K. pneumoniae</i> was cultured also in urine.
87, F	2	<i>Str. pneumoniae</i>	Asthma, coronary disease. Found lying in home, diagnosed with a cerebral infarct in ED, dies feverish and unconscious in stroke unit. Infarct categorized being secondary to infection.
93, F	4	<i>Enterobacter aerogenes</i>	Arteriosclerosis obliterans, renal disease. First episode of infection a month before, gets <i>Clostridium difficile</i> –diarrhoea, arrives to ED with low GCS-points, diagnosed with urosepsis, dies in a normal ward.
94, F	1	<i>H. influenzae</i>	Asthma, coronary disease, pulmonary embolism. Arrives with dyspnoea to ED, dies in high dependency unit to pneumonia and renal failure.

DIC = Disseminated Intravascular Coagulation, DM2 = Diabetes Mellitus type2, ED = Emergency Department, F= Female, GCS = Glasgow Coma Scale, ICU = Intensive Care Unit, M = Male, MODS = Multiple Organ Dysfunction Syndrome

5.2.4 The main underlying disease associated with death of patients in Groups 2 and 3

Table 7 presents the main underlying disease of the 79 patients in Groups 2 and 3. With 30 patients, solid tumour with metastasis was the most common underlying disease associated with death and haematological malignancy was the second common with 10 patients. Thus, malignancies combined were associated with 41% of all deaths in bacteraemia.

Table 7. The main underlying disease associated with the death of the 79 patients in Groups 2 and 3

Underlying disease	n (%)
Solid tumour with metastasis	30 (38)
Haematological malignancy	10 (13)
Liver disease	10 (13)
Neurological or neurosurgical disease	7 (9)
Heart disease	6 (8)
Diabetes mellitus (any type)	4 (5)
Renal disease	2 (3)
Other	3 (4)
No data / not classifiable	7 (9)

5.3 Cell-free DNA (Study II)

5.3.1 Level of plasma cfDNA on days 0 to 4 after admission to the ED

The day 0 plasma cfDNA was analysed from 481 culture-positive cases (469 patients). The median level on day 0 was 1.38 µg/ml (range 0.75 to 38.85). As detailed in Table 8, the median level was higher on the day of admittance in those who died by day 7 compared to those who survived. Survivors had statistically significantly lower median levels on days 1 to 4 as well.

Table 8. Plasma cfDNA levels on day 0 to 4 after admission to ED in all cases and in relation to death by day 7

Days after admission	Plasma cfDNA (µg/ml), median (quartiles)			P-value
	All (n=481)	Non-survivors (n=44)	Survivors (n=437)	
Day 0	1.38 (1.20-1.77)	2.02 (1.51-2.92)	1.35 (1.19-1.66)	<0.001
Day 1 ⁹	1.36 (1.18-1.73)	2.20 (1.57-3.63)	1.33 (1.17-1.64)	<0.001
Day 2 ¹⁰	1.35 (1.16-1.65)	1.87 (1.59-2.92)	1.33 (1.16-1.61)	<0.001
Day 3 ¹¹	1.33 (1.16-1.61)	1.81 (1.48-4.34)	1.32 (1.14-1.58)	<0.001
Day 4 ¹²	1.35 (1.17-1.71)	1.66 (1.49-2.38)	1.33 (1.15-1.68)	0.017
Maximum value	1.49 (1.28-1.91)	2.24 (1.56-4.55)	1.45 (1.27-1.81)	<0.001

5.3.2 Factors affecting the level of cfDNA

The factors affecting the level of cfDNA on the day of admission were analysed (Table 9). The following factors did not have a statistically significant effect on the level and they were excluded from the table: cardiovascular disease, diabetes, chronic kidney disease, solid tumour with metastasis, Gram-positive bacteraemia, and polymicrobial infection. Patients with haematological malignancy had a level that was statistically significantly lower than in cases without such malignancy (median 1.27 µg/ml, IQR 1.12-1.68, $p=0.041$). The level of cfDNA was higher in cases with a positive marker on the severity of the infection.

⁹ Data available on 446 cases

¹⁰ Data available on 389 cases

¹¹ Data available on 300 cases

¹² Data available on 137 cases

Table 9. The characteristics and underlying conditions that had a statistically significant effect on the level of cfDNA on the day of admission

	Plasma cfDNA (µg/ml) on day of admission to the emergency department		<i>P</i> -value
	factor present, median (quartiles)	factor absent, median (quartiles)	
Characteristics and underlying conditions			
Male	1.42 (1.24-1.85)	1.30 (1.16-1.64)	0.002
Age over 60 years	1.39 (1.22-1.80)	1.32 (1.16-1.60)	0.035
Age over 80 years	1.46 (1.25-1.89)	1.35 (1.18-1.69)	0.020
Liver disease	1.91 (1.41-2.15)	1.35 (1.19-1.67)	<0.001
Alcohol abuse ¹³	1.95 (1.47-2.38)	1.34 (1.18-1.64)	<0.001
Severity of the infection			
qSOFA score ≥2 ¹⁴	1.56 (1.26-2.20)	1.34 (1.16-1.64)	<0.001
Severe sepsis	1.61 (1.31-2.23)	1.31 (1.17-1.60)	<0.001
Septic shock	1.96 (1.32-2.83)	1.36 (1.19-1.70)	<0.001
Admitted from ED to ICU	1.95 (1.40-2.89)	1.35 (1.19-1.69)	<0.001
Pitt Bacteraemia Score ≥4 ¹⁵	1.83 (1.34-2.73)	1.35 (1.19-1.68)	<0.001

5.3.3 The prognostic value of cfDNA in comparison to qSOFA

As presented in Table 8, patients that died by day 7 had significantly higher levels of cfDNA compared to those that survived. CfDNA had an AUC of 0.77 (95% CI 0.69 to 0.85) in predicting death by day 7. QSOFA having AUC 0.77 (95% CI 0.70 to 0.85) was comparable with cfDNA. The cut-off value of cfDNA was estimated using ROC curve (Figure 3). The prognostic values of both the cut-off value of 1.69 µg/ml and positive qSOFA are shown in Table 10. In cases that had both positive qSOFA and cfDNA ≥1.69 µg/ml, the specificity in prediction of death rose to 93%, but the sensitivity declined to 61% (AUC 0.77). This high specificity can be seen in the Kaplan-Meier survival curve presented in Figure 4. Combination of positive qSOFA and cfDNA >1.69 µg/ml resulted in a 20-fold risk of death by day 7 compared to those with negative qSOFA and cfDNA <1.69 µg/ml.

¹³ Social or medical problems of alcohol abuse in the past 12 months

¹⁴ Data available on 458 cases

¹⁵ Data available on 474 cases

Statistically significant variables associated with death by day 7 were liver disease, alcohol abuse, cardiovascular disease, Pitt Bacteraemia Score ≥ 4 , cfDNA level ≥ 1.69 $\mu\text{g/ml}$ and positive qSOFA score. In a multivariable analysis on these factors, liver disease and alcohol abuse did not remain significant; other factors remained.

Contrary to cfDNA, C-reactive protein (CRP) on day 0 did not have a significant predictive value for death by day 7 (AUC 0.52, 95% CI 0.43 to 0.60, data on 478 cases).

Figure 3. Receiver operating characteristic (ROC) curve on day 0 level of cfDNA, qSOFA score ≥ 2 , and level of CRP in relation to death by day 7.

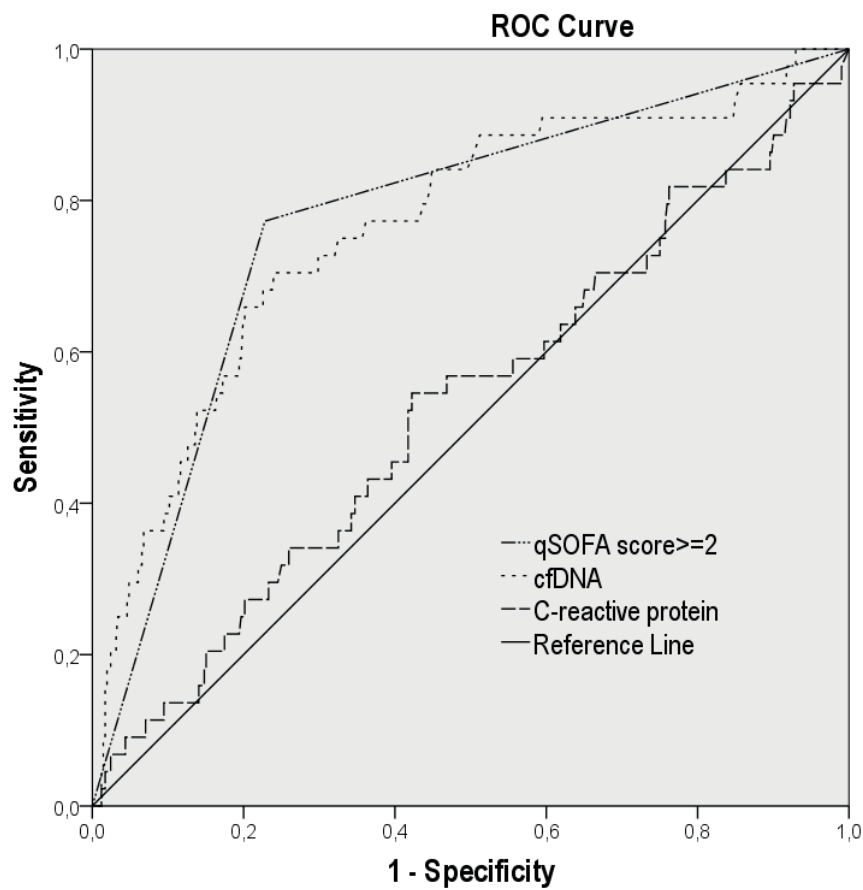


Table 10. Diagnostic values of cfDNA, positive qSOFA, and the combination of them in relation to death by day 7

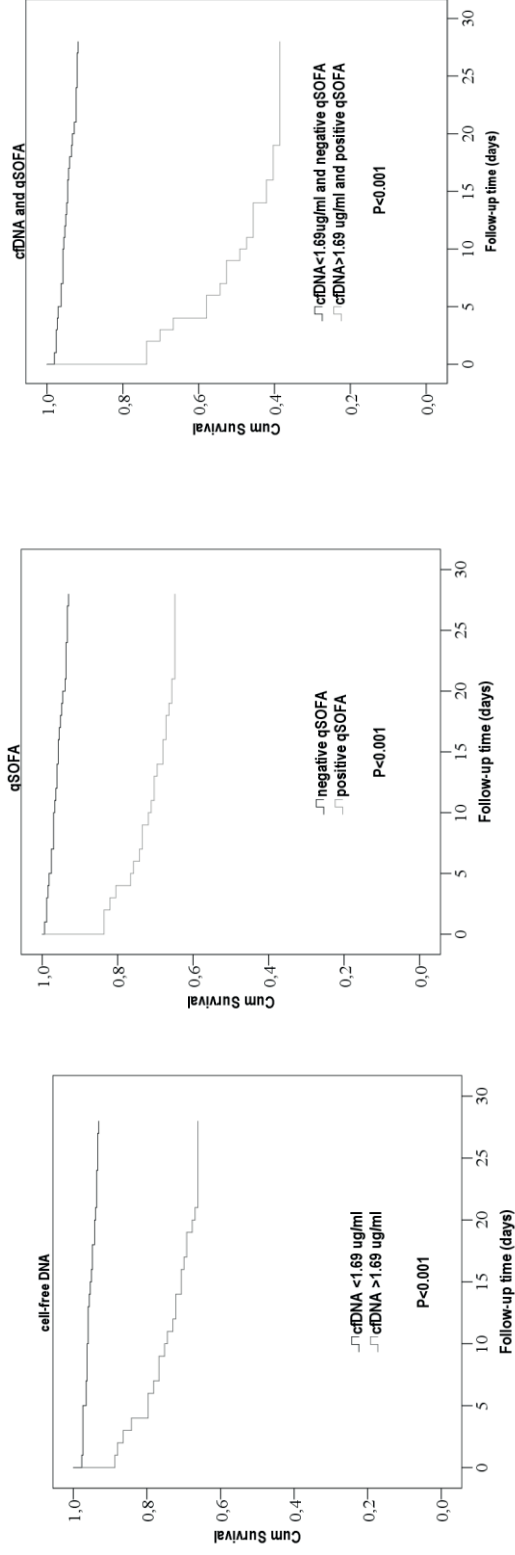
	n (deceased/ survivors)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Odds Ratio 95% CI	AUC with 95%CI	p-values
cfDNA >1.69 µg/ml	134 (31/103)	70.4	76.4	23.1	96.2	7.7 (3.9-15.3)	0.73 ¹⁶ (0.65-0.82)	<0.001
Positive qSOFA ¹⁷	128 (34/94)	77.3	77.3	26.6	97.0	11.6 (5.5-24.3)	0.77 (0.70-0.85)	<0.001
cfDNA >1.69 µg/ml and qSOFA score ≥2 ¹⁸	57 (27/30)	61.4	92.8	47.4	95.8	20.3 (10.0-41.4)	0.77 (0.68-0.86)	<0.001

¹⁶ AUC of cfDNA as a continuous variable was 0.77 (95% CI 0.69 to 0.85)

¹⁷ Data available on 458 cases

¹⁸ Data available on 458 cases

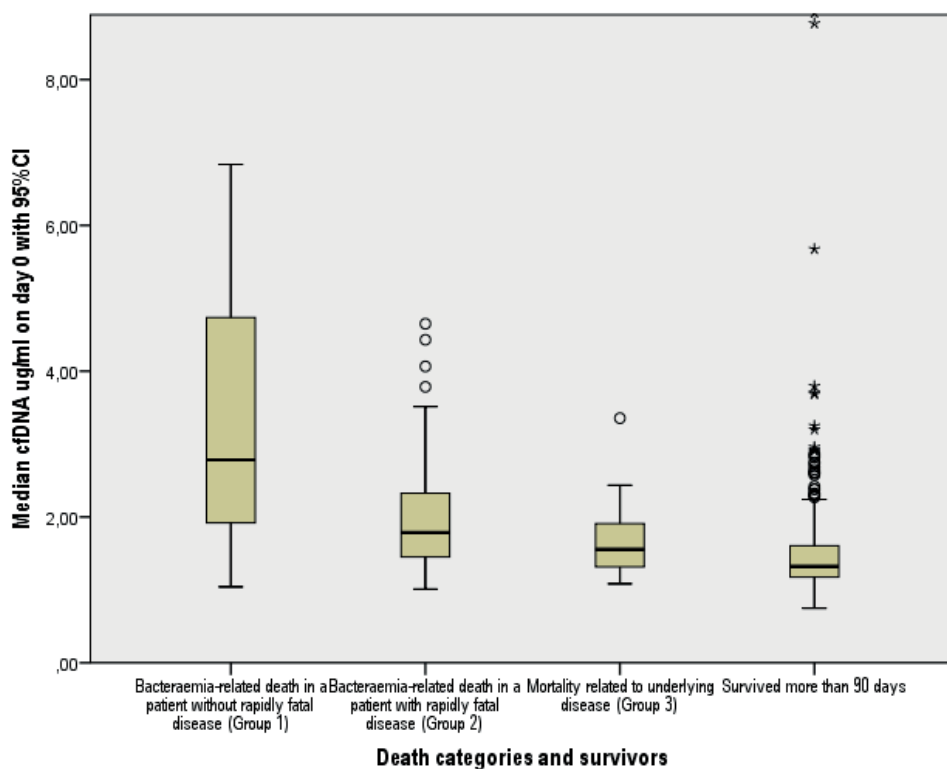
Figure 4. Cumulative day 28 survival in cases with cfDNA > 1.69 µg/ml compared with those with cfDNA ≤ 1.69 µg/ml, qSOFA positive compared with those with qSOFA negative, and cfDNA > 1.69 and qSOFA positive compared with those with cfDNA ≤ 1.69 µg/ml and qSOFA negative.



5.3.4 The level of cfDNA in the three death categories (additional data)

The level of day 0 cfDNA was calculated in the three different categories used in Study I. Among patients who died by day 90, the level was the highest in Group 1 with a median of 2.78 µg/ml (IQR 1.87 to 4.93) and the lowest in Group 3 (median 1.79 µg/ml, IQR 1.43-2.36). The median level was the lowest in survivors. The levels of groups with 95% CI are presented in Figure 5. The difference between the groups is statistically significant ($p < 0.001$).

Figure 5. Median cfDNA levels on day 0 with 95% confidence intervals in the three death categories and in patients that survived more than 90 days



5.3.5 Patients with the highest level of cfDNA

Thrombocyte level was lower than $100 \times 10^9/l$ in 116 cases. These cases had statistically significantly higher level of day 0 cfDNA than cases with level over $100 \times 10^9/l$ (median 1.72 $\mu g/ml$ vs. median 1.33 $\mu g/ml$, $p < 0.001$). Other DIC parameters including fibrinogen, thromboplastin time and D-dimer were not routinely investigated in all study patients. Nevertheless, DIC resulting in amputations was present in three of the top five cases with the highest level of cfDNA on day of admission. The patient with the highest level of cfDNA (38.85 $\mu g/ml$) had a serious pneumococcal infection resulting in multiple amputations. Two other patients in the top five cases had infections caused by *Capnocytophaga canimorsus* with levels of 9.65 $\mu g/ml$ and 8.92 $\mu g/ml$. Their infections also resulted in skin necrosis and amputations. The third and the last patient with bacteraemia caused by *Capnocytophaga canimorsus* also had a high level of cfDNA that was twice the median and in the top five percent of cases (3.05 $\mu g/ml$, median 1.38 $\mu g/ml$).

5.4 PCSK9 (Study III)

5.4.1 PCSK9 levels on days 0-4 after admittance to ED, and levels stratified by different factors

The level of PCSK9 was elevated in bacteraemic patients. The median PCSK9 level on day 0 was 376 ng/ml (IQR 293-483), which is statistically significantly higher compared to the small group of control patients (median 188 ng/ml, IQR 139-264, $p = 0.001$). The concentration of PCSK9 stayed on a high level on days 1 to 4 as well, with medians of 383, 381, 360 and 356 ng/ml, respectively. As presented in Table 11, male cases and cases with liver disease and alcohol abuse had a statistically significantly lower level of day 0 PCSK9. Cases that used statins had a significantly higher level.

Table 11. The level of PCSK9 stratified by different characteristics and underlying conditions (n=481)

Characteristics and underlying conditions	n (%)	Plasma PCSK9 level (ng/ml) on day of admission		P-value
		factor present, median (quartiles)	factor absent, median (quartiles)	
Male	253 (53)	356 (273-472)	405 (310-492)	0.001
Age over 60 years	333 (69)	381 (298-486)	368 (275-478)	0.223
Age over 80 years	107 (22)	379 (304-477)	376 (289-485)	0.804
Cardiovascular disease	155 (32)	388 (299-479)	371 (291-484)	0.868
Diabetes any type	137 (29)	375 (295-468)	379 (292-487)	0.591
Chronic kidney disease ¹⁹	66 (14)	364 (297-440)	379 (292-487)	0.152
Liver disease	49 (10)	284 (176-352)	389 (305-488)	<0.001
Alcohol abuse ²⁰	49 (10)	264 (190-388)	388 (306-489)	<0.001
Solid tumour with metastasis	55 (11)	399 (291-484)	375 (293-482)	0.783
Haematological malignancy	45 (9)	382 (308-485)	376 (291-484)	0.508
Use of oral corticosteroids	92 (19)	415 (306-487)	370 (291-480)	0.125
Use of statins	116 (24)	413 (335-532)	363 (283-468)	<0.001

5.4.2 The level of PCSK9 in relation to the causative bacteria and site of infection

There was no statistically significant difference in the level of PCSK9 between Gram-positive or Gram-negative bacteria (median 381 ng/ml vs. 380 ng/ml, respectively, $p=0.499$). Cases that had bacteraemia caused by *Streptococcus pneumonia* had the highest day 0 level (476 ng/ml, IQR 319-561), and cases with a polymicrobial infection the lowest (311 ng/ml, IQR 235-501). In accordance with the causative microbe, cases whose site of infection was lower respiratory tract had the highest level of PCSK9 compared to other foci (472 ng/ml, IQR 315-557). Cases whose site of infection could not be classified or was unknown had the lowest level (352 ng/ml, IQR 262-474).

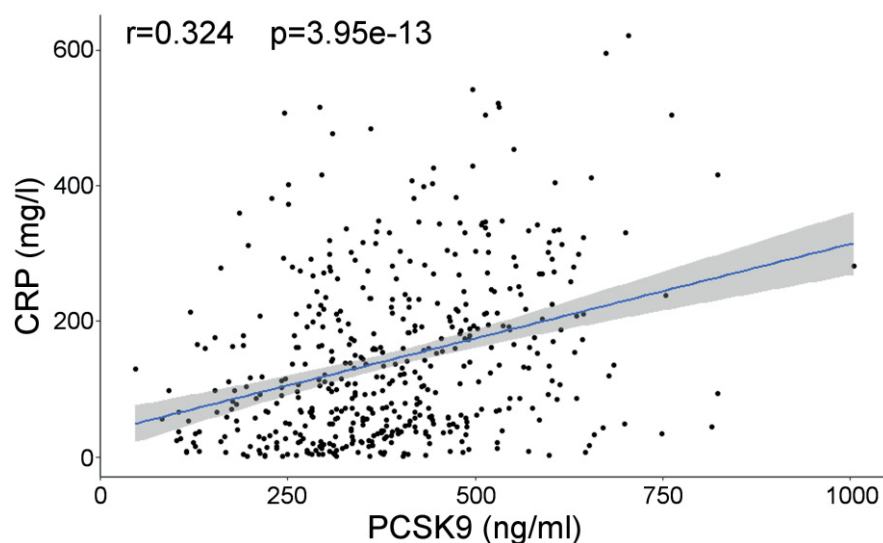
¹⁹ History of creatinine more than 120 $\mu\text{mol/l}$

²⁰ Social or medical problems of alcohol abuse in the past 12 months

5.4.3 PCSK9 as an acute phase reactant

As the level of PCSK9 was higher in this bacteraemia cohort compared to the normal human range, Pearson's product-moment analysis was used to study the possible correlation between PCSK9 and other infection-associated markers. There was no correlation between day 0 level of PCSK9 and day 0 leukocyte number, body temperature, or cfDNA (data not shown), but there was a significant positive correlation to CRP on days 0 to 4 ($p < 0.001$ on all days). The day 0 correlation is illustrated in Figure 6. In bacteraemia caused by *Str. pneumoniae*, the level of PCSK9 and day 0 CRP were the highest of all bacteraemias (PCSK9 median 476 ng/ml, CRP median 212 mg/l).

Figure 6. The correlation between day 0 plasma level of PCSK9 and day 0 C-reactive protein. The shadowed area shows the 95% confidence interval.



5.4.4 The level of PCSK9 in relation to the severity of the bacteraemia and death

As presented in Table 12, PCSK9 level was negatively associated with the severity of sepsis. Nevertheless, among these the only statistically significant indicator was the need of vasopressors ($p=0.026$), and there was a strong trend towards significance in cases that were transferred to ICU ($p=0.05$). One indicator was an exception; cases

with qSOFA score ≥ 2 did not have a lower level but the same as cases with the score < 2 . Patients that died by day 7, day 28 and day 90 all had a statistically significantly lower level of day 0 PCSK9. The 45 patients that died by day 7 had a median level of 306 ng/ml (IQR 257-454), which is still on a higher level than the normal range of 170-220 ng/ml. Patients that died by day 28 and day 90 had a median level of 308 ng/ml and 320 ng/ml, respectively. The exclusion of cases who were using statins did not change these results; the patients that died by day 7 and day 28 had a significantly lower level of PCSK9 nevertheless. In contrast, if cases with liver disease were excluded, the association between low PCSK9 and death by day 7 or day 28 would be insignificant ($p=0.336$ and $p=0.059$).

Table 12. Plasma PCSK9 levels on the day of admission to the emergency department in relation to the severity of the bacteraemia (n=481).

Plasma PCSK9 level(ng/ml) on day of admission				
Severity of the bacteraemia	n (%)	factor present, median (quartiles)	factor absent, median (quartiles)	P-value
qSOFA score ≥ 2 ²¹	128 (28)	378 (263-489)	376 (299-480)	0.564
Need of vasopressor	49 (10)	316 (256-438)	379 (298-486)	0.026
Need of mechanical ventilation	24 (5)	309 (235-593)	377 (395-481)	0.631
Severe sepsis ²²	145 (30)	363 (266-477)	380 (301-486)	0.120
Septic shock ⁴	36 (8)	347 (256-438)	378 (296-486)	0.110
Admitted from ED to ICU	44 (9)	343 (256-431)	379 (297-486)	0.050
Pitt Bacteraemia Score ≥ 4 ²³	58 (12)	344 (262-493)	380 (298-482)	0.323
Death by day 7	45 (9)	306 (257-454)	381 (298-486)	0.022
Death by day 28	71 (15)	308 (242-431)	387 (299-487)	0.001
Death by day 90	101 (21)	320 (249-441)	388 (307-489)	0.001

²¹ Data available on 458 cases

²² According to Sepsis-2 definition

²³ Data available on 474 cases

6 DISCUSSION

6.1 Characteristics of the study cohort

The characteristics concerning age, sex, underlying diseases, and source of infection are similar to what is reported in the literature (Diekema et al. 2003, Lee et al. 2007, Ortega et al. 2007). The all-cause mortality is also similar to other community-onset bacteraemia studies (Lee et al. 2007, Ortega et al. 2007). Distribution of pathogens in this study is slightly different than reported in nationwide statistics as the latter also includes hospital-acquired infections (Jaakola et al. 2018). Nevertheless, two of the most common pathogens are the same (*E.coli* and *Staphylococcus aureus*).

Second generation cephalosporin was the most popular choice of antibiotic (57%, including combination therapies). This antibiotic is in first-line choices in different Finnish guidelines concerning common bacterial foci such as urosepsis and sepsis of unknown focus (Rintala and Karlsson 2017, Urinary tract infections: Current Care Guidelines 2019), and hence the popularity could be justified. MRSA was rare among positive blood-cultures in ED (3 cases) even though at the time of patient gathering it was epidemic in Pirkanmaa region (Jokinen et al. 2015). In this study vancomycin was started to nine cases; more widespread use of empiric vancomycin is not necessary.

The median time before the start of the antibiotic was 174 minutes after arriving to ED. This is a longer time than those reported in sepsis literature (Kumar et al. 2006, Ferrer et al. 2014, Lee et al. 2017). However, one has to remember that there might be publication bias in the reported literature. The median time to the start of antibiotics in culture-positive cases that were transferred to ICU was much less (83 minutes). All the studies that have found that it matters how rapidly the antibiotics are started in the ED are retrospective ones that used complex statistical calculations, and they still might be biased (Klompas et al. 2018). The only prospective study did not find any benefit (Alam et al. 2018). Nevertheless, there should be no delay in ED, especially in cases with septic shock (Liu et al. 2017).

As much as 94 cases (19%) were on oral glucocorticoids upon arrival to the ED. The dose was increased in only 16 cases (3%). Additional 15 cases (3%) got glucocorticoids due to some other indication, such as asthmatic obstruction, and 19

(4%) got hydrocortisone for sepsis. Not all of those who are on oral glucocorticoids need a dose adjustment when they have an infection, but in any case, it needs to be remembered in many of them (van der Goes et al. 2014).

6.2 Sepsis mortality

6.2.1 The new method of categorization and two other methods comparable to it

Even though widely used, the term “sepsis mortality” is vague. Every patient has gone through their unique events before death and sepsis can be a major factor in this story or only a sideshow. It has been known that underlying diseases have an impact on the prognosis of sepsis patients, but the magnitude of this role has been studied less. As an example, it has been known that severe sepsis is a common and deadly complication in cancer patients (Williams et al. 2004). In the current study, a new method was used in which the patients who deceased were categorised into three different groups. Bacteraemia played a major role only in the deaths of patients in Group 1. In Group 2, the bacteraemia was more of a last hit to patients with a poor prognosis, and the bacteraemia had no role in the deaths of Group 3. Group 1 turned out to be the smallest, only 16% of deaths were bacteraemia-related and the patients had a prognosis of more than 6 months. The biggest group (48%) were those whose death was still bacteraemia-related, but these patients had a poor prognosis anyway. In 36% of deaths, bacteraemia was not the immediate cause of death nor a factor in the chain of events leading to death. Among these bacteraemic patients, deaths were associated with cancer in particular; ca. 50% of deaths when solid and haematological cancers were combined. Other examples of deadly underlying diseases were liver disease and neurological disease.

The method has limitations. The stratification of patients was made by clinicians using medical records. Thus, it may be exposed to limited information as well as subjective presumptions of the life expectancy. Only one patient that was stratified to Group 1 was without any underlying disease, and the patients in this Group 1 were as aged as the patients in other groups (median age 77). Hence the stratification should be done by at least two clinicians to improve validity. The method should also be tested in non-bacteraemic sepsis patients before it can be generalized to sepsis patients as a whole.

An almost similar method has been published recently (Kopczynska et al. 2018). The study focused on the attributable fraction of mortality due to sepsis and also stratified the patients into three different groups: “Sepsis-related”; “Possibly sepsis-related” and “Non-sepsis related”. Patients fulfilling the Sepsis-3 criteria both in ED and general wards were included. Instead of clinical judgement, the categorization was based on a pre-specified algorithm which had criteria such as “Death within 14 days of sepsis episode” or “Treated with intravenous antibiotics at the time of index episode”. Out of 166 deaths, 40 deaths were either sepsis-related or possibly sepsis-related. They concluded that 7% (12 cases) of these deaths were directly related to sepsis, a percentage slightly smaller than the percentage of Group 1 in Study I (16%). Of note, 70% of these patients that died had an existing “Do Not Resuscitate” order reflecting serious underlying diseases.

In another recent retrospective cohort study, researchers randomly selected patients that either died in the hospital or were sent to hospice (Rhee et al. 2019). They also used clinicians to review the immediate and underlying causes of death, but also the preventability of the sepsis-associated death using a 6-point Likert scale. They found that sepsis was the most common immediate cause of death and the three most common underlying causes of death were solid cancer, chronic heart disease and hematologic cancer. They concluded that most sepsis-associated deaths were unlikely to be preventable through better hospital-based care. There are exceptions, but in general the same conclusion could be drawn from Study I.

6.2.2 The lack of effect on mortality in clinical sepsis trials

Numerous randomized clinical trials on sepsis treatment have not demonstrated a statistically significant difference in the primary end point (i.e. death) (Marshall 2014). For this reason it has been suggested that other endpoints, such as organ function variables, should be considered instead (Cohen et al. 2015). One explanation for this lack of effect is that sepsis medications tackle only some pathophysiological phenomenon of sepsis, not the pathophysiology of the underlying diseases. In this study, Table 6 presented the healthiest of all bacteraemia patients that died (Group 1 patients). What can be seen from this table is that, with some exception, even the healthiest had plenty of different underlying diseases, and patients sicker than them can be helped even less by a new sepsis drug.

Many of the sepsis trials are conducted in ICUs. There the patient selection is different from other wards; patients should be neither too healthy to benefit of ICU,

nor too sick. In the current study 10% of the cases were transferred to ICU and 44% of these died by day 90. Around half (10 out of 21) of these deceased were categorised to Groups 2 and 3. Hence, the underlying diseases have a major role in the deaths of bacteraemic ICU patients as well.

Furthermore, one has to remember that in some of the deaths that were categorised as belonging to Group 1, sepsis was not the immediate cause of death, only more of an underlying disease. The immediate cause of death was in some cases a complication of sepsis, such as stroke or pulmonary embolism that had occurred already at home. Moreover, 4 out of the 16 patients in Group 1 died of uncontrollable abdominal catastrophe. These are examples of cases in which sepsis medication is probably not of help.

6.2.3 The endpoints of day 7, day 28, day 90 and in-hospital mortality

What endpoint of time to use in sepsis studies has been under debate (Cohen et al. 2001, Vincent 2004, Marshall et al. 2005). There are also discussions that mortality would not always be the best endpoint, but morbidity and quality of life would (Vincent 2004, Marshall et al. 2005). It has been difficult to say at what point death is not related to sepsis anymore (Vincent 2004). This study showed that even death by day 7 after bacteraemia does not mean that the infection is a major factor in the death. In 66% of the deaths by day 7, the major factor in death was the underlying disease and the bacteraemia was only the last hit, if not even that. Even more with deaths by day 28, 79% of the patients belonged to Groups 2 and 3. Lastly, although day 90 mortality is often used as an endpoint in literature, these results show that as much as 84% of the deaths by this day are actually something else than deaths attributable to the infection. The use of day 90 mortality might be justified in the safety issues of new sepsis drugs.

Moreover, the endpoint of in-hospital mortality is a controversial measure, because it may be depending on many factors which are related to hospital policies and not to sepsis itself. As examples, the palliative care might be given either in hospital or at home, and the naming of the facilities that give geriatric care could be either hospital or something else.

6.3 cfDNA

Circulating nucleic acids were discovered already in the 1940s (Mandel and Metais 1948). In this century, the prognostic value of cfDNA in sepsis, stroke and acute myocardial infection has been under investigation. The current study added evidence that cfDNA is of use in bacteraemic ED patients, and the prognostic value is comparable to qSOFA. For example, adding cfDNA level measurement to all patients that have qSOFA score ≥ 2 , a clinician could find patients that are in especially high (20-fold) risk of death. Furthermore, the level was the highest in Group 1 (death category used in Study I) indicating that the infection in itself was a main factor in this elevation, and not that much the underlying diseases.

There are other biomarkers that have been suggested for prognostic use as well. In studies carried out with suPAR, AUC in predicting death by day 28 has been around the same than in this study and in other studies with cfDNA (suPAR 0.79-0.84 vs. cfDNA 0.76-0.84) (Huttunen et al. 2011a, Huttunen et al. 2011b, Uusitalo-Seppala et al. 2012). These results are somewhat better than results from ICU-studies with, for example, secretoneurin, N-terminal pro-B-type natriuretic peptide and chromogranin A which have had AUC in predicting hospital mortality of less than 0.70 (Rosjo et al. 2012, Rosjo et al. 2016).

Recent studies have revealed that cfDNA and DNA-binding proteins appear to have a major importance in the pathogenesis of DIC (Liaw et al. 2016). This study added some evidence that cfDNA is particularly high in septic patients with DIC even though the study could not establish it. Results are indicative that if a patient has a particularly high cfDNA on day 0, there is a chance that the patient might end up having skin necrosis. All three *Capnocytophaga canimorsus* patients had multiple times the median level of cfDNA and a patient with serious pneumococcal infection that ended up in multiple amputations had 20 times the median level. Furthermore, it is also not established whether or not the elevation of cfDNA in patients like these occurs through active secretion from neutrophils or from necrosis. The study investigating the origins of cfDNA in sepsis patients found that it originated from neutrophils (Moss et al. 2018), but in this study the number of cases was small and the possible presence of DIC is not reported.

In general, there are four different categories where biomarkers could be of use: as a diagnostic tool, as a tool for staging of disease severity, for prediction and monitoring of clinical response to an intervention, and as an indicator of prognosis (Selleck et al. 2017, Califf 2018). The best biomarkers are of help in all these categories. Present study, and studies done earlier (Chang et al. 2003, Rainer et al.

2003, Rhodes et al. 2006), have indicated that the (total) level of cfDNA has first and foremost value in prognosis, but further studies might indicate that there could be use in diagnostics and disease severity as well, particularly concerning DIC.

6.4 PCSK9

The bacteraemic patients in this study had approximately twice the level of PCSK9 compared to the normal human range, and this upregulation of PCSK9 continued until day 4. Infections caused by Gram-positive or Gram-negative microbe had the same level even though LPS originating from Gram-negative bacteria has been more commonly linked to PCSK9. A recent study concluded that also LTA originating from Gram-positive bacteria are removed in a LDL-Receptor/PCSK9 dependent manner (Grin et al. 2018), but it could also be that the level of PCSK9 is not dependent on level of these pathogenic lipids.

The level of PCSK9 was statistically significantly higher in pneumococcal infections and lower respiratory infections compared to other microbes and foci. Similarly, the level of CRP was the highest in pneumococcal infections. These results are in line with another result of this study that the level of PCSK9 has a significant positive correlation to CRP. Another way how PCSK9 resembled CRP was that the level was lower in patients with liver disease. Moreover, another study has also suggested PCSK9 as an acute phase reactant as they found a positive correlation with PCSK9 and TNF- α plasma levels in healthy adults (Ricci et al. 2018).

These results were contrasting with some of the previous PCSK9 studies. A Canadian group has presented that the more severe the infection, the higher the level of PCSK9 (Boyd et al. 2016). The results of this study were vice versa; those who had more severe infection had a lower level of PCSK9. The same Canadian group has also presented that patients with PCSK9 loss-of-function genetic variants have improved survival after septic shock (Walley et al. 2014), but another group did not find any protective effect among patients with these genetic variants (Mitchell et al. 2019).

The present study was the first to study mortality and the level of PCSK9 in patients with infection. The study found that the level was significantly lower in patients that died by day 7 and day 28 compared to those that survived the bacteraemia, but still higher than within healthy patients. In other words, reduced plasma PCSK9 response was associated with mortality. As presented in the cfDNA study, CRP does not behave the same way; there is no specific CRP level indicating

increased mortality. The function of CRP is, for example, to activate complement (Thompson et al. 1999), but studies on the function of PCSK9 in infections are still scarce.

6.5 Future considerations

In clinical sepsis trials, some method to get rid of the confounding effect of the underlying diseases is needed so that the effect of sepsis itself would stand out (if existing). For example, in the only prospective study about the timing of the antibiotic (a pre-hospital antibiotic trial), 40% of the patients had a Do Not Resuscitate policy on admission (Alam et al. 2018). Researchers could read the medical records of the patients that were deceased post-hoc and stratify them in groups to find the patients where sepsis was the major factor in death. The results of this subgroup are not that generalizable in the same way as results of a trial with many exclusion criteria, but just a modification of exclusion criteria does not solve the problem. As both the patients and the events before the patients die are unique, pre-specified algorithms are not able to stratify patients to different death categories as well as clinical judgement. The method of using clinical judgment has limitations, but if a post-hoc analysis reveals that the medication (such as antibiotics or activated protein C) or policy (such as timing of the antibiotic) has an effect, further studies could be initiated.

If a future trial establishes that all septic patients with DIC have strongly elevated level of cfDNA, a clinician might already on day 0 find whom to allocate the effort to stop this devastating complication of sepsis. For now, there is no effective treatment to stop the necrosis, but it remains to be seen if this treatment could be, for example, a drug that stops the active secretion of cfDNA from neutrophils or a drug that binds the cfDNA or something else.

Total cfDNA has been shown to have value in the prognosis of the patients. The turnaround time on the laboratory analysis of cfDNA level is approximately 10 min, but it first needs plasma extraction which may limit its use as point-of-care test. It remains to be seen if it will be taken into clinical practise for example in DIC patients. In any case, instead of total cfDNA, there is already one established clinical test that uses cfDNA. Testing foetal cfDNA from maternal blood decreases the need of amniocentesis in the screening of chromosomal deficiencies (Ehrich et al. 2017). This is only one example where the origin of cfDNA could be of clinical use. Especially in cancer research, the so called liquid biopsy is a hot topic (Corcoran and

Chabner 2018). Cell-free circulating tumour DNA (ctDNA) could be of help in all stages of cancer care; in search of a source tissue, in checking the response to treatment etc. Similar to cfDNA, non-coding RNAs (especially microRNAs/miRNAs) are on the rise to diagnostics as well. Together these are called circulating nucleic acids, and the research on them has not stopped at cancer, but continued on to for example heart failure. (Reithmair et al. 2017, Huang et al. 2019). Furthermore, it could be that in the future we might be seeking for a microbial cfDNA from our ED patients. A recent study achieved 94% agreement with blood culture using a next-generation microbial cfDNA sequencing test (Blauwkamp et al. 2019).

The role of both the LDL-receptor and PCSK9 enzyme in clearance of pathogenic lipids was only recently discovered. No definitive answer can be given on the effect of PCSK9 antibody on septic patients before a randomized controlled clinical trial has been performed. They have been safe drugs in cholesterol studies, but even this proof of safety cannot be directly transferred into a different population; i.e. to septic patients.

6.6 Strengths and limitations

The strengths of the study are the relatively high number of cases (497) whose data is not gathered from registries but individually and prospectively, and the including of only culture-positive cases which are more likely to represent true infections than cases with suspected infections that are culture-negative. Another strength is that due to the design of the study, cfDNA and PCSK9 levels could be studied already on day 0.

This study also has some limitations. This was a single-centred cohort concerning only culture-positive cases. These results should not be straightforwardly extrapolated to culture-negative cases with infection. Furthermore, 167 cases were missed due to technical reasons. The potential bias on the material was assessed by reviewing the bacteraemia that were not included in the study. The percentages of the most common bacteraemia (*E. coli* and *Staphylococcus aureus*) did not differ between the study population and the cases that were missed. Among cases included compared to those not included, the percentage of *Str. pneumoniae* was higher (11% vs 4%). This may be due to the short incubation period of *Str. pneumoniae*, which resulted that they were less missed.

7 CONCLUSIONS

The characteristics and outcome of a bacteraemia, the importance of the underlying diseases in deaths of patients with bacteraemia, and the association of cfDNA and PCSK9 with the severity of the bacteraemia can be concluded as follows:

1. Blood culture-positive patient in ED has day 7, day 28 and day 90 all-cause mortality of 9%, 14% and 20%, respectively.
2. Underlying diseases have a major role in bacteraemia mortality. Malignancies are the most common underlying diseases associated with mortality. Patients with bacteraemia who die within 90 days die mostly either of bacteraemia weakening a patient with a rapidly fatal underlying disease, or the underlying disease itself. The bacteraemia-related deaths of patients without rapidly fatal underlying disease occur mostly within 7 days, but they still represent minority in all deaths by day 7.
3. CfDNA, in contrast to CRP, has value in the prognosis of a patient with bacteraemia. Especially when it is combined to positive qSOFA it finds patients with high risk of death. DIC resulting in amputations was common among patients with the highest level of cfDNA.
4. PCSK9 was upregulated in bacteraemic infections. Cases with more severe disease had a lower level of PCSK9 compared to cases with less severe disease. Patients that died had the lowest level, but still higher than the level in healthy patients. PCSK9 resembled an acute phase reactant because the expression was induced in these bacteraemic infections, it correlated significantly with CRP, and the level was lower in cases with liver disease.

8 SUMMARY

In summary, around one third of the cases had qSOFA score of ≥ 2 . Ca. 10% needed care in high dependency units and another 10% were treated in ICUs. The percentage of people that died shortly after the bacteraemia was quite high (9% by day 7), but even the use of this day 7 endpoint does not mean that these patients died of infection. Also, deaths by this endpoint are mostly reflecting the fact that bacteraemia is a common complication of fatal underlying diseases. The endpoint of 90 days should be avoided in sepsis studies as it is almost completely reflecting other deaths than the deaths that are directly related to the infection. The endpoint of in-hospital mortality should be avoided as well.

Both this study and previous results have shown that cfDNA is a prognostic biomarker. Within the current cohort, there were few deaths where infection itself, instead of underlying diseases, played a major role. The main reason why some of these few died was that their bacteraemic sepsis was complicated with DIC. Measuring cfDNA might someday aid clinicians in ED in the diagnosis of this complication.

The current results indicate, in contrast to some of the previous studies, that a higher level of PCSK9 in bacteraemic patients is not associated with more severe disease. In this study those that die after a bacteraemia have a lower level of PCSK9 on the day of admission compared to all bacteraemic patients. It was also showed that PCSK9 resembled acute phase reactants. Studies done with hypercholesterolemia patients have shown that blocking of PCSK9 enzyme lowers the LDL-cholesterol level and reduces the risk of cardiovascular disease. The answer to the question whether or not this blocking improves the survival of sepsis patients remains open.

One explanation why there were so few deaths attributable to sepsis might be that they receive such good care in the study hospital (and probably other hospitals in the western world). Some of the patients die, for example, from the devastating complications of sepsis, i.e. DIC, but otherwise patients that have a prognosis, and have been admitted to ED early enough, survive. In general, there might not be much room for improvement in sepsis survival. However, this will remain so only if there are effective antibiotics in the future as well.

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Ps. Were you those who read only the acknowledgements, or did you have a quick glance at the abstract as well?

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11 APPENDIX

Tiedonkeruulomake

Mikrobiologien osuus

Nimi, löydös, seurantanäyte jne

- 1) Koodi
- 2) ww-numero
- 3) Nimi
- 4) Sosiaaliturvatus
- 5) Aika milloin veriviljelynäyte otettu (muodossa pp.kk.vvvv kk:kk)
- 6) Mikrobin nimi
- 7) Fimlabin käyttämä mikrobikoodi
- 8) Aika milloin ensimmäinen plasmanäyte otettu (sama muoto kuin yllä)
- 9) Milloin 1. seurantanäyte otettu
- 10) Milloin 2. seurantanäyte otettu
- 11) Milloin 3. seurantanäyte otettu
- 12) Milloin 4. seurantanäyte otettu
- 13) Milloin 5. seurantanäyte otettu
- 14) Mikrobiologien huomautukset

Kliinikon osuus

Perustiedot

- 15) Ikä veriviljelyn ottohetkellä
- 16) Sukupuoli
0 Mies
1 Nainen

Perussairaudet (0=ei, 1=kyllä)

- 17) Potilaan sairauskertomuksessa maininta muistisairaudesta, oireisesta aiemmasta aivoinfarktista tai aivoverenvuodosta tai muusta etenevästä aivosairaudesta kuten MS-taudista
- 18) Krooniset vaskulaariset sairaudet MCC, ASO, IC (ei pelkkä HA)
- 19) Tunnettu läppävikä, tekoläppä tai aiempi endokardiitti
- 20) Pitkäaikainen keuhkosairaus ml. astma, COPD, fibroosit jne
- 21) Pitkäaikainen reumatautisairaus ml. RA, SLE jne
- 22) Pitkäaikainen maksasairaus mm. kirroosi, PBC, NASH, mutta ei pelkkä virushepatiitti

- 23) Pitkäaikainen munuaissairaus: HD- tai PD-hoidossa, krea edeltävästi yli 120 tai dU-Prot yli 1 g
- 24) Potilas sairastaa DM ja mitä tyyppiä (pl. IGT tai IFG)
 - 0 Ei
 - 1 DM tyyppi 1
 - 2 DM tyyppi 2
 - 3 DM tyyppi epäselvä tai muu (esim LADA)
- 25) Alkoholin aiheuttamia sosiaalisia tai medisiinisiä ongelmia viimeisen 12 kk aikana
- 26) Muiden päihteiden (mm. kannabis, buprenorfiini, amfetamiini, bentosodiatsepiini) aiheuttamia sosiaalisia tai medisiinisiä ongelmia viimeisen 12 kk aikana
- 27) Potilas sairastaa tai on sairastanut solidia maligniteettia
- 28) Jos kyllä, onko maligniteetti metastasoitunut paikallisia imusolmukkeita pidemmälle?
- 29) Jos kyllä, mikä maligniteetti kyseessä? (vapaa teksti)
- 30) Potilas sairastaa tai on sairastanut hematologista maligniteettia
- 31) Tiedossa oleva MRSA kantaja edeltävästi
- 32) Tiedossa oleva ESBL kantaja edeltävästi
- 33) McCabe luokitus
 - 0 Terve
 - 1 Ei-fataali perussairaus
 - 2 Ultimately fatal (ennuste 0,5-5 v)
 - 3 Rapidly fatal (ennuste <0,5 v)
- 34) Muut olennaiset tiedot: elin- tai kantasolusiirto, HIV, plasmafereesi, KPC tmv kantaja, perna poistettu, hypogamma, tiedossa oleva infektoitunut vierasesine jne (vapaa teksti)

Lääkitys (0=ei, 1=kyllä)

- 35) Potilas on saanut solunsalpaajalääkitystä tai immunosupressanttia (siirännäispotilaat) edeltävän 12 kk aikana
- 36) Potilas on saanut biologista lääkitystä edeltävän 12 kk aikana (TNF-salp ja kaikki muut vastaavat)
- 37) Potilaalla oli sairaalaan tullessa kortisonilääkitys, joka vastaa > tai = 5 mg prednisolonia
- 38) Potilaalla oli sairaalaan tullessa jokin muu DMARD-lääke kuin yllämainitut
- 39) Potilaalla oli antibiootti (ml. sieni- ja viruslääkitys) sairaalaan tullessa
- 40) Jos kyllä, mikä antibiootti oli kyseessä (kirjoita numero)
 - 1 Amoksisilliini, ampisilliini, pivmesillinaami
 - 2 Fenoksimetyylipenisilliini ja vastaavat iv/im muodot
 - 3 Kloksasilliini ja vastaavat
 - 4 Amoxin comp ja vastaavat
 - 5 Tazocin ja vastaavat
 - 6 1. polven kefalosporiini
 - 7 2. polven kefalosporiini
 - 8 3. polven kefalosporiini
 - 9 Karbapeneemi
 - 10 Trimetopriimi

- 11 Sulfonamidin ja trimetopriimin yhdistelmä
- 12 Makrolidi
- 13 Klindamysiini
- 14 Aminoglykosidi
- 15 Fluorokinoloni
- 16 Vankomysiini
- 17 Metronidatsoli
- 18 Nitrofurantoiini
- 19 Linetsolidi
- 20 Daptomysiini
- 21 Sienilääkitys
- 22 Viroslääkitys
- 23 Tetrasykliini, doksisykliini, tigecycliini
- 41) Jos enemmän kuin yhden ab käyttö, mikä on tämä toinen valmiste (samat numerot kuin yllä)
- 42) Muuta olennaista tai huomioitavaa lääkityksestä (esim saanut kasvutekijää tai IVIG-lääkitystä edeltävästi tai APC:tä hoidon aikana) (vapaa teksti)
- 42A) Potilaalla on merkintä statiinin käytöstä ensiapuun tullessa
- 42B) Potilaan statiini oli
 - 1= Simvastatiini
 - 2= Atorvastatiini
 - 3= Pravastatiini
 - 4= Rosuvastatiini
 - 5= fluvastatiini
- 42C) Potilaan statiinin annos (milligrammoissa)

Tietoja Acutan käynnistä, erityisesti keltainen kaavake

- 43) Mistä potilas tulee
 - 0 Kotoa
 - 1 Palvelutalosta, vanhainkodista tms
 - 2 Tk-vuodeosastolta tai muusta sairaalasta
 - 3 Epäselvää
- 44) Tulohetki Acutaan (muodossa pp.kk.vvvv kk:kk)
- 45) Matalin Acutan tulopäivän systolinen verenpaine (mmHg)
- 46) Ym. syst verenpaineen diastolinen arvo (mmHg)
- 47) Jos ym syst arvo on >90 eikä vasopressoreiden tarvetta onko potilaalla silti yli 30 mmHg syst lasku ja yli 20 mmHg diast lasku
- 48) Potilaan korkein mitattu lämpö Acutan tulopäivänä (lukema yhden desimaalin tark)
- 49) Jos potilaalla oli alle 36 asteen lämpö, mikä oli lukema (lukema yhden desimaalin tark)
- 50) Potilaan korkein mitattu hengitystiheys Acutan tulopäivänä
- 51) Potilaan korkein mitattu syke Acutaan tulopäivänä (lukema)
- 52) Jos potilaalla oli mitattu a-astrup tulopäivänä, mikä oli pCO₂ (X,XX kPa) spontaanienhengityksellä

- 53) Jos potilaalla oli mitattu a-astrup tulopäivänä, mikä oli laktaatti (X,X mmol/l)
- 54) Potilaan henkinen tila Acutan tulopäivänä
 0 Orientoitunut (GCS puheosuus 5/5 pist)
 1 Desorientoitunut (GCS puheosuus 4/5 pist)
 2 Tokkurainen (=Stuporous, alentunut tajunta, ääntelyä tai irrallisia sanoja, 2-3/5 pist)
 3 Tajuton (=Comatose, ei ääntelyä, 1/5 pist)
- 55) Oliko potilaalla neutropenia (alle 1 10E9/l) Acutaan tulopäivänä
- 56) Acutaan tulopäivänä potilaalla sairauskertomuksessa merkintä
 -Kirjavasta ihosta
 -Heikentyneestä kapillaaritäytöstä
 -DIC:stä
 -Heikentyneestä virtsan tulosta <0,5 ml/kg
 -ARDS:stä
 -Sydämen uudesta systolisesta dysfunktioista
 -Akuuttista tajunnan häiriöstä
 -EEG häiriöstä
 -Laktaatti on yli sairaalan viitearvon
 -Trombopenia alle 100
- 57) Jos kyllä, mikä/mitkä oli(vat) kyseessä tai muu olennainen statuslöydös esim MOF tai sydänpysähdys ilman menehtymistä (vapaa teksti)
- 58) Täyttyykö potilaalla septinen shokki –kriteeri: Persistoivasti syst RR alle 90 TAI MAP alle 60 mmHg TAI yli 40 mmHg lasku perustasosta JA Lasku asianmukaisesta nesteresuskitaatiosta huolimatta JA Ei muita syitä verenpaineen laskulle
- 59) Annettiinko potilaalle sepsiksen tai sen epäilyn vuoksi kortisonia
 0 Ei
 1 Iv-hydrokortisonia sepsiksen vuoksi
 2 Aiemman kortisoniannoksen nosto
 3 Po- tai iv-kortisonia muulla indikaatiolla esim. heng ahd
- 60) Potilaalle aloitettiin antibiootti tulotekstin tai keltaisen kaavakkeen perusteella tulopäivänä
- 61) Jos kyllä, mikä ab oli kyseessä (kirjoita numero, samat kuin kohta 40)
- 62) Jos potilaalle aloitettiin toinenkin antibiootti, mikä ab alkoi (nro)
- 63) Jos kyllä, mikä oli ensimmäisen antibiootin aloitushetki (muodossa pp.kk.vvvv kk:kk)
- 64) Mihin potilas siirtyi Acutasta (ml. PTO), kirjoita numero (kirjoita 1, jos pt siirtyi nopeasti vo tai valv kautta teholle)
 1 Teho
 2 KARA, KARB, SPÄI-valvonta, KEI1-valvonta, GAS-valvonta, VALS-valvonta
 3 Muu vuodeosasto
 4 Kotiin
 5 Kuoli ensiavussa

Tietoja jatkotapahumista

- 65) Potilas joutui respiraattoriin

- 66) Potilas joutui infektion non-invasiiviseen ventilaatioon (ei aiempaa kotikäyttöä)
- 67) Potilas tarvitsi noradrenaliinia, adrenaliinia, dopamiinia tai dobutamiinia 30 vrk sisään viljelystä
- 0 Ei
- 1 Noradrenaliinia
- 2 Dopamiinia
- 3 Dobutamiinia
- 4 Kombinaatiota, muuta tai epäselvää, mitä sai
- 68) Potilas joutui dialyysiin, kehitti DIC:n, ARDS:n, MOF:n tai muun vakavan infektiokomplikaation 30 vrk:n kuluttua viljelystä (empyeemat tmv abcessit pois lukien)
- 69) Jos potilas menehtyi, mikä oli kuolinpäivä (muodossa pp.kk.vvvv)

Pohdinta

- 70) Kliinikon arvio infektiotokuksesta jälkeensä CDC nosocomial jaottelu pl. disseminoitunut, BSI ja bronkiitti ja lisättyä avoin/tuntematon
- 0 Avoin/tuntematon
- 1 Virtsatiet
- 2 Kirurgisen haavan infektio, ml vierasesineiden infektiot ja prostatabiopsiat
- 3 Pneumonia
- 4 Luiden, bursien ja nivelten infektio, ml epidur abc
- 5 Hermoston infektiot
- 6 Cardiovaskeettisen systeemin infektiot, ml endokardiitti, mediastiniitti ja suonten infektiot
- 7 KNK, SUU tai SIL-alan infektiot, ml sinuiitti, faryngiitti
- 8 GI-alan infektiot ml. suolisto, sappitieperäinen ja peritoniitti
- 9 Lisääntymiselinten infektiot
- 10 Iho, ml. ruusu, selluliitti, nekrotisoiva faskiitti, mastiitti ja ihonalainen paise
- 71) Kliinikon arvio toisesta infektiotokuksesta (samat numerot kuin yllä)
- 72) Kliinikon arvio onko kyseessä terveydenhuollon toimintayksikön antamaan hoitoon liittyvä infektio
- 73) Muuta erityistä huomioitavaa tästä potilaasta (vapaa teksti)

Laboratoriokokeet

Vv-hetki on päivä 0 ja 1. 2. 3. ja 4. seuranta päivä

- 74) Päivän 0 ensimmäinen kokonaisleukosyyttimäärä
- 75) Päivän 1 ensimmäinen kokonaisleukosyyttimäärä
- 76) Päivän 2 ensimmäinen kokonaisleukosyyttimäärä
- 77) Päivän 3 ensimmäinen kokonaisleukosyyttimäärä
- 78) Päivän 4 ensimmäinen kokonaisleukosyyttimäärä
- 79) Onko potilaalla päivinä 0-4 trombosyytit alle 100
- 0 Ei
- 1 Kyllä
- 2 Ei tutkittu

- 80) Päivän 0 ensimmäinen CRP-arvo
- 81) Päivän 1 ensimmäinen CRP-arvo
- 82) Päivän 2 ensimmäinen CRP-arvo
- 83) Päivän 3 ensimmäinen CRP-arvo
- 84) Päivän 4 ensimmäinen CRP-arvo
- 85) Onko potilaalla päivinä 0-4 laktaatti yli sairaalan viitealueen
 - 0 Ei
 - 1 Kyllä
 - 2 Ei tutkittu
- 86) Muuta huomioitavaa laboratoriotutkimuksista (vapaa teksti)
- 87) Päivien 0-4 ensimmäinen prokalsitoniini
- 88) Milloin prokalsitoniini otettu

12 PUBLICATIONS

PUBLICATION

I

Sepsis-related mortality in 497 cases with blood culture-positive sepsis in an emergency department

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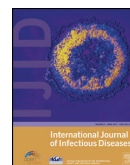
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Sepsis-related mortality in 497 cases with blood culture-positive sepsis in an emergency department

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SUMMARY

Objective: Few studies have sought to establish how often death after sepsis is related to the sepsis and how often underlying diseases have a major role in case fatality.

Methods: In this retrospective cohort study, data were collected on 497 cases with blood culture-positive sepsis in an emergency department (ED).

Results: Sepsis was categorized as severe in 31% of cases; 7% had septic shock. The quick Sepsis-related Organ Failure Assessment score was positive in 136 out of 473 cases (29%). Ninety-eight patients died by day 90; in 16 of these cases (16%) the death was sepsis-related in a patient without a rapidly fatal underlying disease, in 45 cases (46%) the death was sepsis-related in a patient with a rapidly fatal underlying disease, and in 37 cases (38%) the death was unrelated to sepsis. Sepsis-related death occurred in 58 out of 61 cases (95%) by day 28.

Conclusions: Underlying diseases were found to have a considerable role in the death of patients suffering from blood culture-positive sepsis in an ED of a developed country, as only 16% of the deaths by day 90 occurred where death was sepsis-related and the patient had a life-expectancy of more than 6 months. Improving the outcome of sepsis with new treatments is thus challenging. It is possible that day 7 + day 28 mortality is a more appropriate endpoint than day 90 mortality when studying the outcome of sepsis, as this time-span includes most of the patients whose death was related to sepsis.

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Introduction

Sepsis is one of the leading causes of death worldwide. It has been ranked as the eleventh most common cause of death in the USA.¹ Advanced age, immunosuppression, diabetes, and cancer are major risk factors for sepsis.^{2–6} Prognostic factors for the severity and outcome of sepsis include advanced age, type of infection (e.g., methicillin-resistant *Staphylococcus aureus* (MRSA), polymicrobial), number of organ dysfunctions, and adequacy of antimicrobial therapy.^{7–9}

Studies have been performed on sepsis patients in emergency departments (EDs) and intensive care units (ICUs) in order to determine risk factors for mortality^{9–13} and the aetiology of illness.¹⁴ Less attention has been paid to the questions of how often these patients actually die of sepsis, how often sepsis is a

contributory factor in the death of a patient with an advanced underlying disease, and how often the death is independent of sepsis.

In this retrospective cohort study, data were collected on 497 adult cases of blood culture-positive sepsis in the ED of Tampere University Hospital (TAUH). A categorization of causes of death was developed in order to establish how often death was related to sepsis in patients without a rapidly fatal underlying disease (group 1), was related to sepsis by weakening of a patient with a rapidly fatal underlying disease (group 2), and was independent of sepsis but caused by the underlying disease (group 3). It was also sought to determine the best cut-off among the commonly used days for mortality used in sepsis research, i.e. day 7, day 28, or day 90, for mortality related to sepsis (deaths in groups 1 and 2).

Materials and methods

TAUH is a tertiary hospital with a catchment population of approximately 524 700 inhabitants in Pirkanmaa County. The ED of

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the hospital handles patients requiring both basic and specialized emergency care. In specialized care, the majority of patients are internal medicine and surgical patients. Blood cultures are taken routinely from patients with signs or symptoms of systemic infection. The population of the present study comprised 497 adult patients admitted to the ED of TAUH and treated in specialized care, who had blood culture-positive sepsis during the period March 1, 2012 to February 28, 2014. The study was approved by the Ethics Committee of Tampere University Hospital. The need for informed consent was waived, as no additional blood sampling was needed and routine patient care was not modified.

TAUH has a 24-bed ICU which includes a seven-bed high dependency unit (HDU). In this article, 'ICU' refers to both of these. There are four other HDUs (cardiology, pulmonary, surgical, and internal medicine), all taking care of sepsis patients; these are referred to as 'HDUs'.

Blood cultures were collected in BacT/Alert Aerobic (FA Plus) and Anaerobic (FN Plus) blood culture bottles and placed in the automated microbial detection system BacT/Alert 3D (bioMérieux, Marcy l'Etoile, France). All patients with a positive blood culture obtained during specialized care in the ED were identified in the microbiology laboratory serving TAUH (Fimlab Laboratories plc). Patient details, the name of the organism, and the date of blood culture were collected by clinical microbiologists (T.S. and J.A.). Cultures positive for coagulase-negative *Staphylococcus*, *Propionibacterium*, *Micrococcus*, *Bacillus*, and *Corynebacterium*, with detection in a single blood culture bottle and without clinical relevance, were considered to be contaminants and were excluded.

Patients whose routine blood samples taken on admission were no longer available for further studies were also excluded. This could be due, for example, to the fact that the blood culture became positive later than 72 h after the day of admission.

The clinical data for the patients included in the study were gathered retrospectively from the patient records by the principal investigator (J.R.). The site of infection was decided retrospectively by the principal investigator based on clinical judgement. Sepsis was deemed to be healthcare-associated if the symptoms had started more than 48 h after admission to a healthcare institution, or the bacteraemia was related to a surgical operation within the preceding 30 days or some other invasive procedure within the previous 10 days.¹⁵ Data on cause of death were gathered from patient records (and autopsy records when applicable) by two clinicians (J.R. and R.H.) independently. In cases of discrepancy, a meeting was held together with a third clinician (J.S.) and a final decision was made.

Cause of death by day 90 was classified into three different categories: (1) group 1 included cases of sepsis-related mortality in patients without a rapidly fatal underlying disease. The immediate cause of death in this group was sepsis, or sepsis was a factor in a chain of events leading to death, and the patient had a life-expectancy of more than 6 months. (2) Group 2 included cases of sepsis-related mortality in patients with a rapidly fatal underlying disease. In this group, the patient died of sepsis (immediate cause of death, or sepsis was a factor in a chain of events leading to death) by weakening of a patient with a rapidly fatal (<6 months) underlying disease. (3) Group 3 included cases of mortality related to underlying disease. In this group, sepsis was not an immediate cause of death or a factor in a chain of events leading to death.

The categorization was based on clinical decision and the judgement was based on the severity of the patient's underlying disease, the patient's pre-performance, severity of the sepsis, and recovery after the infection. The main underlying disease associated with death was determined retrospectively by the principal investigator.

Diagnoses of sepsis, severe sepsis, and septic shock were made according to consensus definitions.¹⁶ Further, the quick Sepsis-

related Organ Failure Assessment score (qSOFA) was calculated post-hoc according to recently published definitions.¹⁷ The qSOFA score was positive if at least two of the following three criteria were fulfilled in the ED: respiratory rate ≥ 22 /min, altered mentation, and systolic blood pressure ≤ 100 mmHg. The McCabe classification was determined as reported by McCabe and Jackson.¹⁸ The Pitt bacteraemia score was calculated as presented by Korvick et al.¹⁹ IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. Categorical data were analyzed by Chi-square test, or Fisher's exact test when appropriate. Odds ratios (OR) with 95% confidence intervals (95% CI) are also presented.

Results

There were 800 consecutive positive blood cultures in adult patients during the study period. One hundred and thirty-six of these were considered to be contaminants and 167 were excluded for other reasons. A total 497 cases of positive blood culture among 484 patients were thus included. All 497 cases had sepsis. During the study period, 11 patients had sepsis twice on different admissions and one patient had sepsis three times. Of the total study population, 262 (53%) were male and 235 (47%) were female; they ranged in age from 16 to 95 years (median 68 years).

Table 1 provides the demographic data, data on the causative organisms, and data on the underlying diseases stratified into six different categories: groups 1, 2, and 3 (as noted in the Materials and methods section), all patients who died, all patients who survived, and all cases. For 16 out of 98 patients (16%) who died by day 90, death was related to sepsis and the patient did not have a rapidly fatal underlying disease (group 1), i.e. 3% of all 497 sepsis cases. For 45 patients (46% of all deaths by day 90), death was related to sepsis in that it weakened the patient leading to the death, which was in any case expected as the patient had a rapidly fatal underlying disease (group 2). For 37 patients (38%), death by day 90 was unrelated to sepsis and was caused by an underlying disease(s) in the patient (group 3).

Of the group 1 patients, four (25%) had alcohol abuse as an underlying disease (Table 1). One was without any underlying disease. Four of the patients in group 1 were over 80 years of age (25%). Three group 1 patients (19%) were found lying at home with a low level of consciousness and were transferred to hospital. In group 2 patients, the rapidly fatal underlying disease was a solid tumour with metastasis in eight cases (18%) and a haematological malignancy in nine (20%). In group 3 patients, the cause of death was a solid tumour with metastasis in 19 patients (51%) and a haematological malignancy in two (5%). Thus, malignancies were associated with death in 46% of patients in groups 2 and 3 combined. Other common rapidly fatal underlying diseases associated with death among the patients in groups 2 and 3 were liver disease (11%), heart disease (11%), and neurological/neurosurgical disease (10%).

The case fatality rate by day 7, day 28, and day 90 was 9%, 14%, and 20%, respectively. Death occurred by day 7 in 94% of group 1 patients, in 56% of group 2 patients, and in 11% of group 3 patients (Figure 1). All except two cases in group 2 died before day 28. The deaths in group 3 occurred most often between day 29 and day 90 (68%). Ninety-five per cent of all sepsis-related deaths occurred within 28 days after sepsis.

Table 2 gives data on the severity of sepsis stratified into the same categories as used in Table 1. The qSOFA was positive in 136 out of 473 cases (29%). Group 1 had the highest Pitt bacteraemia scores. Forty-eight (10%) cases were transferred from the ED to the ICU and 52 (11%) to HDUs. Thus, the majority of sepsis patients and the majority of qSOFA-positive cases were taken care of in general wards. Out of 449 cases who were treated outside the ICU, 104

Table 1
Characteristics underlying diseases, and causative organisms in relation to the outcome.

	Sepsis-related mortality in patients without a rapidly fatal underlying disease (Group 1) (n = 16)	Sepsis-related mortality in patients with a rapidly fatal underlying disease (Group 2) (n = 45)	Mortality related to underlying disease (Group 3) (n = 37)	All patients who died (n = 98)	Patients who survived (n = 399)	All cases (N = 497)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Median age, years (range)	77 (51–94)	78 (42–93)	70 (44–91)	74 (42–94)	68 (16–95)	68 (16–95)
Male sex	8 (50)	24 (53)	25 (68)	57 (58)	205 (51)	262 (53)
Underlying diseases						
Heart disease	8 (50)	23 (51)	10 (27)	41 (42)	135 (34)	176 (35)
Diabetes mellitus, any type	6 (38)	15 (33)	13 (35)	34 (34)	105 (26)	139 (28)
Neurological	5 (31)	14 (31)	9 (24)	28 (29)	84 (21)	112 (23)
Solid tumour with metastasis ^a	1 (6)	10 (22)	17 (46)	28 (29)	29 (7)	57 (12)
Alcohol abuse ^b	4 (25)	5 (11)	8 (22)	17 (17)	35 (9)	52 (11)
Liver disease	3 (19)	8 (18)	6 (16)	17 (17)	32 (8)	49 (10)
Haematological malignancy	0 (0)	11 (24)	4 (11)	15 (15)	30 (8)	45 (9)
No underlying diseases	1 (6)	0 (0)	0 (0)	1 (6)	70 (14)	71 (14)
Causative organism ^c						
Gram-positive	5 (31)	18 (40)	13 (35)	36 (37)	189 (47)	225 (45)
Gram-negative	9 (56)	19 (42)	14 (38)	42 (43)	181 (45)	223 (45)
<i>Escherichia coli</i> urosepsis	3 (19)	6 (13)	3 (8)	12 (12)	85 (21)	97 (20)
Polymicrobial and anaerobes	2 (13)	8 (18)	10 (27)	20 (20)	28 (7)	48 (10)

^a The most common solid tumour with metastasis was cancer of the gastrointestinal tract (36 cases).

^b Social or medical problems of alcohol abuse in the past 12 months.

^c Excluding fungi (n = 1).

(23%) had severe sepsis and nine (2%) had septic shock. Of those with septic shock, three died in the ED and the remainder had underlying diseases that had progressed too far for them to benefit from treatment in the ICU.

By site of infection, the most common infection was urinary tract infection (131 cases, 26%), followed by intra-abdominal infection (84 cases, 17%), infection of the skin, soft tissues, and bones (72 cases, 15%), and lower respiratory tract infection (54 cases, 11%). The site of infection was unknown in 118 cases

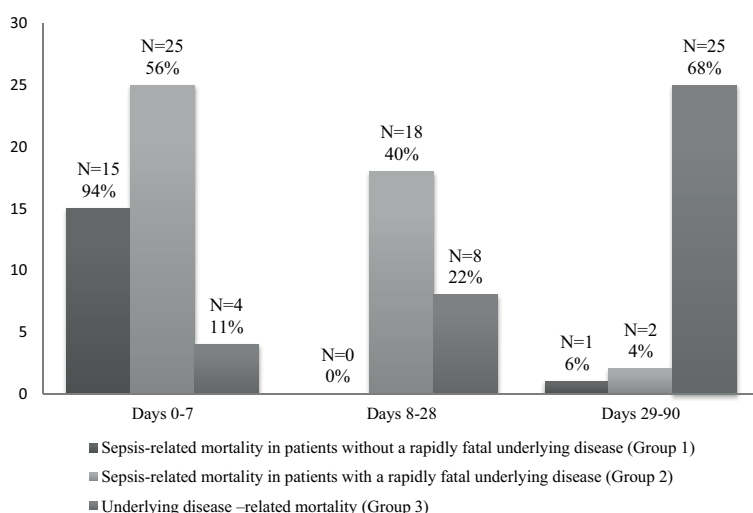


Figure 1. Number of cases in groups 1, 2, and 3 in relation to day of case fatality in a total of 98 patients who died within 90 days after sepsis. The percentage is per category of cause of death.

Table 2

Severity of the sepsis in relation to the outcome.

	Sepsis-related mortality in patients without a rapidly fatal underlying disease (Group 1) (n = 16)	Sepsis-related mortality in patients with a rapidly fatal underlying disease (Group 2) (n = 45)	Mortality related to underlying disease (Group 3) (n = 37)	All patients who died (n = 98)	Patients who survived (n = 399)	All cases (N = 497)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Severe sepsis	16 (100)	30 (67)	13 (35)	59 (60)	93 (23)	152 (31)
Septic shock	9 (56)	9 (20)	4 (11)	22 (22)	15 (4)	37 (7)
qSOFA-positive ^a	13 (81)	26 (59)	16 (46)	55 (58)	81 (21)	136 (29)
Admitted from ED to ICU	11 (69)	7 (16)	3 (8)	21 (21)	27 (7)	48 (10)
Pitt bacteraemia score ^b						
0–1	1 (6)	16 (36)	20 (57)	37 (39)	269 (68)	306 (62)
2–3	3 (19)	17 (39)	10 (29)	30 (32)	93 (24)	123 (25)
≥4	12 (75)	11 (25)	5 (14)	28 (29)	33 (8)	61 (12)

ED, emergency department; ICU, intensive care unit; qSOFA, quick sepsis-related organ failure assessment.

^a Data available for 473 cases (95 who died and 378 who survived).^b Data available for 490 cases.

(24%). Sepsis was healthcare-associated in 81 patients (16%). The previous use of immunosuppressive medications was common: 94 (19%) patients were taking corticosteroids (any dose) and 56 (11%) were on cancer chemotherapy. Antibiotics were started on the day of admission in 93% of patients. The most common antibiotic was cefuroxime (57%), followed by ceftriaxone (18%) and fluoroquinolone (11%). An antibiotic combination was started in 16% of patients. The median time from admission to the start of antibiotics was 174 min (range 0–1269 min).

Of all cases, 426 (86%) had one or more underlying diseases. As shown in Table 1, there were significantly more alcohol abusers in group 1 as compared to those who survived for more than 90 days (4/16 vs. 35/399; OR 3.8, 95% CI 1.2–12.4, $p = 0.04$). There were also significantly more patients with a solid tumour with metastasis and patients with haematological malignancies among those who died by day 90 than among survivors (28/98 vs. 29/399, OR 5.1, 95%

CI 2.9–9.1, $p < 0.01$ and 15/98 vs. 30/399, OR 2.2, 95% CI 1.1–4.3, $p = 0.02$, respectively).

The causative organisms are listed in Table 3. Gram-positive and Gram-negative organisms were equally common. There were few cases with MRSA and extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and no cases with vancomycin-resistant enterococci or carbapenemase-producing *Enterobacteriaceae*.

Discussion

In this study, the case fatality rate by day 7, day 28, and day 90 was 9%, 14%, and 20%, respectively, in 497 cases with blood culture-positive sepsis treated in an ED. The day 7 and day 28 case fatality rates in this study are similar to those reported in a study by Lin et al., who also studied bacteraemic patients in the ED.⁹ In two other studies investigating the day 28 case fatality rate in bacteraemic patients in the ED, Lee et al. reported a rate of 9% and Kao et al. reported a rate of 19%.^{12,20}

Only 16% of all deaths by day 90 in the present study were related to sepsis in patients with a life-expectancy of more than 6 months (group 1). Forty-six percent of deaths occurred in patients with a rapidly fatal underlying disease although death was sepsis-related (group 2), and 38% of deaths were related to the underlying disease (group 3). It is known that underlying diseases have a role in the deaths of sepsis patients,^{2–4,9} but the categorization of the present study gives a picture of the significance of the underlying diseases in these deaths.

Even patients in group 1 had many factors contributing to death, for example alcoholism or very old age. There was only one patient in this group without any underlying disease. It is possible that there were patients in group 1 who sought treatment too late, as three patients in this group were found lying at home with a low level of consciousness. There were also significantly more alcohol abusers as compared to those who survived more than 90 days. Altogether, in this cohort consisting mostly of community-acquired cases seen in a university hospital ED in a developed country, there were few non-survivors who would have had a life-expectancy of more than 6 months and who might have benefited from a novel drug directed against sepsis or septic shock. This may also explain why so many clinical trials in the field of sepsis have failed.²¹

Day 90 mortality has been used as the endpoint in many sepsis studies.^{22–24} However, the present study showed that most (95%) of the patients whose death was sepsis-related died within 28 days after sepsis, while the majority of deaths occurring between day 28

Table 3

Causative organisms.

Organisms	n (%)
Gram-positive	225 (45.3)
<i>Staphylococcus aureus</i>	74 (14.9)
MRSA	3 (0.6)
Coagulase-negative <i>Staphylococcus</i>	11 (2.2)
<i>Streptococcus pneumoniae</i>	52 (10.5)
β-haemolytic streptococci	45 (9.1)
Viridans streptococci	21 (4.2)
Enterococci	17 (3.4)
Other Gram-positive	5 (1.0)
Gram-negative	223 (44.9)
<i>Escherichia coli</i>	159 (32.0)
ESBL- <i>E. coli</i>	9 (1.8)
<i>Klebsiella</i> sp	21 (4.2)
ESBL- <i>Klebsiella</i> sp	0 (0.0)
<i>Pseudomonas aeruginosa</i>	18 (3.6)
Other Gram-negative	25 (5.0)
Others	49 (9.9)
Anaerobes	15 (3.0)
Fungi	1 (0.2)
Polymicrobial	33 (6.6)
All	497 (100)

MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum beta-lactamase.

and day 90 were independent of sepsis. This makes day 28 a more appropriate time-point to investigate sepsis-related mortality, as regulatory agencies have indeed considered.²¹ Nonetheless, only 34% of deaths occurring between day 0 and day 7 were sepsis-related in patients with a life-expectancy of more than 6 months. Thus, even when day 7 and day 28 are used as endpoints for sepsis mortality, deaths independent of sepsis are not ruled out.

This study has some limitations. By reason of the study design, it was not possible to include all blood culture-positive cases admitted to the ED during the study period. The potential representativeness of the material was assessed by reviewing the blood culture findings of those cases that were not included in the study. Altogether there were 167 such cases (contaminants excluded). The percentages of the most common blood culture findings, *S. aureus* and *Escherichia coli*, did not differ between the study population and the cases that were not included. It is thus unlikely that missed cases would have had a major impact on the results. Among cases not included in the material as compared to those included, the percentage of anaerobes and other Gram-negative organisms (see Table 3) was somewhat higher (9.0% vs. 3.0% and 10.2% vs. 5.0%, respectively) and the percentage of *Streptococcus pneumoniae* was lower (4.2% vs. 10.5%). This may be due to the long incubation period of anaerobes and other Gram-negative organisms and the short incubation period of *S. pneumoniae*. Again, assuming that case fatality among missed cases with anaerobes or other Gram-negatives would be approximately the same as among those included in the study, the numbers would be too low to alter the major findings of the study.

Only blood culture-positive cases were included, which would exclude for example those with false-negative blood cultures. This might also have led to a lower number of septic patients with respiratory infection. However, it is also a strength that culture-negative sepsis cases, which are difficult to define, were not included. Hence the material consists more of true infections than patients who were for example only positive for systemic inflammatory response syndrome (SIRS).

The material was gathered in a tertiary level hospital of a developed country with a low incidence of multi-resistant bacteria. These types of results cannot, therefore, be generalized to low-income countries, as has been pointed out by Rello and Leblebicioglu.²⁵ It is possible that the role of the underlying diseases would also have been different if the study patients had been only from ICUs.

The method used to categorize the cause of death was developed by the authors. It encompasses the classification used on the death certificate (immediate cause and underlying cause) and combines it with the McCabe classification of rapidly fatal disease. This has limitations, since in some cases it was not easy to decide whether the patient would have lived for more than 6 months without the sepsis or not. Even group 1 patients were elderly and had many underlying diseases. However, the authors believe that the categorization used is illustrative of the course of events among these patients, and the use of two or three clinicians to provide a judgement gives it sufficient validity.

In conclusion, this study showed that patients with sepsis who die within 90 days after sepsis in a developed country, die mostly either of sepsis by weakening of the patient with a rapidly fatal (<6 months) underlying disease, or of the underlying disease itself. This does not mean that sepsis patients do not need high-quality care. Indeed, the low number of cases of sepsis-related mortality among patients with a life-expectancy of more than 6 months may be attributed to the good quality of the care these patients received. When investigating sepsis-related mortality, day 28 should be used as the endpoint to identify most of the sepsis-related mortality, while a large part of sepsis-independent mortality is ruled out.

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Ethical approval

The study was approved by the Ethics Committee of Tampere University Hospital.

Conflict of interest

JR, JS, TS, JA, and RH report no conflict of interest.

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PUBLICATION

II

Plasma cell-free DNA and qSOFA score predict 7-day mortality in 481 emergency department bacteraemia patients

Rannikko, J., Seiskari, T., Huttunen, R., Tarkiainen, I., Jylhävä, J., Hurme, M., Syrjänen, J. and Aittoniemi, J.

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Plasma cell-free DNA and qSOFA score predict 7-day mortality in 481 emergency department bacteraemia patients

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Abstract. Rannikko J, Seiskari T, Huttunen R, Tarkiainen I, Jylhävä J, Hurme M, Syrjänen J, Aittoniemi J (Tampere University Hospital; University of Tampere; Fimlab Laboratories, Tampere, Finland; Biostatistics, Karolinska Institute, Stockholm, Sweden) Plasma cell-free DNA and qSOFA score predict 7-day mortality in 481 emergency department bacteraemia patients. *J Intern Med* 2018; <https://doi.org/10.1111/joim.12766>

Background. A few studies have shown that both quick Sequential Organ Failure Assessment (qSOFA) score and cell-free DNA (cfDNA) have potential use as a prognostic marker in patients with infection. We studied these two markers alone and in combination to identify those emergency department (ED) patients with the highest risk of death.

Methods. Plasma cfDNA level was studied on days 0 to 4 after admittance to the ED from 481 culture-positive bloodstream infection cases. The qSOFA score was evaluated retrospectively according to Sepsis-3 definitions. The primary outcome was death by day 7.

Introduction

Cell-free plasma DNA (cfDNA), also called extracellular DNA, comprises fragments of DNA that are released from apoptotic and necrotic cells [1]. Elevated levels of cfDNA in blood have been found in various clinical conditions, such as cancer, trauma, stroke and sepsis [2–5]. A few studies have indicated that cfDNA may have value in predicting the mortality of sepsis patients in intensive care units (ICUs) [3, 6–11] and in emergency department (ED) patients [12, 13]. In recent years, cfDNA has also been found to have a role as a link between innate immunity and coagulation [14], and increased levels of cfDNA are found in patients with deep vein thrombosis [15]. To the best of our knowledge, no

Results. CfDNA on day 0 was significantly higher in nonsurvivors than in survivors ($2.02 \mu\text{g mL}^{-1}$ vs. $1.35 \mu\text{g mL}^{-1}$, $P < 0.001$). CfDNA level was high ($>1.69 \mu\text{g mL}^{-1}$) in 134 (28%) of 481 cases, and the qSOFA score was ≥ 2 in 128 (28%) of 458 cases. High cfDNA and qSOFA score ≥ 2 had 70% and 77% sensitivity and 76% and 76% specificity in predicting death by day 7, respectively. High cfDNA alone had odds ratio (OR) of 7.7 (95% CI 3.9–15.3) and qSOFA score ≥ 2 OR of 11.6 (5.5–24.3), but their combination had OR of 20.3 (10.0–41.4) in predicting death by day 7 when compared with those with low cfDNA and qSOFA score < 2 . Amongst the five cases with the highest cfDNA levels, there were three patients with severe disseminated intravascular coagulation.

Conclusion. CfDNA and qSOFA score can be used independently to identify those bacteraemia patients at high risk of death, and combining these two markers gives additional advantage.

Keywords: bacteraemia, biomarker, sepsis.

studies have evaluated cfDNA as a possible marker of disseminated intravascular coagulation (DIC).

The criteria for sepsis were recently changed, and the quick Sequential Organ Failure Assessment (qSOFA) score has been introduced [16]. The qSOFA score was not invented to replace systemic inflammatory response syndrome (SIRS), but instead to help identify patients with suspected infection outside ICUs who are likely to develop complications [17, 18]. The qSOFA score has been shown to be better than SIRS as a prognostic marker of in-hospital mortality in an ED setting [19].

In our earlier study, we evaluated 497 cases with blood culture-positive infection in the ED to

establish how often death was related to sepsis and how often underlying diseases played a major role [20]. In this study, we have measured plasma cfDNA in 481 of these blood culture-positive infection cases and evaluated the use of cfDNA as a prognostic marker in addition to the qSOFA score. We have also evaluated the possible association of high cfDNA to the clinical characteristics of bacteraemia. We used death by day 7 as a primary end-point, as in our earlier study, most of the sepsis-related deaths in patients with a prognosis of more than 6 months occurred early after admission to the ED. Our secondary end-point was death by day 28.

Methods

Tampere University Hospital is a tertiary hospital situated in the Pirkanmaa region of Finland with a catchment population of approximately 524 700 inhabitants. Blood cultures are routinely taken from patients with signs or symptoms of systemic infection. As described in our previous study [20], blood culture-positive cases in the specialized care ED of Tampere University Hospital were selected during the period 1 March 2012 to 28 February 2014. In this study, all cases with an available plasma sample for cfDNA analysis were included. The clinical data of the patients were gathered retrospectively. The primary and secondary end-points were death by day 7 and day 28, respectively.

Blood cultures were collected in BacT/Alert aerobic (FA Plus) and anaerobic (FN Plus) blood culture bottles and placed in an automated microbial detection system BacT/Alert 3D (bioMérieux, Marcy l'Etoile, France). All culture-positive cases were selected by a clinical microbiologist. Cultures positive for coagulase-negative Staphylococcus, Propionibacterium, Bacillus, Micrococcus and Corynebacterium, with detection in a single blood culture bottle and without clinical relevance, were considered to be contaminants and were excluded. Also, positive samples were excluded if the first routine blood sample within the first 24 h after the blood culture collection (day 0) was missed. This could be because the culture became positive later than 72 h after admission.

The level of plasma cfDNA was analysed from blood samples from days 0 to 4. The day 0 sample was gathered mostly at the same time as the collection of the blood cultures, but samples

gathered within 24 h of the collection were included. Qubit dsDNA HS Assay Kit and Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA) were used for quantification of plasma cfDNA. The manufacturer's instructions and protocols were followed at each step. After plasma extraction, the turnaround time spent on the laboratory analysis of cfDNA level was approximately 10 min. The intra-assay coefficients of variation from control samples were between 3.55% and 5.73%. The final result was the average of the two samples.

Diagnoses of sepsis, severe sepsis and septic shock were made according to Sepsis-2 consensus definitions [21]. A criterion for qSOFA score was calculated based on Sepsis-3 definitions [16]. The qSOFA score comprises the following criteria: altered mentation, respiratory rate ≥ 22 and systolic blood pressure ≤ 100 mmHg. In this study, altered mentation was classified based on the verbal section of the Glasgow Coma Scale: if the patient had less than 5 points in this section, the patient was considered to have altered mentation. The Pitt Bacteraemia Score was calculated as presented by Korvick *et al.* [22].

IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. A *P*-value of <0.05 was considered significant. Nonparametric data were analysed by Mann-Whitney *U*-test or Kruskal-Wallis test when appropriate. The Youden index was used to select optimal cut-off analysis. Predictive performance (accuracy) of the cfDNA level and qSOFA score was assessed using the AUC/ROC statistic. A logistic regression model was used to study the independent effect of high cfDNA level on mortality models adjusted for potential confounders. The survival curve was assessed using the Kaplan-Meier method, and survival differences between groups were compared by log-rank test. The study was approved by the Ethics Committee of Tampere University Hospital and the National Supervisory Authority for Welfare and Health. The need for informed consent was waived as no additional blood sampling was needed and routine patient care was not modified.

Results

There were 800 consecutive positive blood cultures during the study period. Contaminants ($n = 136$) were excluded, and 167 blood cultures were

excluded for missing the blood sample from day 0. The first sixteen of the gathered samples went missing later. A total of 481 cases amongst 469 patients were thus included. There were 253 (53%) male cases, 228 (47%) female cases, all were adults (>16 years), and the median age was 68 years. Table 1 provides information on patient characteristics, clinical presentation and microbiology of the infection.

The minimum cfDNA level on day 0 to day 4 was $0.72 \mu\text{g mL}^{-1}$, and the maximum was $66.95 \mu\text{g mL}^{-1}$. The mean level on day 0 was $1.73 \mu\text{g mL}^{-1}$, and the median was $1.38 \mu\text{g mL}^{-1}$. Table 2 and Fig. 1 show levels of cfDNA during days 0 to 4 in all cases and in relation to death by day 7. CfDNA day 0 levels in the nonsurvivors were significantly higher compared with survivors. Furthermore, levels remained at a significantly higher level on day 1 to day 4.

The optimal cut-off value for the plasma cfDNA values on day 0 in predicting death by day 7 was estimated using ROC curve, as illustrated in Fig. 2. CfDNA had an AUC of 0.77 (95% CI 0.69 to 0.85) in predicting death by day 7. A cut-off value of $1.69 \mu\text{g mL}^{-1}$ had sensitivity of 70.4% and specificity of 76.4% in predicting death by day 7 with an AUC of 0.73 (95% confidence interval (CI) 0.65 to 0.82, $P < 0.001$). Plasma cfDNA levels above this cut-off value are hereafter referred to as high and levels below this value are referred to as low. CfDNA level was high in 134 (28%) of 481 cases, and qSOFA score was ≥ 2 in 128 (28%) of 458 cases. A comparison of high cfDNA and qSOFA score ≥ 2 in relation to death by day 7 is given in Table 3. QSOFA score ≥ 2 together with high cfDNA level on day 0 resulted in a 20-fold risk of death by day 7, when compared with those with low cfDNA level and qSOFA score < 2 . Odds ratios of death by day 7 were 7.7 (95% CI 3.9 to 15.3) and 11.6 (95% CI 5.5 to 24.3) for high vs. low cfDNA and qSOFA score ≥ 2 vs. qSOFA score < 2 , respectively (Table 3). Figure 3 presents the Kaplan-Meier survival curves by day 28 for high cfDNA, qSOFA score ≥ 2 and both.

Death by day 28 was a secondary end-point. CfDNA on day 0 was significantly higher in nonsurvivors than in survivors ($1.95 \mu\text{g mL}^{-1}$ vs. $1.33 \mu\text{g mL}^{-1}$, $P < 0.001$). CfDNA had an AUC of 0.76 (95% CI 0.69 to 0.82) in predicting death by day 28. The optimal cut-off value of cfDNA in predicting death by day 28 was the same

Table 1 Patient characteristics, clinical presentation and microbiological data of the study population

	Data
Demographic	
Cases/Patients	481/469
Patients admitted twice/three times	10/1
Gender (female/male)	228/253
Median age, y (range)	68 (16–95)
Chronic medical condition	n (%)
Coronary artery disease, chronic vascular disease or chronic heart failure	155 (32)
Diabetes mellitus	137 (29)
Social or medical problems of alcohol abuse	49 (10)
Solid tumour with metastasis	55 (11)
Haematological malignancy	45 (9)
Severity of the sepsis	n (%)
Sepsis	481 (100)
Severe sepsis (≥ 1 organ failure)	145 (30)
Septic shock	36 (8)
qSOFA score $\geq 2^a$	128 (28)
Case transferred from ED to ICU	44 (9)
Site of infection	n (%)
Urinary	134 (28)
Intra-abdominal	83 (17)
Skin, soft tissue and bones	71 (15)
Pulmonary	48 (10)
Unknown	116 (24)
Causative organism	n (%)
Gram-positive	213 (44)
<i>Staphylococcus aureus</i>	71 (15)
<i>Streptococcus pneumoniae</i>	46 (11)
Gram-negative	222 (46)
<i>E. coli</i>	156 (32)
Others	46 (10)
Anaerobes	15 (3)
Fungi	1 (0.2)
Polymicrobial	30 (6)
Day of case fatality	n (%)
Days 0 to 7	44 (9)
Days 0 to 28	69 (14)
Days 0 to 90	97 (20)

qSOFA, quick Sequential Organ Failure Assessment; ED, emergency department; ICU, intensive care unit.

^aData available on 458 cases.

Table 2 Plasma cell-free DNA (cfDNA) levels during days 0 to 4 after admission to emergency department in all cases and in relation to death by day 7

Days after admission	Plasma cfDNA ($\mu\text{g mL}^{-1}$), median (quartiles)			P-values
	All	Nonsurvivors	Survivors	
Day 0	1.38 (1.20–1.77)	2.02 (1.51–2.92)	1.35 (1.19–1.66)	<0.001
Day 1	1.36 (1.18–1.73)	2.20 (1.57–3.63)	1.33 (1.17–1.64)	<0.001
Day 2	1.35 (1.16–1.65)	1.87 (1.59–2.92)	1.33 (1.16–1.61)	<0.001
Day 3	1.33 (1.16–1.61)	1.81 (1.48–4.34)	1.32 (1.14–1.58)	<0.001
Day 4	1.35 (1.17–1.71)	1.66 (1.49–2.38)	1.33 (1.15–1.68)	0.017
Maximum value	1.49 (1.28–1.91)	2.24 (1.56–4.55)	1.45 (1.27–1.81)	<0.001

cfDNA values available on 481 patients on day 0, 446 patients on day 1, 389 patients on day 2, 300 patients on day 3, 137 patients on day 4 and 481 patients on days 0 to 4 (maximum value).

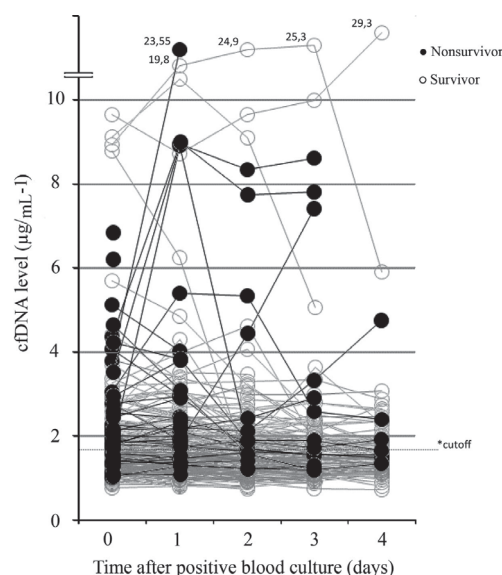


Fig. 1 A line plot diagram showing cell-free DNA (cfDNA) levels during days 0 to 4 after admission to emergency department in 44 patients who died by day 7 (black plots) and 437 survivors (open plots). One case with all cfDNA levels over $30 \mu\text{g mL}^{-1}$ excluded from the figure. *Cut-off, cfDNA value of $1.69 \mu\text{g mL}^{-1}$ had an optimal sensitivity of 70.4% and specificity of 76.4% in predicting day 7 mortality from the day 0 sample based on the AUC-ROC-analysis.

as in death by day 7 ($1.69 \mu\text{g mL}^{-1}$). The results for the secondary end-point (day 28) are shown in Table 3.

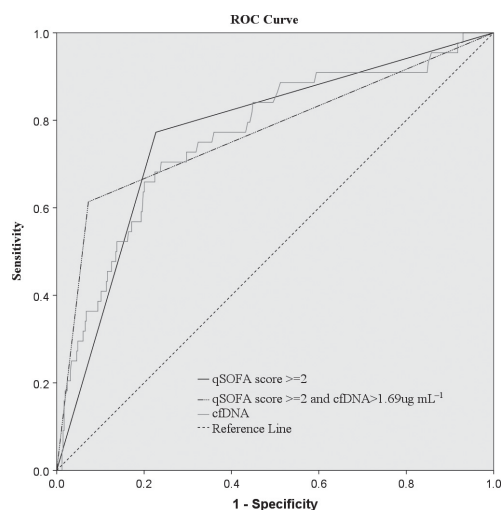


Fig. 2 Receiver operating characteristic (ROC) curve on plasma cell-free DNA level, qSOFA score ≥ 2 and both cfDNA $> 1.69 \mu\text{g mL}^{-1}$ and qSOFA score ≥ 2 measured on day of admission to the emergency department in relation to case fatality by day 7.

The maximum level of cfDNA at day 0 was $38.85 \mu\text{g mL}^{-1}$, and in this patient, it remained between $29.50 \mu\text{g mL}^{-1}$ and $66.95 \mu\text{g mL}^{-1}$ on days 1 to 4. This patient suffered from a severe pneumococcal infection with DIC resulting in multiple amputations. Amongst the five highest cfDNA levels at day 0, there were two other patients with DIC caused by *Capnocytophaga canimorsus* sepsis that resulted in skin necrosis or amputations.

Table 3 Diagnostic values of day 0 cfDNA with cut-off value of $1.69 \mu\text{g mL}^{-1}$, qSOFA score ≥ 2 and both qSOFA score ≥ 2 and cfDNA $> 1.69 \mu\text{g mL}^{-1}$ in predicting death by day 7 and day 28

	<i>n</i> (deceased/survivors)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Odds Ratio with 95% CI	AUC with 95% CI	<i>P</i> -values
Death by day 7								
cfDNA $> 1.69 \mu\text{g mL}^{-1}$	134 (31/103)	70.4	76.4	23.1	96.2	7.7 (3.9–15.3)	0.73 ^a (0.65–0.82)	< 0.001
qSOFA score ≥ 2 ^b	128 (34/94)	77.3	77.3	26.6	97.0	11.6 (5.5–24.3)	0.77 (0.70–0.85)	< 0.001
cfDNA $> 1.69 \mu\text{g mL}^{-1}$ and qSOFA score ≥ 2 ^b	57 (27/30)	61.4	92.8	47.4	95.8	20.3 (10.0–41.4)	0.77 (0.68–0.86)	< 0.001
Death by day 28								
cfDNA $> 1.69 \mu\text{g mL}^{-1}$	134 (45/89)	65.2	78.4	33.6	93.1	6.8 (3.9–11.8)	0.72 ^a (0.65–0.79)	< 0.001
qSOFA score ≥ 2 ^b	128 (45/83)	66.2	78.7	35.2	93.0	7.2 (4.1–12.6)	0.72 (0.66–0.79)	< 0.001
cfDNA $> 1.69 \mu\text{g mL}^{-1}$ and qSOFA score ≥ 2 ^b	57 (35/22)	51.5	94.3	61.4	91.8	17.7 (9.3–33.7)	0.73 (0.65–0.81)	< 0.001

^aSee results for the AUC of cfDNA as a continuous variable.^bData available on 458 cases.

Their cfDNA levels were $9.65 \mu\text{g mL}^{-1}$ and $8.92 \mu\text{g mL}^{-1}$.

Median level of cfDNA at day 0 in cases with qSOFA score < 2 was $1.34 \mu\text{g mL}^{-1}$ (interquartile range (IQR) $1.16 \mu\text{g mL}^{-1}$ to $1.64 \mu\text{g mL}^{-1}$). Ten patients with qSOFA score < 2 died by day 7 with the median day 0 cfDNA level of $1.45 \mu\text{g mL}^{-1}$ (IQR $1.38 \mu\text{g mL}^{-1}$ to $1.90 \mu\text{g mL}^{-1}$). The difference of day 0 cfDNA level between survivors and nonsurvivors in patients with qSOFA score < 2 was statistically insignificant ($P = 0.10$). C-reactive protein (CRP) level on day 0 did not have a significant predictive value for death by day 7 (AUC 0.52 (95% CI 0.43 to 0.60), $P = 0.677$, data on 478 cases). In predicting transfer to ICU within 24 hours of admission, cfDNA level had an AUC of 0.70 (95% CI 0.61 to 0.80, $P < 0.001$) and qSOFA score ≥ 2 0.72 (0.63 to 0.80, $P < 0.001$).

Table 4 presents the day 0 plasma cfDNA levels stratified by various demographic features, underlying conditions and severity of sepsis. The level of cfDNA was statistically significantly higher in the following conditions: liver disease, alcohol abuse, septic shock, admitted from ED to ICU, Pitt Bacteraemia Score ≥ 4 and death by day 7 or day 28. The level of cfDNA was statistically significantly lower in patients with haematologic cancer.

Statistically significant variables associated with death by day 7 in univariate analysis were cardiovascular disease, liver disease, alcohol abuse, MRSA carriage, Pitt Bacteraemia Score ≥ 4 , high cfDNA, qSOFA score ≥ 2 and both qSOFA score ≥ 2 and high cfDNA. The following variables were not associated with death by day 7: sex, age over 60, age over 80, neurological disease, pulmonary disease, rheumatological disease, kidney disease, substance abuse (other than alcohol), metastatic tumour, haematological malignancy, ESBL carriage, gram stain result, E.coli urosepsis or CRP over 100 mg L^{-1} on the first day. The odds ratios of death by day 7 for high cfDNA, qSOFA score ≥ 2 and both adjusted for these variables were 7.7 (95% CI 3.9 to 15.3), 11.6 (95% CI 5.5 to 24.3) and 21.3 (95% CI 10.5 to 43.5), respectively. In multivariable analysis, we included all statistically significantly associated variables in univariate analysis. QSOFA score ≥ 2 , high cfDNA, qSOFA score ≥ 2 combined with high cfDNA, cardiovascular disease and Pitt Bacteraemia Score ≥ 4 remained significant in this model.

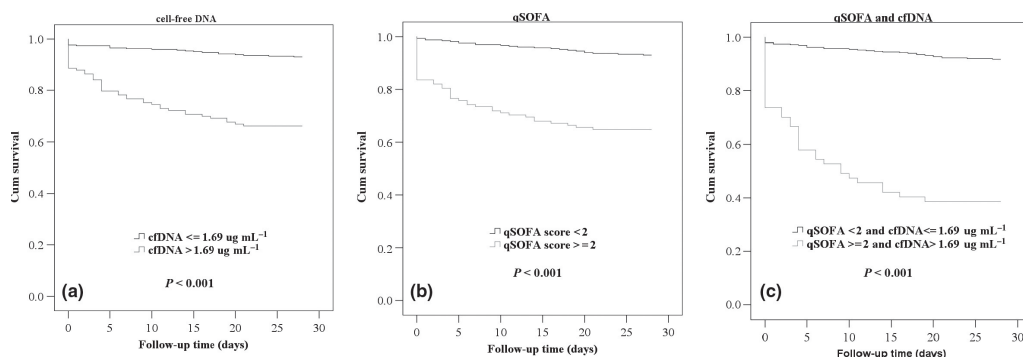


Fig. 3 Cumulative day 28 survival in culture-positive cases with (a) maximum plasma cell-free DNA (cfDNA) $> 1.69 \mu\text{g mL}^{-1}$ compared with those with cfDNA $\leq 1.69 \mu\text{g mL}^{-1}$, (b) qSOFA score ≥ 2 compared with those with qSOFA score < 2 and (c) cfDNA > 1.69 and qSOFA score ≥ 2 compared with those with cfDNA $\leq 1.69 \mu\text{g mL}^{-1}$ and qSOFA < 2 . The survival curve was calculated using the Kaplan–Meier method, and survival differences between groups were compared by log-rank test.

Discussion

The present study shows that qSOFA score and cfDNA identify patients with a high risk of death due to bacteraemia infection. In this study, 34 of 128 (27%) patients with qSOFA score ≥ 2 in the ED had died by day 7. If the patients had both qSOFA score ≥ 2 and cfDNA of more than $1.69 \mu\text{g mL}^{-1}$ on day 0, 47% of them died. Thus, the combination of high cfDNA level and qSOFA score ≥ 2 elevated the risk of death by day 7 from 12-fold to 20-fold.

QSOFA has recently been shown to predict organ failure and in-hospital mortality in 1009 patients with suspected infection [23]. Yet, the authors recommended the use of further confirmatory test. Given the present results, the combination of qSOFA score ≥ 2 and high cfDNA does not improve sensitivity in predicting death by day 7, but it could be an option to further improve specificity. As an illustration, we suggest a two-step approach where qSOFA score ≥ 2 is followed by cfDNA analysis: in the present material comprising 458 sepsis cases, qSOFA score ≥ 2 results in 128 cases at higher risk of death due to bacteraemia infection during the first week. Analysing the cfDNA of these 128 exposes 57 cases with even higher 7-day mortality of 47%. Moreover, the turnaround time spent on laboratory analysis of cfDNA level was approximately 10 min (after plasma extraction). This does not make turnaround time a significant obstacle for clinical use, but due to the need for plasma extraction, no point-of-care test is yet available.

The Sepsis-3 task force did not find relevant change in predictive validity when lactate was added to qSOFA [24]. Clinicians will continue measuring lactate as the result is a numerical value rather than just the value of positive or negative. Especially high levels of lactate are seen in patients with severe infections, and the kinetics of lactate has been shown to have value in the prognosis of sepsis patients [25]. In this study, especially high levels of cfDNA were seen in patients with DIC that resulted in amputations. This is not surprising as a growing body of evidence suggests a role for cfDNA and DNA-binding proteins in the pathogenesis of DIC [14]. Because there were only few patients with DIC in our material, further studies are needed to establish cfDNA as an early marker for DIC. Nevertheless, this finding also suggests that cfDNA could be an option to identify those patients with severe consequences of sepsis.

The AUC of cfDNA in predicting death by day 7 and day 28 was 0.77 and 0.76, respectively. These are somewhat lower than in two studies carried out in ED with soluble urokinase-type plasminogen activator receptor (suPAR) [26, 27]. These studies had an AUC of 0.79 and 0.84 in predicting death by day 30. When using their cut-off values, suPAR seems to be more sensitive in predicting death (76% and 83% vs. 65%) and cfDNA has a slightly better specificity (69% and 76% vs. 78%). In another cfDNA study carried out by our group, these predictors are more or less the same as in the

Table 4 Day of admission plasma cell-free DNA (cfDNA) values stratified by various demographic features, underlying conditions and severity of sepsis

	Plasma cfDNA ($\mu\text{g mL}^{-1}$) on day of admission to emergency department		<i>P</i> -value
	Factor present, median (quartiles)	Factor absent, median (quartiles)	
Characteristics and underlying conditions			
Male	1.42 (1.24–1.85)	1.30 (1.16–1.64)	0.002
Age over 60 years	1.39 (1.22–1.80)	1.32 (1.16–1.60)	0.035
Age over 80 years	1.46 (1.25–1.89)	1.35 (1.18–1.69)	0.020
Cardiovascular disease	1.40 (1.22–1.75)	1.36 (1.19–1.82)	0.822
Diabetes any type	1.38 (1.18–1.70)	1.36 (1.20–1.77)	0.894
Chronic kidney disease ^a	1.38 (1.26–1.73)	1.36 (1.19–1.77)	0.561
Liver disease	1.91 (1.41–2.15)	1.35 (1.19–1.67)	<0.001
Alcohol abuse ^b	1.95 (1.47–2.38)	1.34 (1.18–1.64)	<0.001
Solid tumour with metastasis	1.51 (1.20–1.97)	1.36 (1.19–1.71)	0.142
Haematologic cancer	1.27 (1.12–1.68)	1.37 (1.22–1.76)	0.041
Gram-positive bacteraemia ^c	1.35 (1.17–1.67)	1.38 (1.23–1.80)	0.180
Polymicrobial infection	1.58 (1.25–2.13)	1.36 (1.19–1.75)	0.058
Severity of sepsis			
qSOFA score $\geq 2^d$	1.56 (1.26–2.20)	1.34 (1.16–1.64)	<0.001
Severe sepsis	1.61 (1.31–2.23)	1.31 (1.17–1.60)	<0.001
Septic shock	1.96 (1.32–2.83)	1.36 (1.19–1.70)	<0.001
Admitted from ED to ICU	1.95 (1.40–2.89)	1.35 (1.19–1.69)	<0.001
Pitt Bacteraemia Score $\geq 4^e$	1.83 (1.34–2.73)	1.35 (1.19–1.68)	<0.001
Death by day 7	2.02 (1.51–2.92)	1.35 (1.19–1.66)	<0.001
Death by day 28	1.95 (1.50–2.58)	1.33 (1.18–1.64)	<0.001

ED, emergency department; ICU, intensive care unit.

^aHistory of creatinine more than $120 \mu\text{mol L}^{-1}$.^bSocial or medical problems of alcohol abuse in the past 12 months.^cExcluding polymicrobial ($n = 30$) and anaerobic ($n = 15$) bacteraemia.^dData available on 458 cases.^eData available on 474 cases.

suPAR studies (AUC 0.84, sensitivity 83% and specificity 76%) [12]. As the biochemical mechanism of these two markers differs greatly, it is possible that these markers perform differently depending on the subgroups within septic patients. Cases with suspected DIC might be one subgroup where cfDNA could be of help.

This study has some limitations. Our study was a single-centre tertiary hospital study comprising mostly community-onset infections. Furthermore, our material comprises only culture-positive cases, so one has to be cautious when extrapolating these results to culture-negative cases with infection. In addition, 167 of the culture-positive cases were excluded as the routine blood sample from day 0

was missed. However, the microbiological data of these missed cases were analysed and we did not find any differences that would have altered the findings of this study [20].

In line with previous studies [28–30], the results of the present study show that CRP-level has no value in predicting fatality. A high level of CRP (or leucocytes) is still an important sign for clinicians; at least they have to think could the patient have an infection or sepsis. When sepsis is suspected, a clinician in the ED has to decide, for example, whether the patient needs care in the ICU. A recent study suggested that only clinical impression of condition might be used to make this decision [31], but occasionally clinicians might need some extra

help in making these decisions. Therefore, counting qSOFA score and measuring the level of cfDNA might help the decision-making process.

In conclusion, the present study shows that the combined information on qSOFA score and cfDNA can identify patients with the highest risk of death due to bacteraemia infection. These markers may find those patients admitted to ED with the highest need of care.

Potential conflicts of interest

Juha Rannikko, Tapio Seiskari, Reetta Huttunen, Iina Tarkiainen, Juulia Jylhävä, Mikko Hurme, Jaana Syrjänen and Janne Aittoniemi have no conflict of interest.

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PUBLICATION

III

Reduced plasma PCSK9 response in patients with bacteremia is associated with mortality

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