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EARLY DETECTION OF SEVERE SEPSIS IN THE EMERGENCY ROOM IN ADULTS – CLINICAL UTILITY OF PROGNOSTIC MARKERS

by

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To My Family

ABSTRACT

Raija Uusitalo-Seppälä: EARLY DETECTION OF SEVERE SEPSIS IN THE EMERGENCY ROOM IN ADULTS – CLINICAL UTILITY OF PROGNOSTIC MARKERS

Department of Infectious Diseases, Faculty of Medicine, Doctoral Programme of Clinical Investigation, University of Turku, Turku, Finland and Satakunta Central Hospital, Pori, Finland.Annales Medica, Turku 2014

Early diagnostic and prognostic stratification of patients with suspected infection is a challenge for clinician. We conducted a single-center prospective cohort study to study various biomarkers in detecting severe sepsis. The research cohort in studies I and III comprised 539 patients with suspected infection in the emergency room (ER). In study II there were 525 patients and in study IV 537.

In study I C-reactive protein (CRP) was compared with procalcitonin (PCT) and interleukin-6 (IL-6). In study II plasma bactericidal/permeability-increasing protein (BPI), group IIA phospholipase A2 (PLA_2GIIA), CRP and white cell count (WBC) were evaluated. In study III soluble urokinase-type plasminogen activator receptor (suPAR) and in study IV pentraxin 3 (PTX3) were investiagted.

In study I high PCT and IL-6 were shown to be better markers than CRP as an early alarm sign for the development of severe sepsis. In study II plasma PLA_2GIIA appeared to be slightly better marker for the development of severe sepsis than CRP or WBC. BPI was not useful. In study III high plasma suPAR concentration was shown to be an independent predictor of case fatality and also to be associated with the development of severe sepsis. In study IV high concentration of PTX3 on admission was also shown to be an independent predictor for the development of severe sepsis.

Taking studies I-IV together, PCT emerged as the best marker for severe sepsis and suPAR for case fatality. PTX3 appeared to be a fairly good marker for both, but hardly gives additional information. CRP is still reasonably good, although not a specific marker for bacterial infection, but it has no prognostic value. suPAR proved an interesting marker for organ damage and case fatality.

KEY WORDS: biomarker, case fatality, severe sepsis, CRP, PCT, IL-6, PLA_2GIIA, BPI, suPAR, PTX3, emergency room

TIIVISTELMÄ

Raija Uusitalo-Seppälä: VAIKEAN SEPSIKSEN VARHAINEN TUNNISTAMINEN PÄIVYSTYSPOLIKLINIKALLA – MERKKIAINEIDEN KÄYTTÖKELPOISUUS AIKUISPOTILAIDEN ARVIOINNISSA

Infektiotautioppi, Lääketieteellinen tiedekunta, Kliinisen tutkimuksen tohtoriohjelma, Turun Yliopisto, Turku ja Satakunnan keskussairaala, Pori.
Annales Medica, Turku 2014

Päivystyspotilaan vakavan yleisinfektion eli sepsiksen varhainen tunnistaminen ja taudin vaikeusasteen arviointi on päivystävälle lääkärille tärkeä haaste. Arvioimme prospektiivisessa kohorttitutkimuksessa eri merkkiaineiden hyödyllisyyttä sepsiksen varhaisessa tunnistamisessa ja vaikeusasteen arvioinnissa. Työssä I ja III oli 539 päivystyspotilasta, joilta kliinikko päätti ottaa veriviljelyn sepsistä epäillen. Tutkimuksessa II oli 525 potilasta ja tutkimuksessa IV 537 potilasta.

Tutkimuksessa I plasman C-reaktiivisen proteiinin (CRP) pitoisuuksia verrattiin plasman prokalsitoniinin (PCT) ja interleukiinin (IL-6) pitoisuuksiin. Tutkimuksessa II verrattiin plasman baktersidisen/ permeabiliteettia lisäävän proteiinin (BPI), ryhmän IIA fosfolipaasi A_2:n (PLA_2GIIA) ja CRP:n pitoisuuksia sekä valkosolujen määriä toisiinsa. Tutkimuksessa III arvioitiin liukoisen urokinaasi-tyyppisen plasminogeenin aktivaattorireseptorin (suPAR) ja tutkimuksessa IV pentraksiini 3:n (PTX3) määrityksen käyttökelpoisuutta.

Tutkimuksessa I todettiin päivystystilanteessa mitattujen korkeiden PCT - ja IL-6 - pitoisuuksien ennustavan vaikean sepsiksen kehittymistä paremmin kuin korkean CRP:n. Tutkimuksessa II plasman PLA_2GIIA vaikutti hiukan paremmalta vaikean sepsiksen ennustajalta kuin CRP tai veren valkosolutaso, mutta BPI ei ollut hyödyllinen. Tutkimuksessa III korkea plasman suPAR- pitoisuus osoittautui itsenäiseksi kuolleisuuden riskitekijäksi ja se liittyi myös vaikean sepsiksen kehittymiseen. Tutkimuksessa IV korkea PTX3 - pitoisuus toimi samaan tapaan kuin suPAR.

Kokonaisuutena PCT osoittautui parhaaksi merkkiaineeksi ennustamaan elinhäiriön kehittymistä ja suPAR kuolleisuutta. PTX3 ei tarjonnut merkittävää lisäetua PCT:iin ja suPAR:iin verrattuna. CRP osoitti suhteellisen hyvin bakteeri-infektion esiintymistä, mutta ennusteellista arvoa sillä ei ollut. suPAR on kiinnostava kuolleisuuden ja elinhäiriön kehittymisen merkkiaine.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ACE	Angiotensin converting enzyme
aPPT	Activated partial thromboplastin time
APR	Acute phase reactant
APACHE	Acute physiology and chronic health evaluation
AT	Antithrombin
AUC^{ROC}	Area under the receive operating characteristic curve
BPAP	Bilevel positive airway pressure treatment
BPI	Bactericidal/permeability-increasing protein
BSI	Bloodstream infection
C5a	Complement component 5a (or other complement, e.g. C3a)
C5aR	C5a receptor protein
CARS	Compensatory anti-inflammatory response syndrome
CD 64	Integral membrane glycoproteins 64 (CD 48 respectively)
CRP	C-reactive protein
CI	Confidence interval
CPAP	Continuous positive airvays pressure treatment
DAMP	Danger associated molecular pattern
DIC	Disseminated intravascular coagulopathy
DNA	Deoxyribonucleic acid
ELAM-1	Endothelial leukocyte adhesion molecule 1
ER	Emergency room
gp130	Glycoprotein 130
HMGB-1	High-mobility-group protein B1
ICAM-1	Intercellular adhesion molecule 1
ICU	Intensive care unit
IL-6	Interleukin-6 (IL-1, IL-2, IL-8, IL-10 respectively)
IL-1β	Interleukin-1 beta
LBP	LPS-binding protein
LPS	Lipopolysaccharide, part of the membrane of Gram-negative bacteria
LTA	Lipotheichoid acid, part of the cell-wall of Gram-positive bacteria
mHLA-DR	Monocytic human leukocyte antigen DR
MIF	Macrophage migration inhibitory factor
MODS	Multiple organ dysfunction syndrome
NO	Nitric oxide
OR	Odds ratio
PAMP	Pathogen-associated molecular pattern
PCT	Procalcitonin
PLA_2	Phospholipase A_2
PLA_2GIB	Group IB phospoholipase A_2 (“Pancreatic phospholipase A_2”)
PLA_2GIIA	Group IIA phospoholipase A_2
PRR	Pattern recognition receptor
PT	Prothrombin time
PTX3	Pentraxin 3
SNP	Single nucleotide polymorphism
SOFA	Sequential organ failure assessment
sTNF	Soluble tumor necrosis factor
sTREM -1	Soluble triggering receptor expressed on myeloid cells 1

suPAR	Soluble urokinase-type plasminogen activator receptor
TNF-α	Tumor necrosis factor –alpha
TLR	Toll-like receptor
VIP	Vasoactive intestinal peptide
WBC	White blood cell

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their respective Roman numerals (I – IV)

I. Uusitalo-Seppälä R, Koskinen P, Leino A, Peuravuori H, Vahlberg T and Rintala E: Early detection of severe sepsis in emergency department: Diagnostic value of plasma CRP, procalcitonin and interleukin-6. Scand J Infect Dis 2011; 43: 883 – 890.

II. Uusitalo-Seppälä R, Peuravuori H, Koskinen P, Vahlberg T and Rintala E: Role of plasma bactericidal/permeability-increasing protein, group IIA phospholipase A2, C-reactive protein and white cell count in early detection of severe sepsis in the emergency room. Scand J Infect Dis 2012; 44: 697-704.

III. Uusitalo-Seppälä R, Huttunen R, Tarkka M, Aittonimi J, Koskinen P, Leino A, Vahlberg T and Rintala E: Soluble urokinase-type plasminogen activator receptor (suPAR) in emergency room patients with suspected infection: a prospective cohort study. J Intern Med 2012; 272:247-56.

IV. Uusitalo-Seppälä R, Huttunen R, Aittoniemi J, Koskinen P, Vahlberg T and Rintala E: Pentraxin 3 (PTX3) is associated with severe sepsis and fatal disease in emergency room patients with suspected infection: a prospective cohort study. PLoS One 2013; 8 (1):e53661. doi: 10.1371/journal.pone.0053661. Epub 2013 Jan 14.

1 INTRODUCTION

Sepsis is one of the most common causes of death in hospitalized patients (Angus and Wax 2001). Patients who survive sepsis carry a significant risk of physical and cognitive impairment (Iwashyna et al. 2010). Sepsis not only causes deaths acutely but also increases the risk of death for up to 5 years after the septic episode even after comorbidities are accounted for. The risk of late death during the first year is associated with the severity of the septic episode (Quartin et al. 1997).

The early diagnosis and stratification of sepsis patients is a difficult but essential task in that early intervention and appropriate antimicrobial therapy can reduce mortality and improve prognosis (Garnacho-Montero et al. 2003; Kumar et al. 2006; Zambon et al. 2008). There has been a constant need for biomarkers which could indicate bacterial infection, sepsis or its severity, e.g. in the emergency room (ER) context (Marshall and Reinhart 2009; Pierrakos and Vincent 2010).

C-reactive protein (CRP) has been used for many years in clinical practice (Povoa et al. 2005; Schmit and Vincent 2008), but its specificity in differentiating between infection and non-infectious inflammation has not been satisfactory (Clyne and Olshaker 1999). The usefulness of CRP for the prognosis of sepsis has also been disputed (Silvestre et al. 2009). Among the most widely studied biomarkers of sepsis are procalcitonin (PCT) and interleukin-6 (IL-6). PCT has been proposed as a more specific diagnostic tool (Nakamura et al. 2009) and also a better prognostic marker for sepsis (Luzzani et al. 2003) than CRP. IL-6 is nonetheless important, as is the multifunctional cytokine (Song and Kellum 2005). Despite many published studies PCT or IL-6 has not been widely used in the emergency room (ER) setting, and there are only few prospective studies made from that perspective. New standardized analytical methods have made these parameters available for round-the-clock use, but their role in everyday practice in the ER has remained unclear.

The optimal sepsis marker has been assumed to have a function in the pathogenesis of sepsis (Marshall and Reinhart 2009). Several authors have suggested that bactericidal/permeability-increasing protein (BPI) and group IIA phospholipids A_2 (PLA_2GIIA) might have such a role (Vadas and Pruzanski 1993; Fourcade et al. 1995; Zasloff 2002; Boman 2003). The bactericidal action of BPI is enhanced by PLA_2GIIA (Madsen et al. 1996). PLA_2GIIA again, is regarded as an acute-phase reactant (Ogawa et al. 1992). However, there are few studies on the correlation between the circu-

latory levels of BPI, PLA_2GIIA and the severity of or mortality attending sepsis in the ER setting.

In predicting the outcome of a sepsis patient, the novel analyte soluble urokinase-type plasminogen activator receptor (suPAR) seems to be promising. suPAR has been intriguingly suggested to be "the molecular crystal ball" (Thuno et al. 2009). We sought here establish wether suPAR would be useful marker in predicting the severity or case fatality in our study cohort in the ER involving patients with suspected infection.

In the recent literature pentraxin-3 (PTX3) has also been shown to be a promising candidate as an early diagnostic and prognostic marker of sepsis (Sprong et al. 2009; Mauri et al. 2010; Huttunen et al. 2011). We now assessed its performance in the ER setting in our study population.

The aim here was to evaluate the usefulness of different sepsis biomarkers in the early stratification of patients admitted to the ER with suspected infection in a large cohort of adult subjects. Our work can be seen as a population-based study, as Satakunta Central Hospital has the only secondary care ER and intensive care unit in the Satakunta Hospital District. It also well represents the everyday clinical practice in ERs at least in Finland.

2 REVIEW OF THE LITERATURE

2.1 OVERVIEW ON SEPSIS AND SEVERE SEPSIS

2.1.1 Definitions of SIRS, sepsis and severe sepsis

Sepsis is a clinical syndrome which complicates severe infection. It is characterized by signals of inflammation (vasodilatation, leukocyte accumulation, increased microvascular permeability) occurring in tissues. In the literature definitions of sepsis are somewhat inconsistent, as is the use of terms like septicemia, sepsis or sepsis syndrome. Sometimes sepsis has been defined as blood culture positive infection, sometimes as an inflammatory state without infection at all. By reason of this heterogeneity evaluation of the literature has been difficult. A consensus conference between the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) in 1991 defined the clinical criteria for systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis and septic shock. These definitions were published in 1992, and in most sepsis studies patients are still classified on this basis (Bone et al. 1992).

In the ACCP/SCCM criteria infection was defined as the invasion of normally sterile tissue by organisms; the criteria for infection are discussed in greater detail in section 2.1.2.

The SIRS criteria are shown in Table 1. Two or more criteria are needed for definition. SIRS results from a dysregulated inflammatory response for many reasons, also non-infectious insult such as an autoimmune disorder, pancreatitis, vasculitis, thromboembolism, burns or surgery.

Table 1. The SIRS criteria according to ACCP/SCCM (Bone et al. 1992)

Two or more of the following conditions
1. Body temperature > 38°C or < 36°C
2. Heart rate > 90 beats per minute
3. Respiratory rate > 20 breaths per minute or PaCO2< 4.3 kPa,
4. White blood cell count > 12 x 109/l or < 4 x 10 9/l or >10 % immature (band) forms.

Sepsis is defined as SIRS in the presence of microbiologically proven or clinically suspected infection.

Severe sepsis is defined as sepsis with organ dysfunction, hypoperfusion or hypotension (systolic blood pressure < 90 mmHg or reduction ≥40 mmHg from baseline) due to sepsis (Table 2).

Table 2 The definition of severe sepsis according to modified ACCP/SCCM criteria (Levy et al. 2003).

Sepsis and at least one of the following signs of hypoperfusion or organ dysfunction
Areas of mottled skin **Capillary refilling requires three seconds or longer** **Urine output <0.5 ml/kg for at least one hours or renal replacement therapy** **Lactate > 2 mmol/l** **Abrupt change in mental status** **Abnormal electroencephalographic (EEG) findings** **Platelet count < 100 000 platelet /ml** **Disseminated intravascular coagulation (DIC)** **Acute lung injury or acute respiratory distress syndrome (ARDS)** **Cardiac dysfunction (i.e. left ventricular systolic dysfunction), as defined by echocardiography or direct measurement of cardiac index**

The criteria for ACCP/SCCM have been criticized for non-specificity and excessive sensitivity. In 2001 a new consensus conference reviewed the definitions for sepsis and related conditions. This consensus work was published in 2003 (Levy et al. 2003). Some updating was done, but basically the criteria remained unchanged.

Sepsis is currently classified on a clinical basis (sepsis, severe sepsis, septic shock). The description based on the SIRS criteria largely defines the pro-inflammatory phase of the syndrome, but the current classification fails to take proper account of the overall immune status of the individual patient. According to recent insights into the pathogenesis of sepsis the need for assessment of patients` immune status has emerged as important factor in the stratification of sepsis cases. This aspect is discussed more fully in the Pathophysiology section.

The staging system called PIRO (P=predisposition, I=insult, R=response, O=organ dysfunction) was proposed as a way of stratifying septic patients according to their Predisposing condition, the severity of Infection, the Response to therapy and degree of Organ dysfunctions. The model still awaits validation. Only a few studies have used and further processed the PIRO model (Rubulotta et al. 2009; Howell et al. 2011), but most of the sepsis studies and meta-analyses still use the criteria of ACCP/SCCM.

2.1.2 Definition of infection

The ACCP/SCCM consensus conference in 1991 defined infection as a pathologic process caused by the invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic micro-organisms (Bone et al. 1992). Bacteremia (correspondingly viremia, fungemia and parasitemia) was defined as the presence of viable bacteria in the blood (Bone et al. 1992). According to this definition a positive blood culture is not needed for the diagnosis of sepsis. The blood culture is positive in 20 – 55 % of patients with severe sepsis (Alberti et al. 2002; Valles et al. 2003; Ylipalosaari et al. 2006; Karlsson et al. 2007) and in 17 % of sepsis patients (Brun-Buisson et al. 1996). The blood culture is positive in 8 – 10 % of SIRS patients in the ER setting (Bates et al. 1997; Yanagihara et al. 2010). In one population-based study of bloodstream infections in Finland cultures were positive in 4 % of all blood cultures taken, corresponding to 28 – 44 culture positive bloodstream infections per 1000 blood cultures (Skogberg et al. 2008).

After a consensus conference in 2003, the definitions of infection in the ICU patients were published in 2005 (Calandra and Cohen 2005). Precise criteria were formulated for six most common types of infection in the ICU, i.e. pneumonia, bloodstream infection (and endocarditis), intravascular catheter-related sepsis, intra-abdominal infection, urosepsis and skin and soft tissue infection (surgical wound infections or non-surgical infec-

tion). Each infection was categorized as being microbiologically confirmed, probable or possible. These definitions have not, however, been uniformly adopted in the sepsis literature.

The source of infection or the causative micro-organisms may be important determinants of the outcome of sepsis patients. It has long been known that for example sepsis originating from the urinary tract is associated with a low mortality rate (Roberts et al. 1991). Mortality from sepsis has been shown to be higher if the source of infection is unknown, gastrointestinal or pulmonary, compared to urinary (Krieger et al. 1983). This observation has recently been challenged in a large French cohort study (Zahar et al. 2011), where pathogen species or infection sites were not associated with mortality when the severity of sepsis and early and appropriate antimicrobials were taken into account.

In the Finnsepsis study (Karlsson et al. 2007) the most common sources of severe sepsis were lungs (43 %), abdomen (32%), skin or soft tissue (10 %) and urinary tract (23%). The infection was communityacquired in 58 % of cases. Blood cultures were taken from 68 % patients and were positive in 40 % of cases. Antibiotics were started before blood culture sampling in over onethird of cases. Gram-positive bacteria were found in 58 % and gram-negative in 33 % of positive blood cultures; yeasts were rare (4%) (Karlsson et al. 2007).

In a population-based study of bloodstream infections (BSI) in Finland (Skogberg et al. 2012) the most common blood culture isolates were Escherichia coli (27 %), Staphylococcus aureus (13%), coagulase negative staphylococci (10%) and Streptococcus pneumoniae (9%). More than 33 % of deaths were caused by E.coli and Staph. aureus infections (Skogberg et al. 2012).

2.1.3 Epidemiology of sepsis and severe sepsis

The incidence of sepsis is growing. In the United States in the late 1970s it was estimated that 164 000 cases of sepsis occurred annually. More recently the estimate has been higher, more than 650 000 cases of sepsis every year (Angus et al. 2001; Martin et al. 2003). The same trend has been noted in Europe (Harrison et al. 2006; Esper and Martin 2009) and also in Finland (Skogberg et al. 2008; Skogberg et al. 2012). In one Finnish popula-

tion-based study of blood culture positive bloodstream infections (BSI) 2007 the annual incidence of BSI in Finland was 168 per 100 000 and during 2004-2007 the average annual increase of cases was 4.4 % (Skogberg et al. 2012). Increasing trend in BSI rates in Finland have been shown to associating with changes in blood-culturing activity (Skogberg et al. 2008; Skogberg et al. 2012) and development in blood-culturing methods (Sogaard et al. 2011).

Only a part of the sepsis patients are blood culture positive and the exact number of sepsis cases is not known, but it is clear that the incidence is growing. The rise in blood culture positive sepsis cases is thought to be due to ageing, increasing use of immunosuppressive therapies, increasing surgery with prostethic materials and also more aggressive intensive care treatments (Esper and Martin 2009). The growing proportion of multidrug-resistant infections makes antibiotic policy more difficult and may also increase the incidence and impact of sepsis.

The annual incidence of severe sepsis in the US has been estimated to be as high as 3.0 cases per 1000 population and 2.3 cases per 100 hospital discharges (Angus et al. 2001). This estimate was derived from a large retrospective analysis in the USA including 6.6 million patients and 192 000 severe sepsis periods. The incidence of severe sepsis in ICU patients has varied between 0.51 and 1.1 case per 1000 population (Padkin et al. 2003; Engel et al. 2007) In the Finnsepsis study the incidence of severe sepsis in adults in Finland was estimated to be lower, 0.38 per 1000 population (Karlsson et al. 2007).

2.1.4 Prognosis

Mortality due to sepsis increases according to disease severity: in one earlier study the case fatality figures for SIRS, sepsis, severe sepsis and septic shock were 7, 16, 20 and 46 percent, respectively (Rangel-Frausto et al. 1995). Estimates of mortality due to severe sepsis vary between 27 and 59 per cent (Martin et al. 2003; Padkin et al. 2003,). Rapid intervention and appropriate antimicrobial therapy can reduce mortality and improve the prognosis of patients (Varpula et al. 2007; Zambon et al. 2008; Levy et al. 2010; Zahar et al. 2011). The duration of hypotension before initiation of effective antimicrobial treatment is a critical factor for survival in septic shock (Kumar et al. 2006). In one study the initial antimicrobial therapy

was inappropriate in 20 % of patients and this was associated with a five-fold reduction in survival (Kumar et al. 2009). In the Surviving Sepsis Campaign it was noted that case fatality due to severe sepsis treated in the ICU has been falling due to modern intensive treatment (Levy et al. 2010) and the mortality rate for severe sepsis appears to be decreasing although the amount of sepsis is increasing (Martin et al. 2003; Dombrovskiy et al. 2005; Levy et al. 2010).

In Finland case fatality for severe sepsis has been less than 30 % (Varpula et al. 2007; Karlsson et al. 2009). It has been shown that mortality increases according to the number of organs failing: the failure of one organ is associated with a case fatality rate of 11.5 % and three 34.0 % (Varpula et al. 2007). Among infection patients in the ER it is of the utmost importance to identify those with sepsis or severe sepsis early enough to improve their prognosis.

2.2 PATHOPHYSIOLOGY OF SEPSIS

2.2.1 Host responses to infection

Sepsis consists in complex pathophysiologic processes in which different cell types, tissues and organ systems are involved. The condition is characterized by both innate and adaptive immune reactions. Over the last few decades the understanding of pathogen-host interactions and inflammation has increased (Medzhitov 2008; van der Poll and Opal 2008), but an integrated view of the pathophysiology of sepsis and its critical points is still lacking (Rice and Bernard 2005; Russell 2006). The host response to infection is a complex system which controls bacterial invasion in the first line, simultaneously initiating the repair of affected tissue. It involves the activation of circulating phagocytic cells and the generation of pro-inflammatory and anti-inflammatory mediators. According to the consensus definition sepsis occurs when the response to infection becomes generalized (ACCM/SCCM 1992) (Bone et al. 1992). Coagulation, complement system activation inflammation, and apoptosis (process of programmed cell death) all take part in the sepsis process (Hotchkiss and Karl 2003; Hotchkiss and Nicholson 2006; Medzhitov 2008; van der Poll and Opal 2008). The outcome is determined not only by the features of the pathogen, but also by the host response, which can be extravagant and result in collateral organ and tissue damage (Nathan 2002). A simplified illustration of the inflammatory response is presented in Figure 1, modified from Reinhart (Reinhart et al. 2012).

The host response to infection is initiated when innate immunity cells (macrophages, monocytes, other myeloid cells, and also endothelial cells) recognize and bind to microbial components by different pathways. Pattern recognition receptors (PRRs) on the surface of host immune cells recognize and bind the pathogen-associated molecular patterns (PAMPs) of microbes (Janeway and Medzhitov 2002). Toll-like receptors (TLRs) and lectin receptors on the cell surface recognize many bacterial substances in the extracellular space (Kumar et al. 2011). Earlier endotoxin and lipopolysaccarides (LPS) found in the cell wall of gram-negative bacteria were seen to initiate the sepsis process (Ulevitch and Tobias 1999). Endotoxin is a good example of a PAMP and in fact, the receptor of LPS was the first TLR found in mammals.

Figure 1 (see over). A simplified overview of the course of the inflammatory response, modified from Reinhart (Reinhart et al. 2012). The analytes studied in this thesis are bolded. PAMP (pathogen-associated molecular pattern), DAMP (danger-associated molecular pattern), LPS (lipopolysaccharide, part of the membrane of gram-negative bacteria), LTA (lipotheichoid acid, part of the cell-wall of gram-positive bacteria), HMGB-1 (high-mobility-group protein B1), C5a and C3a (complement components 5a and 3a), C5aR (C5a receptor protein), aPPT (activated partial thromboplastin time), PT (prothrombin time), AT (antithrombin), ELAM-1 (endothelial leukocyte adhesion molecyle 1), ICAM-1 (intercellular adhesion molecule 1), ACE (angiotensin converting enzyme), NO (nitric oxide), VIP (vasoactive intestinal peptide), CRP (C-reactive protein), PCT (procalcitonin), LBP (LPS-binding protein), PTX3 (pentraxin 3), PLA2GIIA (Group IIA phospolipase A2), IL-6 (interleukin-6), IL-8 (interleukin-8), MIF (macrophage migration inhibitory factor), BPI (bactericidal/permeability-increasing protein), suPAR (soluble urokinase-type plasminogen activator receptor), sTNF (soluble tumor necrosis factor), sTREM -1 (soluble triggering receptor expressed on myeloid cells 1), TLR 2 and 4 (Toll-like receptor 2 and 4), mHLA-DR (monocytic human leukocyte antigen DR), CD 64 and CD 48 (integral membrane glycoproteins 64 and 48), DIC (disseminated intravascular coagulation).

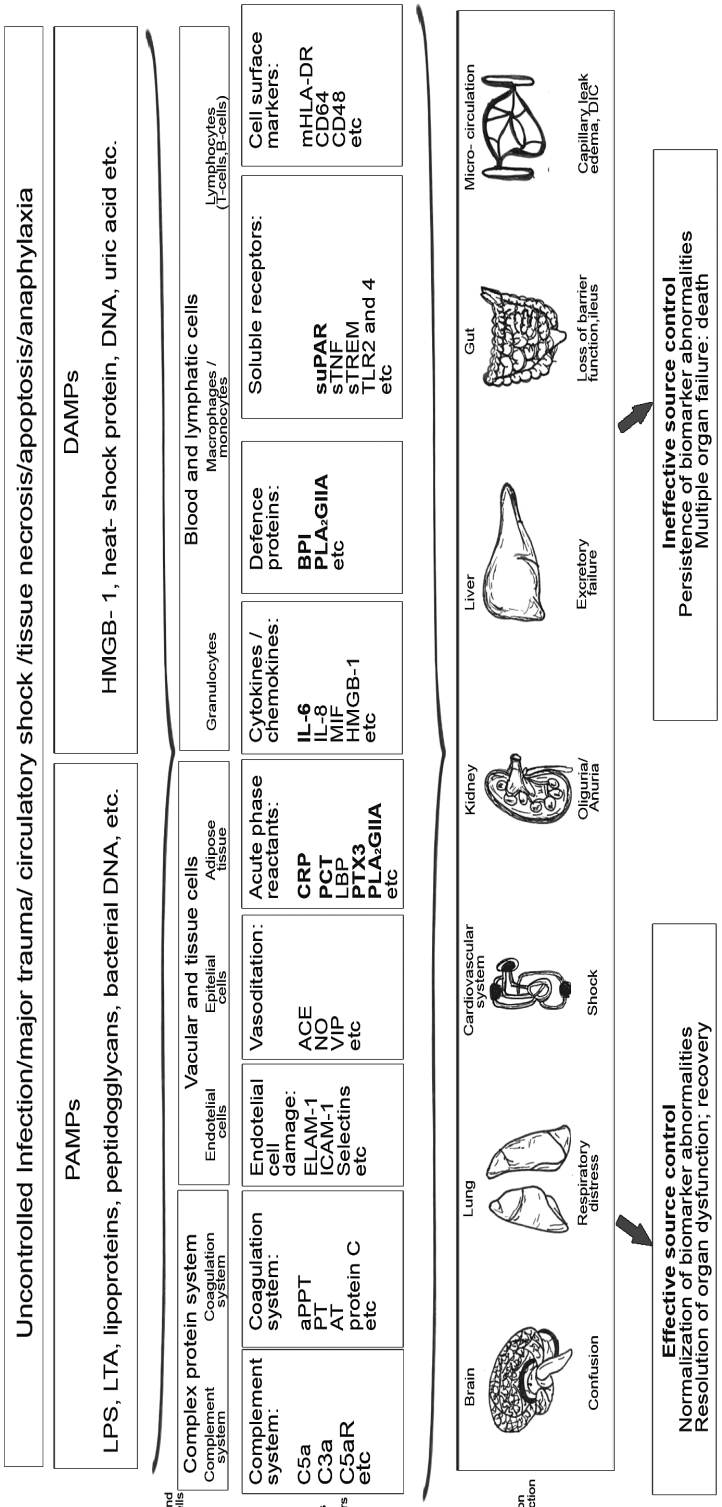


Figure 1. A simplified overview of the course of the inflammatory response, modified from Reinhart (Reinhart et al. 2012). The analytes studied in this thesis are bolded. Abbreviations used here are presented on the previous page.

The binding of micro-organisms to immune cell surface receptors induces the activation of inflammatory, coagulation and complement cascades (Ward 2008; Opal and Patrozou 2009). Polymorphonuclear leukocytes activate adhesion molecules, leading to their aggregation and marginalization to the vascular endothelium. This is facilitated by endothelium expressing adherence molecules which attract leukocytes (Movat et al. 1987). Leukocytes then migrate to the site of injury. The release of mediators by polymorphonuclear leukocytes at the infection focus is responsible for the signs of local inflammation: warmth and erythema due to vasodilatation and hyperemia, and edema due to increased microvascular permeability (Movat et al. 1987).

The normal response to infection is regulated by a mixture of proinflammatory and anti-inflammatory mediators secreted by activated macrophages (van der Poll and Lowry 1995). Major pro-inflammatory cytokines include tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β). Secretion of TNF-α is self-sustaining, while other cytokines and mediators (e.g. IL-6, IL-8, IL-10, and platelet activation factor and interferon gamma) increase the levels of other mediators. The proinflammatory environment leads to recruiting of more polymorfonuclear leucocytes and macrophages (van der Poll and Lowry 1995).

Cytokines which inhibit the production of TNF-α and IL-1β are classified as anti-inflammatory cytokines. They suppress the immune system by inhibiting cytokine production. However their effects may not be solely anti-inflammatory. IL-6, for example, enhances B cell proliferation and immunoglobulin secretion and increases the development of cytotoxic cells (Szabo et al. 1991). The balance of proinflammatory and anti-inflammatory mediators regulates the complex inflammatory process, including adherence, chemotaxis, phagocytosis and bacterial killing. Combinations of cytokines can have additive, inhibitory or synergistic effects and complex molecular mechanisms regulate these processes (Bode et al. 2012). If the mediator balance is suppressed, homeostasis will be restored (Bone 1996). Bone called this a “compensatory anti-inflammatory response syndrome” (CARS), which may follow the hyper-inflammatory phase, especially in patients with severe sepsis (Bone et al. 1997) (Figure 3 on page 28). During this phase multiple organ dysfunctions may be present and the patient is also susceptible to nosocomial infections (Bone et al. 1997; Kollef et al. 2008; Limaye et al. 2008).

Profuse release of pro-inflammatory cytokines (e.g. TNF-α and IL-1β) in patients with sepsis may contribute to the progression of local infection to sepsis (Pinsky and Matuschak 1989; Pinsky et al. 1993). These mediators can cause many physiological changes such as fever, hypotension, leukocy-

tosis, further induction of pro-inflammatory cytokines (e. g. IL-6 and Il-8) and acute phase proteins, and the activation of coagulation and fibrinolysis. TNF-α is thought to have a central role in sepsis. The infusion of TNF-α produces symptoms resembling septic shock (Tracey et al. 1986). High levels of TNF-α in sepsis are due to the binding of endotoxin to LPS-binding protein and later transfer to CD14 on macrophages, which stimulate TNF-α release (Tracey and Lowry 1990).

The polymorphonuclear leukocytes are the central cellular effectors of the primary defense system, developing in the bone marrow where the cytoplasmic granules are syntethized. A diversity of neutrophil subpopulations has been described (Borregaard 1997), and the antimicrobial proteins and peptides appear to be confined to primary azurophilic and specific granules. Bactericdal/permeability –increasing protein (BPI) was identified in human azurophilic granules and shown to be antibacterial towards E. coli and several other gram-negative bacteria (Weiss et al. 1978). BPI plays an important role in the oxygen-independent mechanism for bacterial killing (Weiss and Olsson 1987). BPI together with PLA2GIIA can inhibit the biological effect of bacterial lipopolysaccaride (LPS) and modify the permeability of the cell membrane (Wiese et al. 1997).

The complement system is a multistep protein cascade which helps clear many pathogens (Walport 2001; Walport 2001). It mediates control against infectious agents and links primary and adaptive immunity. The bactericidal action of the complement system consists of classical, alternative and lectin pathways. The important step in complement-mediated bacteriolysis via all these pathways is the deposition of the activated third component of complement (C3b) on bacterial surface. This leads to changes in membrane composition and in cell lysis (Wright and Levine 1981; Taylor and Kroll 1984). Complement also binds to cells have undergone apoptosis. Activation of the complement system plays an important role in sepsis (Walport 2001; Walport 2001). Inhibition of the system reduces inflammation and lessens mortality in animal models (Guerrero et al. 1993; Furebring et al. 2002; Liu et al. 2007).

Extensive cellular injury may occur when the immune response becomes generalized in sepsis. Cellular damage is the precursor to organ dysfunction. The exact mechanism of cellular injury is not fully understood, but tissue ischemia, cytopathic injury and apoptosis have been proposed. The organ failure in severe sepsis resembles the multiple organ dysfunction syndrome (MODS) seen in patients with severe traumatic injury (Ni Choileain and Redmond 2006).

Apoptosis or so-called programmed cell death is the important mechanism by which dysfunctional cells are normally eliminated. It constitues a series of regulated physiologic and morphologic cellular changes leading to cell death. This is also an important process in repairing inflammatory tissue damage once an infection has settled (Hotchkiss and Nicholson 2006; van der Poll and Opal 2008).

Critically ill patients often have systemic activation of both inflammation and coagulation (Levi 2010). During sepsis or SIRS, both endothelial cells and activated mononuclear cels may produce pro-inflammature cytokines that mediate coagulation activation. Activation of the coagulation system and thrombin generalization is dependent on an IL-6-induced expression of tissue factor on activated mononucler cells and endothelial cells (Levi 2010). At same time endothelial-bound anticoagulants, for example protein C system and the antithrombin system, are shutt-off by pro-inflamatory cytokines. Activated coagulation proteases can bind to protease-activated resceptors and intracellular signaling leads to increased production of prooinflammatory cytokines (Levi 2010).

The vascular endothelium is involved in three processes which play roles in sepsis phathophysiology: vascular tone, vascular permeability and coagulation (Schouten et al. 2008). Insufficient oxygen delivery in relation to oxygen consumtption is a typical feature of sepsis. It is believed that a critical cause of organ failure in severe sepsis is abnormal heterogeneity in the function of microcirculatory within tissues for imbalance in the coagulation and fibrinolytic systems, both activated in the course of sepsis (Lehr et al. 2000; Sakr et al. 2004). One possible mechanism for tissue ischemia may also be endothelial lesion, which results from interaction between endothelial cells and activated polymorphonuclear cells (Helliwell et al. 1998). This induces among other things the secretion of lytic enzymes and vasoactive substances (e.g. nitric oxide, endothelin, platelet-derived growth factor and platelet activating factor) into the extracellular medium (Helliwell et al. 1998; Sakr et al. 2004; Trzeciak et al. 2007; Trzeciak et al. 2008) damaging endothelial cells. During sepsis the erythrocytes became more rigid and lose their ability to deform in the systemic microcirculation (Piagnerelli et al. 2003).

There is increasing evidence to show that patients who survive over the initial sepsis process often develop nosocomial infections or may suffer a reactivation of latent viruses (Kollef et al. 2008; Limaye et al. 2008). These observations have led to the hypothesis that the early hyperinflammatory state is followed by a hypoinflammatory phase with marked immunosupression called immunoparalysis (Volk et al. 2000). Immunoparalysis in patients with sepsis is further characterized by an association between low

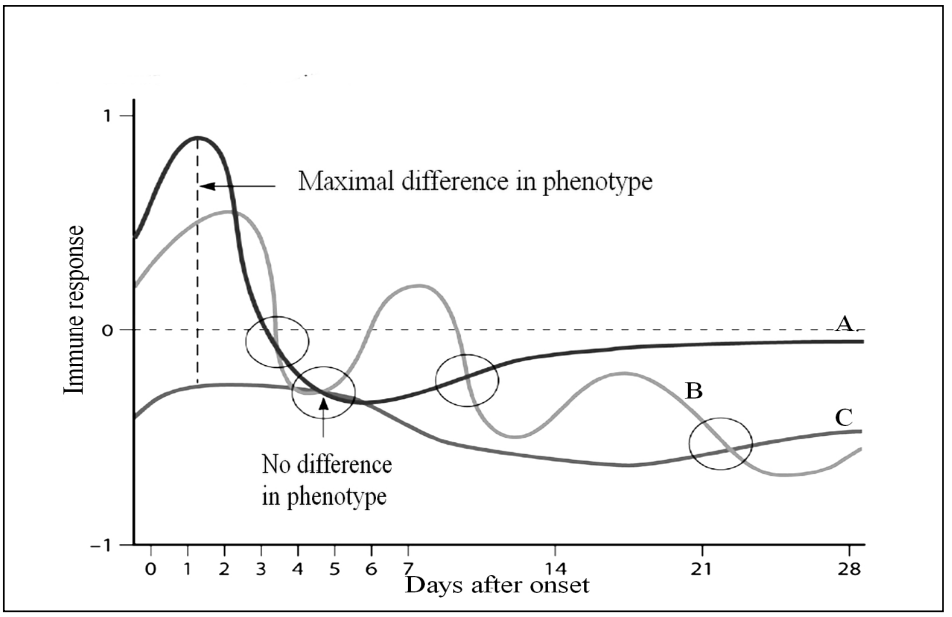
level of monocytic HLA-DR surface expression and immune cell dysfunctions (Wolk et al. 2000). The immunosuppression caused by sepsis is manifested by the loss of the delayed-type hypersensitivity response, a failure to clear the primary infection, and the development of secondary infections (Hotchkiss et al. 2009). Patients who die of sepsis often yield biochemical, cytological and immunohistochemial findings consistent with immunosuppression (Boomer et al. 2011). In Table 3 potential mechanisms of immune suppression in patients with sepsis are presented.

Table 3. Potential mechanisms of immune suppression in patients with sepsis (Hotchkiss and Karl 2003)

Shift from an inflammatory (Th1) to an anti-inflammatory (Th2) response
Anergy
Apoptosis-induced loss of CD4 T cells, B cells, and dendritic cells
Loss of macrophage expression of major-histocompatibility-complex class II and co-stimulatory molecules
Immunosuppressive effect of apoptotic cells

In conclusion, sepsis may not be solely an uncontrolled and overwhelming inflammatory process but may also be associated with an immunosuppressive or anergic state. In autopsy studies in animals and humans who succumbing to sepsis apoptosis of lymphocytes has been observed (Coopersmith et al. 2002; Hotchkiss and Karl 2003). In the majority of immunocompetent patients with sepsis the inflammatory response will involve a more or less pronounced proinflammatory phase, dominated by the activation of innate immune weapons such as leukocytes, monocytes, macrophages, complement and coagulation systems, endothelial or epithelial cell response etc (Figure 1 on page 23). This may or may not be followed by an immunosuppressive phase. The potential for an inflammatory response will vary from patient to patient, as described in the Figure 2 on next page. This variation reflects the overall differences in the response profile to sepsis between individuals and the dynamic nature of sepis (Hotchkiss and Karl 2003; Reinhart et al. 2012). These differences may derive from individual genetic susceptibilities. This is clarified in Section 2.2.3.

The difficulties to understand and control the complex process of severe sepsis have let to several disappointments in therapeutic interventions (Opal et al. 1997; Abraham et al. 1998; Giroir et al. 2001; Warren et al. 2001; Russell 2006; Marti-Carvajal et al. 2012).



A= healthy person severe course of sepsis, B=healthy individual no source control,
C= immune suppressed person

Figure 2. Various immune responders for sepsis (Figure modified from Reinhart (Reinhart et al. 2012).

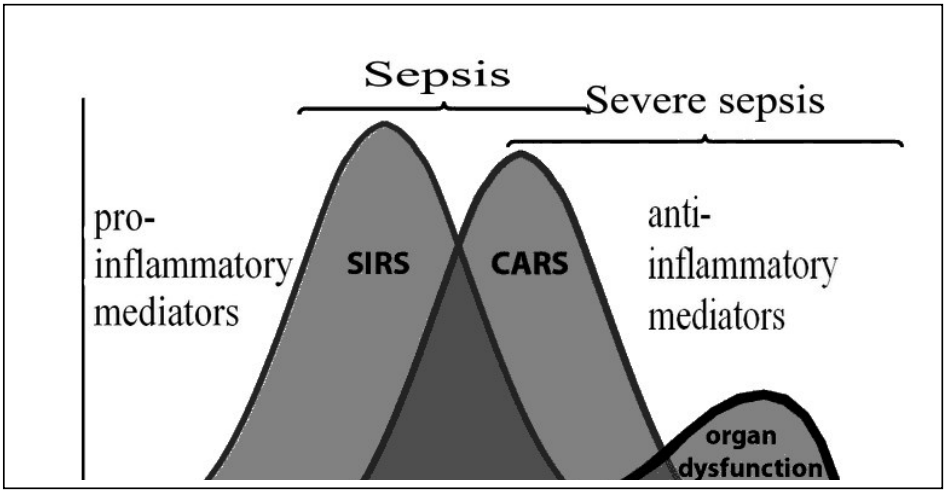


Figure 3. An alternative model for the progression of sepsis to severe sepsis suggesting that the Compensatory Anti-inflammatory Response Syndrome (CARS) begins while the pro-inflammatory SIRS is still present. Modified from Faix (Faix 2013)

2.2.2 Effects of microbes on response

A variety of bacteria, fungi and to some extent also viruses may be reasons for sepsis. Gram-positive and -negative bacteria, fungi and viruses all have different cell-wall molecules which bind to specific receptors on the surface of immune cells. The original model of sepsis was a response to endotoxin, a lipopolysacaride (LPS) found in the cell wall of gram-negative bacteria (Ulevitch and Tobias 1999). LPSs bind to lipopolysacaride-binding protein (LBP) (Schumann et al. 1996). This LPS/LBP complex is then presented to CD14 receptors on neutrophils, endothelial cells, macrophages and monocytes, leading to activation of these cells (Van Amersfoort et al. 2003). LBP could also join with lipoteichoic acid and pepidoclygans of gram-positive bacteria. Lipopeptides present in spirochetes, mycoplasma, and mycobacterium species are also recognized by LBP (Schroder and Schumann 2005). Peptidoglycans of gram-positive bacteria and LPS/LBP complex bind to TLR-2 and TLR-4 respectively (Takeuchi et al. 1999). TLR-2 is activated not only by gram-positive bacteria but also by fungi (Toshchakov et al. 2002). TLR-2 and TLR-4 have been shown to induce nitric oxide synthase II (iNOS) and TNF-α in macrophages by different signaling pathways (Paul-Clark et al. 2006). This activation of TLR leads to production of pro-inflammatory cytokines and activation of the adaptive immune response, but it can also cause both direct and indirect tissue injury in the host (Russell 2006).

Also different bacterial products (e.g. staphylococcal enterotoxin B, toxic shock syndrome toxin-1, Pseudomonas exotoxin A, and M protein of hemolytic group A streptococci) may contribute to the progression of a local infection to sepsis (Opal and Cohen 1999; Hotchkiss and Karl 2003). The same is true of the endotoxin of gram-negative organisms. Endotoxin may cause many features of sepsis when infused into animals (Lin et al. 1994; Evans et al. 1995; Jellema et al. 2002) or human volunteers (von der Mohlen et al. 1995; Jellema et al. 2002; van der Poll and Opal 2008). This includes activation of complement, coagulation and fibrinolytic systems. This may lead to microvascular thrombosis and the production of vasoactive products such as bradykinin (van der Poll and Opal 2008). Elevated plasma endotoxin is associated with shock and multiple organ dysfunctions (Guidet et al. 1994).

Pathogenic strains of bacteria differ from other species by the expression of specific clusters of virulence genes (Merrell and Falkow 2004; van der Poll and Opal 2008). The regulators of these genes have been proposed as potential therapeutic targets in the treatment of sepsis (Merrell and Falkow

2004; van der Poll and Opal 2008). The increasing levels of resistance to antibiotics is an emerging problem for antimicrobial treatment globally and also in Finland. Septic infections caused by antibiotic-resistant bacteria may have higher mortality and morbidity rates than similar infections with antibiotic-susceptible strains (Cosgrove et al. 2003; Schwaber and Carmeli 2007). The impact of antibiotic miss-match on mortality may vary according to the particular bacteria, the infection foci and patient characteristics. However, the selection of an efficient antimicrobial agent is increasingly challenging and therefore early detection of antimicrobial resistance in sepsis is of utmost importance.

2.2.3 Genetic susceptibility

Some individuals may be genetically more prone to the onset of a septic condition during infection. Studies undertaken during the last decade on genetic associations in sepsis have focused on the assessment of single polymorphisms using candidate gene strategy (Chen and Sullivan 2003; Arcaroli et al. 2005). The single nucleotide polymorphism (SNP) is the most common form of gene variation. The total number of common SNPs in the human genome is estimated to be more than 10 million. SNPs are used as genetic markers (Arcaroli et al. 2005). Polymorphism in innate immunity genes result significant interindividual variability in response to sepsis (Holmes et al. 2003). Even the risk of dying from sepsis seem to be more dependent on genetic than on environmental factors (Petersen et al. 2002).

Genome-wide association studies have pinpointed inflammation- and innate immunity-related genes associated with sepsis (Sutherland and Walley 2009; Tang et al. 2010; Wong et al. 2010). Stratification methods based on the genetic background of patients are under development (Wong et al. 2010). In the present work genetic variation was not studied.

2.3 DIAGNOSTIC AND PROGNOSTIC BIOMARKERS OF SEPSIS

2.3.1 Overview of sepsis biomarkers

Diagnostic biomarker of sepsis are used either to identify or rule out sepsis (Dellinger et al. 2008; Marshall and Reinhart 2009) or to identify the etiology of SIRS (e.g. differentiating bacterial from viral infection or bacterial infection from other inflammatory states). The optimal biomarker for the diagnosis and prognostic evaluation should have rapidly increasing circulatory levels in response to infectious stimulus, be analytically stable and readily available in the emergency setting and simple to analyze, and its utility must be well documented. It should also have a reasonable cost-effect ratio, and should have good sensitivity and specificity for sepsis.

Prognostic biomarkers are used in assessing the progression of severe sepsis or organ dysfunction or even case fatality. Biomarkers are also used to guide the antimicrobial treatment. Several biomarkers have been studied either as a therapeutic goal for sepsis or as a diagnostic tool or an indicator of sepsis severity (Dellinger et al. 2008).

Hitherto some 200 different biomarkers have been suggested as diagnostic or prognostic biomarker for sepsis. In a large and relatively recent review of sepsis biomarkers Pierrakos and Vincent summarized 3370 references covering 178 biomarkers (Pierrakos and Vincent 2010). This diversity reflects a complex pathophysiology of sepsis (Marshall et al. 2003). Coagulation, complement system activation, inflammation and apoptosis are all involved in the sepsis process, and separate markers for each system have been proposed (Pierrakos and Vincent 2010). The systemic nature of sepsis and many cell types, tissues and organs involved expand the number of potential biomarkers (Figure 1, page 23).

Most of the biomarkers used in clinical studies have been studied primarily as prognostic indicators and relatively few have been used for diagnosis (Pierrakos and Vincent 2010; Faix 2013). By reason of the large number of parameters it is impossible to evaluate all markers detailed here. After their large review Pierrakos and Vincent concluded that none of the biomarkers has sufficient specificity or sensitivity to be routinely employed in clinical practice (Pierrakos and Vincent 2010). PCT and CRP have been most

widely used, but even these have limited ability to distinguish sepsis from other inflammatory conditions or to predict outcome. The markers evaluated in our studies are discussed in greater detail in the following.

2.3.2 C-reactive protein (CRP)

C-reactive protein (CRP) is an acute phase reactant, a protein synthesized in the liver mainly in response to IL-6, which is produced not only during infection but also in many inflammatory processes (Volanakis 2001; Black et al. 2004).

CRP was discovered by Tillet and Francis as far back as 1930 (Tillett and Francis 1930). It is a classical member of the pentraxin family, characterized as a short pentraxin. It is a 224-residue protein and is an annular pentameric disc in shape. The CRP gene is located on the first chromosome. (Volanakis 2001; Black et al. 2004)

CRP is widely used for the diagnosis of bacterial infections, but its levels in the circulation are also elevated in viral or fungal infections, rheumatic and other inflammatory diseases, malignancies and tissue necrosis or injury (Volanakis 2001; Black et al. 2004). During the acute-phase response, the levels of CRP start to increase within 4-6 hours of acute insult, reaching a peak at 48-72 hours. With resolution, CRP declines with a relatively long half-life of 18 - 20 hours (Volanakis 2001; Black et al. 2004). Clinically it is important to note that CRP levels can be normal in the early stage of serious infection and sepsis. Serial measurement of CRP level has proved a relatively good indicator of the efficacy of treatment (Povoa et al. 2006; Schmit and Vincent 2008).

CRP is a component of the innate immunity response and has both proinflammatory and anti-inflammatory actions (Janeway and Medzhitov 2002; Black et al. 2004; Marnell et al. 2005). It binds to microbial polysaccharides, activating the classical complement pathway. CRP can also bind to phagocytic cells via Fc receptors, suggesting that it can initiate elimination of pathogens and targeted cells by interaction between both humoral and cellular ways of inflammation (Marnell et al. 2005). CRP also has a role in host defence and clearance of necrotic and apoptotic cells (Volanakis 2001). Pro-inflammatory functions of CRP include activation of the complement system, the introduction of monocytes of inflammatory cytokines and tissue factor (Ballou and Lozanski 1992) and shedding of the IL-6 re-

ceptor (Jones et al. 1999). As a consequence the CRP response to tissue injury may sometimes worsen tissue damage.

Various analytical methods are used in measuring the CRP concentration, for example ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutinination. In one meta-analysis on serum procalcitonin and CRP as markers of bacterial infection, means of CRP levels varied widely, with 8 different methods used among the 12 studies included (Simon et al. 2004).

CRP levels vary with age, sex and race (Woloshin and Schwartz 2005). Population studies describe a skewed distribution of the plasma CRP concentration. Approximately 70 % of samples from the reference population have a CRP level under 3 mg/l, but some individuals have minor elevations up to 10 mg/l. It is useful to regard CRP levels from 3 to 10 mg/l as only minor elevations, while values greater than 10 mg/l are to be seen to reflect clinically significant inflammation or infection (Kushner 1982). Values between 3 and 10 mg/l are noted in minor inflammations such as parodontitis but also states like obesity, diabetes mellitus, uremia or even depression or smoking (Kushner et al. 2006). These levels are also considered risk factor for cardiac events (Ridker et al. 2008; Yousuf et al. 2013).

CRP is, at least in Finland, one of the most commonly used laboratory tests in the ER. It is cheap and easy to standardize and has been widely used for the detection of infection and inflammation. Although CRP concentrations tend to be higher in invasive bacterial infections than in viral infections, the ability in differentiating bacterial from viral infection is not very good (Salonen and Vaheri 1981; Young et al. 1991; van der Meer et al. 2005).

CRP does not show sufficient specificity for an accurate diagnosis of sepsis or severe sepsis in the ER setting. Its sensitivity in detecting sepsis is relatively poor (Rintala et al. 1992; Reinhart et al. 2012). Its usefulness as a prognostic factor in sepsis has been disputed (Suprin et al. 2000; Pettilä et al. 2002; Silvestre et al. 2009).

2.3.3 Procalcitonin (PCT)

Procalcitonin (PCT) is a 12.6 kDa polypeptide composed of 116 amino acids (Jacobs et al. 1981; Weglohner et al. 2001) It was first described as a precursor for calcitonin hormone (Roos et al. 1974; Jullienne et al. 1980),

found in the C cells of the thyroid and the pulmonary endocrine cells. Calcitonin has an important role in calcium homeostasis.

In 1993 Assicot and colleagues demonstrated increased PCT concentrations in patients with sepsis and infection (Assicot et al. 1993). Procalcitonin in serum is also elevated for several other reasons, as shown in Table 4.

Table 4. Causes of elevated hyperprocalcalcitonemia, modified from Becker (Becker et al. 2008).

A	**Neuroendocrine tumors** **Medullary thyreoid cancer** **Small cell cancer** **Carcinoid syndrome**
B	**Noninfectious systemic inflammation** **Inhalational injury** **Pulmonary aspiration** **Pancreatitis** **Heat stroke** **Mesenteric infarctation**
C	**Severe local infection** **Bacterial** **Viral** **Parasitic**
D	**Sepsis**
E	**Trauma** **Mechanical injury** **Burns** **Surgery**

In marked systemic inflammatory conditions PCT is produced by various cells e.g. in lung, liver, kidney, fat, muscle, stomach and intestine (Müller et al. 2001). High PCT levels during infection are not followed by an increase in calcitonin or calcium levels in serum (Snider et al. 1997). This may be due to changes in the promoter of the PCT gene, responding to intestinal translocation of lipopolysaccharide or other bacterial constitutes, or by a secondary proinflammatory cytokine stimulus such as TNF-α (Domenech et al. 2001).

In animal models, the levels of PCT in the circulation are rapidly elevated after injection of bacteria or bacterial toxins. The PCT response has been shown to be faster than the CRP response. The plasma half-life of PCT is up to 24 hours. Measurements of PCT in healthy individuals have shown

very low levels (<0.05 ng/ml) (Snider et al. 1997; Nijsten et al. 2000). In Finland SI-unit µg/l is usually used when reporting PCT concentration.

In measuring the procalcitonin concentration in serum or plasma many methods have been used. Procalcitonine assays measure not exclusively procalcitonin but also at least one other constituent of this prohormone (Becker et al. 2010). The most widely studied assay to date has been the LUMItest (Brahms Henningsdorft, Germany). It is an immunoluminometric (ILMA) assay. LUMItest is rather insensitive and cannot thus detect mild elevations (Becker et al. 2010). Other ILMA assays, "ProCa-S" (Brahms) and "PCT sensitive" (Brahms) have proved more sensitive. In 1995 a new more sensitive ELISA test was developed for the aminoterminus of PCT which also detects the intact PCT prohormone (Nylen et al. 1995), and its utility in evaluating SIRS and sepsis has been reported in several publications (Snider et al. 1997; Whang et al. 1998; Ammori 2003). Newer commercial tests like the Kryptor PCT assay (Brahms) using time-resolved amplified cryptate emission and a new procalcitonin assay from Roche (ECLIA) are sufficiently sensitive and ideal for large numbers of determinations (de Wolf et al. 2009). One previous study (Prieto and Alvarez 2009) reported an approximately 15 % negative bias when comparing ECLIA from Roche to the Brahms Kryptor PCT assay. It is thus important to specify the method used for laboratory analysis when proposing cut-off limits for clinical use. New analytical methods have made it possible to use PCT measurement also in everyday practice in the emergency room.

PCT has been extencively studied as a sepsis biomarker in recent years. Several earlier works comparing PCT, IL-6 and CRP have demonstrated that PCT is the best parameter for identifying sepsis (Pettilä et al. 2002; Balc et al. 2003; Bell et al. 2003; Aikawa et al. 2005), but whether PCT is more sensitive and specific than CRP for the diagnosis of sepsis is still discussed. In an attempt to clarify the situation a meta-analysis by Uzzan and associates reviewed and analysed 25 studies with a total of 2966 patients (Uzzan et al. 2006). A subanalysis was made of 15 studies to compare the diagnostic ability of CRP versus PCT. The results of the meta-analysis found a global diagnostic accuracy odds ratio for PCT of 15.7 (95 % confidence interval 9.1 – 27.1) and of 5.4 (3.2 – 9.2) for CRP. The large studies included tended to find lower estimates for PCT sensitivity and specificity than smaller studies. Despite the limitations the investigators concluded that "PCT represents a good biological diagnostic marker of sepsis, severe sepsis and septic shock" and "should be included in diagnostic guidelines for sepsis and in clinical practice in intensive care units" (Uzzan et al. 2006). In another meta-analysis Tang and associates were more critical (Tang et al. 2007). They studied the accuracy of PCT in sepsis diagnosis and collected 672 studies, of which only 18 were considered suitable for

analysis. The high rate of rejection was due to failure provide evidence of infection in sepsis patients. As it is generally accepted that detection of bacteremia is not a demand for the clinical diagnosis of sepsis, the rejection of studies has prompted criticism of their conclusion that PCT cannot reliable classify sepsis from SIRS in critically ill patients (Reinhart and Brunkhorst 2007; Becker et al. 2008).

Most studies made have included only blood culture-positive episodes or only patients admitted to ICU. In the ER setting stepwise multivariate logistic regression analysis has shown that plasma PCT is a good marker of sepsis severity compared with 15 other clinical, biochemical and bacteriologic variables tested (Viallon et al. 2008). PCT has also been found to be significantly higher in patients with bacteraemia and septic shock than in other patients when studied in the ER (Chan et al. 2004).

Altogether six meta-analyses have been made on the diagnostic accuracy of PCT to detect infection in different patient populations; four of them identified PCT as useful for the diagnosis of clinically or microbiologically documented infection (Simon et al. 2004; Uzzan et al. 2006; Mofidi et al. 2009; Vouloumanou et al. 2011). One meta-analysis found only moderate benefit (Jones et al. 2007) and one, as already noted, was more critical (Tang et al. 2007).

The PCT concentration has been observed to increase with increasing severity of sepsis and organ dysfunction (Giamarellos-Bourboulis et al. 2002). This has led to interest in using PCT as a prognostic marker. In one fairly large study with 472 ICU patients it was found that a high maximum PCT and an increase in PCT value were both independent predictors of 90-day mortality in contrast to levels of CRP and WBC (Jensen et al. 2006). In a smaller Finnish study (N=61) there was a significant difference in PCT values between survivors and non-survivors on day 1 and 2 after admission to the ICU (Pettilä et al. 2002); however there were similar significant differences for IL-6 levels and Acute Physiology and Chronic Health Evaluation (APACHE) II and sequential organ failure assessment (SOFA) scores. Only the APACHE II and male gender were found to be independent predictors of death in multivariate analysis.

Recently PCT has been intensively studied as a biomarker for antimicrobial stewardship. In several studies it has been shown that PCT measurement can be successfully used in the guidance of antibiotic therapy (Hochreiter et al. 2009; Bouadma et al. 2010). A meta-analysis reviewing a large number of clinical studies conducted to validate the role of PCT in antibiotic guidance programs (Schuetz et al. 2011; Schuetz et al. 2012), and in the future this may be the major utility of PCT. In our papers this aspect was not studied.

2.3.4 Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is an important and multifunctional cytokine with a wide range of biological activities. It helps to regulate immune reactivity, acute-phase response, inflammation, oncogenesis and hematopoesis (Song and Kellum 2005). IL-6 is derives from a single gene encoding a product of 212-amino acid peptide, with a molecular weight of about 22-27 kDa. The human IL-6 gene has been mapped to chromosome 7 (Song and Kellum 2005).

Weissenbach and associates described a molecule they named interferon-ß2 (Weissenbach et al. 1980). A variety of other names were given to this molecule (e.g. B-cell differentiation factor, T-cell replacing factor) until in 1989 it came to be known as IL-6. The first observations of IL-6 in the serum of sepsis patients were reported in same year (Helfgott et al. 1989; Waage et al. 1989).

IL-6 is principally synthesized by endothelial cells, fibroblasts and moncytes/macrophages in inflammation and infection but also for instance stress, trauma and tissue injury (Song and Kellum 2005; Cuschieri et al. 2010). Production of IL-6 can be triggered by many stimulators such as bacterial endotoxins, viruses, fungi and cytokines (Song and Kellum 2005). IL-6 gene expression is regulated by steroid hormones and dexametasone can down-regulate its production (Woloski et al. 1985).

The IL-6 and IL-6 receptor complex bind to two glycoprotein 130 molecules (gp130). This entire complex then activates a Janus kinase or signal transducer and activator of transcription and mitogen-activated protein kinase cascade. This cacade contributes to the subsequent activation of acute-phase proteins. The gp130 molecyle is a common cytokine signal transducer which can also be activated by many other factors. Without gp130 IL-6 is basically inert (Song and Kellum 2005).

As a sepsis mediator, IL-6 has a variety of actions on different types of target cells, but the liver seems to be its major target organ (Song and Kellum 2005). In the liver IL-6 induces acute-phase proteins such as CRP and LBP. IL-6 is elevated in the plasma earlier than the other acute-phase proteins. It was shown in one experimental study that IL-6 increases substantially in the serum of healthy volunteers after intravenous administration of a small amount of endotoxin, peaking as fast as 2 hours post injection (Fong et al. 1989). In comparison, peak values of PCT are seen after 6-8 hours and CRP after 36-50 hours. The biological half-life of IL-6 is approximately

20-30 minutes (Song and Kellum 2005). IL-6 may also serve as the link between inflammation and thrombosis in sepsis (Bernardo et al. 2004).

In healthy adults, IL-6 plasma levels are low, below 10 pg/ml. In sepsis IL-6 levels can rise above 10 000 pg/ml, even over 100 000 pg/ml. Due to the heterogeneity of IL-6 plasma concentrations intra- and inter-individually, a cut-off value for risk stratification is difficult to set. Data from clinical studies suggest that the majority of sepsis patients have increased IL-6 plasma levels, and this is associated with the severity of the disease and outcome (Hack et al. 1989; Casey et al. 1993; Gogos et al. 2000; Bozza et al. 2005). Constantly high IL-6 levels are associated with multiple organ dysfunction (Pinsky et al. 1993; Pettilä et al. 2002) and death (Patel et al. 1994; Tanaka et al. 1996; Pettilä et al. 2002). An early decrease in IL-6 level in septic patient may be an indicator of a better prognosis and effective treatment. However, IL-6 is not specific for sepsis; high concentrations of IL-6 have also been demonstrated in multiple trauma patients (Cuschieri et al. 2010), in rheumatoid arthritis and other rheumatic diseases (Arend and Gabay 2004). IL-6 has also shown to be a good prognostic marker for septic neonates (Kuster et al. 1998; Martin et al. 2001; Ng et al. 2006). Neverthless, physiologic significance of elevated IL-6 concentrations remains unclear. In an animal model (a mouse model with acute septic peritonitis) IL-6 levels predicted survival and were also able to target those mice which could benefit most from treatment (Osuchowski et al. 2009). Results are too contradictory to conclude whether IL-6 inhibition or administration provides benefit during sepsis. It has been shown in an animal model that lack of IL-6 does not influence mortality in sepsis (Remick et al. 2005). On the other hand, animal studies have sown that inhibition of IL-6 production or IL-6 blockade results in better outcome of sepsis (Gennari and Alexander 1995; Riedemann et al. 2003), but this could not be proved in human studies (Vyas et al. 2005).

In earlier studies up to 1992, plasma IL-6 was measured by bioassays using the growth of a hybridoma B cell line or protein synthesis by a hepatoma cell line (Song and Kellum 2005). Subsequently radioimmunoassay and enzyme-linked immunosorbent assay have been used to evaluate IL-6. Immunologic assays cannot measure IL-6 activity, only the presence of IL-6 antigen, and therefore biological activity may not correlate. Commercially available assays can evaluate IL-6 in plasma or serum. These tests have made it possible to measure IL-6 more easily, also in the ER setting, but its role in everyday practice in the emergency room remains unclear.

2.3.5 Group IIA phospolipase A_2 (PLA_2GIIA)

Phospholipases A_2 (PLA_2) are a family of enzymes which hydrolyze phospholipids to fatty acids and lysophospholipids. Mammalian PLA2 are divided into three broad categories secreted PLA_2 and cytosolic PLA_2 (calcium dependent and independent) (Nevalainen et al. 2008). Cytocolic PLA_2s plays roles in cellular signaling and prostanoid metabolism and secreted PLA_2s participate in the first-line antimicrobial defense against bacteria and other pathogens (Nevalainen et al. 2008). Group IIA phospolipase A2 (PLA_2GIIA) is a calcium-dependent, low molecular weight (13.99 kDA) enzyme which is highly cationic and optimally active at neutral pH (Dennis 1997; Koduri et al. 2002). The gene coding for it is located on chromosome I (Tischfield et al. 1996).

PLA_2GIIA is regarded as an acute-phase protein which is expressed in high levels in the plasma of patients with inflammatory diseases and infe2ction (Green et al. 1991; Nevalainen 1993; Vadas et al. 1993; Grönroos et al. 2002). PLA_2GIIA is an enzyme which catalyzes the hydrolysis of cell membrane phospholipids. It is syntethized in many gland cells and can be measured in mucosal secretions, including Paneth cells in the intestinal mucosa, lacrimal glands and tears, and prostatic gland cells and seminal plasma (Nevalainen et al. 1993; Nevalainen et al. 1994; Huhtinen et al. 2002). The liver (hepatocytes) has been proposed to be a major cellular source of circulating PLA_2GIIA in the human (Crowl et al. 1991), although other sites such as blood platelets (Kramer et al. 1989) and Paneth cells (Corke et al. 2001) have also been envisaged.

PLA_2GIIA is regarded as an important mediator of inflammatory reactions, linking local and systemic processes (Pruzanski and Vadas 1991; Pruzanski 2005). The transcription of the PLA_2GIIA gene is induced by inflammatory cytokines such as IL-1, IL-6 and TNF-α (Pruzanski and Vadas 1991; Pruzanski 2005). PLA_2GIIA has an important role in the host defense mechanisms against bacterial invasion. As far back as 1970s it was noted that that PLA_2GIIA has bactericidal properties. First it was shown that together with BPI, PLA_2GIIA could kill gram-negative bacteria (Weiss and Elsbach 1977; Elsbach et al. 1979; Elsbach et al. 1994). PLA_2GIIA requires other defense factors such as complement activation (Madsen et al. 1996) or BPI to penetrate the lipopolysaccaride capsule of gram-negative bacteria and thus enable the phospholipid hydrolysis of the bacteria (Elsbach et al. 1979; Elsbach et al. 1994). It is known that gram-positive bacteria are more sensitive to PLA_2GIIA than gram-negative bacteria (Nevalainen et al. 2008).

PLA_2GIIA is able to penetrate the peptidoglycan envelope of gram-positive bacteria and to gain access to the cell phospholipids. Gram-positive bacteria are more vulnerable to killing by PLA_2GIIA during the growth phase when the bacterial cells are dividing than in the stationary phase (Foreman-Wykert et al. 1999; Grönroos et al. 2005). Evidence shows that PLA_2GIIAis directly lethal to bacteria such as Staphylococcus aureus and Listeria monocytogenes in human acute-phase serum (Grönroos et al. 2005).

It was hypothesized that PLA_2GIIA might participate in the pathophysiology of sepsis and septic shock, and that the inhibition of PLA_2GIIA activity might be a beneficial therapeutic strategy. However, treatment with a specific inhibitor of PLA_2GIIA has failed to improve the clinical outcome in patients with severe sepsis (Zeiher et al. 2005).

Several methods have been used to measure the PLA_2GIIA concentration. Matsuda and colleagues used radioimmunoassay (RIA) methods using a monoclonal antibody. Thereafter that ELISA (Vadas et al. 1992) and TR-FIA method (Nevalainen et al. 1992) were developed. Nevalainen subsequently published a modified TR-FIA methods (IFMA) for PLA_2s (PLA_2GIIA and phospholipaase 2 group 1B (PLA_2GIB) (Nevalainen et al. 2005). They found that PLA_2GIB and PLA_2GIIA were present in serum samples from control individuals at very low levels, and that elevated levels were found in the sera of patients with acute pancreatitis (PLA_2GIB) and septic infections (PLA_2GIIA). Immunologic determinations of PLA_2GIIA concentrations in body fluids and tissue extracts are highly sensitive and specific methods. Immunoassays of PLA_2GIIA are based on the specific immunoreaction between the antibody to purified PLA_2GIIA and the enzyme present in the sample. There is some variation in the reference for normal PLA_2GIIA values depending on the methodology, but according to reviews the normal serum and plasma concentration of PLA_2GIIA in man is usually below 10 μg/l (Nevalainen et al. 2005). Nowdays also a commercial kit for detection of PLA_2GIIA excists, but the method has been used only in scientific publications not in clinic.

The serum levels of PLA_2GIIA and CRP correlate in several acute and chronic diseases and in postoperative states (Vadas et al. 1993; Grönroos et al. 1994; Haapamäki et al. 2006). This was also observed in a small study with 46 patients with various types of infections (Rintala and Nevalainen 1993). The level of PLA_2GIIA has been shown to correlate with the severity of sepsis and septic shock (Green et al. 1991; Vadas et al. 1992). The prognostic value of PLA_2GIIA has been studied in the intensive care unit in patients with multiple injuries or sepsis (Dajak et al. 2006). In this study PLA_2GIIA was not associated with survival in the sepsis group. The utility

of PLA_2GIIA as a diagnostic or prognostic marker in the ER setting with patients with suspected infection remained unclear.

2.3.6 Bactericidal/permeability-increasing protein (BPI)

Bactericidal/permeability-increasing protein (BPI) is a 55-kDa cationic protein contained within granules of polymorphonuclear leukocytes (Elsbach and Weiss 1995; Elsbach and Weiss 1998). It has an elongated, boomerang-like shape which consists of two domains. The antibacterial and LPS-neutralizing activity of BPI is localized in the N-terminal half of the protein (Ooi et al. 1991), whereas the C-terminal half accelerates the opsonic activity of the molecule, i.e. its capability to promote phagocytosis (Iovine et al. 2002).

In humans BPI is expressed mainly in the bone marrow in myeloid precursors of neutrophils (granulocytes) and stored in the azurophilic granules of polymorfonuclear leukocytes (Weiss et al. 1978; Weiss and Olsson 1987; Gullberg et al. 1997) and to a lesser extent in eosinophils (Calafat et al. 1998). Expression of BPI has also been detected in human gastrointestinal epithelial cells (Canny et al. 2002), dermal fibroblasts (Reichel et al. 2003), genital tract cells (Canny et al. 2006), and epithelial cells of the excretory lacrimal gland ducts (Peuravuori et al. 2006). Human BPI belongs to a family of lipid-interactive/binding proteins, with structural similarity to lipopolysaccaride-binding protein (LBP) (Bingle and Craven 2004), a liver-derived plasma component which delivers LPS to its receptor host cells. The BPI and LBP genes were both localized on human chromosome 20 (Gray et al. 1993) and their genomic structures resemble each other (Hubacek et al. 1997).

BPI is a potent cytotoxic agent for gram-negative bacteria. It is released during neutrophil activation, and neutralizes soluble and membrane-bound endotoxin. BPI inhibits the biological effect of bacterial LPS and alters the permeability of the bacterial cell membrane (Wiese et al. 1997). Direct binding of BPI to the bacterial envelope is critical for its antimicrobial action, and it has high affinity to the lipid A moiety of LPS (Gazzano-Santoro et al. 1992). The lipid A region is common to LPSs, thus BPI is able to neutralize endotoxin from a broad array of gram-negative pathogens and inhibit LPS-dependent inflammatory responses. The binding of the protein leads to changes in membrane current, influencing membrane permeability to hydrophobic molecules. It also changes the transmembrane potential,

which influences channel gating and causes membrane dysfunction (Wiese et al. 1997). The bactericidal action of BPI is markedly accelerated by PLA_2GIIA (Madsen et al. 1996)

In several animal models, recombinant BPI (rBPI) and its amino-therminal derivate ($rBPI_{21}$) were protective against lethal and also sub-lethal challenges with gram-negative bacteria and endotoxin (Lin et al. 1994; Evans et al. 1995; Jellema et al. 2002). Small empirical studies in humans (von der Mohlen et al. 1995; Jellema et al. 2002) have shown a significant protective effect of BPI, but in a large-scale controlled trial evaluating a rBPI fraction for treatment of meningococcal sepsis in children no effect on survival in the treatment group was shown (Levin et al. 2000; Giroir et al. 2001).

There are only a few small studies on the correlation between the circulatory levels of BPI and severity and mortality in sepsis. In these studies levels of BPI were shown to correlate positively with sepsis severity and mortality (Calvano et al. 1994; Rintala et al. 2000; Berkestedt et al. 2010).

First quantitave ELISA method for BPI (then called 57 kDa cationic antimicrobial protein, CAP 57) was invented by Pereira et al allredy in yer 1989 (Pereira et al. 1989). 1994 White and coworkers used ELISA method and noticed that BPI should be measured from EDTA plama instead of serum for avoiding artifacts caused by the destruction of PMNs and release of BPI during coagulation (White et al. 1994). In studies done in Turku in-house time-resolved fluoroimmunoassay (TR-FIA) method has been used as described by Häggblom et al (Häggblom et al. 1996) and later (Rintala et al. 2000; Nupponen et al. 2002; Peuravuori et al. 2006). Nowdays several commercial human BPI ELISA tests are available using solid-phase enyme linked immunosorbent assay based on the sandwich principle (for example Hyman BPI, Hycult®biotec, Hycult Biotec, Uden, the Netherlands).

2.3.7 Soluble urokinase-type plasminogen activator receptor (suPAR)

The urokinase-type plasminogen activator system consists of protease, a receptor (uPAR) and inhibitors (Ossowski and Aguirre-Ghiso 2000). The receptor (uPAR) was cloned in 1990 (Roldan et al. 1990). In the followingg year soluble urokinase-type plaminogen activator receptor (suPAR) was identified (Ploug et al. 1991). uPAr is a glycoprotein released during inflammation and infection (Ossowski and Aguirre-Ghiso 2000). It interacts with several molecules mediating immune system signals (Ossowski and Aguirre-Ghiso 2000).

uPAR is expressed on various cell types, including neutrophils, lymphocytes, monocytes, macrophages, endothelial and malignant cells in response to chemotaxis-inducing stimuli (e.g. interleukins) (Ossowski and Aguirre-Ghiso 2000; Thuno et al. 2009) After cleavage by proteases from the cell surface into soluble form, suPAR can be found in the blood and other organic fluids (Rabna et al. 2010; Backes et al. 2011; Eugen-Olsen 2011; Tzanakaki et al. 2012). suPAR exists in three forms possessing different properties related to their structural differences (Thuno et al. 2009). suPAR takes part in various immunological functions, including adhesion, migration, chemotaxis, proteolysis, immune activation, tissue remodelling, invasion and signal transduction (Ossowski and Aguirre-Ghiso 2000). Plasma suPAR concentrations are believed to represent the degree of immunoactivation (Koch et al. 2011)

suPAR can be measured in organic fluids e.g. blood (plasma or serum), cerebrospinal fluid, broncoalveolar lavage or urine using a monoclonal antibody double sandwich enzyme-linked immunosorbent assay (ELISA) (Thuno et al. 2009; Backes et al. 2011; Eugen-Olsen 2011; Tzanakaki et al. 2012). suPAR levels in healthy persons are low and stabile throughout the day and are not induced by fasting. In one previous small study the median level of suPAR in healthy adult serum was as low as 1.5 ng/ml (range 1.2 – 1.9 ng/ml, N=44 (Stephens et al. 1997). In a population of over 2038 apparently healthy Danish subjects aged 41 – 71 years, the median level of suPAR in plasma was reported to be 3.9 ng/ml (Sehestedt et al. 2011) measured using suPARnostic ® Standard Kit, ViroGates A/S, Birkearod, Denemark) (Sehestedt et al. 2011).

In a previous study involving 151 SIRS patients, plasma suPAR levels showed only limited value for the diagnosis of bacterial infection (Kofoed et al. 2007). AUC values for CRP, PCT and suPAR were 0.81, 0.72 and 0.50 respectively. In contrast one recent paper including 85 patients with SIRS and comparing the same parameters concluded that suPAR was useful in the differential diagnosis of bacterial infection among patients with SIRS (Yilmaz et al. 2011).

High suPAR levels have been shown to predict disease severity and outcome in various infections such as bacteremia (Wittenhagen et al. 2004; Huttunen et al. 2011; Mölkanen et al. 2011), bacterial meningitis (Østergaard et al. 2004), human immunodeficiency virus (HIV) (Sidenius et al. 2000; Lawn et al. 2007), tuberculosis (Eugen-Olsen et al. 2002) and malaria (Ostrowski et al. 2005). High suPAR has also been shown to predict poor outcome in patients with SIRS (Kofoed et al. 2008). High suPAR concentrations have been associated with admission to the ICU and survival in critically ill patients (Koch et al. 2011).

It has been suggested that suPAR serum concentrations are not directly related the presence and severity of bacterial infection but rather to the presence and severity of organ dysfunction. In critically ill patients suPAR has been shown to be associated with hepatic and renal failure (Koch et al. 2011; Donadello et al. 2012). In patients with chronic liver diseases, suPAR levels were elevated and closely reflected the severity of liver dysfunction, chirrosis and prognosis in these patients (Zimmermann et al. 2011).

It has been proposed that suPAR has a role in the pathogenesis of focal segmental glomerulosclerosis. In an experimental animal model Wei and colleagues have demonstrated that circulating suPAR enters the glomerulus and binds ß3 integrin, which normally anchors podocytes to the glomerular basement membrane (Wei et al. 2011). High plasma levels of suPAr lead to increased ß3 integrin activation, causing podocyte effacement and renal scarring (Wei et al. 2011).

Low-grade inflammation is thought to contribute to the development of cardiovascular diseases, type-2 diabetes mellitus, cancer and mortality. In a Danish MONICA 10 cohort study (N=2602, follow-up 1993-2006) plasma suPAR was shown to have an association with incident cancer, cardiovascular disease, type-2 diabetes mellitus and mortality in the general population (Eugen-Olsen et al. 2010). In another Danish study, an elevated plasma suPAR concentration was also shown to be associated with subclinical organ damage and cardiovascular events independently of traditional risk factors or high sensitive CRP (Sehestedt et al. 2011).

2.3.8 Pentraxin 3 (PTX3)

Pentraxins are multi-functional pattern-recognition proteins interacting with pathogen components. Pentraxin 3 (PTX3) is a distant relative of CRP. It was identified in the 1990s as an early induced gene in endothelial cells and macrophages. Pentraxins are highly conserved proteins with a cyclic multimeric structure. PTX3 is a long pentraxin with an N-terminal domain coupled to the C-terminal pentraxin domain (Garlanda et al. 2005; Bottazzi et al. 2009). PTX3 and CRP have different gene organizations and localization, ligand recognition, producing cells and inducing signals (Introna et al. 1996; Basile et al. 1997; Bottazzi et al. 1997).

PTX3 is an inflammatory mediator produced by various cell types in peripheral tissue (for example macrophages, dendritic cells, endothelial cells, ovarian granulose cells, fibroblasts, adipocytes and smooth muscle cells) in response to proinflammatory signals such as TNF-α and IL-1β (Garlanda et al. 2005; Mantovani et al. 2008; Bottazzi et al. 2009). It plays an important role in the early phase of inflammation: it recognizes microbial moieties, activates the classical pathway of complement and facilitates recognition by macrophages and dendritic cells (Garlanda et al. 2005). PTX3 has an important role in the regulation of the innate immune response by contributing to the opsonization and clearance of apoptotic or necrotic cells (Bottazzi et al. 2009). PTX3 can be both protective or deleterious for the host, depending on the type of injury and on PTX3 levels (Manfredi et al. 2008).

PTX3 can be measured using solid-phase enzyme –linked immunosorbent assays for th quantitatvie measurements (Mantovani et al. 2008; Yamasaki et al. 2009). Several commercial methods are nowdays available (for example (Quantikine® DPTX 30; R&D Systems Inc., Minneapolis, USA).

PTX3 concentrations in healthy persons are normally lower than 2 ng/ml and increase dramatically under inflammatory and infectious condition (Mantovani et al. 2008; Yamasaki et al. 2009). Previous clinical studies have shown that the levels of PTX peak earlier than CRP (Sprong et al. 2009; de Kruif et al. 2010; Vänskä et al. 2011).

PTX3 is not a specific marker of bacterial infection, elevated plasma PTX3 concentrations being seen in various conditions. High PTX3 levels have correlated with poor outcome in several different situations such as cardiovascular diseases (Matsui et al. 2010; Inoue et al. 2012), lung cancer (Dia-

mandis et al. 2011) and polymyalgia rheumatica (Pulsatelli et al. 2010). A high level of PTX3 has also been seen to predict the severity of disease in dengue virus infection (Mairuhu et al. 2005), epidemic nephropathy (Outinen et al. 2011) and leptospirosis (Wagenaar et al. 2009).

Several studies have shown that PTX3 can predict the severity of bacterial infection (Muller et al. 2001; Sprong et al. 2009; Mauri et al. 2010; Huttunen et al. 2011). In one previous paper PTX3 predicted culture-positive bloodstream infection and severe sepsis (the need for ICU treatment, longer hospital stay and acute congestive heart failure) in febrile patients admitted to the emergency room (de Kruif et al. 2010). In ICU patients the concentrations of PTX3 have been associated with severity of infection (Muller et al. 2001; Mauri et al. 2010).

In one previous study the maximum PTX3 level on days 1 – 4 after bacteremia diagnosis (Huttunen et al. 2011) was shown to be a predictor of case fatality. Another recent work has revealed that high concentrations of plasma PTX3 persisting over the first five days after the onset of severe sepsis are associated with mortality, but not the PTX3 value on day 1 (Mauri et al. 2010).

3 AIMS OF THE STUDY

Early detection of severe sepsis and the clinical utility of prognostic markers in adult patients with suspected infection in the emergency room: a single-center prospective cohort study

The specific aims in studies I-IV were:

I. to determine the value of plasma CRP, PCT and IL-6 for early detection of severe sepsis

II. to study the utility of BPI, PLA_2GIIA, WBC and CRP for early detection of severe sepsis

III. to assess the diagnostic and prognostic value of plasma suPAR for severe sepsis and case fatality

IV. to study the prognostic utility of plasma PTX3 for severe sepsis and case fatality

4 PATIENTS AND METHODS

4.1 STUDY DESIGN AND PATIENT INCLUSION

This was a single-center prospective follow-up study. Publications I – IV were part of a project identifying severe sepsis and the prognostic utility of laboratory parameters in an unselected patient population in an emergency room (ER) setting. Patients were recruited at Satakunta Central Hospital, a 350-bed secondary care hospital on the western coast of Finland serving the Satakunta Hospital District with a population of 240 000 inhabitants. The study cohort consisted of adult patients admitted to the ER on suspicion of infection, from whom a clinician had decided to take samples for blood cultures. Enrolment took place over a 14-month period in the years 2004 – 2005. To ensure written informed consent and interview within 24-48 h, only patients admitted between Sunday 7 a.m. and Wednesday 3 p.m. were enrolled. Before initiating the study, a pre-evaluation of the target population was conducted to ensure the representativeness of the cohort. This assessment covered 1551 consecutive patients from whom blood cultures had been taken in the emergency department in the year 2003. The rate of positive blood cultures in this pre-evaluation was 8.3 % and case fatality by day 28 after admission was 6.7%. No significant differences were noted between patients in respect of study days and other days or between the study and pre-evaluated populations in respect of age, gender, rate of positive blood cultures or mortality rate. Each patient was taken into account only once.

Blood samples for the study were taken upon admission concurrently with the blood culture samples. Blood was collected into two 10 ml EDTA tubes (plasma) and two 7-ml serum tubes (serum). The EDTA tubes were kept on ice until centrifugation. Plasma and serum were transferred in 1 – 2-ml aliquots to CryoPure® (Sarstedt, Germany) tubes. These were stored at - 70ºC until assayed.

A structured interview was undertaken by the investigator or research nurse 24-48 hours after admission.Highest body temperature, lowest blood pressure, highest pulse and respiratory rates were recorded daily on days 1-7. Symptoms and clinical signs, Glasgow coma scale, risk factors for sepsis, underlying diseases, medications and diagnosis at admission were recorded, likewise duration of stay in intensive care and in hospital. All antimicrobial agents used during one week befor admission were also recorded. Potential organ failure (respiratory, cardiovascular, renal, hematological,

hepatic or central nervous system), overall case fatality and sepsis-attributable case fatality were recorded. Final diagnoses, source of infection and trauma or other possible reasons for inflammation were taken from medical reports. A follow-up check was made by phone 3 months, 6 months and one year after enrolment.

On study days blood cultures were taken in ER for suspected infection altogether from 1109 patients. From 500 patients the study samples were not unfortunately taken. From those 500 missed patients blood cultures were positive with 37 patients (7.4%).

Blood samples for the studies were taken from 609 patients. Fifty-five patients (or close relatives) refused to participate, and their blood samples were destroyed. Fifteen were excluded from the analysis: one due to a missing blood sample at admission, 11 due to incomplete data for reliable classification (whether they had bacterial infection or not) and three had SIRS and organ dysfunction but no bacterial infection (one with epidemic nephropathy and two with myocardial infarction). The final study material consisted of 539 patients in studies I and III. In study II fourteen patients were excluded due to missing WBC on admission (N=525) and in paper IV two patients due to missing blood sample for PTX analysis (N=537). Patient inclusion in studies I - IV is shown in the Figure **4**.

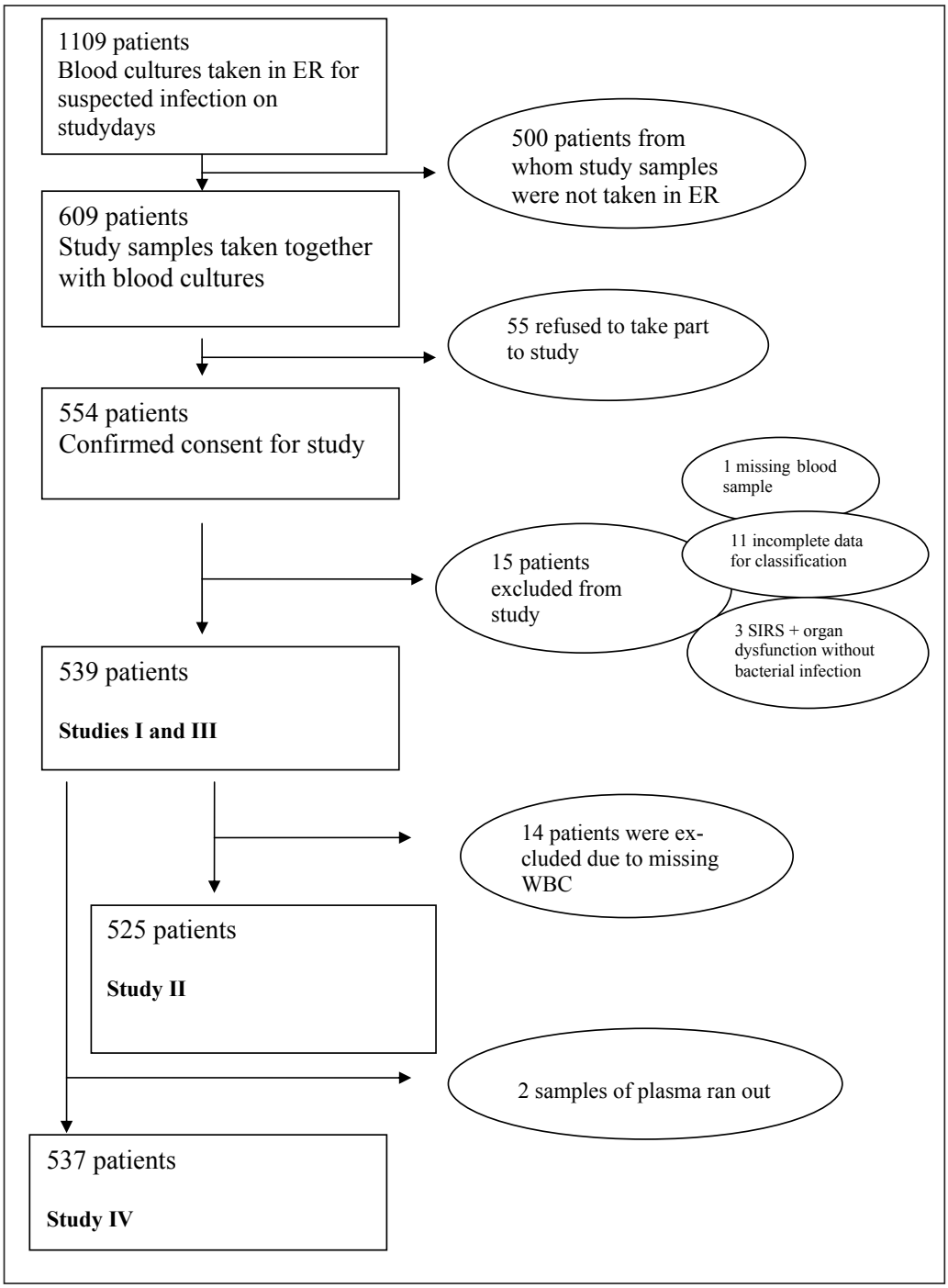


Figure 4. Patients inclusion in studies I-IV.

4.2 DEFINITIONS

Table 5 shows criteria and classification of patients to the study groups in studies I – IV on the basis of the American College of Chest Physicians / Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference definitions (Bone et al. 1992), using three criteria: systematic inflammatory response syndrome (SIRS), bacterial infection (documented or probable), and sepsis-associated organ dysfunction. SIRS was defined as at least two of the following conditions: 1. body temperature > 38°C or < 36°C, 2. heart rate > 90 beats per minute, 3. respiratory rate > 20 breaths per minute or PaCO2< 4.3 kPa, 4. white blood cell count > 12 x 109/l or < 4 x 10 9/l or >10 % immature (band) forms.

Bacterial infection was taken as documented if there was either pathogenic bacterial growth (for example Staphylococcus aureus) in at least one blood culture or in normally sterile tissue or if the same less pathogenic bacterium (for example Staphylococcus epidermidis) was detected in two different samples. Bacterial infection was rated probable if the clinician suspected bacterial infection and either an infection focus had been confirmed clinically and/or radiologically or antimicrobial treatment had been started and the response to treatment supported the diagnosis of bacterial infection.

Table 5. Criteria and classification of patients according to study groups

Study group		Criteria	Studies I and III	Study II	Study IV
Group 1	**No SIRS[a], no bacterial infection**	**Patients with no SIRS (less than two SIRS criteria at admission +/- 24 hours [b]), nor documented [c] or probable [d] bacterial infection**	**59**	**58**	**59**
Group 2	**Bacterial infection, no SIRS**	**Patients with documented or probable bacterial infection, but no SIRS (less than two SIRS criteria at admission +/- 24 hours)**	**68**	**63**	**67**
Group 3	**SIRS, no bacterial infection**	**Patients with SIRS (at least two SIRS criteria at admission +/- 24 hours), but no documented or probable bacterial infection**	**54**	**53**	**54**
Group 4	**Sepsis**	**Patients with sepsis (SIRS and documented or probable bacterial infection but no organ dysfunction due to sepsis)**	**309**	**302**	**308**
Group 5	**Severe sepsis**	**Patients with severe sepsis (sepsis with signs of organ failure, i.e. disturbed perfusion, metabolic acidosis, oliguria or neurological disorders)**	**49**	**49**	**49**
Total			**539**	**525**	**537**

[a] SIRS (Systemic Inflammatory Response Syndrome): At least two of the following conditions. 1. Temperature > 38°C OR < 36°C, 2. Heart rate > 90 beats per minute. 3 Respiratory rate > 20 breaths per minute or PaCO2<32 mmHg (4,3 kPa) 4. White blood cell count > 12 X 10^9/l or < 4 X 10^9/l or >10 % immature (band) forms)

[b] Measured and documented SIRS criteria (f. ex. temperature) were accepted 24 hours before or after admission

[c] Documented bacterial infection: Microbiologically confirmed bacterial infection (either pathogenic bacterial growth in blood culture or in normally sterile tissue or the same usually less pathogenic bacterium (e.g. Staphylococcus epidermidis) in two different samples)

[d] Probable bacterial infection: A clinician has suspected bacterial infection AND either the infection focus was confirmed clinically and/or radiologically OR antimicrobial treatment was started and the response to treatment supports bacterial infection

4.3 PATIENTS

4.3.1 Patient characteristics and underlying diseases

Characteristics and underlying diseases of patients are shown in the following Table 6. There were 311 males and 228 females.

Table 6. Characteristics and underlying diseases of patients

Characteristics **N**	**Studies I and III** **539**	**Study II** **525**	**Study IV** **537**
	median (range)	**median (range)**	**median (range)**
Age , years	**61 (18 – 100)**	**64 (18 – 99)**	**64 (18 – 100)**
BMI [a], kg/m^2	**27.0 (14.7 – 67.6)**	**27.0 (14.7 – 67.6)**	**27.0 (14.7 – 67.6)**
	N (%)	**N (%)**	**N (%)**
Male gender	**311 (57.7)**	**304 (57.9)**	**310 (57.7)**
Alcohol abuse [b]	**25 (4.6)**	**25 (4.8)**	**25 (4.7)**
Smoking (current smoker)	**126 (23.4)**	**123 (23.4)**	**126 (23.5)**
Diabetes (type 1 and 2)	**82 (15.2)**	**78 (14.9)**	**81 (15.1)**
Malignancy (solid or hematological)	**95 (17.6)**	**78 (14.9)**	**95 (17.7)**
Rheumatic diseases	**50 (9.3)**	**48 (9.1)**	**50 (9.3)**
Chronic renal insufficiency [c]	**18 (3.3)**	**18 (3.4)**	**18 (3.4)**
Cardiovascular disease [d]	**289 (53.6)**	**283 (53.9)**	**289 (53.8)**
COPD or asthma [e]	**108 (20.0)**	**106 (20.2)**	**108 (20.1)**
Operation six months previously	**75 (13.9)**	**72 (13.7)**	**75 (14.0)**
Device [f]	**82 (15.2)**	**79 (15.0)**	**82 (15.3)**
Continuous medication [g]	**390 (72.4)**	**380 (72.4)**	**389 (72.4)**
Continuous cortisone treatment [h]	**59 (10.9)**	**58 (11.0)**	**59 (11.0)**

[a] body mass index. Data available on 391,/383 /390 patients.
[b] alcoholism was diagnosed or patient had previously been treated for alcohol-induced disease
[c] plasma creatinine concentration constantly more than 170 µmol/l (5 patients were receivingchronic dialysis treatment)
[d] continuous medication for cardiovascular disease (i.e. hypertension, artherosclerosis or other cardiovascular disease)
[e] continuous medication for asthma or COPD
[f] joint or heart valve prosthesis or pace-maker (does not include dental implants)
[g] continuous medication for a chronic disease
[h] continuous systemic cortisone treatment (daily dose more than 10 mg of oral prednisolone)

4.3.2 Bacterial etiology of sepsis

Blood cultures were positive in 8.7 – 8.9 % of patients as expected on the basis of pre-evaluation and the literature. The summary of blood cultures are presented in table below (Table 7). The most common bacteria in blood were E. coli (N=12), Streptococcus pneumoniae (N=10), and Staphylococcus species (N=9). All blood culture positive findings are shown in Table 8 on next page.

Although blood cultures were intended to be taken before starting antimicrobial treatment, about one quarter of patients had received antibiotics before blood cultures were taken, in most cases before admission to hospital. All antimicrobials were recorded during one week before admission.

Table 7. Summary of blood cultures

Characteristic	Pre-evaluation	Missed patients[a]	Studies I and III	Study II	Study IV
Number of patients, whom blood cultures taken	**1551**	**500**	**538** [b]	**524** [b]	**536** [b]
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Positive (clinically significant [c]**)**	**129 (8.3)**	**37 (7.4)**	**47 (8.7)**	**47 (8.9)**	**47 (8.8)**
Positive (contamination [d]**)**	**11 (0.7)**	**NS** [e]	**4 (0.7)**	**3 (0.6)**	**4(0.7)**
Blood cultures taken after antimicrobial treatment started	**NS** [e]	**NS** [e]	**136 (25.3)**	**131 (25.0)**	**136 (25.4)**

[a] Missed patients: patients from whom study samples were not taken in ER although blood cultures were taken
[b] blood cultures were not taken for one patient (for technical reasons)
[C] either pathogenic bacterial growth in blood culture or the same usually less pathogenic bacterium (e.g. Staphylococcus epidermidis) in two different samples
[d] less pathogenic bacterium (e.g. Staphylococcus epidermidis) only in one blood sample
[e] Not studied

Table 8. Blood culture findings according to study groups (Studies I and III). N=538

	All patients N=538 (%)	Group1 N=59 (%)	Group2 N=67 (%) [a]	Group 3 N=54 (%)	Group 4 N=309 (%)	Group 5 N=49 (%)
Results of Gram´s staining of bacterial pathogen in blood culture						
Purely gram positive	**25 (4,6)**	**0**	**0**	**0**	**22 (6,5)**	**5 (10,2)**
Purely gram negative	**15 (2,8)**	**0**	**0**	**0**	**7 (2,3)**	**8 (16,3)**
Mixed gram positive and gram negative	**7 (1,3)**	**0**	**0**	**0**	**6 (1,9)**	**1 (2,0)**
Blood culture findings (in bracket contaminations) [b]						
Stafylococcus aureus	**4**	**0**	**0**	**0**	**2**	**2**
Other staphylococcal species	**5 (+3)**	**(1)**	**0**	**0**	**3 (+2)**	**2**
Streptococcus pneumoniae	**10**	**0**	**0**	**0**	**10**	**0**
Other streptococcus species	**6**	**0**	**0**	**0**	**4**	**2**
Listeria monocytogenes	**1**	**0**	**0**	**0**	**1**	**0**
Enterococcus species	**2**	**0**	**0**	**0**	**2**	**0**
Anaerobic gram-positive species	**3**	**0**	**0**	**0**	**3**	**0**
E.coli	**12**	**0**	**0**	**0**	**8**	**4**
Klebsiella species	**7**	**0**	**0**	**0**	**4**	**3**
Pseudomonas species	**2**	**0**	**0**	**0**	**2**	**0**
Enterobacter species	**4**	**0**	**0**	**0**	**3**	**1**
Neisseria meningitidis	**1**	**0**	**0**	**0**	**0**	**1**
Anaerobic gram-negative species	**5 (+1)**	**0**	**0**	**0**	**3 (+1)**	**2**

4.3.3 Types of infection

In studies I and III 369 patients (68.5 %) had a community-acquired infection and 112 (20.8 %) a health-care-associated infection; 58 (10.8 %) had no infection at all. Only 13 patients (2.4 %) had proven viral infection, but viral infection was suspected clinically in 172 patients (31.9%), either alone or with bacterial infection. There were no HIV-infected patients, and no fungemia or parasitic infections in our study material.

4.3.4 Sources of infection

Table 9 shows the different sources of infection in studies I and III. Most common foci were in the lungs (195 patients), the gastrointestinal tract (75 patients), the urinary tract (59 patients, one had gynecological infection), skin and soft tissue (40 patients) and visceral organs (other than gastrointestinal) 30 patients. One patient may have had more than one focus and also sources of viral infections are shown here if known.

4.3.5 Clinical data and outcome of patients

Table 10 presents clinical data and outcome of patients in studies I and III according to study groups. The 28-day case fatality was 6.1 %. In the sepsis group the 28-day case fatality was 3.2 % and in the severe sepsis group 28.6 %.

In studies II and IV the missing patients were all in no-sepsis groups and none of these excluded patients died nor were treated in the ICU.

Table 9. Sources of infection (N=539). One patient may have had more than one focus (Study I and III)

	All patients N=539 N (%)	Group 1 N=59 N (%)	Group 2 N=68 N (%)	Group 3 N=54 N (%)	Group 4 N=309 N (%)	Group 5 N=49 N (%)
Upper respiratory tract [a]	**40**	**6**	**8**	**10**	**15**	**0**
Lower respiratory tract [b]	**195**	**2**	**25**	**5**	**137**	**26**
Urinary and gynecological infection	**60**	**0**	**10**	**0**	**43**	**8**
Gastro-intestinal [c]	**75**	**2**	**7**	**2**	**53**	**11**
Other visceral organs [d]	**30**	**0**	**3**	**1**	**24**	**2**
Central nervous system	**4**	**1**	**0**	**1**	**0**	**2**
Cardiac [e]	**5**	**1**	**0**	**0**	**3**	**1**
Skin and soft tissue	**40**	**0**	**12**	**0**	**24**	**4**
Bone and articular	**10**	**0**	**3**	**0**	**6**	**1**
Postoperative infection	**15**	**0**	**2**	**0**	**12**	**1**
Catheter infections	**6**	**0**	**2**	**0**	**3**	**1**
Viral syndromes with infection focus [f]	**7**	**3**	**1**	**2**	**0**	**1**
All focuses of infection	**487**	**15**	**73**	**21**	**320**	**58**

[a] pharyngitis, tonsillitis, sinusitis
[b] pneumonia, pleuritis
[c] infection focus in mouth, esophagus, ventricle, intestinal
[d] infection focus in gall bladder, bile duct, liver, pancreas, spleen
[e] pericarditis, myocarditis, endocarditis
[f] viral exanthema, epidemic nephropathy

Table 10. Clinical data and outcome of the study patients (N = 539). Studies I and III.

Character	All patients N= 539 N (%)	Group 1 N=59 N (%)	Group 2 N=68 N (%)	Group 3 N=54 N (%)	Group 4 N= 309 N (%)	Group 5 N=49 N (%)
Mortality (28 day)	**33 (6.1)**	**2 (3.4)**	**4 (5.9)**	**3 (5.6)**	**10 (3.2)**	**14 (28.6)**
Death due to sepsis	**23 (4.3)**	**0**	**0**	**0**	**10 (3.2)**	**13 (26.5)**
Treated in ICU	**42 (7.8)**	**2 (3.4)**	**0**	**3(5.6)**	**8 (2.6)**	**29 (59.2)**
Mechanical ventilation	**14 (2.6)**	**1 (1.7)**	**0**	**0**	**0**	**13 (26.5)**
CPAP/BPAP treatment[a]	**22 (4.1)**	**0**	**0**	**0**	**5 (1.6)**	**17 (34.7)**
Hemofiltration / acute dialysis	**16 (3.0)**	**0**	**0**	**1 (1.9)**	**0**	**15 (30.6)**
Inotropic treatment	**19 (3.5)**	**1 (1.7)**	**0**	**0**	**1 (0.3)**	**17 (34.7)**
Hypotension[b]	**28 (5.2)**	**1 (1.7)**	**0**	**0**	**1 (0.3)**	**26 (53.1)**
GCS < 15[c]	**26 (4.8)**	**0**	**0**	**0**	**0**	**26 (53.1)**
DIC[d]	**8 (1.5)**	**0**	**0**	**0**	**0**	**8 (16.3)**

[a] Continuous positive airvays pressure or bilevel positive airway pressure treatment
[b] Systolic blood pressure of <90 mmHg or a reduction of 40 mmHg from baseline. No response to 500 ml intravenous fluid replacement.
[c] Glasgow Coma Scale score <15 attributable to sepsis
[d] Disseminated intravascular coagulopathy

4.4 LABORATORY METHODS

4.4.1 CRP (Studies I – IV)

CRP in plasma was measured with an immunoturbidimetric assay by Modular P800 automatic analyzer (Roche Diagnostics GmbH).

4.4.2 PCT (Studies I, III, IV)

PCT in plasma were measured with immunochemiluminometric assays (ECLIA) by Modular E170 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

4.4.3 IL-6 (Studies I and III)

IL-6 in plasma was measured with immunochemiluminometric assays (ECLIA) by Modular E170 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

4.4.4 PLA_2GIIA (Study II)

The concentrations of PLA_2GIIA in EDTA plasma were measured by time-resolved fluoroimmunoassay as described elsewhere (Nevalainen et al. 1992). The 42 reference blood samples for PLA_2GIIA and BPI were taken during blood donations at the Finnish Red Cross Transfusion Service in

Turku, Finland (28 men and 14 women, mean age 45 years, median age 45 years, age range 21-64 years). The level of PLA_2GIIA in the 42 control EDTA plasmas from healthy blood donors was 5.3 µg/l ± 0.4 (mean ± SD, range 1.7-10.6 µg/1, median 5.2 µg/l). Estimations of PLA_2GIIA comparatore values were made using a 95% reference interval with the 2.5th and 97.5th percentiles, giving a reference interval of 1.8-10.5 µg/l for these 42 samples from randomly selected blood donors.

4.4.5 BPI (Study II)

The concentrations of BPI in EDTA plasma were measured by time-resolved fluoroimmunoassay as described by Nuupponen and associates (Nupponen et al. 2002). The 42 references were taken during blood donations at the Finnish Red Cross Transfusion Service in Turku, Finland (28 men and 14 women, mean age 45 years, median age 45 years, age range 21-64 years). The mean BPI concentration in the 42 control individuals was 7.1 ± 5.7 µg/1 (mean ± SD, range 0.9-24.1 µg/1, median 5.2 µg/1). BPI measurement with 95% reference interval with the 2.5th and 97.5th percentiles gave a reference interval of 1.35-23.0 µg/l.

4.4.6 suPAR (Study III)

Plasma suPAR levels were determined using a commercial double monoclonal antibody sandwich enzyme immunoassay (suPARnostic® Standard Kit, ViroGates A/S, Birkerød, Denmark).

4.4.7 PTX3 (Study IV)

The PTX3 concentration in EDTA plasma was determined using a commercial solid-phase enzyme-linked immunosorbent assay (ELISA) (Quantikine® DPTX 30; R&D Systems Inc., Minneapolis, USA).

4.5 STATISTICAL METHODS

4.5.1 Statistical methods (Studies I and II)

Statistical analysis was made using the SAS System for Windows, release 9.2 (SAS Institute Inc., Cary, NC, USA). P-values less than 0.05 were considered statistically significant. Chi-square test was used to compare differences between study groups in categorical variables. Continuous variables were compared using non-parametric Mann-Whitney U test as appropriate. Receiver operating characteristic (ROC) analysis was used to evaluate how well different parameters (PCT, IL-6 and CRP in study I and BPI, PLA_2GIIA, CRP and WBC in study II) discriminated severe sepsis patients from others. Differences in AUC (area under ROC curve) between these parameters were compared using the nonparametric approach (DeLong et al. 1988).

Univariate logistic regression was applied using each parameter as a predictor for severe sepsis. Analyses were also adjusted for confounding factors. A confounding factor was included in adjusted models if there was a significant difference between the study groups in this factor and the number of cases in the groups was sufficient. Multivariate analyses were adjusted for confounding factors using each parameter separately and together as predictor. Correlations were calculated using Pearson`s correlation coefficients. Parameters were log-transformed for logistic models and correlation analysis due to skewed distributions.

4.5.2 Statistical methods (Studies III and IV)

An SPSS package (version 15) was used for statistical analyses and a two-sided p-value < 0.05 was taken as cut-off for statistical significance. Categorical data were analyzed by Chi-square test or Fisher´s exact test when appropriate, and nonparametric continuous data by Mann-Whitney U-test or Kruskal-Wallis test. A logistic regression model was used to study the independent effect of plasma suPAR in study III and PTX3 in study IV on

mortality and severe sepsis models adjusted for potential confounders. Odds ratios (ORs) were expressed with their 95% confidence intervals (CI) when appropriate. The accuracy of maximum suPAR value in predicting severe sepsis and case fatality was evaluated using ROC curves (Boyd 1997). In this method, a test which is perfect has 100% sensitivity and no false-positives (1-specificity=0) and will have an area under the curve (AUC) of 1.0, whereas a test of no diagnostic value would have an AUC of 0.5. The 95% confidence intervals were calculated. The Youden index with the highest sum of sensitivity and specificity was used to select the optimal cut-off for analysis. Correlations between parameters were analyzed using Spearman's rank analysis.

4.6 ETHICAL CONSIDERATIONS

The study was approved by the Ethical Reviewer Board of Satakunta Hospital District. Written informed consent was obtained from patients or first-degree relatives.

5 RESULTS

5.1 CRP (Studies I - IV)

CRP is a widely used marker for bacterial infection and inflammation and was thus assessed as a comparatore value in studies I - IV. The levels of CRP according to study groups were studied in paper I. The concentrations of plasma CRP on admission are presented in Figure 5. Median levels and ranges according to study groups are put together with all studied parameters and presented in Table 17 on page 90 at the end of the results section.

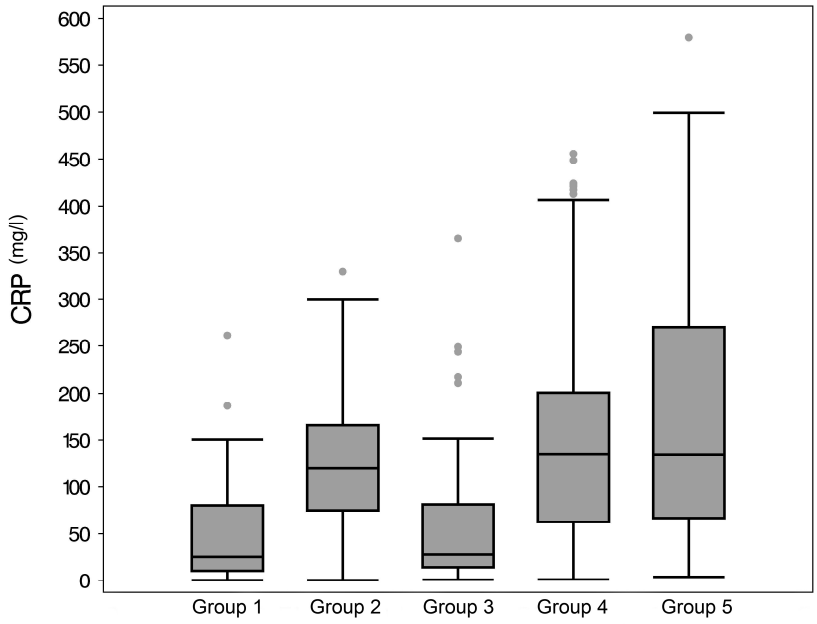


Figure 5. Median levels of plasma C-reactive protein (CRP) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

In paper I plasma CRP was studied together with PCT and IL-6 in early detection of severe sepsis. In univariate logistic regression analysis using CRP as a predictor of severe sepsis, the odds ratio (OR) for CRP was 1.33 (95 % confidence interval (CI) 1.01 – 1.75, p=0.045). In ROC analysis as differentiating between patients with severe sepsis (Group 5) and patients without severe sepsis (Groups 1 - 4) the AUC for CRP was 0.60 (95 % CI 0.51 – 0.69, p = 0.027).

In study I in multivariate logistic regression analysis CRP was not an independent predictor of severe sepsis. The confounders used in study I were: (1) continuous medication for cardiovascular disease (hypertension, arteriosclerosis or other cardiovascular disease); (2) continuous systemic cortisone treatment (daily dose more than 10 mg oral prednisolone); (3) continuous acetylsalicylic acid medication; (4) antimicrobial treatment 1 week previously, (5) viral infection and (6) inflammation focus documented. Age or sex had no difference between study groups and were not included to multivariate regression analysis.

In ROC analysis when distinguishing patients with confirmed or probable bacterial infection (Groups 2, 4 and 5, N=426) from those without bacterial infection (Groups 1 and 3, N=113) AUC for CRP was 0.79 (95 % CI 0.74 – 0.84, p<0.001).

In study III plasma CRP was studied together with PCT, IL-6 and suPAR and in study IV with PCT and PTX3. In both studies case fatality on day 28 was also in central focus as suPAR and PTX3 have proved to be promising predictors for case fatality. The optimal cut-off levels for parameters studied for predicting severe sepsis and case fatality were evaluated using ROC curves and Youden`s index. At a CRP concentration of 158 mg/l the specificity for predicting severe sepsis was 70%, but the sensitivity was only 47%. The cut-off level for case fatality for CRP was not evaluated as CRP was not a predictor for survival in univariate analysis. The optimal cut-off levels of parameters studied are presented in Table 16 on page 89.

In Figure 6 results of ROC analyses in predicting different perspectives (bacterial infection, sepsis, severe sepsis and case fatality (d28) for CRP, PCR, and IL-6 are presented. Parameters studied in papers I-IV are taken together in Table 17 at the end of the result section.

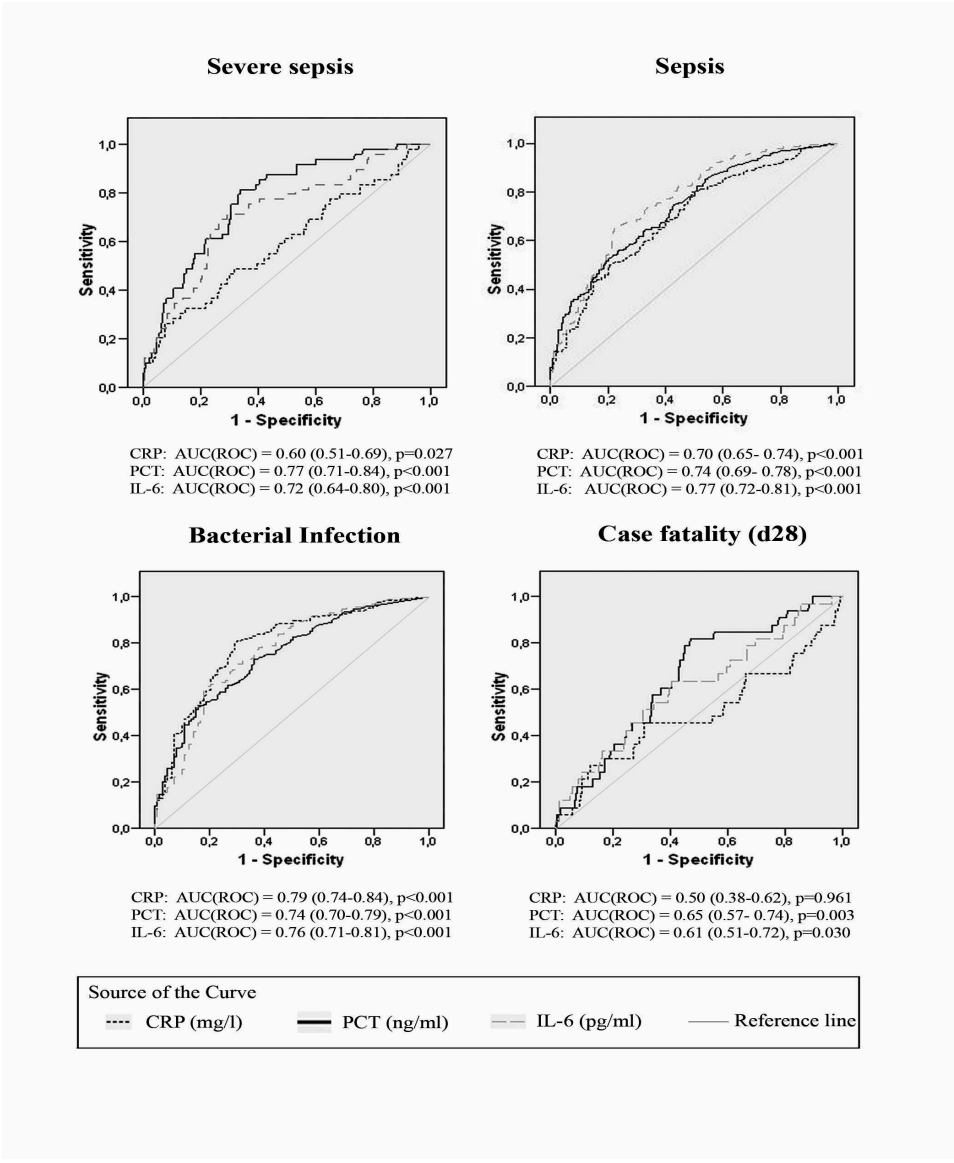


Figure 6. Receiver operating characteristic (ROC) curve for plasma levels of C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6) detected on admission in relation to severe sepsis, sepsis, bacterial infection and case fatality (d28) in patients with suspected infection. AUC^{ROC} (95% confidence interval), $p < 0.001$.

5.2 PCT (Studies I, III and IV)

PCT was studied in paper I and described as a comparatore value in studies III and IV. In the latter two studies PCT was also analyzed as a prognostic marker for case fatality (d28).

Levels of plasma PCT at admission are presented in Figure 7 and together with other parameters in Table 17 on page 90. Median levels of PCT were significantly higher in the severe sepsis group than in the other groups (Groups 1 - 4) and also in those groups bacterial infection was present (Groups 2, 4 and 5, N=426) compared within groups without bacterial infection (Groups 1 and 3, N=113), $p<0.001$.

In study I plasma PCT was studied together with IL-6 and CRP as a predictor of severe sepsis, sepsis and bacterial infection. In logistic regression analysis using PCT as predictor of severe sepsis OR for PCT was 1.58 (95 % CI 1.37 – 1.82, $p < 0.001$). In multivariate logistic regression analysis PCT remained a significant independent predictor for severe sepsis also after adjusting for significant confounders, as did also IL-6. There was no significant difference between PCT and IL-6 in AUC values. The confounders included here were: (1) continuous medication for cardiovascular disease (hypertension, arteriosclerosis or other cardiovascular disease); (2) continuous systemic cortisone treatment (dailydose more than 10 mg oral prednisolone); (3) continuous acetylsalicylic acid medication; (4) antimicrobial treatment 1 week previously, (5) viral infection and (6) inflammation focus documented. Age or sex had no difference between the study-groups and were not therefore included to multivariate regression analysis in study I.

As noted above, in papers III and IV optimal cut-off values for the studied parameters were evaluated. The best limit level for PCT for severe sepsis was 0.30 ng/ml, showing a sensitivity of 82% and a specificity of 66% and for case fatality (d28) 0.19 ng/ml (sensitivity 82% and specificity 53%). The optimal cut-off levels are presented in Table 17 on page 89 .

Figure 6 shows ROC curves for PCT together with IL-6 and CRP in different contexts: in bacterial infection, sepsis, severe sepsis, and case fatality (d 28). In study III PCT was studied as a comparatore value for suPAR and in study for PTX3. The summary data of all studies are presented in 18 at the end of the results section (on page 88).

In studies III and IV first univariate and then multivariate logistic regression analysis were made and high PCT remained an independent predictor for both case fatality and severe sepsis also after adjusting for statistically significant confounders. The confounders used in papers III and IV were those statistically significantly differed between survivours and and non-survivours (Table 12 on page 78) and patients with or without severe sepsis (Table 13 on page 79). When studing case fatality (d28) confounders were age over 60 years, alcohol abuse (alcoholism was diagnosed or patient had been treated for alcohol-induced disease previously), diabetes (types 1 and 2) and continuous cortisone treatment (daily dose over 10 mg oral prednisolone)). When evaluating severe sepsis statistifically significant confoudders were alcohol abuse and continuous corrtisone treatment, not age or sex.

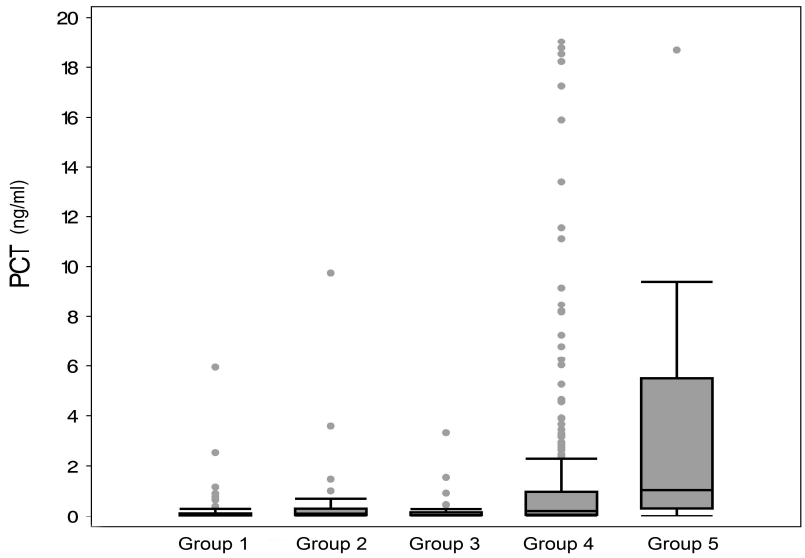


Figure 7. Median levels of plasma procalcitonin (PCT) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

5.3 IL-6 (Studies I and III)

IL-6 was evaluated in study I and used as a comparatore value and assessed as a prognostic marker in study III. The levels of IL-6 plasma concentrations in the five study groups are presented in Figure 8 and together with other studied parameters in Table 17 on page 90. Like PCT levels the median concentration of IL-6 were significantly higher in the severe sepsis group than in the other groups and also in those groups whese bacterial infection was present (Groups 2, 4 and 5, N=426) compared with groups without bacterial infection (Groups 1 and 3, N=113).

In study I plasma IL-6 was studied together with PCT and CRP as a predictor of severe sepsis, sepsis and bacterial infection. In logistic regression analysis using IL-6 as predictor of severe sepsis OR for IL-6 was 1.54 (95% CI 1.32 – 1.80, $p < 0.001$). In multivariate logistic regression analysis also IL-6, like PCT, remained a significant independent predictor of severe sepsis also after adjusting for confounders. There was no significant difference between PCT and IL-6 values.

As noted above, in paper III optimal cut-off values for the studied parameters were evaluated. The best cut-off value for IL-6 for severe sepsis was 172 pg/ml, with a sensitivity of 69% and a specificity of 73% and for case fatality (d28) 93.6 pg/ml with a sensitivity 64% and a specificity 60%. The optimal cut-off levels are presented in Table 16 on page 89.

Figure 6 on page 65 shows ROC curves in different contexts: in detection severe sepsis, sepsis, bacterial infection and case fatality (d 28). In study III IL-6 was assessed as a comparatore value for suPAR. The summary data of all studies are presented in Figure 18 at the end of the results section (on page 88).

As described earlier in study III multivariate logistic regression analyses were made and high IL-6 ($\geq$ 172 pg/ml) remained an independent predictor for severe sepsis, OR 2.99 (95% CI 1.48 – 6.04, p=0.002), but not for case fatality. High IL-6 remained an independent predictor for severe sepsis also after adjusting for potential confounders.

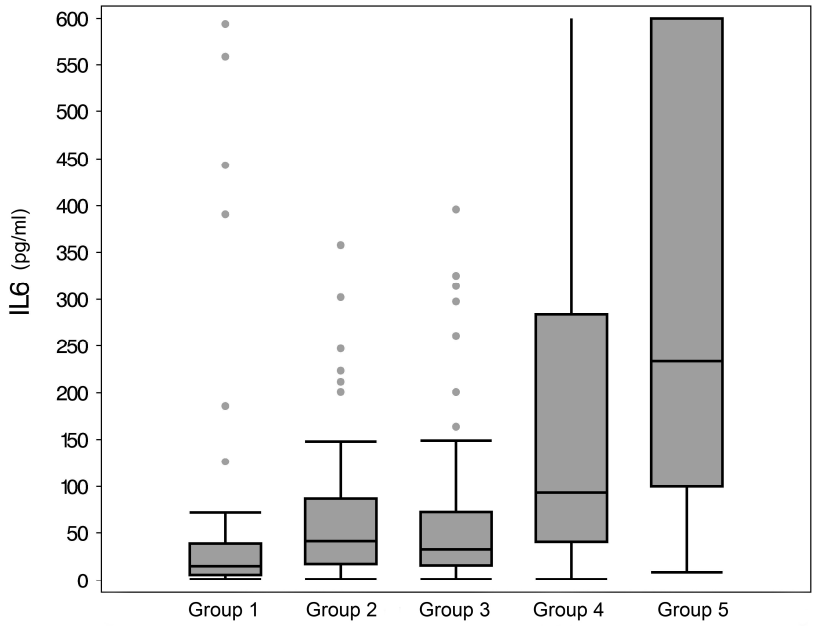


Figure 8. Median levels of plasma interleukin-6 (IL-6) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

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5.4 PLA_2GIIA (Study II)

PLA_2GIIA was measured in study II. Plasma PLA_2GIIA concentrations are shown in Figure 9 and together with other parameters in Table 17 on page 90. The levels of PLA_2GIIA differed significantly between severe sepsis (Group 5) and other patients (Groups 1 – 4) (p=0.001).

In the logistic regression analysis using PLA_2GIIA as predictor in discriminating severe sepsis patients from other patients OR for PLA_2GIIA was 1.48 (95% CI 1.20 – 1.81, $p < 0.001$). In multivariate logistic regression analysis when all studied parameters (BPI, PLA_2GIIA, WBC and CRP)

were included together in a logistic model PLA_2GIIA remained a significant predictor for severe sepsis (OR 1.37, 95 % CI 1.05 – 1.78, p=0.019). Table 11 on page 72 show the results of multivariate logistic regression analysis in respect of the independent predictive value of measured parameters for severe sepsis after adjusting for statistically significant confounders. Only PLA_2GIIA remained a significant independent predictor for severe sepsis in that analysis.

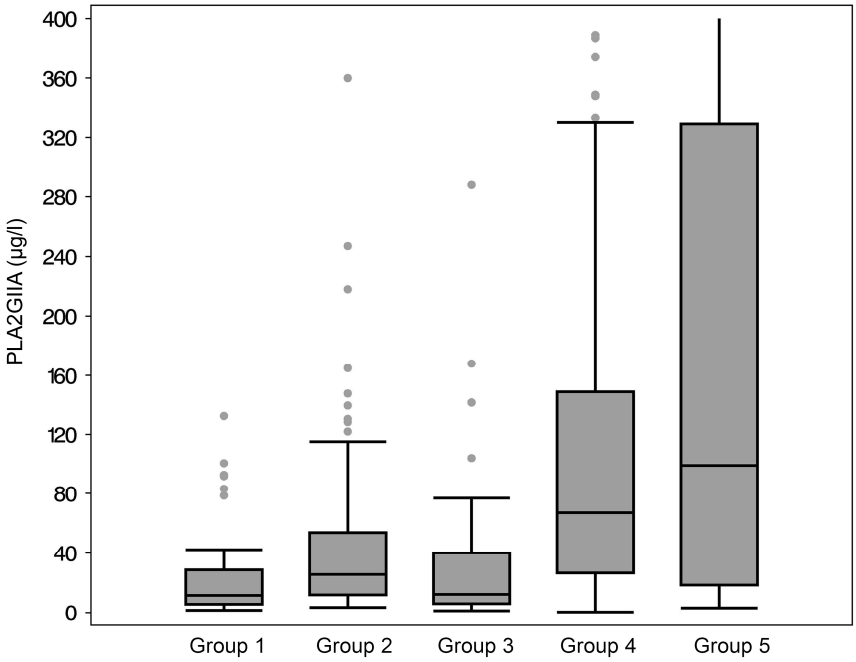

Figure 9. Median levels of plasma Group IIA phospoholipase A_2 (PLA_2GIIA) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

Figure 11 on page 72 gives the results of AUC^{ROC} analysis in different contexts (severe sepsis, sepsis, bacterial infection and case fatality (d 28). According to this AUC^{ROC} analysis PLA_2GIIA seemed to have some value in differentiating sepsis or bacterial infection from other patients. In predicting sepsis AUC for PLA_2GIIA was best, 0.75 (95 % CI 0.70 – 0.79, $p<0.001$), but no statistical difference was noted between AUC^{ROC} s from PLA_2GIIA and CRP. In detecting bacterial infection CRP had the best

AUC^{ROC} , but there was no statistical difference between PLA_2GIIA and CRP. When studying PLA_2GIIA as a prognostic marker evaluating survivors and non-survivors on day 28 AUC^{ROC} was only 0.53 (95% CI 0.42 – 0.63, p=0.632).

Comparing studied markers to each other (Figure 18 on page 88) using AUC^{ROC} analysis PLA_2GIIA seemed to equal with PCT and CRP in detecting sepsis or bacterial infection.

5.5 BPI (Study II)

BPI was measured in study II. Figure 10 shows median levels of BPI in plasma according to study group. BPI and other studied parameters are presented together in Table 17 on page 90. The levels of BPI differed significantly between severe sepsis (Group 5) and other patient groups (Groups 1 – 4) (p=0.001). The BPI/WBC ratio was also studied, but there was no difference between severe sepsis and others in this respect (p=0.531).

In the logistic regression analysis using BPI as predictor in discriminating severe sepsis from other patients OR for BPI was 2.66 (95 % CI 1.54 – 4.60, p=0.001), but in multivariate logistic analysis when all studied parameters PLA2GIIA, WBC and CRP were included together in a logistic model, BPI was not an independent predictor for severe sepsis (Table 11 on page 72).

The results of AUC^{ROC} analysis in different contexts (severe sepsis, sepsis, bacterial infection and case fatality (d 28) are presented in Figure 11. BPI was not a good marker in any of these respects. AUC^{ROC} for BPI for case fatality was only 0.54 (0.44-0.63), p=0.498.

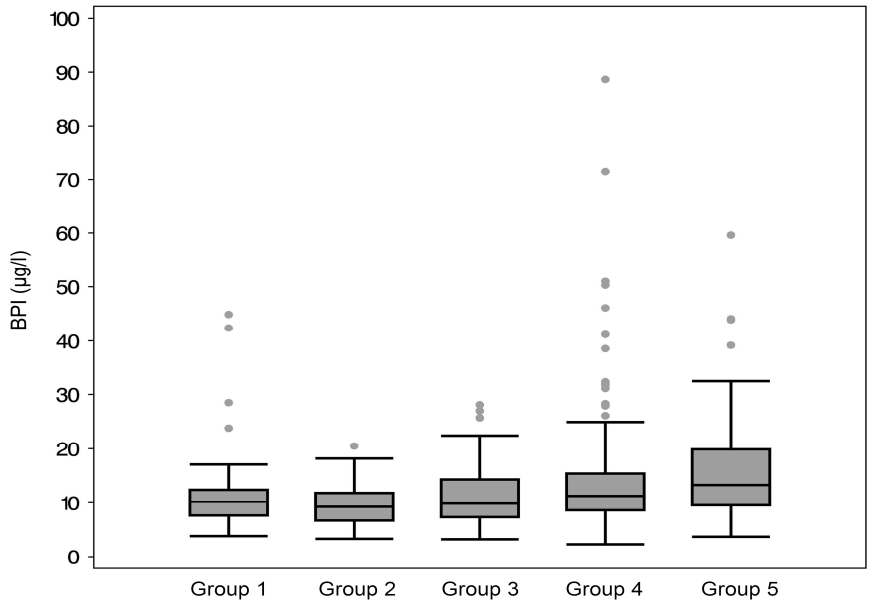

Figure 10. Median levels of plasma Bactericidal/permeability-increasing protein (BPI) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

Table 11. Multivariate logistic regression analysis in assessing the independent predictive value of PLA2GIIA, BPI, CRP, and WBC for severe sepsis. Parameters were log-transormed for analysis. Analysis was made after adjusting for significant confounders and all parameters were included together in the logistic model..

Parameter	Odds ratio (95% confidence limits)	p-value
PLA2GIIA	1.42 (1.08 – 1.86)	0.013
BPI	1.52 (0.75 – 3.10)	0.250
CRP	1.02 (0.73 – 1.42)	0.902
WBC	1.74 (0.86 – 3.51)	0.125

[a] The statistically significant confounders were:
1. Alcoholism. Patient had alcoholism diagnosis or had been treated for alcohol induced disease previously
2. Continuous medication. Patient used regularly at least one medicine for chronic disease
3. Antimicrobial treatment one week previously
4. Continuous systemic cortisone treatment (Patient used continuous systemic cortisone treatment, daily dose more than 10 mg oral prednisone)

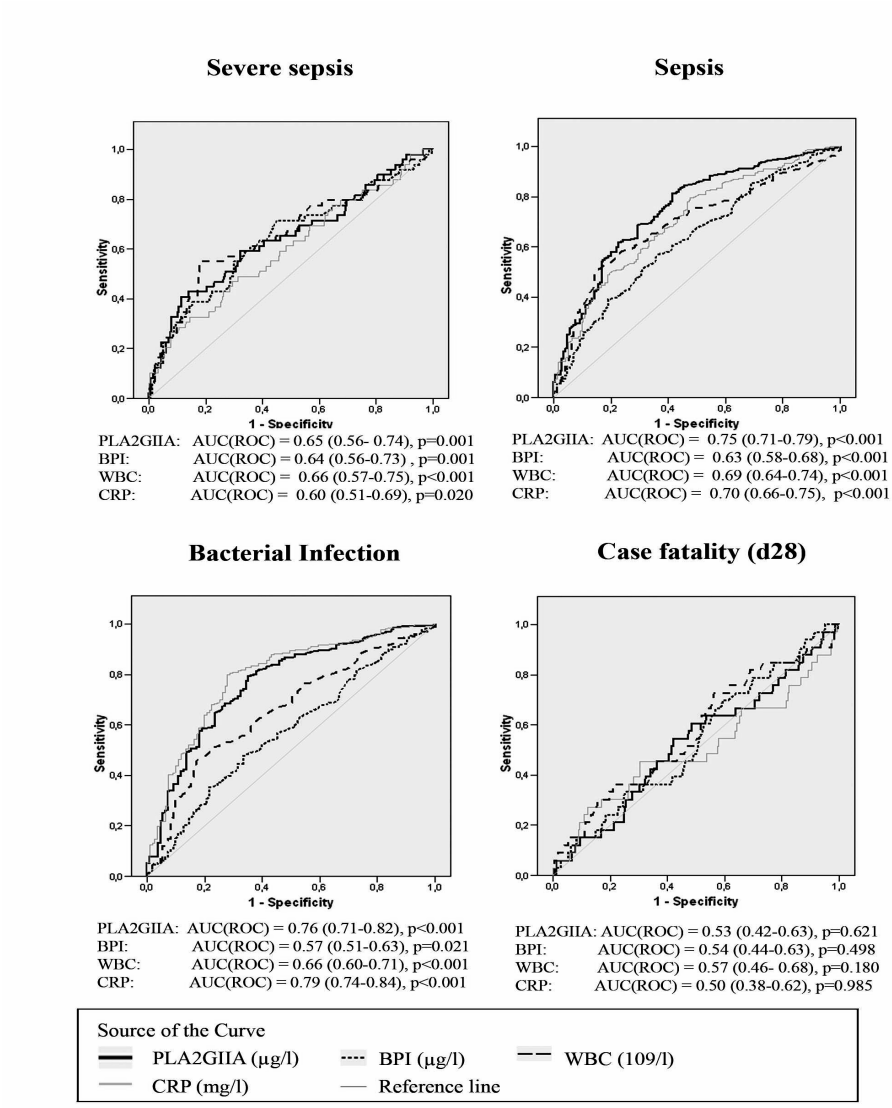

Figure 11. Receiver operating characteristic (ROC) curve for plasma levels of Group IIA phospoholipase A2, (PLA2GIIA), bactericidal/permeability-increasing protein (BPI), C-reactive protein (CRP) and white blood cell count (WBC) detected on admission in relation to severe sepsis, sepsis, bacterial infection and case fatality on (d28) in patients with suspected infection. AUC^{ROC} (95% confidence interval), p<0.001 (Study II).

5.6 suPAR (Study III)

suPAR was studied in paper III. The median suPAR levels in plasma with 25 % percentiles according to study group are presented in Figure 12 and together with other studied parameters in Table 17 on page 90. The levels were significantly higher in non-survivors compared to survivors (8.3 vs. 4.9 ng/ml, $p<0.001$) and in patients with severe sepsis compared to other groups (7.9 vs. 4.8 ng/ml, $p<0.0001$). No significant difference was detected between the mean suPAR levels in assessing patients with or without bacterial infection or with or without sepsis.

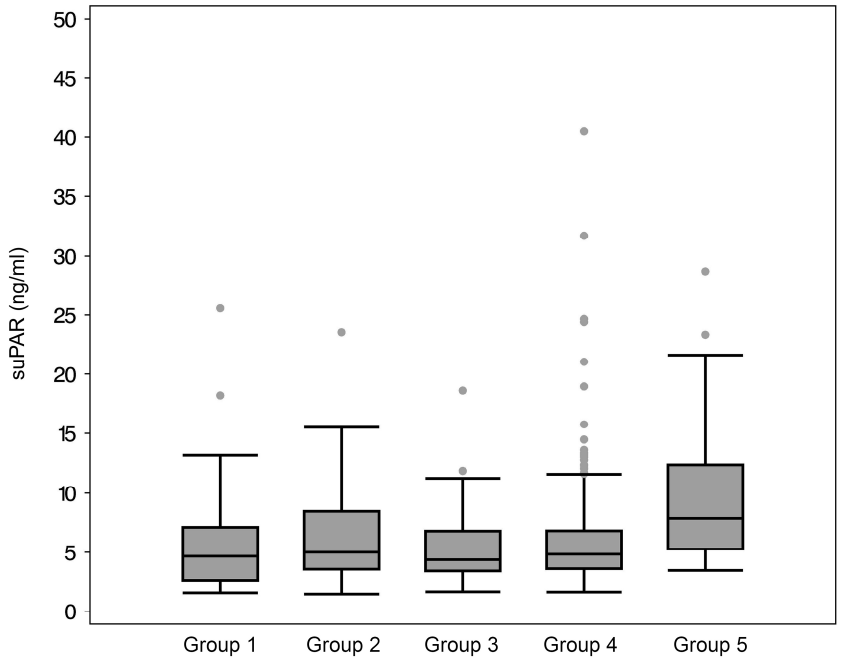

Figure 12. Median levels of plasma soluble urokinase-type plasminogen activator receptor (suPAR) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

suPAR levels in patients on admission stratified by underlying conditions, demographic parameters and clinical findings are shown in paper III, Table 3. suPAR was significantly higher in older (<60 years) than younger patients (5.6 ng/ml vs 4.0 ng/ml, p<0.001), diabetic patients than non-diabetic (6.5 ng/ml vs 4.8 ng/ml, p<0.001), patients with chronic renal insufficiency (10.0 ng/ml vs 4.9 ng/ml, p=0.004), cardiovascular disease (5.8 ng/ml vs 4.3 ng/ml, p<0.001 or continuous cortisone treatment (7.5ng/ml vs 4.8 ng/ml, p<0.001) than without these underlying conditions. suPAR was also significantly higher in patients, who needed ICU stay (8.1 ng/ml vs 4.5 ng/ml, p<0.001), vasopressors (8.0 ng/ml vs 4.9 ng/ml, <0.001), mechanical ventilation 6.6 ng/ml vs 5.0, p<0.001), or C-PAP/bi-PAP treatment (6.2 ng/ml vs 5.0 ng/ml, p=0.017) or developed disseminated intravascular coagulation (DIC) 16.2 vs 5.0, p<0.001) during 28 days period.

Figure 13 presents the correlations of the biomarkers studied in paper III. Plasma suPAR levels correlated positively with markers of inflammation (PCT, IL-6 and CRP) and renal dysfunction (creatinine), but there were no significant associations between WBC or platelet count and suPAR. Liver function tests were not systematically measured and therefore possible associations between them and suPAR could not be determined.

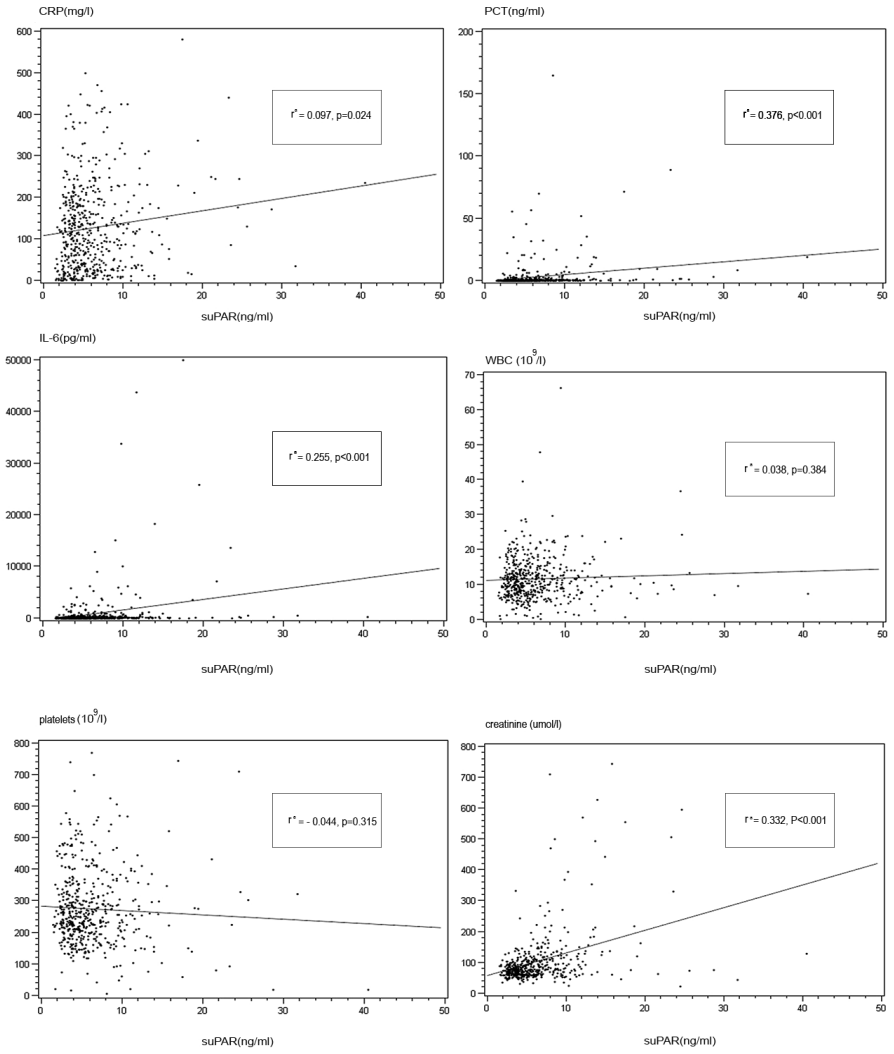

Figure 13. Correlation of plasma soluble urokinase-type plasminogen activator receptor (suPAR) and C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), white blood cell count (WBC), platelet count (platelets) and plasma creatinine on admission. r [a] Spearman`s rank correlation coefficient. N=539 Data available on 523 (WBC and platelets) and 487 patients (creatinine)

Figure 14 shows ROC curves in different contexts: in detection of severe sepsis, sepsis, bacterial infection and case fatality (d 28). AUC^{ROC} for prediction of case fatality was 0.79 (95% CI 0.72 - 0.86, p<0.001), and 0.75 for severe sepsis (0.68 - 0.81, p< 0.001). The optimal cut-off level for predicting fatal disease was estimated using ROC curves and Youden`s index and this cut-off level was used to classify patients into those with high and low suPAR values. At a cut-off level of 6.4 ng/ml suPAR had 76% sensitivity and 69% specificity for fatal diseases. High suPAR levels on admission were associated with many endpoints indicative of severe disease or organ dysfunction. For predicting the development of severe sepsis during 28 days the cut-off level of suPAR concentration was 6.6 ng/ml; sensitivity and specificity for severe sepsis were 67% and 72%. Optimal cut-off-levels are presented in Table 16 on page 89

Clinical characteristics of patients and case fatality (d28) are presented in Table 12. High suPAR, PCT and IL-6 levels, age over 60 years, alcohol abuse, diabetes and continuous systemic cortisone treatment were associated with case fatality, while the levels of CRP and WBC were not. Results of the corresponding analysis for severe sepsis are presented in Table 13.

In multivariate logistic regression analysis, high suPAR remained an independent predictor of case fatality (OR 3.86, 95 % CI 1.63 - 9.11, p=0.002) and severe sepsis (OR 3.11, 95 % CI 1.56 – 6.22, p=0.001) after adjusting for potential confounders (Study III, Tables 5 and 6).

Table 12. Clinical characteristics of patients and case fatality (d28). Statistical differences between survivours and non-survivours were tested using Pearson Chi Square (category) and Mann Whitney U test (continuous variables).

Characteristic	All patients N=539 N (%)	Non-survivors (d28)N=33 N (%)	Survivors (d 28) N=506 N (%)	p-value
Male sex	311 (57.7)	17 (51.5)	294 (58.1)	0.458
Age > 60 years	313 (58.1)	26 (78.8)	287 (56.7)	0.013
BMI [a] ≥ 30	120 (22.3)	4 (25.0)	116 (30.9)	0.614
Alcohol abuse [b]	25 (7.0)	4 (12.1)	21 (4.2)	0.035
Smoking (current smoker)	126 (23.4)	8 (24.2)	118 (23.3)	0.903
Diabetes (type 1 and 2)	82 (15.2)	9 (27.3)	73 (14.4)	0.047
Malignancy (solid or hematological)	95 (17.6)	9 (27.3)	86 (17.0)	0.133
Rheumatic diseases	50 (9.3)	5 (15.2)	45 (8.9)	0.230
Chronic renal insufficiency [c]	18 (3.3)	3 (9.1)	15 (3.0)	0.058
Cardiovascular disease [d]	289 (53.6)	23 (69.7)	266 (52.6)	0.056
COPD or asthma [e]	108 (20.0)	5 (15.2)	103 (20.4)	0.469
Operation six months previously	75 (13.9)	5 (15.2)	70 (13.8)	0.832
Device [f]	82 (15.2)	8 (24.2)	74 (14.6)	0.136
Continuous medication [g]	390 (72.4)	30 (90.9)	360 (71.1)	0.014
Continuous cortisone treatment [h]	59 (10.9)	8 (24.2)	51 (10.1)	0.012
Optimal cut-off level *				
suPAR [i] ≥ 6.4 (ng/ml)	183(34.0)	25 (75.8)	158 (31.2)	<0.001
PCT [j] ≥0.19 (ng/ml)	264 (52.7)	27 (81.8)	237 (46.8)	<0.001
IL-6 [k] ≥ 93.6 (pg/ml)	225 (41.8)	21 (63.6)	204 (40.3)	0.009
CRP [l] ≥ 157.7 (mg/l)	171 (31.7)	15 (45.5)	156 (30.8)	0.080

[a] body mass index. (kg/m^2). Data available on 391 patients.
[b] alcohol abuse was diagnosed or patient had previously been treated for alcohol-induced disease
[c] plasma creatinine concentration constantly more than 170 μmol/l (5 patients had chronic dialysis treatment, no difference between the study groups)
[d] continuous medication for cardiovascular disease (i.e. hypertension, arteriosclerosis or other cardiovascular disease)
[e] continuous medication for asthma or COPD
[f] joint or heart valve prosthesis or pace-maker (does not include dental implants)
[g] continuous medication for a chronic disease
[h] continuous systemic cortisone treatment (daily dose more than 10 mg of oral prednisolone)
[i] plasma soluble urokinase-type plasminugen activator receptor
[j] plasma procalcitonin
[k] plasma interleukin-6.Data available on 538 patients.
[l] plasma C-reactive protein).

* Optimal cut-off level for predicting fatal disease was estimated using ROC curves and Youden`s index

Table 13. Clinical characteristics of patients with and without severe sepsis. Statistical differences between groups were tested using Pearson Chi Square (category) and Mann

Characteristic	All patients N=539 N (%)	Severe sepsis N=49 N (%)	Other patients N=490 N (%)	p-value
Male sex	311 (57.7)	30 (61.2)	281 (57.3)	0.600
Age > 60 years	313 (58.1)	28 (57.1)	285 (58.2)	0.890
BMI [a] >=30	120 (30.7)	11 (31.4)	109 (30.6)	0.921
Alcohol abuse [b]	25 (4.6)	8 (16.3)	17 (3.5)	0.001
Smoking (Current smoker)	126 (23.4)	11 (22.4)	115 (23.5)	0.872
Diabetes (type 1 and 2)	82 (15.2)	11 (22.4)	71 (14.5)	0.139
Malignancy (solid or hematological)	95 (17.6)	4 (8.2)	91 (18.6)	0.068
Rheumatic diseases	50 (9.3)	6 (12.2)	44 (9.0)	0.453
Chronic renal insufficiency [c]	18 (3.3)	4 (8.2)	14 (2.9)	0.049
Cardiovascular disease [d]	289 (53.6)	29 (59.2)	260 (53.6)	0.413
COPD or asthma [e]	108 (20.0)	10 (20.4)	98 (20.0)	0.946
Operation six months previously	75 (13.9)	6 (12.2)	69 (14.1)	0.723
Device [f]	82 (15.2)	7 (14.3)	75 (15.3)	0.850
Continuous medication [g]	390 (72.4)	42 (85.7)	348 (71.0)	0.028
Continuous cortisone treatment [h]	59 (10.9)	12 (24.5)	47 (9.6)	0.001
Optimal cut-off level *				
suPAR [i] ≥ 6.6 (ng/ml)	170 (31.5)	33 (67.3)	137 (28.0)	<0.001
PCT [j] ≥0.30 (ng/ml)	205 (38.0)	40 (81.6)	205 (38.0)	<0.001
IL-6 [k] ≥ 172 (pg/ml)	165 (30.7)	34 (69.4)	131 (26.8)	<0.001
CRP [l] ≥ 158	171 (31.7)	23 (46.9)	148 (30.2)	0.016

a body mass index. (kg/m2). Data available on 391 patients.
b alcohol abuse was diagnosed or patient had been previously treated for alcohol-induced disease
c plasma creatinine concentration constantly more than 170 µmol/l (5 patients had chronic dialysis treatment, no difference between the study groups)
d continuous medication for cardiovascular disease (i.e. hypertension, arteriosclerosis or other cardiovascular disease)
e continuous medication for asthma or COPD
f joint or heart valve prosthesis or pace-maker (does not include dental implants)
g continuous medication for a chronic disease
h continuous systemic cortisone treatment (daily dose more than 10 mg of oral prednisolone)
i plasma soluble urokinase-type plasminugen activator receptor
j plasma procalcitonin
k plasma interleukin-6.Data available on 538 patients.
l plasma C-reactive protein

* Optimal cut-off level for predicting fatal disease was estimated using ROC curves and Youden`s index

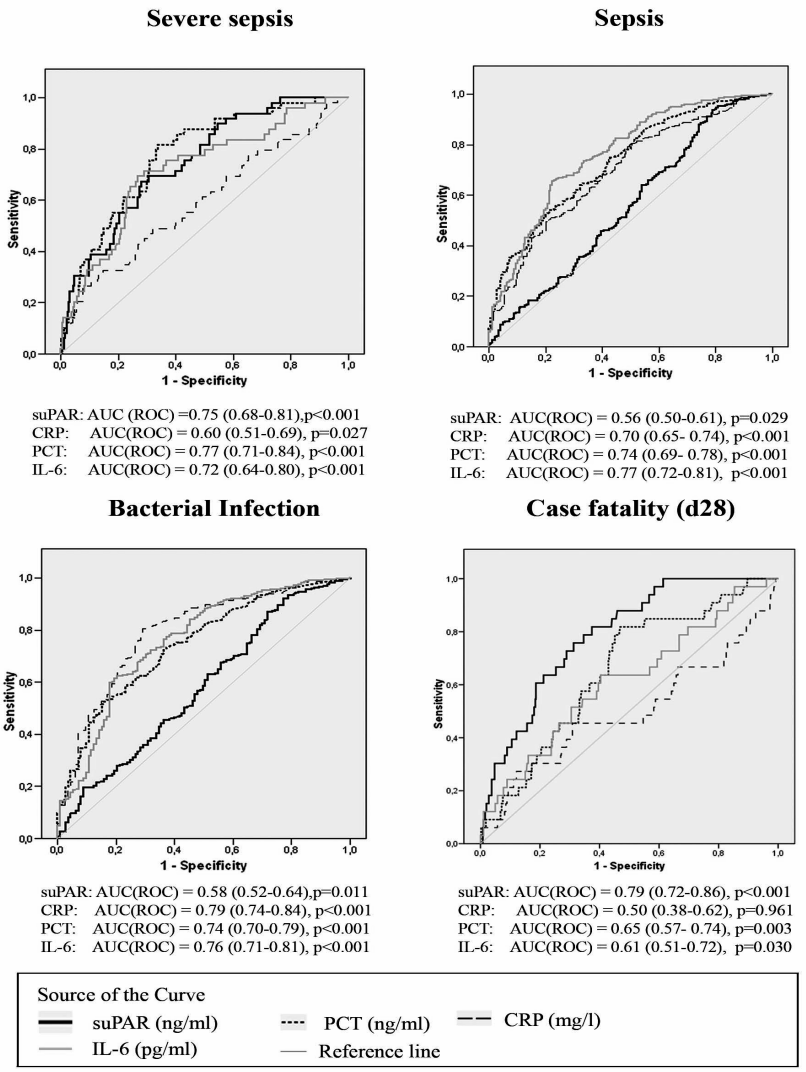

Figure 14. Receiver operating characteristic (ROC) curve for plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR), procalcitonin (PCT), interleukin-6 (IL-6) and C-reactive protein (CRP) detected on admission in relation to severe sepsis, sepsis and bacterial infection in patients with suspected infection. AUC^{ROC} (95% confidence interval), p<0.001 (Study III)

5.7 PTX3 (Study IV)

PTX3 was evaluated in paper IV. The median levels of PTX3 in plasma according to study groups are presented in Figure 15 and together with other parameters in in Table 17 on page 90. The median PTX3 were significantly higher in patients with severe sepsis compared to other groups (16.7 vs 4.9 ng/ml, $p < 0.001$) and in non-survivors compared to survivors (day 28 fatality) (14.1 vs 5.1 ng/ml, $p < 0.001$). No significant difference was detected between the mean PTX3 levels among patients with or without bacterial infection or with or without sepsis.

PTX3 levels in patients on admission stratified by underlying conditions, demographic parameters and clinical findings are shown in paper IV, Table 3. PTX3 was significantly higher in older patients (<60 years) than in younger (6.3 vs 4.0 ng/ml, $p<0.001$), obese (BMI≥30) than non-obese (6.5 vs 4.4, $p=0.017$), cardiovascular disease (6.4 vs 4.5 ng/ml, $p<0.002$) or continuous cortisone treatment (7.6 vs 5.0 ng/ml, $p<0.022$) than without these underlying conditions. PTX3 was also significantly higher in patients, who needed ICU stay (11.6 vs 5.2 ng/ml, $p<0.001$), vasopressors (14.2 vs 5.3 ng/ml, <0.015), or developed disseminated intravascular coagulation (DIC) 46.2 vs 5.3 ng/ml, $p<0.001$) during 28-day period.

The Figure 16 presents the correlations of biomarkers studied in paper IV. Plasma PTX3 levels correlated positively with PCT and CRP and creatinine concentration and also with WBC. A weak negative correlation was shown with platelet count and PTX3.

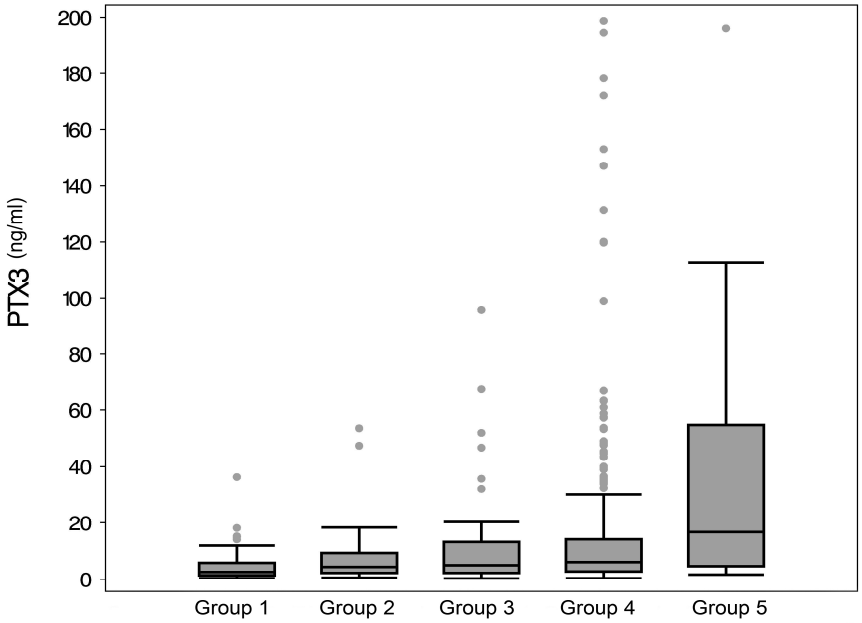

Figure 15. Median levels of plasma pentraxin 3 (PTX3) with 25 % percentiles at admission in the five study groups. Group 1: No SIRS, no bacterial infection; Group 2: Bacterial infection, no SIRS; Group 3: SIRS, no bacterial infection; Group 4: Sepsis and Group 5: Severe sepsis.

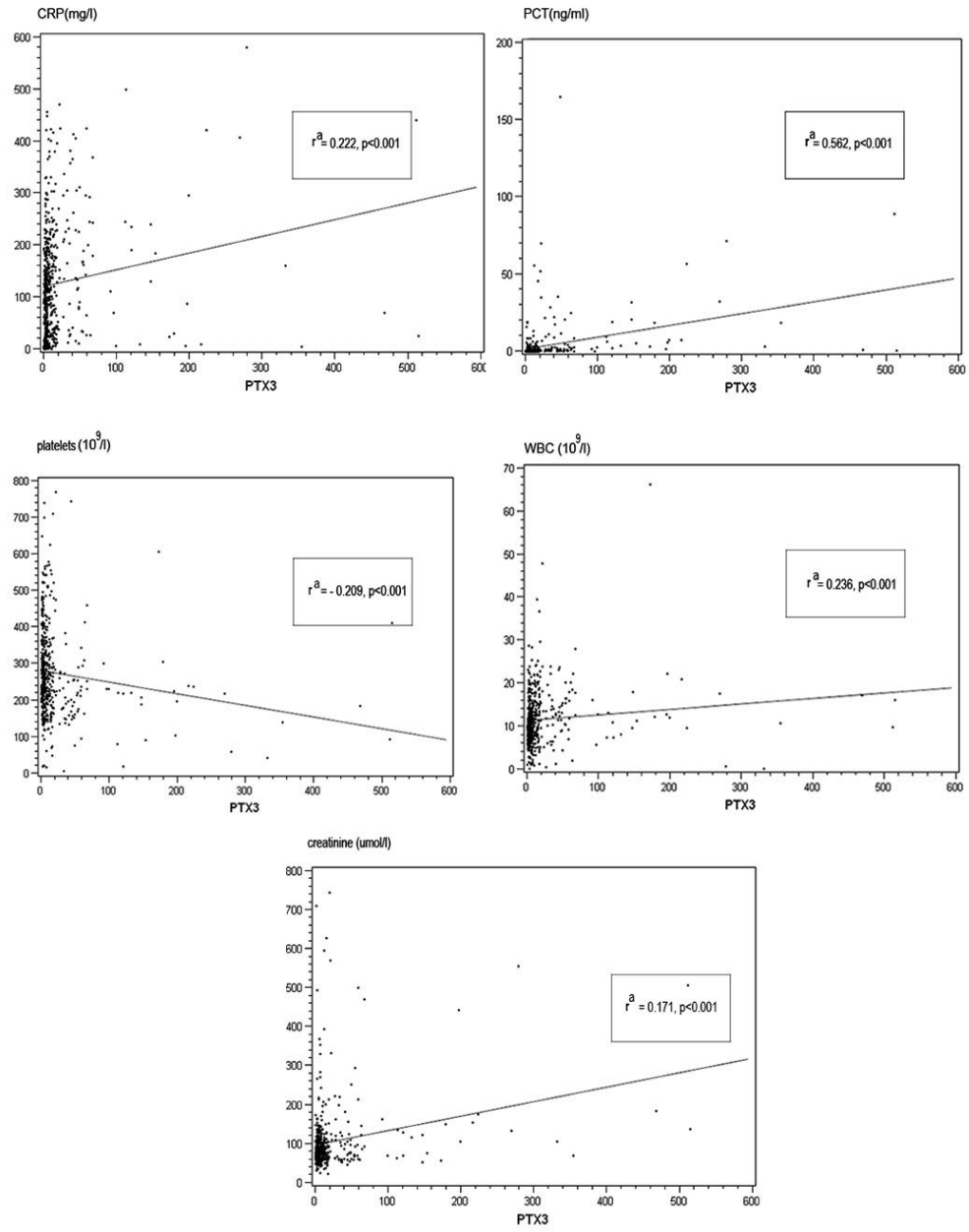

Figure 16. Correlation of plasma pentraxin 3 (PTX3) and C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), platelet count (platelets) and plasma creatinine on admission. r [a] Spearman`s rank correlation coefficient. N=537 Data available on 523 (WBC and platelets) and 487 patients (creatinine)

Figure 17 shows ROC curves in different contexts: in detection of severe sepsis, sepsis, bacterial infection and case fatality (d 28). AUC^{ROC} for prediction of case fatality was 0.69 (95% CI 0.58 - 0.79, $p<0.001$, and 0.73 for severe sepsis (0.66 - 0.81, $p< 0.001$).

The optimal cut-off level for predicting fatal disease (day 28) and for the development of severe sepsis during days 0-28 were estimated using ROC curves and Youden`s index. At a cut-off level of 7.7 ng/ml, PTX3 had 70% sensitivity and 63% specificity for fatal diseases (d28). For predicting the development of severe sepsis on days 0- 28 the cut-off level for PTX3 concentration was 14.1 ng/ml, with sensitivity 80 % and specificity 63% (Table 16 on page 89).

In a univariate model high PTX3 values predicted severe sepsis when used as grouping variable applying the optimal cut-off level as a deviding line as described. So also did high PCT and CRP. Alcohol abuse and continuous cortisone treatment were also associated with severe sepsis. These parameters were combined in the multivariate model when evaluating the independent predictive value of PTX3 for severe sepsis. The results are shown in table Table 1.

When studying the case fatality on day 28 high PTX3 and high PCT, age over 60 years, alcohol abuse, diabetes and continuous systemic cortisone treatment were shown to be significant factors in the univariate model. The results from this multivariate analysis are shown in table 16 on next page.

Table 14. Multivariate logistic regression analysis evaluating the independent predictive value of pentraxin 3 (PTX3), procalcitonin (PCT) and C-reactive protein (CRP) for severe sepsis. The optimal cut-offs for these parameters were counted using ROC curve analysis and Youden`s index. Parameters were taken for analysis together with statistically significant confounders (N=537)

Character	Odds ratio	95 % Confidence limits	p
PTX3 ≥ 14.1 ng/ml	**3.02**	**1.50 – 6.01**	**0.002**
PCT ≥ 0.30 ng/ml	**5.55**	**2.37 – 13.00**	**< 0.001**
CRP ≥ 158 mg/l	**1.11**	**0.56 – 2.20**	**0.775**
Alcohol abuse [a]	**4.88**	**1.69 – 14.09**	**0.003**
Continuous cortisone treatment [b]	**4.20**	**1.82 – 9.70**	**< 0.001**

[a] alcoholism was diagnosed or patient had previously been treated for alcohol-induced disease
[b] continuous systemic cortisone treatment (daily dose more than 10 mg of oral prednisolone

Table 15. Multivariate logistic regression analysis evaluating the independent predictive value of pentraxin 3 (PTX3) and procalcitonin (PCT) for 28-d case fatality. The optimal cut-offs for parameters were estimated using ROC-curve analysis and Youden`s index. Parameters were taken to analysis together with statistically significant confounders (N=537)

Character	Odds ratio	95 % Confidence limits	p
PTX3 ≥ 7.7 ng/ml	**2.37**	**1.04 – 5.38**	**0.040**
PCT ≥ 0.19 ng/ml	**3.51**	**1.35 -- 9.15**	**0.010**
Age > 60 years	**3.02**	**1.11 – 8.19**	**0.030**
Alcohol abuse [a]	**6.01**	**1.60 – 22.67**	**0.008**
Diabetes (type 1 and 2)	**2.13**	**0.89 – 5.05**	**0.088**
Continuous cortisone treatment [b]	**2.28**	**0.93 – 5.59**	**0.073**

[a] alcoholism was diagnosed or patient had been treated for alcohol induced disease previously
[b] continuous systemic cortisone treatment (daily dose more than 10 mg of oral prednisolone)

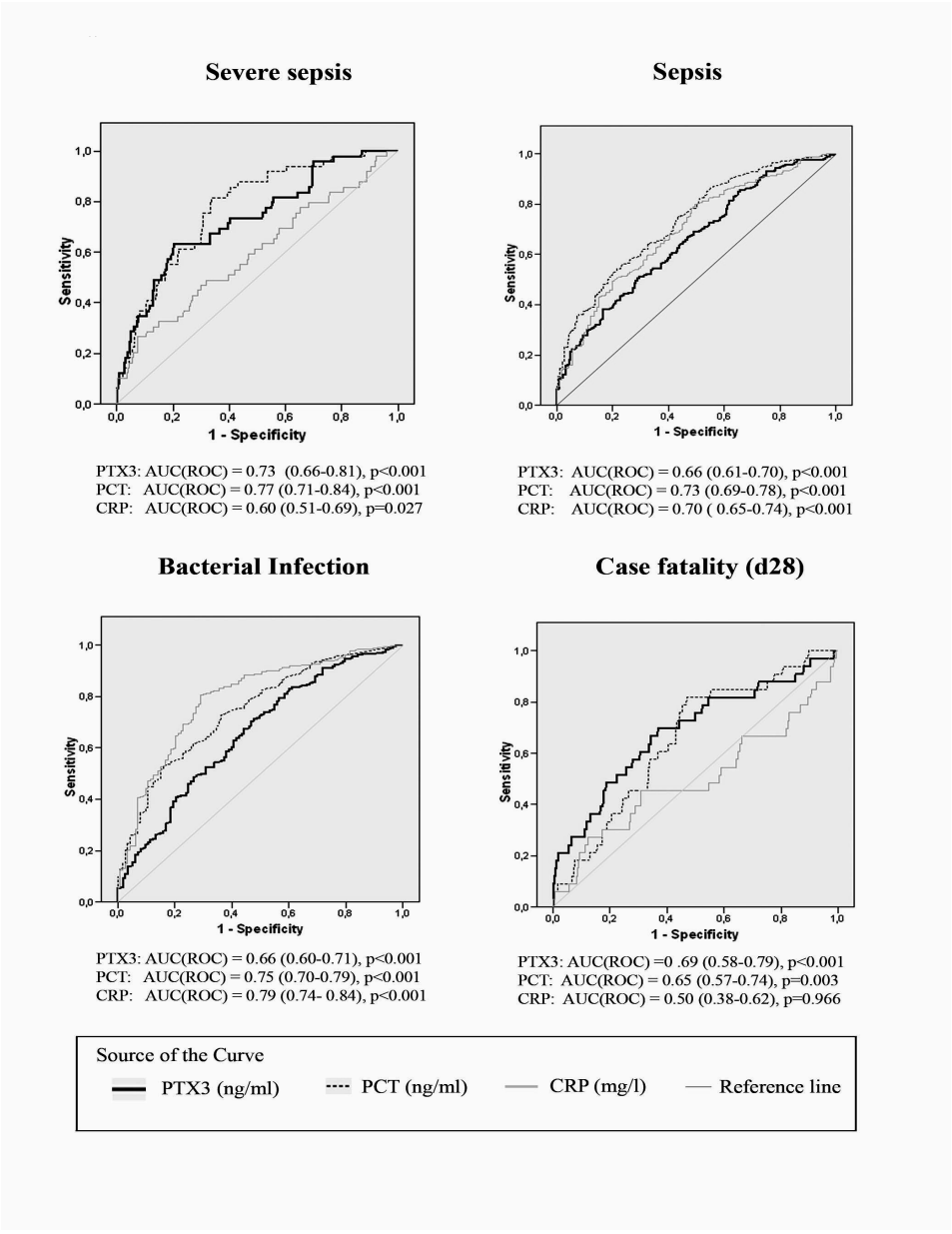

Figure 17. Receiver operating characteristic (ROC) curve for plasma levels of pentraxin 3 (PTX3), procalcitonin (PCT), and C-reactive protein (CRP) detected on admission in relation to severe sepsis, sepsis, bacterial infection and case fatality (d28) in patients with suspected infection. AUC^{ROC} (95% confidence interval), p<0.001 (Study IV).

5.8 Results in summary

Taken together the main findings in the studies I – IV are presented in Figure 18. According to AUC^{ROC} analysis PCT emerged as the best marker of severe sepsis, PLA_2GIIA for sepsis, CRP for bacterial infection and suPAR for case fatality (d28). Optimal cut-off values are presented in Table 16 on page 89.

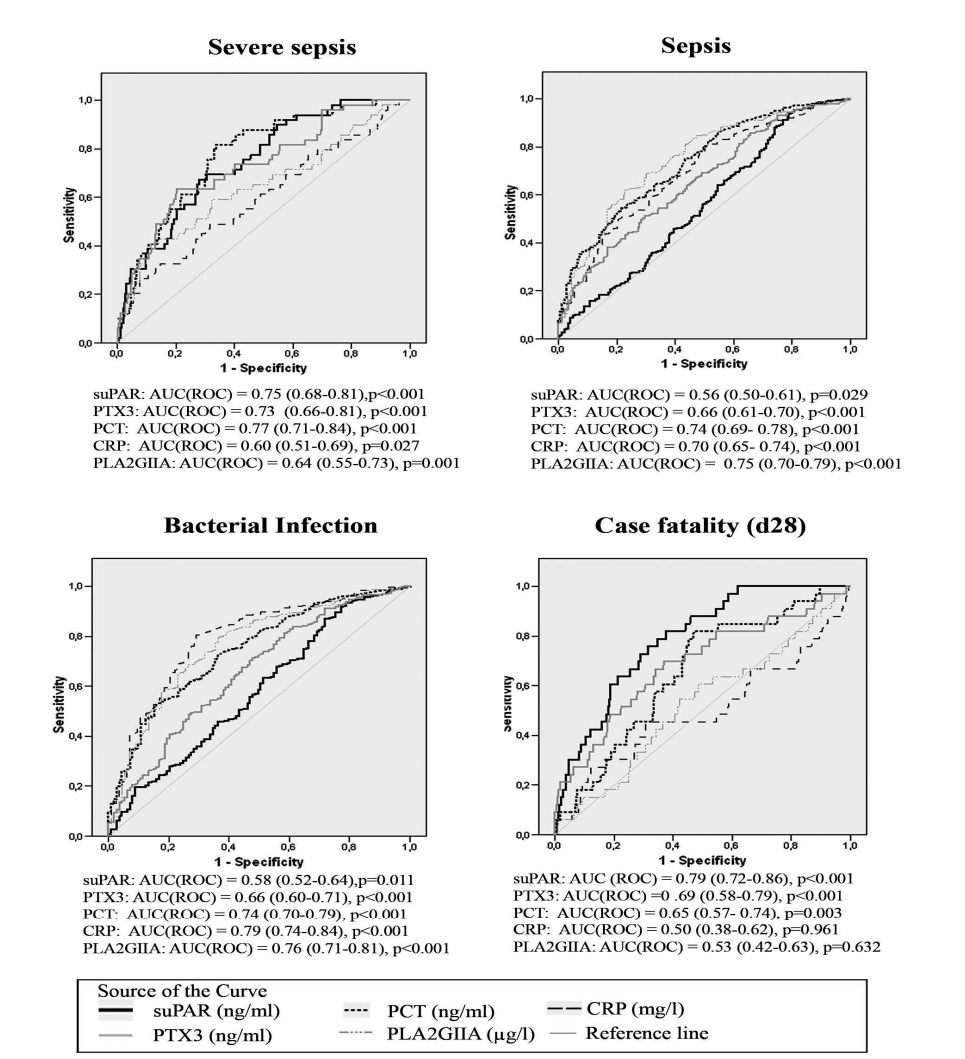

Figure 18. Summary figure. Receiver operating characteristic (ROC) curve for plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR), pentraxin 3 (PTX3), procalcitonin (PCT), Group IIA phospoholipase A2, (PLA2GIIA), and C-reactive protein (CRP) detected on admission in relation to severe sepsis, sepsis, bacterial infection and case fatality (d28) in patients with suspected infection. AUC^{ROC} (95% confidence interval), p<0.001.

Table 16. Optimal cut-off values for C-reactive protein (CRP), procalcitonin (PCT), interleukin 6 (IL-6), soluble urokinase-type plasminogen activator receptor (suPAR), and pentraxin 3 (PTX3) measured on admission for prediction of severe sepsis or case fatality on day 28 (d28) (Studies III – IV). Optimal limit levels were estimated using ROC curves and Youden`s index.

Studied marker on admission	Predicting severe sepsis between days 0 and 28			Predicting case fatality (d28)		
	Optimal cut-off level	Sensitivity	Specificity	Optimal cut-off level	Sensitivity	Specificity
CRP	158 mg/l	47%	70%	-		
PCT	0.30 ng/ml	82%	66%	0.19 ng/ml	82%	53%
IL-6	172 pg/ml	69%	73%	93.6 pg/ml	64%	60%
suPAR	6.6 ng/ml	67%	72%	6.4 ng/ml	76%	69%
PTX3	14.1 ng/ml	80%	63%	7.7 ng/ml	70%	63%

Table 17. Parameters at admission in the five study groups

Parameter	N	No SIRS, no bacterial infection median (range)	Bacterial infection, no SIRS median (range)	SIRS, no bacterial infection median (range)	Sepsis median (range)	Severe sepsis median (range)
Study I	**539**					
CRP (mg/l)		25.5 (0.2 - 261.6)	119.3 (0.2 - 330.3)	27.8 (0.7 - 365.4)	135.0 (1.0 - 455.8)	134.5 (3.8 - 580.0)
PCT (ng/ml)		0.05 (0.02 - 5.95)	0.13 (0.02 - 9.75)	0.07 (0.02 - 3.34)	0.23 (0.02 - 165.16)	1.05 (0.03 - 88.96)
IL-6 (pg/ml)		15.3 (1.5 - 653)	41.3 (1.5 - 2 637)	32.7 (1.5 - 3 552)	93.5 (1.5 – 43 790)	233.4 (9.0 – 50 000)
Study II	**525**					
BPI (µg/l)		10.2 (3.8 - 44.9)	9.2 (3.3 - 20.5)	9.8 (3.2 - 28.1)	11.3 (2.3 - 88.7)	13.3 (3.7 - 59.6)
PLA_2GIIA (µg/l)		11.5 (1.5 - 567.0)	25.5 (3.3 - 360.0)	12.2 (1.2 - 488.0)	67.0 (0.4 - 1841.0)	99.0 (3.0 - 2170.0)
CRP (mg/l)		25.0 (0.2 - 261.6)	118.3 (0.2 - 330.3)	27.4 (0.7 - 365.4)	133.7 (1.0 - 455.8)	134.5 (3.8 - 580.0)
WBC (10^9/l		8.1 (4.5 - 29.6)	8.5 (4.4 - 20.1)	9.8 (1.1 - 28.7)	11.9 (0.1 - 66.2)	15.2 (0.7 - 47.8)
Study III [a]	**539**					
suPAR (ng/ml)		4.7 (1-5 – 25.6)	5.0 (1.5 – 23.6)	4.4 (1.6 – 18.6)	4.8 (1.6 – 40.5)	7.9 (3.5 – 28.7)
Study IV [b]	**537**					
PTX3 (ng/ml)		2.6 (0.4 – 36.3)	4.4 (0.6 – 53.6)	5.0 (0.3 – 95.9)	6.1 (0.4 – 514.7)	16.7 (1.7 – 510.9)

6 DISCUSSION

6.1 STUDY DESIGN

The object here was to assess the early detection of severe sepsis and the clinical utility of prognostic biomarkers in an emergency room setting in Satakunta Central Hospital, serving 240 000 inhabitants and having the only intensive care unit in the area. Our study cohort was fairly large and seemed adequate for a study of adult emergency room patients with a suspicion of infection. Clinicians unrelated to the study made their decision to take blood cultures from study patients according to the suspicion of infection and according to national and local guidelines of the treatment of sepsis. The study samples were taken upon admission concurrently with the blood cultures. For practical purposes (acquiring patient´s permission), patients were enrolled only from Sunday to Wednesdays; 55 patients refused to take part and were excluded. Amounth of missed study samples on study days was large, 500 patients, but however, compared with our prior evaluation of 1551 consecutive patients no difference was noted between the study and target population at least with regard to age, sex, the rate of positive blood cultures, and mortality rate. We therefore think our study population to be representative and rather unselected. Patient enrolment took place over a 14-month period from 2004 to 2005 in Satakunta Central Hospital. Collection of material being laborious and time-consuming we were obliged to compromise on the original target of 600 – 800 subjects. Even so, our study is, as far as we know the largest made in an emergency room setting.

Patients were divided into 5 study groups retrospectively on the basis of ACCP/SCCM Consensus Conference definitions in 1992 (Bone et al. 1992), using three criteria: SIRS, bacterial infection (documented or probable), and sepsis-associated organ dysfunction. Only part of the patients had the marks of severe sepsis on admission. We excluded 15 patients from the analysis: one due to a missing blood sample at admission, 11 with incomplete data for classification (bacterial infection or not) and 3 for SIRS and organ dysfunction but no evidence for bacterial infection (1 with epidemic nephropathy and 2 with acute myocardial infarction). The definition of bacterial infection and sepsis are well in line with prior literature. The criteria for ACCP/SCCM have been criticized for non-specificity and excessive sensitivity (Levy et al. 2003). The description based on the SIRS criteria largely defines the proinflammatory phase of the syndrome, but the

current classification dos not accurately reflect the overall immune status of the individual patient. According to novel insights into the pathogenesis of sepsis the need for an assessment of patients' immune status has appeared important in the stratification of cases (Huttunen and Aittoniemi 2011; Reinhart et al. 2012).

The frequency of positive blood cultures in our studies were at the same level as expected according to our prior analysis, where the blood culture was clinically significantly positive in 8.3 %, in our studies 8.7 – 8.9 %. Among missed patients on study days blood cultures were positive with 7.4 % (37 / 500 patients). It is also in line with published literature (Skogberg et al. 2008; Yanagihara et al. 2010). In our studies about a quarter of patients had received antimicrobial treatment prior to admission and before blood cultures were taken. This probably reflects well the real situation in the ER at least in Finland. The distribution of blood culture findings and infection foci were as expected (Karlsson et al. 2007). Resistant microbes in blood culture findings are a rising problem, but in Satakunta they were rare at the time when patient enrollment took place in years 2004 – 2005 and no multi-resistant bacteria were found. The delay starting the antimicrobial therapy was not analyzed here although it is known that rapid and appropriate antimicrobial treatment can reduce mortality and improve the prognosis of patients (Varpula et al. 2007; Zambon et al. 2008; Levy et al. 2010; Zahar et al. 2011).

The case fatality rate by day 28 in our studies was low, as expected. In studies I and III case fatality was 7.6 %, corresponding well to the proportion in the pre-evaluation cohort (6.7 %). When comparing severe sepsis patients in our study to the Finnsepsis Study patients (Karlsson et al. 2007), our cohort appears representative. Hospital mortality in the Finnsepsis study was 28.3 % and in our study 28-day case fatality in the severe sepsis group was 28.6 %. Also the distribution of sources of infection was consistent in both studies. In the Surviving Sepsis Campaign it was noted that case fatality due to severe sepsis had fallen to the same level as in our severe sepsis group (Levy et al. 2010).

As in real life, also in our studies adult patients with suspected infection in the ER were a heterogeneous group having many underlying diseases and medications. There were thus many possible confounders which we carefully sought to control. There were no significant differences between the five study groups with regard to either age or sex. Obesity (BMI >30 kg/m^2) and smoking were not associated with any subgroup. No difference was noted between the study groups in respect of diabetes, malignancies, rheumatic diseases, chronic renal insufficiency, chronic lung diseases (COPD or asthma), operations done 6 months previously, or transplanta-

tions. There were no human immunodeficiency (HIV) patients in our study population. There were significantly more patients with cardiovascular diseases in the group 2 and also in 5 than in other groups. In the severe sepsis group (group 5) there were more patients with alcohol abuse (a diagnosis of alcoholism or previous treatment in hospital for alcohol-induced disease) and continuous systemic cortisone treatment (daily dose over 10 mg prednisone/prednisolone).

Results of univariate regression analysis and statistical differences between patients with or without severe sepsis and survivals versus non-survivals are presented in Table 13 and Table 12 univariate analysis it was shown that apart from biomarkers studied, alcohol abuse and continuous systemic cortisone treatment also predicted severe sepsis, but e.g. age or sex or cardiovascular disease did not. When predicting case fatality (d28) age older than 60 years, alcohol abuse, diabetes, and systemic cortisone treatment were associated with fatality. These observations seem to be clinically relevant and were taken into account when making multivariate logistic regression analysis and adjusting the results for confounders.

In our studies we analyzed only one plasma sample taken upon admission, and did not conduct follow-up measurements. The sample was taken as early as possible at admission to the hospital. However, the weakness of this study – and of sepsis studies in general - was that we were not able to reliably control the time frame how soon the test were made after the onset of sepsis. Therefore, it is difficult to know at what point in the natural course of the disease an individual patient stands. The various possible courses of the immune response to infection and sepsis make it hard to determine the significance of a single test result. It is possible that serial measurements could have been more useful in this respect.

In our studies we compared study groups to each other and in different perspectives and had not “normal controls” in our protocol, except in paper II where normal controls were used for the evaluation of BPI and PLA2GIIA measurements. Because laboratory methods used here were well established, we think that normal controls were not needed in our study design.

6.2 CRP (Studies I - IV)

CRP is one of the most widely used laboratory markers in the emergency departments in Finland. Its analytical methods are cheap and readily available in automatic analyzators around the clock. The measurement of CRP levels are much used when infection or sepsis is suspected (Gabay and Kushner 1999; Volanakis 2001; Black et al. 2004). The levels of CRP rise in infection and inflammation, but its utility in assessing the severity and prognosis of sepsis has been disputed (Suprin et al. 2000; Pettilä et al. 2002; Silvestre et al. 2009; Al-Subaie et al. 2010; Silvestre et al. 2010). The usefulness of CRP is hampered by its reponse time. Therefore, it must be borne in mind that in the early course of sepsis CRP can be still normal, as it peaks later than IL-6 or PCT.

In our studies CRP was used as a reference marker and its utility was compared with other markers in various contexts. In our first study we evaluated the usefulness of plasma CRP, PCT and IL-6 in the early detection of severe sepsis in an ER setting. It was shown that CRP can be used to predict severe sepsis in the emergency room, but PCT and IL-6 proved superior in predicting severe sepsis. In multivariate logistic regression analysis evaluating the independent predictive value of the parameters studied, CRP did not remain a significant independent predictor of severe sepsis, whereas PCT and IL-6 did. In study III the optimal cut-off level for CRP in predicting severe sepsis was 158 mg/l with 47 % sensitivity and 70 % specificity. It is clear that the value of CRP in predicting the severity of sepsis is rather low, sensitivity being a about the same level as tossing a coin. Our results support earlier findings demonstrating PCT to be a better marker than CRP for sepsis severity (Balc et al. 2003; Aikawa et al. 2005; Tschaikowsky et al. 2011). This aspect is discussed more in the chapter of PCT.

In our study, CRP seemed to be equal to PCT or IL-6 in evaluating bacterial infection in patients without bacterial infection or sepsis as against no-sepsis. According to AUC^{ROC} analysis, CRP seemed to be even slightly better marker for bacterial infection than PCT and IL-6, but no statistical difference between AUCs for bacterial infection or sepsis was noted. It is well known that CRP is not a specific marker of bacterial infection, but is also elevated in non-infectious inflammatory conditions (Volanakis 2001; Black et al. 2004; Meisner et al. 2006). It has been shown in many studies that CRP is elevated in many inflammatory states or tissue damage as in auto-

immune and rheumatic diseases (Eberhard et al. 1997), myocardial infarction or after surgery (Meisner et al. 1998). The role of CRP in differentiating bacterial from viral infection is not clear, although concentrations tend to be higher in invasive bacterial infection than in viral infections (Suprin et al. 2000; van der Meer et al. 2005). In our study PCT or IL-6 were not better markers for bacterial infection than CRP. In one meta-analysis, the diagnostic accuracy of PCT was better than that of CRP among patients hospitalized due to suspected bacterial infection (Simon et al. 2004). On the other hand one study has shown that CRP, IL-6 and LPS are better diagnostic markers for infection and sepsis than PCT in patients admitted to a department of internal medicine. In this study in question PCT was superior as a severity marker (Gaini et al. 2006).

In studies II and IV the case fatality on day 28 was assessed. It was shown that CRP had no value in predicting case fatality on day 28 and therefore no cut-off value for case fatality was counted. This is in line with several previous studies (Suprin et al. 2000; Pettilä et al. 2002; Silvestre et al. 2009; Silvestre et al. 2010).

In our work median levels of plasma CRP in the sepsis and severe sepsis groups did not differ from each other, but CRP levels were significantly higher in groups with bacterial infection than without. Similar findings have been made in several studies previously (Al-Nawas et al. 1996; Meisner et al. 1999; Castelli et al. 2004). For clinical use ranges are large and there is considerable overlapping between groups, which makes the utility of single measurement vulnerable.

We showed that CRP is not a good prognostic marker for severe sepsis, nor for case fatality (d28) in patients with suspected infection in an ER setting but it was a feasible although not specific marker for bacterial infection. Serial measurements of CRP are part of the routine in clinical care of infection patients. Although not specific for infection, CRP is still a practical method in the follow-up of the treatment. In Finland CRP is very widely used and its limitations are fairly well known. CRP is a practical comparator when studying other biomarkers. Low price and good standardization support its continued use in clinical practice, but according to our results and several previous studies CRP is not a good prognostic marker in patients with suspected infection in an ER setting.

6.3 PCT (Studies I - IV)

The role of PCT - calcitonin precursor - in the host defence against invading microbes has been unresolved. However, the level of PCT in circulation has shown to increase in various inflammatory and infectious conditions. Therefore, in addition to CRP, PCT is one of the most widely studied parameters for a diagnostic and prognostic marker in sepsis. Most studies have been made among ICU patient, not in the ER setting, and include various pre-selective elements e.g. only patients having positive blood cultures. The utility of PCT has been controversial (Simon et al. 2004; Uzzan et al. 2006; Tang et al. 2007), and the interpretation of some studies is difficult on account of variations in PCT assays and predictive cut-off points and different patient populations.

In our studies, PCT was measured using automatic analyzer and a commercial method suitable for around-the-clock use. In our first study comparing PCT, CRP and IL-6, PCT emerged as a good marker in the early diagnosis of severe sepsis at admission, but the difference between PCT and IL-6 was not statistically significant. In multivariate logistic regression analysis PCT remained a significant independent predictor of severe sepsis also after adjusting for significant confounders. In studies III and IV the optimal cut-off level for PCT in predicting severe sepsis was 0.30 ng/ml, with a sensitivity of 82 % and a specificity of 66 %. The sensitivity of PCT in predicting severe sepsis in our study was reasonably good, but its specificity was rather poor for clinical use. In multivariate analysis in both studies III and IV PCT still remained an independent predictor of severe sepsis.

Our results are in line with those of previous studies. The majority of previous works have found PCT to be a better marker of severe sepsis than CRP (Balc et al. 2003; Aikawa et al. 2005). PCT shows a more favorable kinetic profile than CRP for early detection of sepsis and also for daily monitoring of treatment response, being shown to have the capacity to discriminate between sepsis and SIRS without sepsis (Brunkhorst et al. 1999; Brunkhorst et al. 2000; Muller et al. 2000; Harbarth et al. 2001).

Here CRP and PCT were equal predictors of bacterial infection but in many previous studies and also in meta-analysies PCT has been the more sensitive and specific for bacterial infection (Simon et al. 2004; Uzzan et al. 2006; Tang et al. 2007). It has also been shown that PCT may be helpful in predicting the blood culture positivity in critically ill patients (Nakamura et al. 2009) and recently also in the ER setting (Riedel et al. 2011). In ac-

cord with our findings, in one previous work with 196 patients, IL-6 and CRP appeared to be superior to PCT as diagnostic markers for infection in patients admitted to a department of internal medicine in Denmark (Gaini et al. 2006).

Here CRP and PCT were also equal in detecting sepsis in patients without sepsis. This is in line with another study made in an emergency department, which found that PCT, IL-6 and CRP moderately well discriminated infected from non-infected patients (Tsalik et al. 2012).

In studies III and IV the prognostic aspect of case fatality on day 28 was studied together with CRP, IL-6 and suPAR (study III) and CRP and PTX3 (study IV). It was shown that PCT is a predictor of case fatality on day 28 with an optimal cut-off level of 0.19 ng/ml with 82% sensitivity but only 53% specificity. In multivariate logistic regression analysis PCT remained an independent predictor for case fatality likewise in many previous studies (Pettilä et al. 2002; Jensen et al. 2006). In a study by a Ruiz-Alvarez et al. PCT did not predict mortality, although CRP, SOFA-score, age and gender did (Ruiz-Alvarez et al. 2009).

Internationally PCT measurement has been included in routine clinical practice and guideline recommendations in the case of critically ill patients, not alone but together with clinical judgment taking account of patient and therapy-related factors which might interfere with the initial course of PCT (Dellinger et al. 2008; O'Grady et al. 2008). In the ER setting the utility of PCT is still more contradictory and the cost-benefit is not clear. In our study I we concluded that no single measurement of any biomarker would be reliable for ruling out sepsis, severe sepsis or bacterial infection for the lack of satisfactory sensitivity and difficulties in handling the time frame in respect of how soon the markers are taken after the onset of sepsis. Recently, however, it has been debated whether biomarkers are better to rule sepsis in or out (Reinhart et al. 2012; Faix 2013). PCT can be elevated in many non-infective conditions, and is thus probably better used to rule out than rule in systemic bacterial infection. If PCT is low the probability that the patient would have bacterial infection or sepsis is small. The same is true with CRP, but false-negative results can occur if samples are taken too early. Although we did not study this aspect, repeated tests should probably be performed at 6 – 12 h intervals (Shehabi and Seppelt 2008; Bouadma et al. 2010).

6.4 IL-6 (Studies I and III)

IL-6 is a central cytokine with important roles in the infection or inflammation process (Song and Kellum 2005). It has been evaluated as a sepsis marker in many studies. New analytical methods have made it possible to use it in diagnostics around-the-clock.

In study I median concentrations of IL-6 were significantly higher in the severe sepsis group than in the other groups and also in those groups where bacterial infection was present compared with groups without. In logistic regression analysis in studies I and III IL-6 appeared to be a predictor for severe sepsis. In multivariate logistic regression analysis in study I both IL-6 and PCT remained significant independent predictors for severe sepsis also after adjusting for confounders. As noted earlier there was no significant difference between PCT and IL-6 values. The best cut-off value for IL-6 for severe sepsis was 172 pg/ml showing 69% sensitivity and 73 % specificity (Study III). In study III after multivariate logistic regression analysis high IL-6 was not a predictor for case fatality, but as mentioned above high IL-6 remained an independent predictor for severe sepsis also after adjusting for potential confounders.

Previous reports comparing IL-6 and PCT with regard to the diagnosis of sepsis are conflicting. Gaini and associates demonstrated IL-6 to be superior to PCT as a diagnostic marker for infection and sepsis (Gaini et al. 2006), but the majority of papers have identified PCT as a more reliable biomarker of sepsis compared to IL-6 (Muller et al. 2000; Harbarth et al. 2001; Aikawa et al. 2005). A group under Mokart found both IL-6 and PCT as early markers of postoperative sepsis after major surgery (Mokart et al. 2005). It is well known that levels of IL-6 increase earlier than PCT (Dahaba and Metzler 2009) and therefore sometimes IL-6 can be the better marker if the patient is very acutely ill. Also with neonates IL-6 has proved a good marker for sepsis and sepsis severity (Kuster et al. 1998; Martin et al. 2001; Ng et al. 2006).

It has been hoped that analysis of cytokines would help towards a better understanding of the pathogenesis and possibly also therapeutic interaction, but until now attempts have been disappointing. IL-6 has thought to be an early and sensitive alarm marker for systemic inflammation and sepsis. In our study I it was equal to PCT as a predictor of severe sepsis, but taking our results together the measurement of IL-6 concentration hardly has additive prognostic or diagnostic value when compared with other studied pa-

rameters on admission in patients with suspected infection. However, it has been disputed that the measurement of several cytokines combined (muliplex cytokine arrays) would gain more information and increase spesificity in acute care context.

6.5 PLA2GIIA (Study II)

PLA_2GIIA has been considered interesting marker for sepsis because of its role in inflammation and innate host defence against microbial invasion (Pruzanski and Vadas 1991; Pruzanski 2005; Nevalainen et al. 2008). We sought here to establish the usefulness of plasma PLA_2GIIA measurements on admission and compared values of PLA_2GIIA with BPI, CRP and WBC on admission in cases of suspected infection in the early detection of severe sepsis. In study II plasma PLA_2GIIA was higher in the severe sepsis group than in other patients on admission. A positive correlation between the concentrations of PLA_2GIIA and CRP and PLA_2GIIA and BPI was shown. These results support previous findings. The levels of PLA_2GIIA and CRP have been reported to correlate the severity of inflammation in critically ill surgical patients (Grönroos et al. 1994), in patients with inflammatory bowel disease (Haapamäki et al. 1999; Haapamäki et al. 2006), with rheumatoid arthritis and with sepsis (Green et al. 1991).

In study II an elevated plasma PLA_2GIIA concentration was shown to be a better marker of severe sepsis on admission than CRP or BPI or WBC. Plasma PLA_2GIIA was shown to be the independent predictor in our study of severe sepsis after adjustment for confounders recognized in the study. The concentration of PLA_2GIIA has previously been shown to correlate with the severity of septic shock (Green et al. 1991; Vadas et al. 1992). Cut-off values for detecting severe sepsis were not determined in study II and as in further studies other parameters such as PCT, suPAR and PTX3 were shown to be better predictors of severe sepsis in our population, this is not considered to be relevant.

In further analysis it was shown that PLA_2GIIA had some value in differentiating sepsis patients from other patients and patients with bacterial infection from patients without. In our study all classical acute-phase proteins (CRP, PCT and PLA_2GIIA) seemed to be almost equal in separating bacterial infection from patients without bacterial infection or sepsis from patients without sepsis . These parameters (CRP, PCT and PLA_2GIIA) were also shown to be better markers for bacterial infection and sepsis than su-

PAR or PTX3, which were evaluated in studies III and IV. In one previous study with 46 patients with different kinds of infection it was also shown that concentrations of PLA_2GIIA in serum were markedly elevated in patients with sepsis and also with blood-culture-negative infections and less in patients having viral infection (Rintala and Nevalainen 1993). In that study the serum levels of PLA_2GIIA correlated with levels of CRP, and were taken to correlate with the microbiological etiology.

In accord with one previous study (Dajak et al. 2006) here likewise plasma PLA_2GIIA was not a predictor for case fatality. Dajak and colleagues made their study in the ICU. In our study II we made an outcome analysis, but because only 33 patients died we concluded that the number of non-surviving patients was too small to publish the results. However, when studying suPAR and PTX3 in papers III and IV, it was proved possible to evaluate case fatality on day 28 also in our material although the number of non-survivors was rather small. For this reason we here undertook further analysis, but plasma PLA_2GIIA was not useful as a prognostic factor for case fatality.

All in all, plasma PLA_2GIIA appeared to be a better predictor for severe sepsis than CRP, BPI or WBC in study II, but our further studies brought out better markers for that purpose. In further analysis plasma PLA_2GIIA was shown to have more or less the same capacity for detecting bacterial infection and sepsis as CRP and PCT. Nowdays the comercial kit for detecting PLA2GIIA is available but it has has not gained similar position as CRP or PCT in everyday laboratory practice.

6.6 BPI (Study II)

Because BPI - together with PLA2GIIA - has a role in the innate immunity and host defence against invading microbes, the measurement of the BPI levels in circulation has been attractive target in the search for good sepsis markers. Moreover, the BPI/neutrophil ratio has been thought to be an indicator of neutrophil activation in microbial invasion (Weiss et al. 1978; Boman 2003).

Plasma BPI was measured in study II. Levels were significantly higher in severe sepsis than other patient groups. There was a modest positive correlation between BPI and WBC (r= 0.49) and only a weak correlation between BPI and PLA_2GIIA (r=0.20), whereas no correlation with BPI and

CRP. Similar correlations have been reported elsewere (Rintala et al. 2000).

Unfortunately in our study the neutrophil count was not studied at admission, and we had to use WBC instead of neutrophils. The BPI/WBC ratio was studied, but there was no difference between severe sepsis patients and other patients. In the AUC analysis and ROC contrast test, the BPI/WBC ratio could not differentiate severe sepsis patients from others. We had to exclude 14 patients due to missing WBC count. It is unlikely that this exclusion significantly affected the results.

In AUC analysis and in the logistic regression analysis using BPI as predictor in discriminating severe sepsis patients from others BPI was able to make this distinction. But in a model where confounding factors were included BPI was not an independent predictor for severe sepsis while PLA_2GIIA was (Table 11). This is to our knowledge the only study when BPI and PLA_2GIIA have been assessed in such a large patient cohort upon admission to hospital. In previous studies BPI has been shown to correlate positively with sepsis severity (Calvano et al. 1994; Rintala et al. 2000; Berkestedt et al. 2010).

For this thesis some further analysies were made. In AUC^{ROC} analysis BPI had an AUC value of 0.63 for sepsis, but according to this analysis all parameters studied (PLA_2GIIA, CRP and WBC) were better markers for sepsis than BPI. As a marker of bacterial infection or case fatality BPI had no value. Our results differ from previous findings where BPI also correlated with case fatality (Calvano et al. 1994; Rintala et al. 2000; Berkestedt et al. 2010).

Our results do not support its routine use in evaluating the severity of sepsis or case fatality on admission in patients with suspected infection.

6.7 suPAR (Study III)

Plasma levels of suPAR are thought to be related to immune cell activation and the measurement of suPAR has been identified as a potential biomarker for prognosis in several clinical settings (Thuno et al. 2009). The number of studies done previously is limited and little is known as to the use of this rather new marker in the emergency room setting.

The findings in study III show that high plasma suPAR levels may be used to predict case fatality and severe sepsis in adult patients admitted to the emergency department with suspicion of infection. We showed that suPAR values were higher in non-survivors than in those who survived (8.3 vs 4.9 ng/ml, $p<0.001$) in all subgroups. High levels of suPAR remained an independent predictor of case fatality and also of severe sepsis after adjustment for potential confounders. High suPAR levels were strong predictors of 28-d (and even 90-d and 1 year case fatality) and allowed better risk stratification compared to PCT, IL-6 and CRP. Serum suPAR at a cut-off level of 6.4 ng/ml showed 76 % sensitivity and 69 % specificity for fatal disease within 28 days.

Recent studies in patients with bacteremia and sepsis have demonstrated that high suPAR is a predictor of disease severity and case fatality (Wittenhagen et al. 2004; Huttunen et al. 2011; Mölkanen et al. 2011). High suPAR concentrations have been associated with admission to the ICU and survival in critically ill patients (Koch et al. 2011). Koch and associates showed that serum levels of suPAR were high already at admission to the ICU and levels remained elevated through the first week of ICU treatment (Koch et al. 2011). In our study suPAR was measured at the earliest possible time-point in the hospital treatment in ER. It was shown that suPAR may already be used to predict case fatality also in an unselected group of patients with suspected infection in the ER. In addition to a high suPAR, a high level of PCT was also an independent predictor for case fatality, but suPAR seemed to be superior to PCT. In accord with our study, Kofoed and group showed high suPAR to predict outcome in patients with SIRS (Kofoed et al. 2008).

Here suPAR levels were higher in severe sepsis patients than in others (7.9 vs 3.9 ng/ml, $p<0.001$). High levels of suPAR, PCT and IL-6 but not CRP at admission remained independent predictors of severe sepsis. The predictive value of suPAR in identifying patients with severe sepsis was fairly similar to that of PCT and IL-6, although in AUC^{ROC} analysis PCT was the best marker for severe sepsis. At a cut-off level for severe sepsis 6.6 ng/ml suPAR had 67% sensitivity and 72% specificity.

It has previously been shown that suPAR is not a specific marker for bacterial infection. A high suPAR level has proved to be a precursor of mortality in patients with malaria (Ostrowski et al. 2005), human immunodeficiency virus (HIV) (Sidenius et al. 2000; Lawn et al. 2007) and tuberculosis (Eugen-Olsen et al. 2002). In our study suPAR levels did not differ between the four other study groups without severe sepsis. In AUC^{ROC} analysis (Figure 14) it is easy to see that suPAR had no diagnostic value in detecting bacterial infection or sepsis in our material. This is well in line with results

from most previous studies (Kofoed et al. 2007; Koch et al. 2011; Savva et al. 2011) and also with one recent systematic review of suPAR as a biological marker in patients with systemic inflammation or infection (Backes et al. 2012). However, in one other recent study including 85 patients with SIRS and comparing the utility of suPAR, PCT and CRP, the authors concluded that suPAR is useful in the differential diagnosis of bacterial infection among patients with SIRS (Yilmaz et al. 2011). The results from our study confirm that high suPAR levels are related to the presence and severity of organ dysfunction, not bacterial infection. Previously with ICU patients suPAR has been an independent indicator of the presence and severity of hepatic and renal failure (Koch et al. 2011; Donadello et al. 2012)

We identified many factors and underlying diseases associated with high suPAR plasma concentration at admission: age over 60 years, diabetes mellitus, rheumatic disease, chronic renal insufficiency and cardiovascular disease. On the other hand there were no differences in suPAR levels between patients with or without a history of alcohol abuse, smoking, obesity or malignancy. It has been previously shown that suPAR levels are higher in the elderly (Ossowski and Aguirre-Ghiso 2000), and in patients with renal insufficiency, rheumatic and cardiovascular disease (Slot et al. 1999; Pawlak et al. 2007; Eugen-Olsen et al. 2010; Wei et al. 2011). suPAR levels have previously been reported to be elevated in patients with a history of alcohol abuse or liver disease (Wittenhagen et al. 2004; Huttunen et al. 2011). Zimmerman and associates have recently shown that suPAR is a biomarker for the diagnosis of liver cirrhosis and alcohol-associated liver disease (Zimmermann et al. 2011). Here we did not measure liver function systematically and only 25 patients out of 539 had a history of alcohol abuse. Our criteria for alcohol abuse were quite strict (alcoholism diagnosed previously or patient had been previously treated for alcohol-induced disease at hospital).

The cut-off levels for case fatality in our study were lower than in previous works on patients with bacteraemia and sepsis (Wittenhagen et al. 2004; Huttunen et al. 2011; Mölkanen et al. 2011), but in two studies including SIRS patients (Kofoed et al. 2008; Yilmaz et al. 2011) the cut-off levels were closer to those in our study. This indicates that the suPAR levels vary in different study populations, the basal conditions of the patients, and the methods used to measure suPAR. More and larger studies would be needed to establish whether our results on prediction of the outcome of patients could be expanded to ER patients for triage for example upon ICU admission or more comprehensive monitoring, as high suPAR levels in the emergency room may predict the need for more intensive therapeutic measures or treatments. Further interventional studies are needed in which cut-off levels are used in decision-making for triage.

6.8 PTX3 (Study IV)

PTX3 is an acute-phase protein whose plasma levels increase rapidly in many inflammatory conditions including sepsis (Mantovani et al. 2008; Bottazzi et al. 2009; Yamasaki et al. 2009). Recently the number of publications on PTX3 has been growing, but little is known as to the utility of PTX3 in stratification of patients in the ER setting.

In study IV the median PTX level was higher in the severe sepsis group than in others and in non-survivors compared with survivors. AUC^{ROC} in prediction of severe sepsis was 0.73 and 0.69 in predicting case fatality by day 28. The optimal cut-off level for plasma PTX3 concentration in predicting severe sepsis was 14.1 ng/ml, showing 63 % sensitivity and 80 % specificity, and the optimal cut-off level for case fatality (d28) was 7.7 ng/ml showing 70 % sensitivity and 63 % specificity. High PTX3 remained an independent predictor for both severe sepsis and case fatality also after adjustment for potential confounders.

In accord with our findings PTX3 has been shown to be an early biomarker for sepsis severity. In a previous meningococcal study it was observed that the levels of PTX3 peak already during the first hours after admission (Sprong et al. 2009). In one recent Finnish study conducted at the onset of febrile neutropenia in hematologic patients it was noted that PTX3 reached the maximum earlier than CRP (Vänskä et al. 2011) and in a previous bacteremia study PTX3 levels were high in the acute phase and normalized on recovery (Huttunen et al. 2011).

Here PTX3 values were significantly higher in the elderly (patients over 60 years) and in those with obesity (BMI $\geq$ 30), cardiovascular diseases and continuous systemic cortisone treatment (daily dose over 10 mg oral prednisolone) compared to those without these risk factors. There was no difference in PTX3 levels in patients having solid cancer or hematological malignancies.

In previous studies, the utility of PTX3 as a prognostic marker of sepsis have been compared with CRP. However, PTX3 has not previously been compared with PCT, although in many papers PCT has been shown to be a better prognostic marker than CRP (Luzzani et al. 2003; Nakamura et al. 2009; Uusitalo-Seppälä et al. 2011). In study IV PCT seemed superior in predicting severe sepsis and PTX3 in case fatality.

In one previous study with febrile patients admitted to the emergency department high PTX3 was shown to predict culture-positive bloodstream infections and severe disease (the need for ICU treatment, longer hospital stay and acute congestive heart failure) (de Kruif et al. 2010). In our material, however, PTX3 was a poorer diagnostic tool than CRP or PCT in separating bacterial infection or sepsis from those without bacterial infection or sepsis. In accord with our results earlier studies have shown that PTX3 is not a specific marker for bacterial infection, but a high PTX3 concentration has seen to predict the severity of viral infections such as dengue (Mairuhu et al. 2005) or epidemic nephropathy (Outinen et al. 2011). High plasma PTX3 concentrations are also seen in various inflammatory conditions without infection. High PTX3 levels have proved to correlate with poor outcome, for example in cardiovascular diseases (Garlanda et al. 2011), lung cancer (Diamandis et al. 2011) and polymyalgia rheumatica (Pulsatelli et al. 2010).

Our results are in line with those in previous studies assessing the utility of PTX3 and CRP in evaluating the severity of infection, bacteremia or sepsis (Muller et al. 2001; He et al. 2007; Sprong et al. 2009; Mauri et al. 2010; Huttunen et al. 2011). In critically ill ICU patients the levels of PTX3 have correlated with the severity of disease (Muller et al. 2001; Mauri et al. 2010). High PTX3 has been found to be an early predictor of shock in severe meningococcal diseases (Sprong et al. 2009). Our findings confirm previous results on PTX3 in the early detection of severe disease already in the ER. PTX3 may be used to predict many variables demonstrating severe sepsis, i.e. need for ICU stay, hypotension, acute renal insufficiency and need for mechanical ventilation already in the emergency room. Thus, PTX3 may help in stratifying patients in order to target resources effectively.

We showed high PTX3 concentrations to be independent predictors of case fatality by day 28 when plasma samples were taken on admission as early as possible from patients with suspected infection. In one previous study the maximum PTX3 value on days 1-4 after a bacteremia diagnosis was observed to be a predictor of case fatality (d28) (Huttunen et al. 2011). Another paper has shown that high levels of plasma PTX3 persisting over the first five days after the onset of severe sepsis and septic shock are associated with mortality, but in contrast to the present findings, not the PTX3 value on day 1 (Mauri et al. 2010). Although PTX3 peaks early it would probably be better to take PTX3 measurements later or as a serial measurement, but then the marker is no longer the kind of early test, we were looking for. In this sense suPAR seems to be a more interesting and better parameter for further investigations.

In our study only a weak negative correlation between PTX3 levels and platelet count was shown. Previously it has been reported that PTX3 can up-regulate tissue factor in activated monocytes - a link between inflammation and clotting activity (Napoleone et al. 2004). This correlation has also been shown in previous sepsis studies (Sprong et al. 2009; Mauri et al. 2010). PTX3 may be involved in the pathological coagulation process in sepsis. A high PTX3 concentration may reflect the role of pentraxins in the clearance of apoptotic cells (Manfredi et al. 2008).

The findings in study IV show that high PTX3 levels on admission can be used to predict severe sepsis and case fatality in patients admitted to the ER with suspected infection, but in both respects we found better markers. The optimal PTX3 cut-off point for severe sepsis in our study was about the same level as one would expect from earlier studies, but the optimal cut-off for day 28 case fatality was surprisingly low, giving too low a specificity for clinical work. Evaluating the results from study IV and study III together, suPAR seem to be better prognostic factor in ER than PTX3 and PCT seems to have more value in the early diagnosis or prediction of severe sepsis than PTX3. As a diagnostic tool PTX3 is poorer than CRP and PCT.

6.9 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The most interesting finding in our studies was the possible use of suPAR as a prognostic factor in ER patients. It is tempting to think that measurment of suPAR could be used for triage to determine which patients require more intensive monitoring in ICU. Reliable risk stratification and prediction of outcome could possibly be used as an instrument for decision-making and identification of patients at low risk possible suitable for outpatient treatment. Conclusions should be drawn with caution and further interventional studies are needed. We studied patients with suspected infection but as Koch and Tache suggest in the editorial concerning our study III, it would be interesting to further investigate medical ER patients even without suspected infection and establish whether suPAR could be used as a simple method basis for risk stratification of medical ER patients (Koch and Tacke 2012).

In the future investigators dealing with prognosis, prediction of sepsis will probably be based on microarray technology, making the investigation of a large number of potential prognostic biomarkers possible at the same time. For effective use of this method, however, more must be learn about the precise mechanisms and possible therapeutic applications, otherwise this approach has been compared to “dynamite fishing” (Novotny 2010). Evaluations providing insight into the immunologic mechanisms in sepsis in vivo are thus important.

The high costs of new analytes must also be borne in mind especially if we would wish to use them on large scale in the ER setting. The results of our studies are collected to table Table 18.

Table 18. The feasibility and costs of the parameters in studies I-IV in diagnosis of bacterial infection, prediction of severe sepsis or case fatality on days 1-28 according to our studies. C-reactive protein (CRP), procalcitonin (PCT), interleukin 6 (IL-6), soluble urokinase-type plasminogen activator receptor (suPAR), and pentraxin 3 (PTX3) were measured on admission.

	CRP	**PCT**	**IL-6**	**PLA_2GIIA**	**BPI**	**suPAR**	**PTX3**
Ethiology: Bacterial infection or not	++	+	+	+	-	+/-	+
Severity: Severe sepsis or not	-	++	++	+/-	+/-	+	+
Fatality: Case fatality by day 28	-	-	+/-	-	-	++	+
Costs	**low** *	**high** **	**high** **	**high** **	**high** **	**high** **	**high** **
Commercial kit available	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**
In clinical use	**yes (widely)**	**yes**	**yes**	**no**	**no**	**possibly**	**no**

++ According to our AUC^{ROC} analysis the best parameter in detecting ethiology, severity or case fatality on day 28 ($p<0.001$)
+ According to our AUC^{ROC} analysis the parameter has some value in predicting ethiology, severity or case fatality on day 28 ($p<0.001$)
+/- According to our AUC^{ROC} analysis the parameter has low capacity in detecting ethiology, severity or case fatality on day 28 ($p=0.001$)
- According to our AUC^{ROC} analysis no use in detecting ethiology, severity or case fatality on day 28 ($p>0.001$)

* Average costs approximately 1.3 – 1.5 euro in clinical use
** Average costs approximately 25 – 45 euro in clinical use

7 CONCLUSIONS AND SUMMARY

I. PCT and IL-6 appeared to be superior to CRP in differentiating severe sepsis patients from other patients and predicting severe sepsis already at admission. A single measurement is not reliable in ruling out bacterial infection, sepsis or severe sepsis, but together with the patient's clinical picture these parameters may be helpful. High PCT or IL-6 can be the alarm signal, serving for early diagnosis of severe sepsis, but in view of the costs of analysis the extensive use of either PCT or IL-6 in the ER setting for patients with suspected infection cannot be suggested.

II. Plasma PLA_2GIIA appeared to be a better marker of severe sepsis at admission than CRP. Study supported the measurement of plasma PLA_2GIIA in the initial diagnostic approach to patients suspected of having severe sepsis at admission to hospital. Despite the important role of BPI in innate host defense, its plasma levels at admission could not differentiate severe sepsis patients better than did PLA_2GIIA and CRP. Due to the relatively high costs and other better test neither PLA_2GIIA nor BPI can be suggested for clinical use in the ER setting.

III. The plasma suPAR level served as a prognostic marker in patients with suspected infection admitted to the emergency department. Plasma suPAR was an independent predictor of case fatality and was also associated with severe sepsis. Of the four potential markers assessed (suPAR, PCT, IL-6 and CRP), suPAR was the best marker for case fatality and PCT was the best predictor of severe sepsis. suPAR is an interesting marker for case fatality and organ damage and should be studied more intensively as a prognostic tool in the ER setting.

IV. High levels of PTX3 in plasma can be used as a prognostic marker in patients with suspected infection admitted to the emergency room. High PTX3 was an independent predictor of severe sepsis between day 0 and 28 and case fatality by day 28 after admission. Due to the relatively high costs and other better markers found (CRP for bacterial infection, PCT for severe sepsis and suPAR for case fatality) PTX3 can not be suggested for clinical use in the ER setting.

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Raija Uusitalo-Seppälä

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