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Matti Vänskä

Biomarkers of Sepsis in Neutropenic Hematological Patients

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MATTI VÄNSKÄ

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ABSTRACT

Neutropenic infections are the leading treatment-related cause of death among patients receiving intensive chemotherapy for acute myeloid leukemia (AML) and in autologous stem cell transplant (ASCT) recipients. Particularly high mortality is associated with gram-negative bacteremia and septic shock. New tools are needed for the timely diagnosis of infectious complications to improve the prognosis of neutropenic hematological patients.

Sepsis biomarkers have mostly been studied in non-neutropenic patients. C-reactive protein (CRP) is used as an indicator of infection also in patients with neutropenic fever despite its nonspecific nature and slow kinetics. Procalcitonin (PCT) is a potential biomarker for severe infections with some evidence in support for use in patients with neutropenic fever. Interleukins-6 and -10 (IL-6, IL-10) have been studied in several infectious conditions, but the results are conflicting, and their usefulness as clinical biomarkers is unsolved. Pentraxin 3 (PTX3) and soluble urokinase-type plasminogen activator receptor (suPAR) are promising experimental biomarkers in early prediction of severe infections, but with only few previous studies in patients with neutropenic fever.

This prospective study evaluated the usefulness of these biomarkers in hematological patients with neutropenic fever. Plasma PCT, PTX3, IL-6, IL-10, and suPAR were measured at the onset of fever and in the next three mornings at hematological ward from 103 patients with AML or ASCT. During the period of neutropenic fever daily CRP levels, heart rate, blood pressure, body temperature, blood culture findings, and the complications of sepsis were registered. The incidence of bacteremia was 19%, gram-negative bacteremia 6%, gram-positive bacteremia 13%, and septic shock 5%. Infection-related mortality during the hospital stay was 3%.

PTX3 increased faster than CRP and predicted septic shock and bacteremia at the onset of fever. However, the optimal cut-off for PTX3 was much higher in patients with non-Hodgkin lymphoma (NHL) and ASCT than in patients with AML. PCT increased rapidly and showed sustained capacity to predict gram-negative bacteremia and complicated course of neutropenic fever. Also IL-6 and IL-10 were associated with complications at the onset of fever but the increased levels of complicated courses decreased rapidly, during 1 or 2 days. The combination of IL-10 and PCT increased predictive capacity from the onset of fever to day 1. Day 1 suPAR and maximal suPAR levels were associated with gram-negative bacteremia and septic shock, but the performance was inferior to PCT.

PTX3 indicated bacteremia and predicted septic shock in neutropenic fever at the onset of fever, but the decision level should be adjusted according to the malignancy. IL-6 and IL-10 predicted complications on the first days of neutropenic fever but only PCT showed sustained diagnostic capacity throughout the study period. Early prediction of complications could possibly be improved by using IL-10 and PCT as a combination.

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TIIVISTELMÄ

Akuuttiin myelooiseen leukemiaan (AML) ja autologisten kantasolusiirtojen (ASCT) tuella annetuissa intensiivihoidoissa neutropeeniset infektiot ovat johtava hoitoon liittyvien kuolemien syy. Gram-negatiiviseen bakteremiaan ja septiseen sokkiin liittyy erityisen suuri kuolleisuus. Hematologisten potilaiden neutropeenisen kuumeen ennustetta voitaisiin ehkä parantaa tunnistamalla suuren riskin potilas merkkiaineella varhaisessa vaiheessa.

C-reaktiivinen proteiini (CRP) on käytetyin tulehdusmerkkiaine myös neutropeenisessa kuumeessa huolimatta sen suhteellisesta hitaudesta ja epäspesifisyydestä. Prokalsitoniinista (PCT) on runsaasti tutkimustietoa infektiodiagnostiikassa, mutta sen käyttö ei ole vakiintunut neutropeenisessa kuumeessa. Interleukiineja 6 ja 10 (IL-6, IL-10) on tutkittu myös neutropeenisilla potilailla. Pentraksiini 3 (PTX3) ja liukoinen urokinaasityyppinen plasminogeeniaktivaattorin reseptori (suPAR) ovat lupaavia kokeellisia sepsiksen merkkiaineita.

Tässä prospektiivisessa tutkimuksessa mitattiin hematologisella osastolla hoidetun 103 AML- ja ASCT-potilaan PCT-, PTX3-, IL-6-, IL-10- ja suPAR-tasot neutropeenisen kuumeen noustessa ja kolmena seuraavana aamuna. Kuumejakson aikana kirjattiin päivittäiset CRP-pitoisuudet, syke, verenpaine, lämpö, veriviljelylöydökset ja sepsiksen komplikaatiot.

Bakteremian ilmaantuvuus oli 19%, gram-negatiivisen bakteremian 6%, gram-positiivisen bakteremian 13% ja septisen sokin 5%. Infektiokuolleisuus oli 3% sairaalajakson aikana. PTX3 nousi nopeammin kuin CRP, mutta ei ennustanut CRP-pitoisuutta paremmin komplikaatioita koko potilasryhmässä tutkittuna. PTX3-pitoisuuden päätöksentekoraja oli riippuvainen perussairaudesta: selvästi korkeampi non-Hodgkin lymfoomaa (NHL) sairastavilla ASCT-potilailla kuin AML-potilailla.

PCT ennusti neutropeenisessa kuumeessa bakteremiaa, gram-negatiivista bakteremiaa ja septistä shokkia kuumeen noususta lähtien säilyttäen ennustearvonsa koko kolmen päivän tutkimusjakson ajan. Myös IL-6:n ja IL-10:n pitoisuudet ennustivat komplikaatioiden kehittymistä. SuPAR-pitoisuus ensimmäisenä aamuna ja sen maksimiarvo kolmen ensimmäisen päivän aikana ennustivat komplikaatioita mutta heikommin kuin esimerkiksi PCT.

Neutropeenisen kuumeen huonon ennusteen merkkiaineena PTX3:n käytön rajoitteeksi osoittautui merkkiainetason riippuvuus perussairaudesta. SuPAR näyttää puolestaan muita merkkiaineita heikommalta diagnostisilta ominaisuuksiltaan. Nopeat merkkiaineet IL-6 ja IL-10 palaavat vaikeissakin taudinkuvissa lähtötasoihinsa kliinisen työn tarpeisiin nähden tarpeettoman nopeasti. PCT-pitoisuus toimii tutkituista merkkiaineista luotettavimmin ennustaen neutropeenisen kuumeen komplikaatioita, erityisesti gram-negatiivista bakteremiaa ja septistä shokkia, ja sitä voitaisiin käyttää nykyistä laajemmin. IL-10 käytettynä yhdessä PCT:n kanssa saattaisi parantaa komplikaatioiden ennustettavuutta.

Yleinen suomalainen asiasanasto: Hodgkinin tauti; akuutti myelooinen leukemia; verenmyrkytys; septinen sokki; sytokiinit; tulehdus; merkkiaineet

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List of the original publications

This dissertation is based on the following original publications:

I Vänskä M, Koivula I, Hämäläinen S, Pulkki K, Nousiainen T, Jantunen E, Juutilainen A. High pentraxin 3 level predicts septic shock and bacteremia at the onset of febrile neutropenia after intensive chemotherapy of hematologic patients. Haematologica 96: 1385-1389, 2011.

II Juutilainen A, Vänskä M, Pulkki K, Hämäläinen S, Nousiainen T, Jantunen E, Koivula I. Pentraxin 3 predicts complicated course of febrile neutropenia in haematological patients, but the decision level depends on the underlying malignancy. Eur J Haematol 87: 441-447, 2011.

III Vänskä M, Koivula I, Jantunen E, Hämäläinen S, Purhonen AK, Pulkki K, Juutilainen A. IL-10 combined with procalcitonin improves early prediction of complications of febrile neutropenia in hematological patients. Cytokine 60: 787–792, 2012.

IV Vänskä M, Purhonen AK, Koivula I, Jantunen E, Hämäläinen S, Pulkki K, Juutilainen A. Soluble form of urokinase-type plasminogen activator receptor as a diagnostic and prognostic marker in hematological patients with neutropenic fever. Leuk Lymphoma 55: 718-721, 2014.

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Contents

Abbreviations

AKI	Acute kidney injury
ALI	Acute lung injury
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
APC	Antigen-presenting cell
APP	Acute-phase protein
APR	Acute-phase response
ARDS	Acute respiratory distress syndrome
ASCT	Autologous stem cell transplantation
AUC	Area under the curve
BSI	Blood-stream infection
C1-9	Complement components
CARS	Compensatory anti-inflammatory response
CoNS	Coagulase-negative staphylococci
CRP	C-reactive protein
DAMP	Damage-associated molecular pattern, alarmin
DIC	Disseminated intravascular coagulation
ELISA	Enzyme-linked immunosorbent assay
FN	Febrile neutropenia, neutropenic fever
FUO	Fever of unknown origin
GvHD	Graft-versus-host disease
HL	Hodgkin lymphoma
HR	Heart rate
ICU	Intensive care unit
IL-6	Interleukin-6
IL-10	Interleukin-10
LPS	Lipopolysaccharide, endotoxin
MAC	Membrane attack complex
MAP	Mean arterial pressure
MBL	Mannose-binding lectin
MM	Multiple myeloma
MODS	Multiple organ dysfunction syndrome
MOF	Multiple organ failure
NF-κB	Nuclear factor-kappa beta
NHL	Non-Hodgkin lymphoma
NLR	Nod-like receptor
NO	Nitric oxide
PAMP	Pathogen-associated molecular pattern
PASS	The Procalcitonin and Survival Study
PCT	Procalcitonin
PICS	Persistent inflammation, immunosuppression, and catabolism syndrome
PRR	Pattern-recognition receptor
PTX3	Pentraxin 3
ROC	Receiver operating characteristic (curve)
SBP	Systolic blood pressure
SCT	Stem cell transplantation
SIRS	Systemic inflammatory response syndrome
suPAR	Soluble urokinase-type plasminogen activator receptor
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
TRM	Treatment-related mortality
WBC	White blood cell

1 Introduction

Neutropenic period after intensive chemotherapy for hematological malignancies is very often complicated with fever, usually of infectious origin. Severe sepsis is relatively common in patients with acute myeloid leukemia (AML) treated with intensive chemotherapy and is associated with a high mortality (1). Also among autologous stem cell transplant (ASCT) recipients, complicated course of neutropenic fever is an important cause of mortality, especially in lymphoma patients (2). Rapid and effective measures are needed to reduce mortality in sepsis of neutropenic patients. Efficient tools for assessing the risk of developing complications are, however, limited (3).

Biomarkers may be helpful in the evaluation of sepsis patients, both for diagnostic and prognostic purposes (4). An ideal biomarker of neutropenic fever would react promptly and would be specific for bacterial infections. Its concentration should stay elevated long enough to be measured reliably in a reasonable time frame. The method for the measurement should be fast, accessible, reproducible, and comparable in various patient groups. A single biomarker seldom fulfills all these expectations (4). Instead, improved prediction of complicated course of neutropenic fever could probably be achieved by using several biomarkers, some markers revealing excessive host response and evolving organ dysfunction, while others may indicate microbial etiology.

C-reactive protein (CRP) is widely used as an indicator of bacterial infection and for the evaluation of treatment response (5). However, it is not specific for bacterial etiology, it increases relatively slowly, and it peaks with a delay of around 2 days. In hematological patients elevated CRP may also be caused by the malignancy or the treatment (1, 2, 5, 6).

Procalcitonin is another biomarker already in clinical use, and has also been studied in patients with neutropenic fever (3, 7). It has several proposed advantages as a sepsis biomarker (8) but also some limitations (9).

Two key cytokines, a pro-inflammatory interleukin-6 (IL-6) and mainly anti-inflammatory interleukin-10 (IL-10), have been studied as biomarkers of sepsis. There is some evidence of their predictive value in patients with neutropenic fever, but the data is inconclusive, and no guidelines for the clinical interpretation of IL-6 and IL-10 concentrations have been presented (3, 4).

Pentraxin 3 (PTX3), a molecule related to CRP, is a promising prognostic biomarker in sepsis (10, 11). Because of its distinct origin and kinetics it may have some advantages over CRP (12). There is only one previous study of PTX3 in patients with neutropenic fever (13).

Soluble urokinase-type plasminogen activator receptor (suPAR) is a molecule reflecting both inflammatory and blood clotting systems. It is often elevated in chronic diseases, and predicts mortality in the general population (14, 15). It is a potential prognostic marker in sepsis (16) but only a few studies in neutropenic hematological patients have been published (17, 18).

We conducted a study with serial measurements of these potential early biomarkers of sepsis in adult hematological patients with neutropenic fever during a three-year period in a hematology ward. The aim was to analyze the usefulness and kinetics of these biomarkers as diagnostic and prognostic tools in patients with neutropenic fever.

2 Review of the Literature

2.1 HOST RESPONSE IN ACUTE INFECTION

Host reaction to pathogens invading through natural barriers like skin and mucosa is initiated by molecular signals: endogenous damage-associated molecular patterns (DAMP), or alarmins, and exogenous pathogen-associated molecular patterns (PAMP) (19-21). PAMPs are a heterogenous group of highly conserved structures found in pathogens, which in general are not present in human tissues. During evolution, mechanisms for rapid detection of these components have evolved, which has a fundamental role in the initiation of host response against invading microbes (19-21). DAMPs are substances leaking or actively released from host cells under stress or damage caused by infection but also by sterile inflammation like in trauma or ischemia (19, 21). The recognition of PAMPs and DAMPs is performed by numerous pattern recognition receptors (PRR), including toll-like receptors (TLR) on the surface of host cells (19-21) and soluble pentraxins (22, 23). Binding of PAMP or DAMP with PRR arouses innate immune mechanisms including cellular phagocytosis, activation of complement and coagulation systems, and in the case of TLR, cytokine production through nuclear factor kappa B (NF-κB) signaling (19-21, 23, 24). A classic example of PAMP is lipopolysaccharide (LPS, endotoxin), a wall constituent of gram-negative bacteria, which is detected by TLR4 (19-21).

Early steps of the immune response to infection are depicted in *Fig.* 1. Immune cells located in tissues, typically macrophages, detect and destroy pathogens and damage-derived material following DAMP-PRR interaction. These phagocytes then initiate the inflammatory reaction by secreting cytokines according to their polarization (20, 25) and by presenting antigens to lymphocytes in lymph nodes (20, 21, 26). Cytokines have several local and systemic effects, including induction of release of acute phase proteins (APPs) from tissues and recruitment of neutrophils from the circulation to the infection site by increased expression of adhesion molecules (20, 21, 26, 27). Neutrophils have a key role in the innate immune system. They kill and remove invading microbes by, e.g., phagocytosis (21, 27). The acute phase response (APR) including production of APPs from liver and other tissues is caused by pro-inflammatory cytokines, essentially IL-1, IL-6 and tumor necrosis factor-alpha (TNF-α) (20, 28, 29). Classical APPs are the pentraxins CRP, serum amyloid A and PTX3, which serve mainly as opsonizing agents (12, 30), ferritin, coagulation factors and complement components (20, 31, 32). APPs may be divided into two categories. Type I APPs are secreted after stimulation by IL-1 and TNF-α, while type II APPs are secreted following stimulation by IL-6 (20, 31). Clinical signs and symptoms of systemic inflammation, e.g., rise in the body temperature, malaise, and several metabolic alterations are caused by APR (20, 31).

Complement components (C1-9) are mostly present in the extracellular space in a non-active form and are activated in a cascade-like manner (21, 33). The key components of complement can opsonize pathogens, activate the following cascade, and ultimately lead to formation of membrane attack complex (MAC), which perforates the outer membrane of the target cell or microbe (20, 21). The complement cascade can be activated through three well-described pathways. Recognition of the specific PAMP mannose by mannose-binding lectin (MBL) initiates complement activation through the lectin pathway, while the classical pathway is activated by interaction of C1q with antigen-antibody complex (20, 21). Contact with micro-organisms can activate complement directly through the alternative pathway (21, 33, 34).

Also the pentraxins CRP and PTX3 can opsonize detrimental material and activate complement (22, 30, 32). C3a, C4a, and particularly C5a are fragments of complement components known as anaphylatoxins capable of inducing degranulation of mast cells and promoting chemotaxis but also initiating mechanisms inhibiting immune reactions (35).

Changes occurring in vascular endothelium are essential in the pathophysiology of sepsis. Expression of markers of endothelial activation like adhesion molecules has been associated with sepsis severity in clinical studies (36). The complement and inflammatory systems interact with the coagulation cascade and platelets become activated (21, 33, 37, 38). Pro-coagulation material in the circulation is increased by the APR (31). Tissue damage and microbial material like LPS cause expression of tissue factor and initiates intravascular coagulation further aggravated by inflammatory cytokines (e.g. IL-6) and acute-phase proteins (APP) (39). Increased coagulation activity can lead to occlusion of small arteries and to ischemic damage, exposing intracellular material and collagen and, thus, predisposing to further clot formation (26, 33, 37, 40). Vascular content, fluids, plasma molecules, and blood cells entering tissues result in edema and deposition of, e.g., coagulation factors and immune cells at the affected site (41). Vasodilatation, increased vascular permeability and fluid extravasation caused by bradykinin and nitric oxide (NO) eventually lead to slowdown of the blood flow; decline in blood pressure and tissue perfusion is seen and described in patients with severe infections (42).

Antigen-presenting cells (APCs) transport pathogen-derived antigens to be presented to lymphocytes, T-lymphocyte activation typically taking place in lymph nodes draining from the anatomical site of the infection. Successful antigen presentation activates cytotoxic T-lymphocytes and natural killer (NK) cells, further increases cytokine production of several immune cells, and activates B-lymphocytes to transform into active plasma cells producing specific antibodies (26). The inflammatory stimulus also leads to activation of inhibitory mechanisms to avoid exaggerated reactions and severe harm to the host. These mechanisms are complex and still under discussion. However, it is clear that a central anti-inflammatory cytokine involved is IL-10, which is induced by TNF-α and inhibits it through negative feedback (26, 43-45).

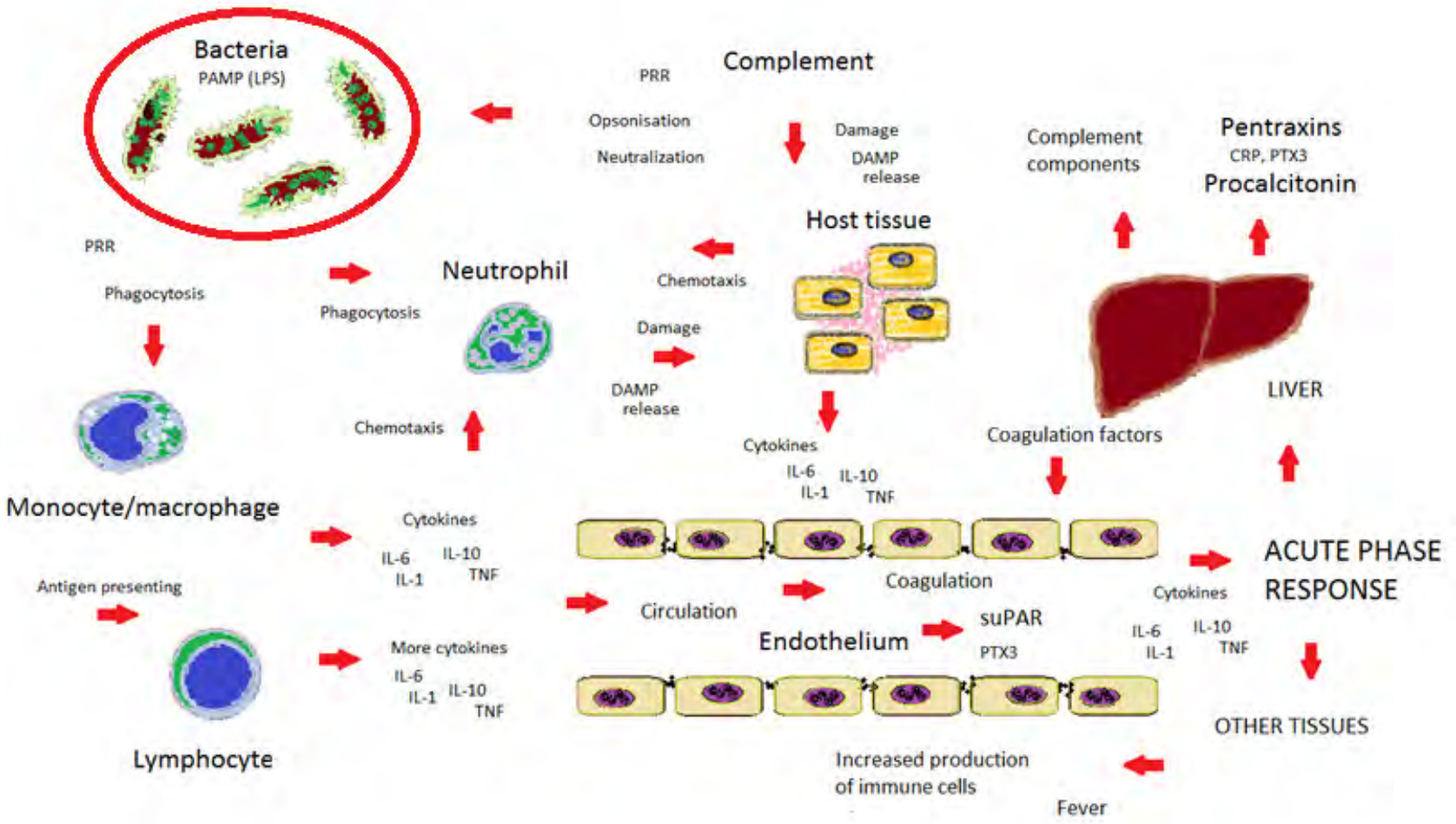

Figure 1. Simplified presentation of the early steps of immune response in bacterial infection. *PAMP*, pathogen-associated molecular pattern; *LPS*, lipopolysaccharide; *PRR*, pattern recognition receptor; *DAMP*, damage-associated molecular pattern; *CRP*, C-reactive protein; *PTX3*, pentraxin 3; *IL*, interleukin; *TNF*, tumor necrosis factor; *suPAR*, soluble urokinase-type plasminogen activator; *host tissue*, tissue at the site of the primary infection, including, e.g., epithelial cells, fibroblasts, resident macrophages, and collagen.

2.2 SEPSIS AND ITS COMPLICATIONS

'…in hectic fever, in the beginning of the malady it is easy to cure but difficult to detect, but in the course of time, not having been either detected or treated in the beginning, it becomes easy to detect but difficult to cure.'

- Niccolo Machiavelli in "The Prince" (Il Principe, 1513)

2.2.1 SIRS, sepsis, severe sepsis, and septic shock

The systemic inflammatory response syndrome *(SIRS)* can be induced by several processes besides infection, e.g., trauma and pancreatitis. It is characterized by detectable signs of systemic inflammation including altered body temperature (>38°C or <36°C), tachycardia (HR >90/min), tachypnea (breathing frequency >20/min or partial blood carbon dioxide pressure <32mmHg) and an increase or decrease in the number of white blood cells (WBC >12,000/μl or <4000/μl or >10% immature forms) (46), although more detailed and more sensitive criteria have also been suggested (47). Sepsis is a condition of SIRS (at least two of the criteria above) with a known or suspected infectious origin (46).

The systemic response to a severe infection is at the beginning generally appropriate to fight against the pathogen but sometimes vital-organ functions are compromised by an excessive host reaction. Severe sepsis is defined as sepsis with signs of organ dysfunction and septic shock as sepsis with hypotension (SBP <90 mmHg or a decrease ≥40 mmHg in SBP from baseline) and signs of perfusion abnormalities, refractory to adequate fluid administration (46).

Sepsis, including its complications severe sepsis and septic shock, is common occurring in around 2% of all hospitalizations and up to 30% of all intensive care unit (ICU) admissions (48). The overall incidence is 25 – 100/100000 inhabitants for severe sepsis and at least three times higher for sepsis (48, 49). Reported mortalities are 10 - 30% for sepsis, 20 - 55% for severe sepsis, and 40 - 80% for septic shock (48, 50, 51). In patients with severe sepsis, e.g. liver failure and older age have been associated with increased early mortality (49, 52).

In an extensive Finnish prospective study of 4500 successive ICU admissions the incidence of ICU-treated severe sepsis was 0.38 per adult population of 1,000, and mortalities were 15.5% and 28.8% for ICU and hospital stay, respectively, and 1-year mortality was 40.9%. The most common organ failures were respiratory failure (86.2%) and septic shock (77%) followed by renal failure (20.6%) (53).

2.2.2 Pathogenesis of the complications of sepsis

The pathophysiological events leading to organ dysfunction in sepsis are not fully understood, but several mechanisms have been suggested (54) (*Fig.* 2). Decreased perfusion and intravascular coagulation results in depletion of oxygen and nutrients in tissues. Oxidative stress produces local acidosis and damages mitochondria while immune mechanisms involving neutrophils and complement directly damage host cells. At the same time protective and repair mechanisms become dysfunctional (34, 35, 55, 56). Inflammatory cytokines produced excessively in sepsis can have also harmful effects on host tissues and cause apoptosis of immune cells (33, 57). Following dysfunction and failure of multiple organ systems (MODS, MOF), mortality increases substantially in sepsis, and the number of failing organs correlates with mortality (54, 58, 59).

In a sepsis patient hypotension that cannot be corrected by fluid resuscitation alone is a hallmark of septic shock. This is the result of decreased peripheral resistance and impaired cardiac systolic function. Sepsis-induced myocardial dysfunction is apparently caused by mitochondrial dysfunction, complement activation, depressing inflammatory substances like NO and IL-6, and dysregulation rather than hypoperfusion in the myocardium (33-35, 41, 54, 60-65).

Acute kidney injury (AKI) is another major complication of sepsis and its presence and course have significant effect on mortality (51, 66). Hypotensive episodes are associated with the emergence and progression of AKI in sepsis (67), but renal dysfunction and damage is also seen in the absence of systemic hypoperfusion due to changes in renal microcirculation (68). Sepsis-induced AKI is a complex multifactorial process in which oxidative stress and immunologic factors like complement activity have roles in addition to macro- and microcirculatory changes (34, 69, 70).

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are commonly associated with sepsis and are also associated with a poor prognosis (71). Lungs are damaged by leukocytes and uncontrolled coagulation (72). Complement components can also be produced in the lung alveoles in addition to the liver. Complement activity probably has a central role in the development of respiratory failure in sepsis when regulatory mechanisms do not work properly (34, 73). Accumulation of fluids, leukocytes, and platelets in lungs causes deterioration in gas exchange and can result in pulmonary edema (55, 72, 73).

Coagulation abnormalities of sepsis culminate in disseminated intravascular coagulation (DIC), in which thrombosis and bleeding occur simultaneously as the extensive inflammatory stimulus activates blood clotting, and coagulation factors are eventually depleted (39, 40). Thrombosis and bleeding contribute to organ damage. Patients with DIC are at a high risk of organ failure and death (38, 40). Coagulopathy can be aggravated in sepsis through impaired coagulation factor production by the liver and leakage of vascular contents into surrounding tissues (39). Malignancies can also cause DIC by themselves without an infection (74).

There is growing evidence that in critical illnesses like sepsis the changes in gut permeability can lead to exacerbation of the clinical situation and to organ failure. Alterations in the mucous layer allow toxic substances and pathogens from the intestinal lumen to be dislocated into the host. This further induces immunological reactions (75) that can be aggravated by impaired removal of bacterial material from the circulation by dysfunctional liver and its macrophages (76).

In addition to acute neurological complications like sepsis-associated encephalopathy, critical illness neuropathy, and myopathy, also long-term disabling cognitive impairment are frequently reported in patients with severe sepsis (77, 78). Moreover, hormonal imbalance is common in sepsis. Altered secretion and responsiveness to stress hormones (e.g. catecholamines, cortisol) and neurohormones (e.g. adrenocorticotropic hormone, vasopressin) have systemic effects and can add to hemodynamic problems and contribute to development of septic shock (76, 79, 80). Furthermore, the inflammatory system and autonomic nervous system are closely interconnected, and in sepsis disturbances take place in both of these systems (55).

Regulatory processes following secretion of anti-inflammatory cytokines like IL-10 are initiated already in early sepsis and prevent harmful effects of excessive inflammation but sometimes add to complications (81). Patients who have died due to sepsis have been reported to be in a profound immunosuppression (82) and a high IL-10 level has been associated with lethal outcome in sepsis (81). The condition with reduced number and activity of different leukocyte populations has been called a compensatory anti-inflammatory response (CARS) to the hyper-inflammatory state of early sepsis (83) or persistent inflammation, immunosuppression, and catabolism syndrome (PICS) (84),

characterized by increased susceptibility to new infections, impaired repair mechanisms, and poor prognosis (55, 75, 82, 84-86).

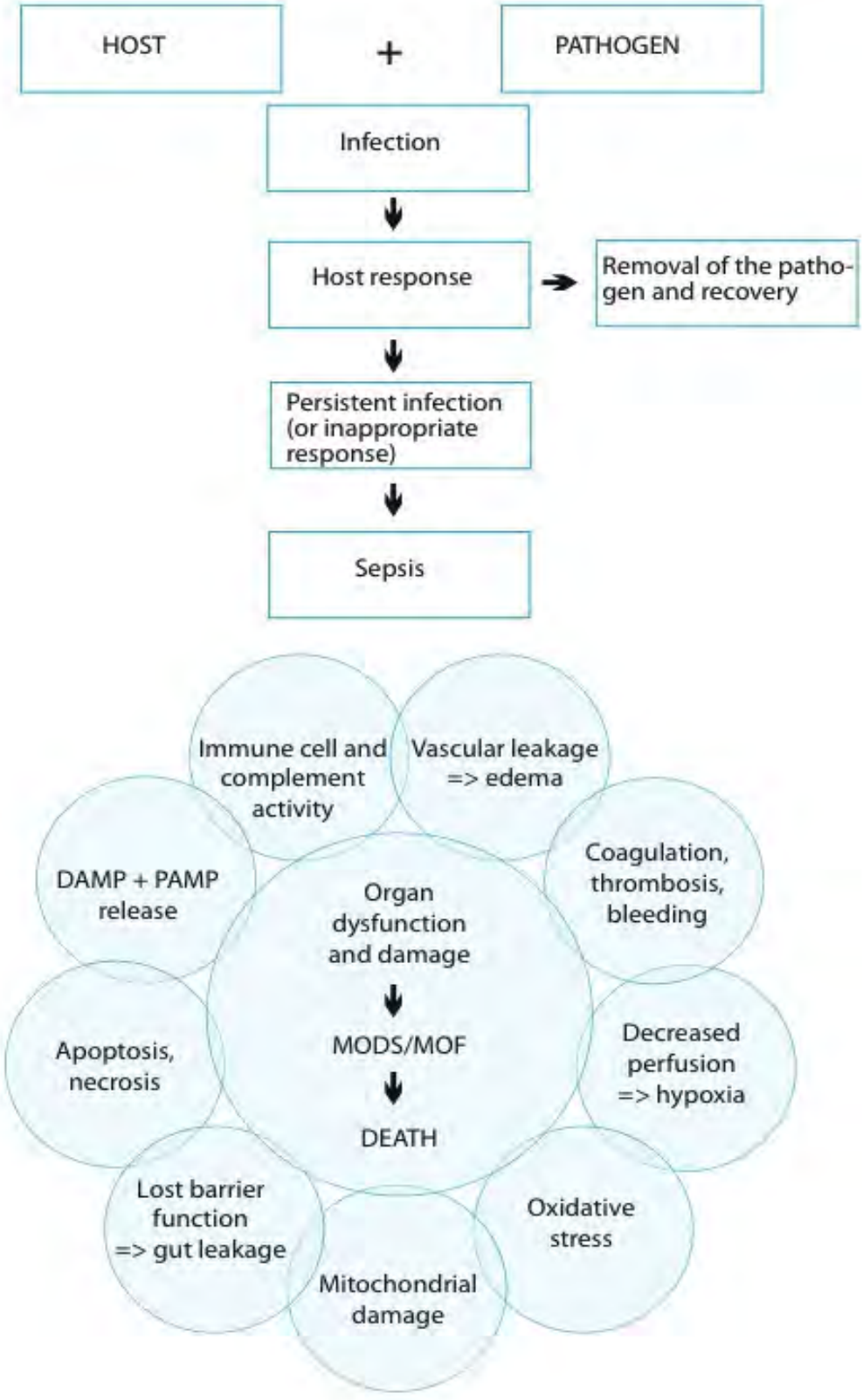

Figure 2. Development of the complications of sepsis

2.3 NEUTROPENIC FEVER

2.3.1 Clinical features and management

In ideal conditions the host is capable of rapidly clearing invading pathogens. The situation is different in a neutropenic patient and can be worsened by conditions usually present in neutropenia, damage to mucosa by cytotoxic therapies, hospital environment, immunosuppressive medication, and indwelling vascular catheters. Chemotherapy aimed at treatment of hematological malignancy causes neutropenia, which is frequently associated with fever even without an obvious infection (6, 87-89). Chemotherapy affects also other parts of the immune system, e.g., immunoglobulin production and function, T-lymphocytes and macrophages, and disrupts natural mucosal barriers. These patients are therefore at exceptionally high risk of potentially lethal infections (87, 90). Absolute neutrophil count (ANC) and the duration of neutropenia are associated with the frequency, rapidity, and severity of infections. Patients with prolonged (>3 weeks) and severe neutropenia (ANC $<0.1 \times 10^9$/L) are at high risk of bacterial infections (87, 88, 90).

The duration and severity of neutropenia can be diminished by the use of granulocyte growth factors when applicable (87), possibly leading to fewer hospitalizations and increased overall survival (91). The management of neutropenic fever consists of prophylactic, empiric, and targeted antibiotics, rapid diagnostics, and supportive care (87). The Infectious Diseases Society of America (IDSA) guideline (92) underlines rapid clinical evaluation and microbiological investigations at the start of neutropenic fever, and prompt initiation of broad-spectrum intravenous antibiotics, reducing sepsis-related mortality to less than 5% (87). The recommended empirical regimens for patients at high risk are combination therapy with a broad-spectrum betalactam with an aminoglycoside, or a single-drug therapy with a carbapenem. Vancomycin is used only if methicillin-resistant staphylococci are present (87).

Local guidelines in Finnish hospitals for prophylaxis and management of neutropenic fever mostly follow international ones. However, there are no established policies regarding, e.g., prophylaxis with fluoroquinolones, which might carry a risk of possible increase in resistant strains, or empirical use of aminoglycosides, leading to a potential risk of nephrotoxicity, as suggested by a systematic review of randomized controlled trials (93).

The antibiotic strategies are under considerable strain because of issue of increasing antibiotic resistance and scarcely presented new antibacterial drugs. Thus, a recent European consensus statement stresses critical use of broad-spectrum antibiotics like carbapenems in neutropenic fever and suggests early discontinuation of antibiotics in cases with apparent non-infectious etiology or prompt resolution of fever (94). Studies so far published do not provide clear evidence supporting de-escalation of antibiotic treatment in neutropenic fever based on biomarker levels.

2.3.2 Epidemiology

The overall incidence of bacteremia in neutropenic fever is around 20 - 30% (2, 95-98). Some neutropenic infections are probably prevented by prophylactic antibiotic use (87, 88). Especially quinolones have been shown to reduce all-cause and infection-related mortality with a reduction also in febrile episodes and infections (99). In stem cell transplant recipients a prophylactic antibiotic therapy with intravenous ceftriaxone at the onset of neutropenia, regardless of fever, reduced significantly infections with a trend of reduced infection-related and all-cause mortality (100). Prophylactic use of broad-spectrum antibiotics, however, increases resistant bacterial strains (98, 101, 102).

The pathogen spectrum found in patients with neutropenic fever have evolved from mostly gram-positive, especially *Staphylococcus aureus,* in the 1950s and early 1960s to

mostly gram-negative bacteria from the gastrointestinal tract in late 1960s and early 1970s, until yet another change when coagulase-negative staphylococci (CoNS), *Streptococcus viridans*, and *Enterococcus* emerged in the 1990s. This was probably due to more frequent use of indwelling catheters and antibiotic prophylaxis aimed at gram-negative bacteria (87, 98, 103). During this and the previous decade gram-negative bacteria have re-emerged and antibiotic resistance has increased. However, resistance patterns vary markedly between reports (87, 88, 96, 101).

Invasive fungal infections, mainly by *Candida spp* and *Aspergillus spp*, are typical in patients with prolonged neutropenia. Antifungal treatment is usually initiated if the patient is not responding to empiric broad-spectrum bacterial antibiotics in 3 - 7 days (87, 88, 90, 104). Besides bacterial and fungal infections also viral infections are seen in patients with neutropenic fever (105, 106), but still a considerable proportion of the episodes remain fever of unknown origin (FUO) without any clinical or microbiological evidence of infection and lower risk of complications than, e.g., in bacteremias (1, 2, 89, 104, 105, 107-110).

The reported infection-related mortality in post-chemotherapy neutropenic fever in patients with solid tumors and hematological malignancies in up-to-date studies is mostly between 5-10% but there is significant variation according to constitution of the patients, malignancies treated, and treatments used. Low mortalities have been observed, e.g., in children with solid tumors and higher mortalities, e.g., in elderly patients with lung cancer (95, 97, 101, 110-113). The highest mortality in neutropenic fever is nevertheless associated with gram-negative bacteremia, caused, e.g., by *Pseudomonas aeruginosa*, invasive fungal infections, and septic shock (52, 88, 89, 95, 98, 105, 109, 113-115).

2.3.3 Neutropenic fever in AML patients

The problems following chemotherapy, neutropenia and subsequent infections, are especially pronounced in patients undergoing serial intensive treatments, like in case of AML. The neutropenic episodes are also especially long in patients treated for AML (116).

A retrospective Finnish study of 84 AML patients with intensive induction and consolidation courses included 280 episodes of neutropenic fever with positive blood culture findings in 59% of the episodes (gram-positive 34%, gram-negative 25%). *Blood-stream infections* (BSI) were significantly more frequent than in neutropenic fever in general (1). The most common isolates were *Staphylococcus epidermidis* (18%) and *E. coli* (10%) by the group, respectively. After the first induction course the incidence of severe sepsis was 13% and the infection-related mortality was 5%. Gram-negative bacteria were common in blood cultures of patients with severe sepsis. (1)

The chemotherapy regimen used in AML, and possibly some other factors, have impact on the incidence and cause of bacteremia in neutropenic fever. A nation-wide prospective study of the Finnish Leukemia Group (1992-2001) including 327 *de novo* AML patients with 956 intensive treatment cycles reported 456 positive blood cultures (48%). The incidence of BSI rose with every cycle. BSI were mostly gram-positive in the first cycles but the proportion of gram-negatives rose as the treatment progressed, from 22% in the cycle I up to 47% in the cycle IV. Also in that study CoNS was the most common finding, though proportion varied between the courses. There were altogether 35 infection-related deaths, which were 3% of the episodes and 9% of the patients (116).

In an Italian retrospective study of 81 AML patients with 181 episodes of febrile neutropenia, levofloxacin prophylaxis was used. Blood cultures were positive in 29% of the neutropenic fever episodes after the induction chemotherapy and in 51% of the episodes after consolidation. Outcome was lethal in 3% of the episodes of neutropenic fever (7% of the patients); most frequently because of invasive fungal infections. After the induction regimen 80% of the positive blood cultures were gram-positive, while after

consolidation regimens gram-negative cultures predominated with a proportion of 72%. Several resistant bacterial strains were seen (102).

In 29 patients treated for AML with 39 episodes of febrile neutropenia, the incidence for any bacteremia was 26%, 18% for gram-negative, and 8% for gram-positive bacteremia (111). Altogether 38% of the episodes were clinically or microbiologically diagnosed infections without bacteremia, while 33% were diagnosed as FUO. Infection-related mortality was 5%.

Among 224 newly diagnosed Italian AML patients undergoing intensive treatment the incidences of gram-negative bacteremia, gram-postive bacteremia, invasive fungal infections, and FUO were 13%, 21%, 4%, and 65% during induction, and 20%, 19%, 2%, and 35% during consolidation, respectively (115). Mortalities for gram-negative bacteremia were 10% and 14%, for gram-positive bacteremia 8% and 5%, and for invasive fungal infections 60% and 80% during induction and consolidation, respectively.

2.3.4 Neutropenic fever in ASCT recipients

In a Finnish retrospective study with ASCT recipients no antibiotic prophylaxis was used. Blood cultures were positive in 26% of 265 episodes of neutropenic fever and gram-positive findings were more common than gram-negative (2). *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the most frequent isolates. The incidence of severe sepsis was 5% in neutropenic fever, and the overall infection-related mortality was 3%, both higher among non-Hodgkin lymphoma (NHL) patients than the others (2).

Two Spanish prospective studies of altogether 720 ASCT patients were analyzed recently (117). The overall incidence of early bacteremia was 20%. The length of neutropenia longer than 9 days was a risk factor for any bacteremia and gram-negative bacteremia. Prophylaxis with fluoroquinolones was a risk factor for gram-negative bacteremia while total parenteral nutrition and deep neutropenia were risk factors for gram-positive bacteremia.

In a Brazilian study 97% of 115 ASCT recipients developed neutropenic fever. A pathogen was found in 54% of the first and 10% of the second blood cultures. Gram-positive blood cultures accounted for 30% of all the episodes, gram-negatives for 27%, and fungal isolates for 1%. The most frequent isolate was CoNS (20%), and a catheter was the most common infection source. Altogether eight patients (7%) died of infection, four with BSI and three of them with gram-negative bacteremia (89).

In another study with 314 patients receiving high-dose therapy and ASCT, the rate of neutropenic fever was 92%. Altogether 39% of the febrile episodes were microbiologically documented infections, mostly BSI, 9% clinically diagnosed infections, and 52% were FUO. Gram-negative bacteremia occurred in 30% of the episodes. Infection was fatal in 12 cases and BSI in seven cases of which six with gram-negative and one with gram-positive bacteremia. Other fatal infections were *Aspergillus fumigatus* invasive pneumonia, pneumonia, and septic shock (109).

A large Finnish retrospective survey of 1482 ASCT recipients retraced early treatment-related mortality (TRM) in 1990 - 2003 (118). The patients were treated mostly for hematological malignancies, most commonly for NHL (n = 542) and MM (n = 528). The overall TRM was 2.8% with a median time to death of 38 days from ASCT. Altogether 26 deaths were caused by organ toxicity, and 16 of the deaths were infection-related, 7 fungal, 6 bacterial, and 3 viral infections. Among the patients with highest risk for early TRM were NHL patients with a risk of 4.4%.

Of 476 ASCT recipients in Spain, 95% developed fever during a 60-day study period (112). Of these 29% were clinically documented infections, 17% microbiologically documented infections, 14% bacteremias, and 54% FUO. *CoNS* and *E. coli* were the most common isolates (25% each) and the infection-related mortality was 5%.

2.4 BIOMARKERS OF SEPSIS AND NEUTROPENIC SEPSIS

In the search for new biomarkers in the diagnosis and prognosis of sepsis, over one hundred molecules have been studied so far, each of these having different properties, and all with a limited sensitivity and specificity (4). In patients with neutropenic fever the studies and biomarkers studied are fewer, and the results are inconsistent for the most part (3, 7).

In hematological patients with neutropenic fever after intensive chemotherapy the risk for complications is high. These patients are therefore treated in hospital with intravenous antibiotics. It is difficult to identify those who will develop severe, life-threathening complications like severe sepsis or septic shock. Early diagnostic and prognostic tools to improve the care of patients in neutropenic fever are needed. Among others, CRP, PCT, and interleukins have been studied as biomarkers but no guidelines for their use exist.

2.4.1 C-reactive protein

As early as in 1930, a precipitation of pneumococcal polysaccharide "C-fraction" was detected in patients with pneumonia (119). Later the responsible protein was identified and named C-reactive protein (120). As a member of the pentraxin family, C-reactive protein (CRP) is ring-shaped and composed of five sub-units (121) making it highly stable (30, 122). Recently, however, a monomeric form of CRP has been found, and it seems to be associated with increased prothrombotic activity (22). CRP is produced primarily by the liver induced by IL-6 as a part of the APR (123, 124). CRP binds to molecules in bacteria, fungi and parasites promoting their elimination by activating complement through the classical pathway, and acts as an opsonin for phagocytes (5, 23, 30, 125). Many other effects, especially pro- and anti-inflammatory, and effects activating coagulation and fibrinolysis have been linked with CRP making it a more diverse actor in human immunology than was previously thought (22). CRP synthesis starts in 6-8 h and the peak concentrations are achieved 36-50 h after the onset of infection (22). The concentration of CRP eventually decreases after successful therapy for infection (22) but usually after a gap of several days. CRP concentration in healthy people is usually <1 mg/L and 99% of the concentrations are under 10 mg/L (5, 30). In several acute conditions elevated CRP concentrations, sometimes up to 500 mg/L, are frequently seen (30).

CRP is probably the most widely used inflammatory marker in medicine applied in a wide variety of conditions (5, 8). Though CRP seems to be indicative of infection and tends to be higher in bacterial than in viral infections it is unspecific and not diagnostic for, e.g., bacteremia (5, 8, 22). In sepsis CRP is associated with disease severity, organ failure, and mortality but great controversy exists in the published data (4, 8, 11, 22, 126, 127). Cut-off values suggested for CRP in sepsis diagnosis are mostly between 50 - 100 mg/l (5).

Production of CRP in infection does not seem to be markedly affected by neutropenia as such (5, 128), nor by most chemotherapy treatments (129), or even by graft-versus-host disease (GvHD) (105, 130). However, hematological malignancies like multiple myeloma, and some cytotoxic therapies like high-dose cytarabine, can induce CRP production and raise fever even without ongoing infection (6, 131-133). Significant rises in concentrations of CRP are usually seen around 2-3 days after the onset of the infection also in neutropenic patients (1).

The evidence for the diagnostic and predictive usefulness of CRP in neutropenic fever is altogether conflicting. In neutropenic fever of patients with hematologic or solid malignancies high CRP levels have been associated with complications, sometimes also with sepsis and microbiologically documented infection (129, 134-137). Several studies, nevertheless, report that CRP has poor diagnostic value for infection or sepsis in patients with neutropenic fever (138-141) and especially lacks specificity for bacteremia (131, 142,

143). A cut-off level 40 mg/L has been used for CRP in distinguishing bacterial infections in neutropenic fever (141) and 120 mg/L for lethal outcome (105).

In AML patients with neutropenic fever severe sepsis was associated with peak CRP and CRP on days 2 – 3 (1). In neutropenic fever of hematologous stem cell transplant recipients high CRP (≥120 mg/L) was associated with a lethal outcome (114) but in another study with ASCT recipients with neutropenic fever CRP levels coincided with rather than predicted development of complications (2). CRP is a non-specific and slow marker for prediction of complications in neutropenic fever.

2.4.2 Pentraxin 3

Pentraxin-3 (PTX3) is a soluble PRR identifying pathogenic structures and regulating immune reactions like phagocytosis and complement activity. It is a member of the pentraxin family and the prototype of long pentraxins (12). PTX3 is produced by a variety of cells, including endothelial and epithelial cells, fibroblasts, and cells of myeloid origin. Secretion is induced by early proinflammatory cytokines such as TNF-α and IL-1, but also by direct contact with microbes (32, 144, 145). There is some evidence of PTX3 having a protective role against infections caused by *Pseudomonas aeruginosa* and *Aspergillus fumigatus,* and defects in PTX3 synthesis or function are associated with increased mortality (146, 147). According to experimental studies PTX3 may also protect from AKI and ARDS/ALI (32).

Levels of PTX3 in healthy people are between 0 and 3 ng/ml (147-149) and peak concentrations, up to 200 - 800 ng/ml, are reached after 6 – 8 h in acute conditions like sepsis, much earlier than those of CRP (12, 147). PTX3 is, however, elevated in a range of non-infectious conditions like rheumatoid arthritis, vasculitis, renal disease, and hematological diseases like myeloproliferative neoplasms (139, 147). Glucocorticoid treatment modifies the PTX3 response, leading to a net increase in blood levels (10, 12).

High PTX3 concentrations are associated with the severity of illness, with organ dysfunction, and with a poor outcome in acute conditions including sepsis (150-152). In patients admitted to an intensive care unit with severe meningococcal disease, PTX3 was an early indicator of shock (153). Moreover, in patients with bacteremia PTX3 was an independent prognostic marker during the first days, better compared with CRP (11).

In one study PTX3 differentiated patients with SIRS from healthy controls (148). In SIRS the median PTX3 was 71 ng/ml and high levels on admission to ICU were associated with sepsis, severe sepsis, septic shock, and 90-day mortality (148). In emergency room patients with suspected infection high levels of PTX3 on admission predicted severe sepsis and death (10). That study reported a median PTX3 of 2.6 ng/ml in patients without bacterial infection or SIRS, 6.1 ng/ml in patients with uncomplicated sepsis, and 16.7 ng/ml in those with sepsis-associated organ dysfunction. The levels were particularly high in patients with DIC or MOF (median 46.2 ng/ml) (10). In yet another study with emergency department patients high PTX3 predicted severe disease, was elevated in patients treated at ward (median 14 ng/ml), and was highly elevated in patients treated at ICU (median 44 ng/ml) (149). In that study PTX3 also correlated with BSI and the best cut-off was 16.1 ng/ml.

Only one previous PTX3 study in patients with neutropenic fever is available (13). In 26 episodes of neutropenia in 11 patients treated for hematological malignancies, PTX3 levels were normal in 10/11 episodes of non-febrile and non-infected neutropenia and in 5/5 episodes of invasive aspergillosis, despite frequent mucositis (median 1.39 ng/ml in both groups). In the other 10 infections the median was 7.2 ng/ml, significantly higher in comparison with the other two groups (13).

2.4.3 Procalcitonin

Procalcitonin (PCT) is a peptide prohormone cleaved by from preprocalcitonin and further cleaved to produce the active hormone calcitonin (154). Calcitonin is produced primarily by thyroid C cells and has various physiological effects, including inhibition of osteoclast activity in bone and maintaining calcium homeostasis (155, 156). Several other tissues, particularly pulmonary neuroendocrine cells, contain and release calcitonin and its precursors during infection and markedly elevated levels have been detected in conditions like chronic lung diseases, malignant tumors, as well as in acute inflammatory states like sepsis (157). Highly elevated calcitonin precursor levels have been provoked by TNFα injection in experimental models, strongly suggesting PCT induction by early proinflammatory cytokines (157).

PCT is one of the most studied biomarkers in sepsis patients and is considered to have advantages over CRP and other biomarkers because of its more favorable kinetics (4, 8, 158). Normal PCT level in non-infected people has been reported to be 0.030 - 0.036 ng/ml. Cut-off level of 0.5 ng/ml is used for distinguishing sepsis from non-infectious SIRS (159) but also higher values have been proposed (158, 160). PCT concentrations elevate 4-12 h after an infectious stimulus and decrease with a half-life of about 24 h when effective treatment is instituted making it a potent indicator of response to treatment (8, 158, 159). Moreover, a decrease of more than 50% in PCT concentration in the first 72 h was associated with a favorable course of severe sepsis in ICU patients compared to those with a lower decrease (161). A systematic review of PCT algorithms for antibiotic therapy observed a reduction in antibiotic use without deleterious effect on clinical outcomes (162). However, a later randomized controlled trial comparing PCT-guided management to standard therapy in ICU patients failed to improve survival and reported an increase in organ-related complications in the study group (9). Results from other studies assessing PCT in directing antibiotic use are awaited (ClinicalTrials.gov Identifiers NCT00832039 and NCT01139489).

PCT is not markedly elevated at the beginning of neutropenia alone (131) or in post-chemotherapy mucositis without severe infection (114, 141, 163). High-dose cytarabine does, however, raise PCT level above 0.5 ng/ml in some patients, though the elevation is not as clear as with CRP (6). PCT does not seem to be influenced by GvHD in hematological patients with neutropenic fever after chemotherapy and stem cell transplant (163), albeit one study with few GvHD cases reported conflicting results (114). Elevated PCT has been shown to associate with severe infections like bacteremia in patients with neutropenic fever (111, 135, 137, 139, 164-167) especially with gram-negative bacteremia (131, 163, 168, 169). Higher PCT predicts complications in neutropenic fever (170, 171); interestingly also in episodes classified as FUO (172). Late PCT peak during persistent fever in neutropenia has been associated with invasive fungal diseases (104), though PCT is not considered reliable in diagnosis of candidemia (8). Decreasing PCT levels have been associated with a favorable outcome in neutropenic fever and *vice versa* (169, 170, 173), but controversy exists (174). PCT cut-off level 0.5 ng/ml has been used for detection of bacteremia or other deep-seated infections also in patients with neutropenic fever (104, 111, 164).

One study in hematological patients with neutropenic fever after intensive chemotherapy (175) reported, however, that PCT was not useful in diagnosing bacteremia or evolving severe infective complications. Another study with patients with neutropenic fever after stem cell transplant did not find a significant association between PCT levels and mortality (105, 114).

2.4.4 Interleukin-6

Material from pathogens and tissue injury is detected by immune cells through pattern recognition receptors (PRRs) leading to intracellular NFkB signaling and secretion of cytokines, e.g., IL-6. These cytokines then initiate several systemic events, the APR, including secretion of APPs from the liver (28, 31, 176). Thus, the secretion of pro-inflammatory cytokines like IL-6 precedes the secretion of APPs, e.g., CRP and PCT. IL-6 is perhaps the most potent pro-inflammatory cytokine and activates a very wide range of events. It is produced by monocytes/macrophages, but also by many other cells (20, 21, 176, 177). IL-6 concentrations rise as fast as 2 h after the onset of the infection and stay elevated longer than, e.g., TNFα or IL-1 (178).

The action of IL-6 is mediated through binding to soluble and cell-bound receptors. IL-6, IL-6 receptor and gp130 form a complex inducing protein synthesis through JAK/STAT and MAPK pathways (29, 41, 177). Cell membrane-bound IL-6 receptors are found on the surfaces of hepatocytes and WBCs. These induce inflammatory responses to infection and hematopoiesis, growth and differentiation of B- and T-lymphocytes, and later antibody production and NK-cell activity (41, 176). Soluble IL-6 receptors in turn, after binding with IL-6, can activate most types of cells and have been associated with pathological inflammatory processes like rheumatoid arthritis (29, 177). IL-6 participates also in the pathogenesis of several hematological and other malignancies, like multiple myeloma (133, 177).

IL-6 has a role in the pathophysiology of sepsis by enhancing inflammation and coagulation, and it has been associated with hemodynamic collapse in patients with sepsis and septic shock (41, 81, 176). Normal range for healthy adults below 10 pg/ml has been suggested (178). The levels rise in inflammation and associate with those of CRP (29, 133). Genetic polymorphisms may alter the levels of IL-6 and may increase susceptibility to some infections but the evidence is not univocal (179, 180).

According to previous studies, IL-6 has potential in the early diagnosis of bacterial infections, sepsis, and severe sepsis (127, 181, 182). High IL-6 is also associated with lethal outcome in sepsis, severe sepsis, and septic shock (158, 183-186). In non-neutropenic patients the reported levels of IL-6 in infectious conditions vary from 20 pg/ml in mild viral infections to around 200-300 pg/ml in severe sepsis and septic shock (181, 182, 186).

In neutropenic fever after chemotherapy for solid cancers or hematological malignancies early IL-6 measurement can differentiate bacteremia from FUO (135, 137, 138, 187-189). High IL-6 levels in patients with neutropenic fever are also associated with severe complications like septic shock and death (105, 114, 142, 171, 190, 191). IL-6 is also higher in neutropenic fever patients with gram-negative bacteremia than in those with gram-positive bacteremia or no bacteremia (142, 190, 192, 193).

In neutropenic fever complicated by gram-negative bacteremia the peak levels of IL-6 are reached 4 h after the start of the fever (193). Overall, the IL-6 peak is reached 1-2 days before that of CRP (192). Moderately elevated IL-6 has also been reported in malignancies even before chemotherapy and during the afebrile neutropenic period, but still rises during neutropenic fever (132, 133). There is considerable variation in the reported IL-6 concentrations in patients with febrile neutropenia: from 30 pg/ml in early FUO, to 1000 pg/ml and higher in severe sepsis and gram-negative bacteremia (137, 138, 189, 192, 194). For patients with neutropenic fever the suggested cut-offs for detecting sepsis and severe sepsis are mostly between 200 and 300 pg/ml (137, 138, 189, 190, 194).

Also reports showing no or limited diagnostic or prognostic value for IL-6 in sepsis and neutropenic fever have been presented (131, 166, 174, 195). Although IL-6 has a central role in the pathogenesis of sepsis, its role in clinical use as a sepsis biomarker remains unclear (158).

2.4.5 Interleukin-10

When a pro-inflammatory response to infection is initiated, also regulatory processes become activated. These are thought to protect the host from the damage caused by excessive immunological activity like is seen in autoimmune diseases (45, 196, 197). One of the central anti-inflammatory mediators regulating and modifying inflammatory responses is IL-10, synthesized and secreted after TLR ligation by monocytes/macrophages and subsets of T- and B-lymphocytes, but also to some extent by neutrophils and endothelial cells (196, 198, 199). Levels of IL-10 correlate with those of pro-inflammatory TNF-α, which induces its production (43). The effect of IL-10 is mediated through binding to its receptors IL-10R1 and 2, which can activate JAK/STAT-pathways and regulate pro-inflammatory cytokine production through inhibition of NF-κB–signaling (45, 198). The most important effects of IL-10 are inhibition of macrophages and Th1-type response, stimulation of Th2-type cells, and B-cell activation, resulting in diminished TNF-α production and antigen presentation, while clearance of antigens and development of tolerance is favored (43, 197, 200). Genetic variability has been reported in the synthesis of IL-10, its receptors, and secretion. These polymorphisms can lead to decreased or increased total activity and possibly predispose to complications of sepsis (45, 179, 196).

IL-10 levels are higher in patients with severe sepsis than in uncomplicated sepsis (186, 201) and higher in septic shock than in cardiogenic shock (202). High IL-10 level is also associated with short- and long-term sepsis mortality (185, 186, 201), as is high IL-10/TNF-α ratio (81). In patients with *Staphylococcus aureus* bacteremia high IL-10 levels at admission identified all eight out of 59 patients who eventually died, with a cut-off value of >7.8 pg/mL (203). One study reported following median IL-10 levels in non-neutropenic patients: 5 pg/ml in sepsis, 5.4 pg/mL in severe sepsis, and 14.1 pg/mL in septic shock (186).

Though IL-10 is partly produced by neutrophils (199) neutropenia does not seem to affect IL-10 levels in sepsis markedly. A study reported similar IL-10 levels in neutropenic and non-neutropenic bacteremic patients and the levels were significantly higher in bacteremic than in non-bacteremic patients (204). Parallel results were reported from a small study with children with neutropenic fever where the median IL-10 was significantly higher in children with bacteremia or clinical sepsis than in those with FUO (205), with a best cut-off value 18 pg/ml. The concentrations of IL-10 in neutropenic patients with gram-negative bacteremia are reported to peak 6 h after the start of the fever (193) and concentrations are higher in gram-negative bacteremia than in gram-positive bacteremia or no bacteremia at all (143, 193).

According to some reports IL-10 in patients with neutropenic does not predict survival (174) or separate viral and bacterial infections from each other (181). The evidence is inconsistent and the study settings have varied, making interpretation difficult.

2.4.6 SuPAR

Conversion of plasminogen to active plasmin causes degradation of fibrin and is a central part of the coagulation-fibrinolysis system, also activated in severe infections. Soluble urokinase-type plasminogen activator receptor (suPAR) is cleaved from respective receptors bound in cell membranes (uPAR), a cofactor in the plasminogen activation (206). SuPAR has a role in infection control by promoting chemotaxis, and it is present in blood and other tissues. The levels rise in conditions causing low-grade inflammation like cancer, chronic infections, and cardiovascular diseases. It seems to predict morbidity and mortality in the general population, but also in acutely ill patients, including those with sepsis (14, 15, 158, 207-212).

Normal suPAR levels are usually 1-3 ng/ml in healthy people (206, 212). Factors such as age, obesity, and, e.g., smoking increase concentrations (14, 213). High suPAR levels, 10

ng/ml and higher, are frequently seen in patients with SIRS and concentrations decrease during recovery (207, 211-216). However, it is not considered very useful in distinguishing sepsis from other causes of SIRS (16, 210), even though there are studies reporting some diagnostic value in infections (214-216). Very high suPAR levels have, however, been reported in association with distinct non-infectious conditions like in liver disease and renal failure (207, 212, 213). Altogether, suPAR is not specific for infections or sepsis and it seems to provide little additional value for diagnostic purposes when compared with more traditional sepsis markers like CRP and PCT (16).

While unsatisfactory as a diagnostic marker and significantly affected by basal conditions, suPAR seems to be prognostic in several studies in variable populations, including sepsis patients (16, 209-212). The proposed cut-off values for predicting mortality are mostly between 6 and 12 ng/ml in conditions like bacteremia (16, 158, 206, 209, 211, 213). Elevated suPAR levels have also been associated with several hematological diseases, especially multiple myeloma (217) and acute leukemia (18, 218). Increased suPAR levels were also seen during conditioning treatment before stem cell transplantation in hematological patients (18). This could make its interpretation difficult in acute infections in neutropenia, especially if baseline levels have not been measured.

SuPAR has previously been studied in neutropenic fever of hematological patients in only a few studies. The first study consisted of 40 patients with 50 episodes of neutropenic fever and of 68 healthy controls. The study reported mean serum suPAR levels of 3.9 (+/- 1.5) ng/ml in the control group and 5.8 (+/- 2.7) ng/ml in hematological patients a day before febrile neutropenia. SuPAR levels rose faster than those of CRP and PCT (17). SuPAR in the first day of neutropenic fever was significantly higher (12.34 vs. 7.62 ng/ml) in patients with documented infection than in those with FUO and the later decrease correlated with a response to treatment. The optimal cut-off was determined as 5.87 ng/ml for prediction of infection. Only two of the patients died (17).

In another small study with 20 patients mostly with acute leukemia who had undergone allogenic stem cell transplantation early suPAR was not associated with bacteremia or with GvHD but it was associated with lethal outcome (18).

3 Aims of the Study

The purpose of this study was to search for early biomarkers for complicated course of neutropenic fever in hematological patients receiving intensive chemotherapy by exploring kinetic behavior of these biomarkers. The specific study aims were:

1. To evaluate the value of pentraxin-3 in predicting complications of neutropenic fever in hematological patients (I).

2. To determine a suitable cut-off level for pentraxin-3 in AML and ASCT patients to indicate the risk of a complicated course in neutropenic fever (II).

3. To study if procalcitonin, interleukin-6, and interleukin-10 are early predictive indicators for complicated course of neutropenic fever (III).

4. To investigate if the soluble form of urokinase-type plasminogen activator receptor (suPAR) is a prognostic marker in hematological patients with neutropenic fever (IV).

4 Patients and Methods

4.1 STUDY DESIGN

This was a prospective study in hematological patients at an adult hematology ward of a tertiary care hospital. The patients were enrolled if the following criteria for inclusion were met: *1*) age from 18 to 70 years, *2*) acute myeloid leukemia or high-dose chemotherapy supported by ASCT, and *3*) willingness to participate in the study. After the enrollment the first episode of neutropenic fever from each patient was included in the study. Blood samples were taken at the onset of neutropenic fever (*day 0*) and in the three following mornings (*days 1-3*) and stored (*Fig. 3*), while clinical data and some laboratory results, e.g., CRP and blood culture findings, were collected from the patient documents. Measurements of PCT, PTX3, IL-6, IL-10, and suPAR were done from frozen samples of serum or plasma. Associations between the clinical course and early biomarker levels were then explored. The endpoints of the study included bacteremia, gram-negative or gram-positive bacteremia, septic shock, and 90-day mortality (suPAR) or death during the same hospital stay (other biomarkers). Complicated neutropenic fever was defined as septic shock/gram-negative bacteremia, or septic shock/any bacteremia.

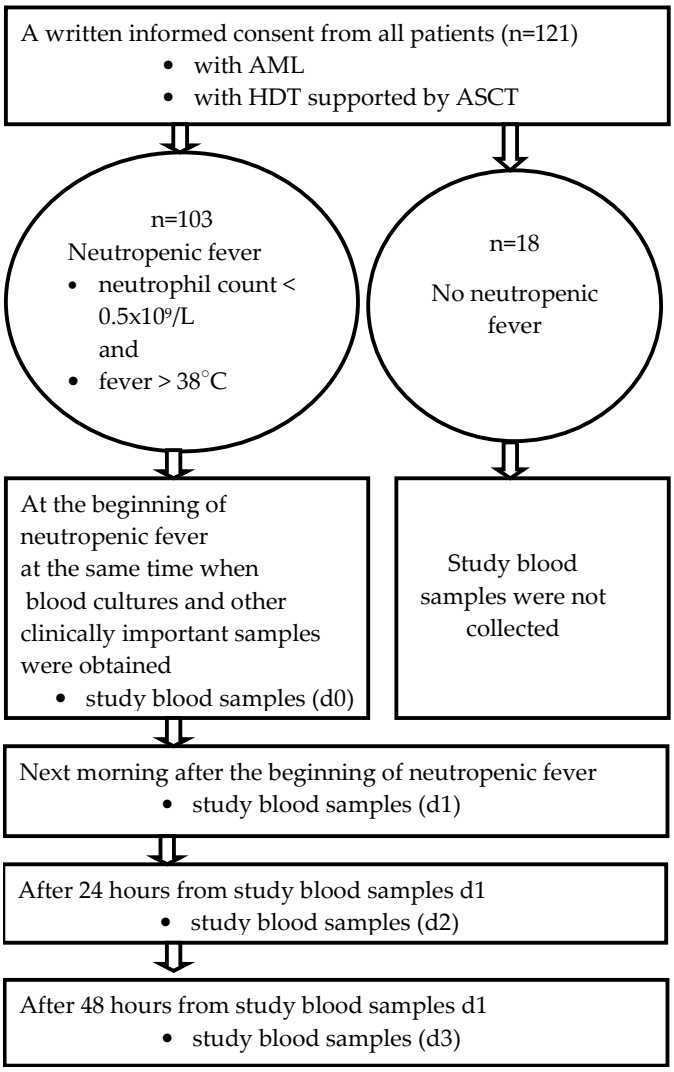

Figure 3. The study protocol. AML, acute myeloid leukemia; ASCT, autologous stem cell transplantation; HDT, high-dose therapy.

4.2 PATIENTS

The study population consisted of 103 adult hematological patients with an episode of neutropenic fever after intensive chemotherapy. In the original studies the number of patients included varied between 67 and 100 depending on the study setting and available data (*Table 1*). All patients involved were treated for hematological malignancies at the Kuopio University Hospital between 1.12.2006 - 30.12.2009 and enrolled if they met the inclusion criteria (see 4.1.). The two major groups were patients with acute myeloid leukemia (AML) and patients receiving high-dose chemotherapy supported by autologous stem cell transplantation (ASCT). The latter group included mostly patients with multiple myeloma (MM), non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (HL), and single patients with other diagnoses.

Table 1. Patients in the original studies (I-IV). AML, acute myeloid leukemia; ASCT, autologous stem cell transplant; NHL, non-Hodgkin lymphoma.

Study	**I**	**II**	**III**	**IV**
Patients	100	67	100	99
AML	32	32	32	32
ASCT	68	35 (all NHL)	68	67
Men	61	43	61	60
Women	39	24	39	39

All patients were treated with intensive chemotherapy. AML patients received chemotherapy according to the Finnish Leukaemia Group prospective AML-2003 protocol, and ASCT recipients according to local policies. Chemotherapy protocols used were carmustine, etoposide, cytarabine, and melphalan (BEAM); high-dose melphalan (HD-MEL); carmustine, etoposide, cytarabine, and cyclophosphamide (BEAC); idarubicine, cytarabine, and thioguanine (IAT); intermediate-dose cytarabine and idarubicin (IdAraC-Ida); high-dose cytarabine and idarubicin (HDAraC-Ida); or mitoxantrone and high-dose cytarabine (Mito-HDAraC).

Patients with NHL and ASCT were given ciprofloxacin prophylaxis from January 2008 onwards and most ASCT recipients also received granulocyte colony stimulating factor after stem cell infusion. AML patients did not receive antibacterial prophylaxis and only a few were given growth factors as part of the supportive care for prolonged neutropenia and infection. Most patients were given prophylactic fluconazole treatment.

All patients were closely monitored and treated at the hematology ward during the neutropenic period and their condition was immediately evaluated when the body temperature started to rise. It was thus possible to collect blood samples and assess the clinical situation at the onset of neutropenic fever. Blood cultures and chest x-rays were taken routinely at the beginning of the fever. Special attention was paid to the monitoring of the patients during febrile neutropenia. Patients were re-assessed daily for signs and sources of infection, and, e.g., body temperature, urine output, blood pressure and pulse, and peripheral arterial blood oxygenation was measured and documented regularly.

Empirical antimicrobial therapy, with a betalactam and an aminoglycoside was initiated when fever (>38°C) or other signs of infection were detected during neutropenia. Vancomycin was used only in cases of infection in central venous catheter or *CoNS* in blood cultures. Additional analysis was done if needed, e.g., later blood cultures in cases of prolonged fever despite empirical therapy.

4.3 METHODS

4.3.1 Definitions

The definitions are adapted from the Society of Critical Care Medicine and American College of Chest Physicians (SCCM/ESICM/ACCP/ATS/SIS) International Sepsis Definitions Conference 2001 (47) and from the Infectious Diseases Society of America (IDSA) 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer (219).

SIRS: systemic inflammatory response syndrome (47)
Sepsis: SIRS as a response to confirmed or strongly suspected infection (47)
Bacteremia: The presence of living bacteria in the blood (46)
Neutropenia: absolute neutrophil count (ANC) <500 cells/μL, or expected to decrease to <500 cells/μL during the next 48 h (219)
Fever: single oral temperature ≥38.3°C (101 F) or ≥38.0°C (100.4 F) sustained a 1-h period (219)
Severe sepsis: sepsis complicated by organ dysfunction (47)
Septic shock: sepsis with acute circulatory failure (=persistent hypotension) (47)
Persistent hypotension: systolic arterial pressure <90 mmHg, mean arterial pressure <60 mmHg, or a reduction in systolic blood pressure >40 mmHg from the baseline, despite adequate volume resuscitation and in the absence of other causes of hypotension (47)

4.3.2 Ethical considerations

The study followed the declaration of Helsinki and had an approval of the Ethic committee of the Kuopio University Hospital (number 100/2006). All patients enrolled gave a written informed consent.

4.3.3 Data collection

Detailed information concerning the clinical course and outcome of the illness and treatment, and laboratory results was collected from patient documents to a data collection form, specially compiled for this purpose. The pre-existing medical conditions, given therapies including medication and, e.g., stem cell transplantations were recorded. The data collected included duration of neutropenia and fever, possible admission to the ICU, arterial blood oxygenation, blood pressure, heart rate, alterations in, e.g., cognitive functions, respiratory rate, urine output, and body temperature suggesting developing complications like severe sepsis and septic shock were registered along with imaging study findings. Also fluids given during the first days of neutropenic fever were recorded on daily basis.

4.3.4 Laboratory methods

Blood cultures were taken at the beginning of the fever and later according to clinical need. The analysis was performed with an automated 9240 system with a 7-day incubation

period for aerobic and anaerobic bottles and 42-day incubation period for F/Lytic bottles (Becton Dickinson, Sparks, MD, USA). Samples for blood cultures were taken from venous blood two or three at a time with one hour intervals, and were examined for aerobic and anaerobic microbes.

A single blood culture was considered positive only if seen clinically relevant as a cause of infection. A pathogen considered to be a common skin contaminant (e.g. *CoNS*) was seen relevant only if it was found in two successive cultures or if there was an ongoing skin or catheter infection.

Blood samples for later analysis were taken at the onset of fever and on the following three mornings. CRP was analyzed daily as a routine procedure, other samples were stored frozen in -20°C until analysis. All the laboratory analyses were performed by Eastern Finland Laboratory Centre (ISLAB, Kuopio, Finland).

Serum **C-reactive protein (CRP)** was measured with a Konelab 60i Clinical Chemistry Analyzer (Helsinki, Finland, sensitivity 0.25 mg/mL) or Cobas 6000-analyzer (Hitachi, Tokyo, Japan). The between-run variations were 2.3–4.3%. The upper reference limit of serum or plasma CRP of a healthy reference population was <10 mg/L at the time.

Plasma **pentraxin 3 (PTX3)** was measured with a sandwich-type ELISA (R&D Systems, Minneapolis, MN, USA). The sensitivity of this assay was 0.12 μg/L. The samples were analyzed in batches. The upper reference limit was 1.2 μg/L for PTX3 in healthy individuals (studied by the manufacturer). The intra-assay precision was 3.8% (mean concentration 2.6 ng/L, n = 20) and inter-assay precision was 6.2% for a pooled plasma sample (mean concentration 2.8 ng/L, n = 40).

Interleukins 6 and 10 (IL-6 and IL-10) were analyzed from EDTA plasma samples with a enzyme-linked immunoassay from R&D Systems. The sensitivity of the IL-6 assay was 0.7 ng/L. The intra-assay precision was 4.2% (mean concentration 16.8 ng/L, n = 20) and inter-assay precision was 6.4% for a pooled plasma sample (mean concentration 17.2 ng/L, n = 20). For IL-10 the sensitivity was 3.9 ng/L, the intra-assay precision was 5.0% (mean concentration 23.9 ng/L, n = 20), and inter-assay precision was 7.3% for a pooled plasma sample (mean concentration 23.2 ng/L, n = 20).

Procalcitonin (PCT) was analyzed from plasma samples with a Cobas 6000-analyzer (Hitachi, Tokyo, Japan), and the sensitivity of the assay was 0.06 μg/L. The intra- and inter-assay precisions for PCT analyses were 1.4% and 3.0% for 0.46 μg/L of PCT and 1.1% and 2.6% for 9.4 μg/L of PCT, respectively.

Analysis of plasma **soluble urokinase-type plasminogen activator receptor** (**suPAR)** was conducted with a suPARnostic double monoclonal antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (sensitivity 0.1 ng/mL; ViroGates, Birkerød, Denmark). Intra- and inter-assay precisions were 1.3% and 2.3% at 3.7 μg/L.

4.3.5 Endpoints

PTX3 and CRP were evaluated in relation to each other and to endpoints of bacteremia and septic shock in study I and to the combined endpoint of bacteremia or septic shock in study II. Also in-hospital mortality was registered. CRP, IL-6, IL-10, and PCT were evaluated in study III for prediction of the endpoint of complicated course of febrile neutropenia defined as positive blood culture and/or septic shock. Also the combinations of these biomarkers were evaluated in the prediction of the complications in the study III. In study IV suPAR was assessed for prediction of any bacteremia, gram-negative or gram-positive bacteremia, septic shock, and 90-day mortality and their combinations. SuPAR was also compared to CRP, PCT, IL-6, and IL-10 in study IV.

4.3.6 Statistical methods

SPSS versions 14.0 (Study I), 17.0 (Study II), and 19.0 (Studies III and IV) of Windows (SPSS, Chicago, IL, USA) were used for the statistical analysis. A p-value <0.05 was considered significant (Studies I-IV). Continuous variables like biomarker concentrations were expressed as means with standard errors (Study II), as medians with a range between minimum and maximum (Studies I, II, III), or as medians with a range between quartiles (Study IV). Logarithmic transformation was done to correct the skewed distribution for parametric tests if necessary (Studies I, II, III). Categorical variables like groups defined by endpoints were given as absolute counts and percentages (Studies I-IV). Differences between the groups were considered as between-subject factors and day-to-day differences as within-subject factors for repeated measures analysis (Study I). Graphical presentations were provided on selected occasions.

For continuous variables the non-parametric Mann-Whitney U-test was used to detect differences between the groups (Studies I-IV). Associations of categorical variables with endpoints were explored with chi-square (Studies I, II, III) and linear-by-linear tests (Study I). The non-parametric Spearman's correlation test was used to evaluate correlations between, e.g., concentrations of two biomarkers (Studies I, III, IV). Youden's index (sensitivity + specificity – 1) for the optimal cut-off level was calculated (Studies II, III, IV). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were defined for selected cut-off values (Studies II, III, IV). Receiver operating characteristic (ROC) curves and area under the curve (AUC) values were given for evaluation and comparison of the diagnostic value of the biomarkers for defined endpoints (Studies I, III, IV). General linear model for repeated measures was applied to analyze differences of biomarker levels at different time-points between the groups by endpoint (Studies I and IV). Significances of differences between biomarker levels in classes by severity of complications were evaluated with the Joncheere-Terpstra test (Study I). Logistic regression analysis, adjusted for age, gender, and malignancy, was applied in studies I and III.

5 Results

5.1 CLINICAL COURSE AND OUTCOME OF FEBRILE NEUTROPENIA

Of the 103 patients three (3%) died during the same hospital stay, all because of infectious complications (Studies I-III), the 90-day mortality was 6% (Study IV). The incidence of septic shock was 5%. One patient died due to respiratory failure associated with severe influenza A infection (pandemic H1N1 2009) and the other two deaths were due to septic shock. Blood cultures were positive in 19% and negative in 81% of the episodes of neutropenic fever. Gram-positive bacteria were found in 13% and gram-negative rods in 6% of the episodes. Gram-positive pathogens isolated were *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus hemolyticus*, *Streptococcus pneumoniae*, *Streptococcus viridans*, *Streptococcus mitis, Streptococcus oralis*, and *Enterococcus faecium*. Gram-negative pathogens were *Escherichia coli*, *Klebsiella pneumonia*, and *Enterobacter cloacae*. *Table 2* shows the endpoints in the original studies.

Table 2. Complications and outcomes in the study populations (Studies I-IV), % (n)

Study	**I** (n=100)	**II** (n=67)	**III** (n=100)	**IV** (n=99)
Mortality	3% (3) (Hospital stay)	4% (3) (Hospital stay)	3% (3) (Hospital stay)	6% (6) (90 days)
Complicated course of febrile neutropenia	Bacteremia or septic shock 21% (21)	Bacteremia or septic shock 21% (14)	Bacteremia or septic shock 21% (21)	Gram-negative sepsis, septic shock or death within 90 days 13% (13)
Septic shock	5% (5)	7% (5)	5% (5)	4% (4)
ARDS	1% (1)	1% (1)	1% (1)	1% (1)
Positive blood culture finding	19% (19)	18% (12)	19% (19)	19% (19)
Gram-negative blood culture finding	6% (6)	6% (4)	6% (6)	6% (6)
Most common Gram-negative species	*Escherichia coli* (4)	*Escherichia coli* (2)	*Escherichia coli* (4)	*Escherichia coli* (4)
Gram-positive blood culture finding	13% (13)	12% (8)	13% (13)	13% (13)
Most common Gram-negative species	*Staphylococcus epidermidis* (6)	*Staphylococcus epidermidis* (3)	*Staphylococcus epidermidis* (6)	*Staphylococcus epidermidis* (6)

5.2 BIOMARKERS AND COMPLICATIONS OF FEBRILE NEUTROPENIA

5.2.1 C-reactive protein

In the group with septic shock CRP level was higher than in the group without septic shock at every time-point (Study I) (*Fig.* 4). CRP at the onset of neutropenic fever and maximal CRP were associated with the combined endpoint of gram-negative bacteremia and/or septic shock in ROC analysis and logistic regression analysis (Study III) (*Table 3, Table 4*). However, CRP levels on day 0 were not associated with any bacteremia or with gram-negative bacteremia alone (Study I), and diagnostic value of CRP for all the complications was weaker than that of PCT, IL-10, and IL-6 (Study III) (*Table 3, Table 4, Table 5*).

In Joncheere-Terpstra test for groups of increasing severity of complications CRP had statistical significance on days 0, 1, and 2 (Study I). Daily increases in CRP were not statistically significant for the complications and the optimal cut-off for CRP in predicting complicated course of neutropenic fever was 84 mg/L (Study III) (*Table 5*). Doubling of standardized value of CRP on day 0 increased the risk of complications to 1.9-fold (95% CI 1.2 - 3.0) (Study III) *(Table 4)*.

5.2.2 Pentraxin 3

Bacteremic patients and patients with septic shock had higher PTX3 levels than those without these complications (*Fig. 4*). Two patients dying of septic shock had very high PTX3 levels, one with a concentration of 2000 μg/L (maximum detectable) on day 0 already, the other having continuously rising levels, 25.2 μg/L, 32.9 μg/L, 157 μg/L, and 2000 μg/L on days 0, 1, 2, and 3, respectively. The patient dying of influenza A due to respiratory failure had intermediate and rising PTX3 concentrations throughout the study period (31.1-55.9 μg/L). PTX3 on day 0 had no statistically significant association with age, sex or co-morbidities (Study I).

PTX3 level on day 0 predicted septic shock, bacteremia, and the combination of these two endpoints in the ROC curve analysis (*Fig. 5*). In the repeated measure analysis (days 0 - 3) PTX3 was significantly higher in patients with bacteremia and in patients with evolving septic shock. Also in groups by increasing severity of complications (non-complicated neutropenic fever, bacteremia without septic shock, and septic shock) PTX3 had statistical significance in Joncheere-Terpstra test on day 0 and day 1 (Study I).

During febrile neutropenia PTX3 levels in patients with AML and in those with NHL and ASCT were associated with both the underlying malignancy and the presence of complications, bacteremia or septic shock. Levels were higher in patients with NHL than in patients with AML, highest in NHL patients with complications, and lowest in AML patients without complications (Study II).

In the ROC curve analysis PTX3 predicted a complicated course of febrile neutropenia in both groups, in AML with an AUC of 0.79 (95% CI 0.61 − 0.97, $p = 0.009$), and in NHL with an AUC of 0.94 (95% CI 0.86 − 1.03, $p = 0.004$) (*Fig. 6*). The optimal PTX3 cut-off level determined by the Youden's index for complicated course was higher in patients with NHL (115 μg/L) than in patients with AML (11.5 μg/L). PTX3 predicted complications of febrile neutropenia in combined analysis based on separate cut-offs, with sensitivity of 0.86, specificity of 0.83, PPV of 0.57, and NPV of 0.96. Taken together PTX3 was better than CRP in predicting complications of febrile neutropenia when background malignancy was taken into account (Study II).

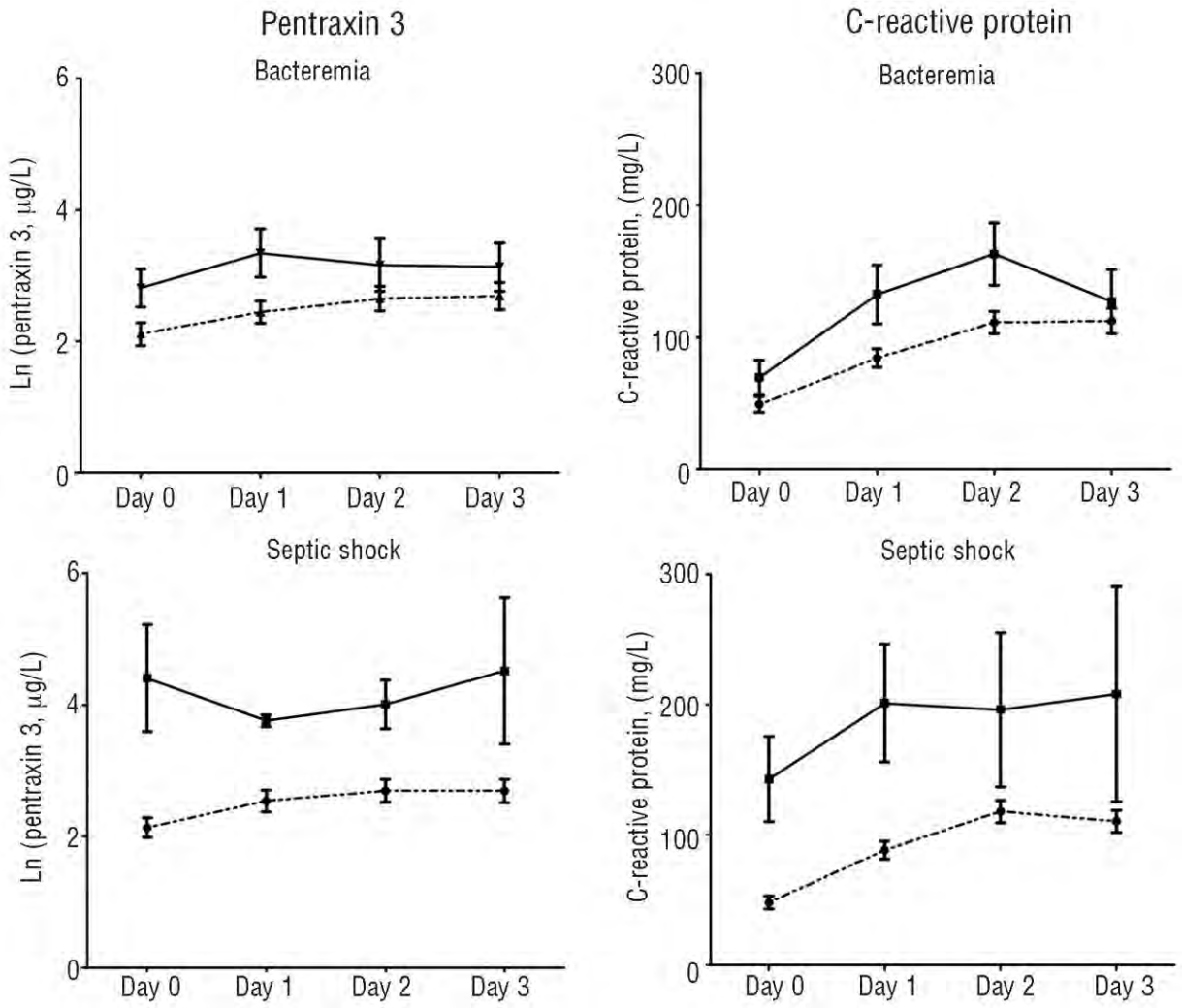

Figure 4. Pentraxin 3 (PTX3) and C-reactive protein (CRP) in bacteremia and septic shock from the onset of fever until day 3. Groups by presence (continuous lines) or absence (dashed lines) of bacteremia (upper panel) and septic shock (lower panel). Means with standard errors (SE), note logarithmic scale for PTX3.

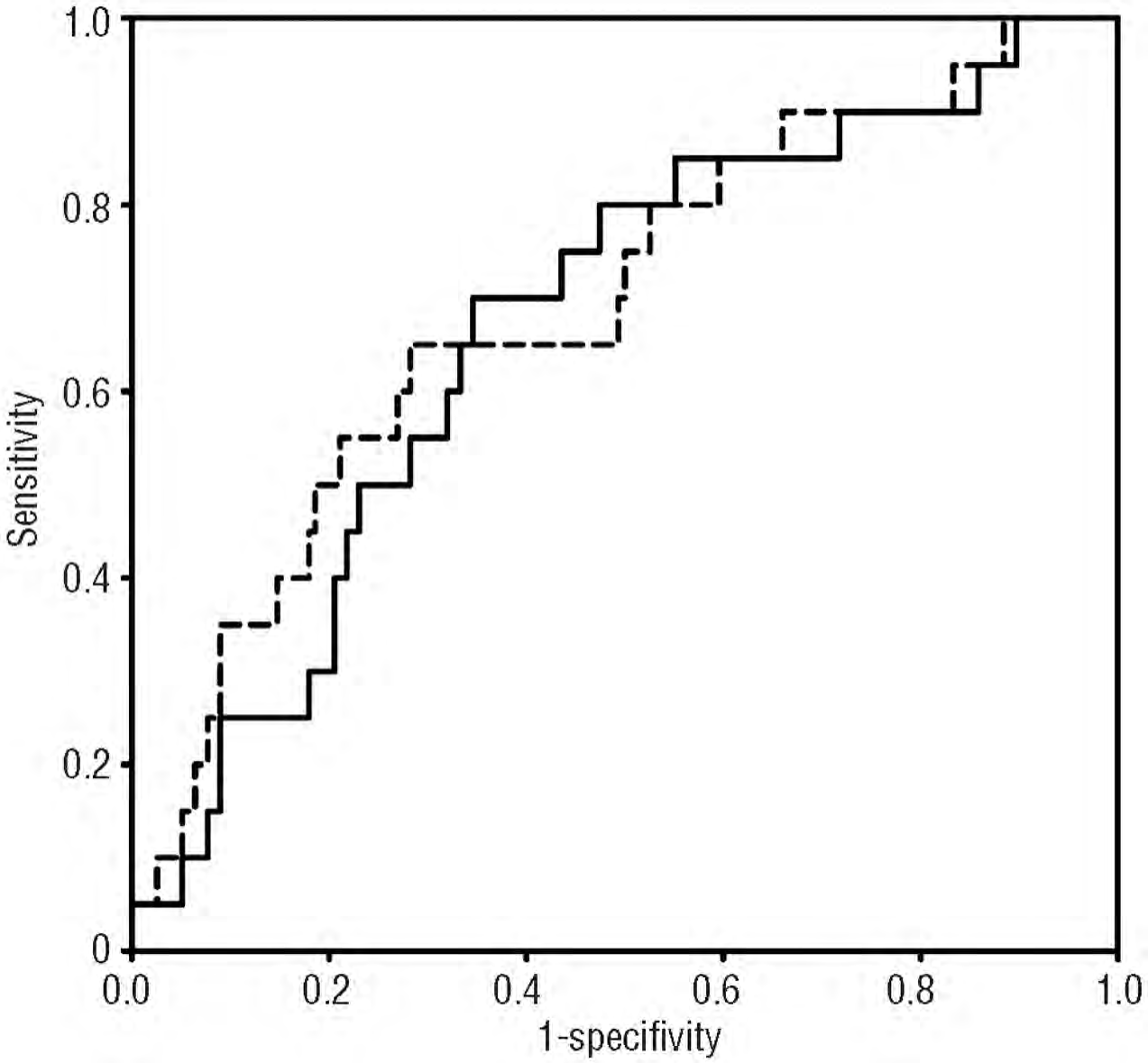

Figure 5. The receiver operating characteristic curve with pentraxin 3 (PTX3) (continuous line) and CRP (dashed line) on day 0 for predicting bacteremia or septic shock. The area under the curve for PTX3 was 0.68 (95% CI 0.56 − 0.81), *p*-value 0.011 and for CRP 0.69 (0.56 − 0.82), *p*-value 0.008 (Study I). *P*-values stand for testing the null hypothesis that the area under the curve equals 0.50. AUC, area under the curve.

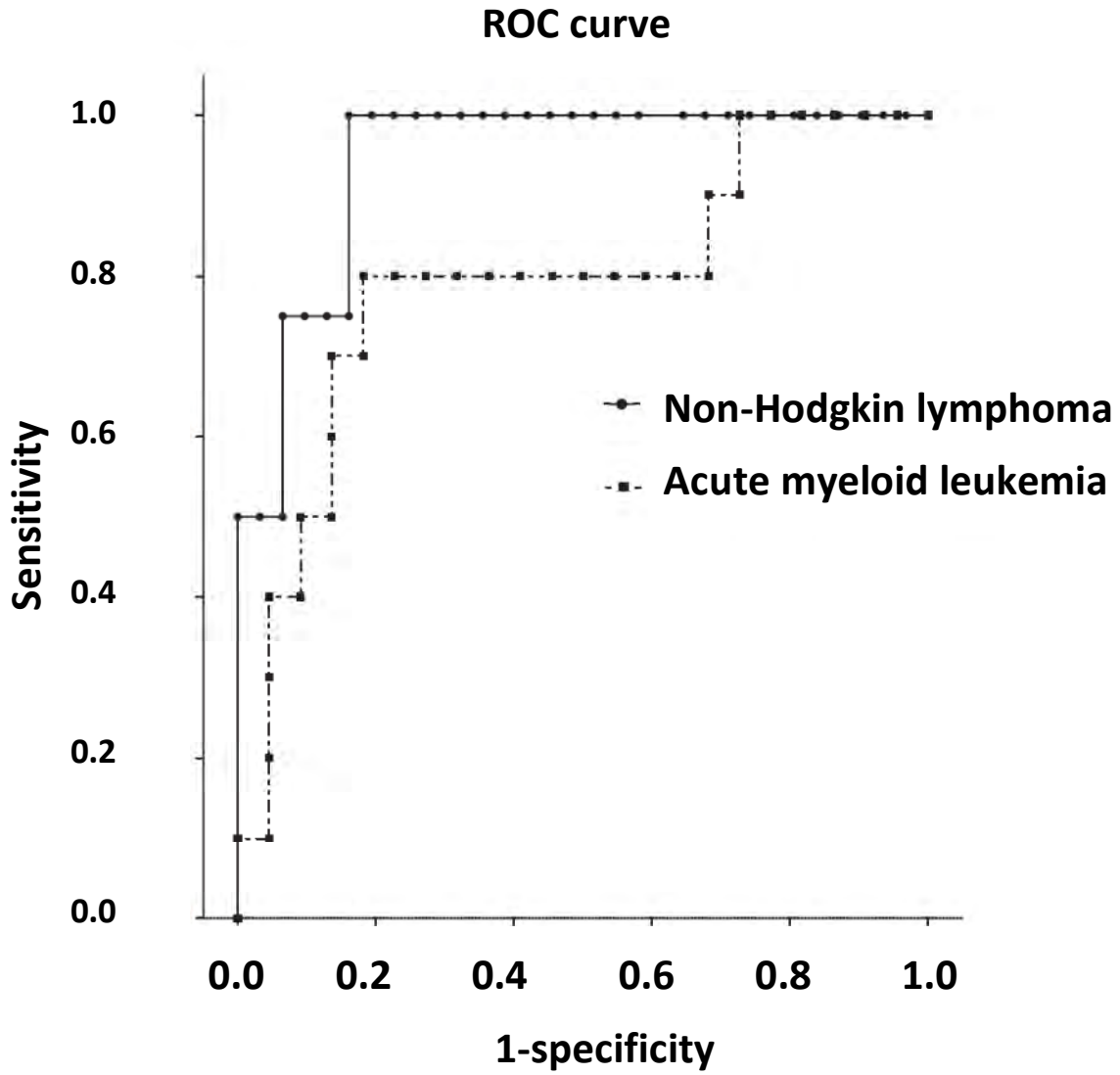

Figure 6. The ROC curve of PTX3 (day 0) for complications of neutropenic fever by malignancy. The AUC for AML (dashed line) was 0.79 and for NHL (continuous line) was 0.94 (study II). AUC, area under the curve.

Table 3. Biomarkers in prediction of complicated neutropenic fever of 100 patients (study III). Interleukin-6 (IL-6), interleukin-10 (IL-10), procalcitonin (PCT), and C-reactive protein (CRP) levels at the onset of fever and peak values, ROC analysis per endpoint. *P*-values stand for testing the null hypothesis that the area under the curve equals 0.50. AUC, area under the curve; CI, confidence interval.

	At the onset of fever		From day 0 to day 3 (maximal value)	
	AUC (95% CI)	*p*-value	AUC (95% CI)	*p*-value
Bacteremia, n = 19				
IL-6	0.66 (0.51–0.81)	0.033	0.68 (0.55–0.82)	0.013
IL-10	0.75 (0.62–0.88)	0.001	0.72 (0.58–0.85)	0.003
PCT	0.72 (0.58–0.85)	0.004	0.74 (0.64–0.85)	0.001
CRP	0.65 (0.51–0.79)	0.052	0.63 (0.48–0.78)	0.081
Gram-negative bacteremia, n = 6				
IL-6	0.76 (0.59–0.93)	0.032	0.75 (0.53–0.97)	0.039
IL-10	0.90 (0.78–1.00)	0.001	0.92 (0.82–1.00)	0.001
PCT	0.75 (0.48–1.00)	0.041	0.81 (0.64–0.99)	0.010
CRP	0.61 (0.39–0.83)	0.370	0.75 (0.50–1.00)	0.043
Septic shock, n = 5				
IL-6	0.85 (0.69–1.00)	0.008	0.92 (0.79–1.00)	0.001
IL-10	0.89 (0.79–0.98)	0.004	0.89 (0.79–0.99)	0.003
PCT	0.91 (0.81–1.00)	0.002	0.98 (0.95–1.00)	<0.001
CRP	0.90 (0.82–0.99)	0.003	0.80 (0.59–1.00)	0.025
Gram-negative bacteremia or septic shock, n = 10				
IL-6	0.80 (0.67–0.93)	0.002	0.83 (0.67–0.99)	0.001
IL-10	0.90 (0.82–0.98)	<0.001	0.92 (0.84–0.99)	<0.001
PCT	0.82 (0.65–1.00)	0.001	0.90 (0.78–1.00)	<0.001
CRP	0.74 (0.57–0.91)	0.013	0.76 (0.58–0.94)	0.007

5.2.3 Procalcitonin

Procalcitonin predicted complicated course of febrile neutropenia (gram-negative bacteremia or septic shock) at all time-points with a strong statistical significance, as did the increase from day 0 to day 1. This was not seen with CRP, IL-6, or IL-10. In logistic regression analysis with standardized variables the maximal PCT concentration showed the strongest association with complicated course of febrile neutropenia compared to maximal IL-6, IL-10, and CRP. Only the association of IL-10 with complications at the onset of fever was superior to PCT (*Table 4*) (Study III).

In the ROC curve analysis PCT at the onset of fever and maximal PCT were associated with any bacteremia, gram-negative bacteremia, septic shock, and to the combination of gram-negative bacteremia or septic shock (Study III) (*Table 3*).

According to logistic regression analysis (*Table 4*), IL-10 performed better than PCT on day 0 but with maximal values from day 0 to day 3 PCT was best. Doubling of standardized d 0 value of PCT increased the risk of complication to 4.3-fold (95% CI 1.7 - 11.4) *(Table 4, Fig. 7)*.

For prediction of a complicated course of febrile neutropenia (gram-negative bacteremia or septic shock) the best cut-off level as determined with Youden's index was ≥0.13μg/L for PCT on days 0 and 1 (*Table 5*). The predictive power was better for a combination of PCT and IL-10 (cut-off ≥37ng/L) on d0-1 (sensitivity 0.95, specificity 0.85, PPV 0.56, and NPV 0.98) (Study III) (*Fig. 8*). Also Youden's index on day 0 - 1 for prediction of gram-negative bacteremia or septic shock was best for the combination of IL-10 and PCT (0.80).

5.2.4 Interleukin-6

The level of IL-6 on day 0 and day 1 predicted a complicated course of febrile neutropenia (gram-negative bacteremia or septic shock). IL-6 at the onset of fever and maximal IL-6 were associated with any bacteremia, gram-negative bacteremia, septic shock, and the combination of gram-negative bacteremia or septic shock in the ROC curve analysis (Study III) (*Table 3*). No correlations with 90-day mortality were observed for IL-6 in study IV.

Maximal IL-6 and IL-6 at the onset of fever showed an association with the complicated course of neutropenic fever in logistic regression analysis with standardized variables. The associations of IL-6 were weaker than the associations of PCT for both maximal and day 0 levels and weaker than the association of IL-10 on day 0, but stronger than the association of maximal IL-10 and both values of CRP (Study III) (*Table 4*).

The optimal cut-off for IL-6 was ≥330 ng/L (*Table 5*). An increase or decrease in IL-6 from day 0 to day 1 was not associated with evolving complications. Youden's index of IL-6 on day 0 - 1 from the onset of fever for gram-negative bacteremia or septic shock was 0.52. Doubling of the standardized value of IL-6 on day 0 increased the risk of complications to 2.9-fold (95% CI 1.4 - 6.0) (Study III) (*Table 4, Fig. 7*).

5.2.5 Interleukin-10

The level of IL-10 at the onset of fever (day 0) and on day 1 predicted a complicated course of febrile neutropenia (gram-negative bacteremia or septic shock). In the ROC curve analysis the maximal IL-10 and IL-10 at the onset of fever were associated with any bacteremia, gram-negative bacteremia, septic shock, and to the combined endpoint of gram-negative bacteremia or septic shock (Study III) (*Table 3*).

In logistic regression analysis with standardized variables IL-10 at the onset of fever showed strongest association with a complicated course of febrile neutropenia compared to earliest PCT, IL-6, and CRP values. Doubling of standardized value of IL-10 on day 0 increased the risk of complications to 3.1-fold (95% CI 1.6 - 5.9) (Study III) (*Table 4, Fig. 7*).

IL-10 performed best with a cut-off value of ≥37 ng/L (sensitivity 0.71, specificity 0.82, PPV 0.52, and NPV 0.92) (*Table 5*). The combination of PCT (with a cut-off of ≥0.13 μg/L) with IL-10 slightly increased prognostic value compared to their performance as single biomarkers (*Fig. 8*). The level of IL-10 normalized in two days on average. The changes in IL-10 levels from the onset of fever to day 1 were not associated with complications (Study III). In study IV there was a correlation between 90-day mortality and IL-10 on days 2 and 3. Youden's index of IL-10 on day 0 - 1 from the onset of fever predicting gram-negative bacteremia or septic shock was quite high (0.79) (Study III) (*Table 5*).

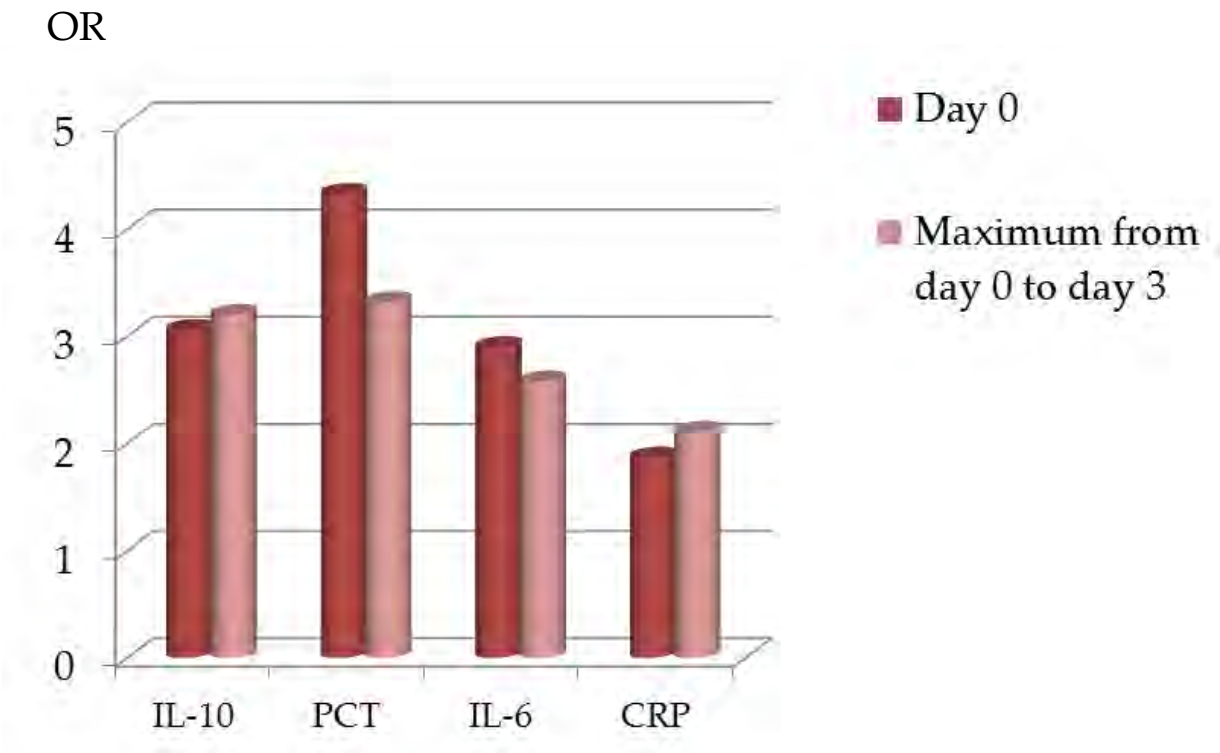

Figure 7. Odds ratios (OR) for interleukin-6 (IL-6), interleukin-10 (IL-10), procalcitonin (PCT), and C-reactive protein (CRP) for complications of neutropenic fever (study III). Logistic regression, age, sex, and hematological malignancy (AML vs. ASCT) included in the multivariate analysis. Values standardized for IL-10, PCT, IL-6 and CRP. See also *Table 4*.

Table 4. Interleukin-6 (IL-6), interleukin-10 (IL-10), procalcitonin (PCT), and C-reactive protein (CRP) for complications of neutropenic fever (bacteremia and/or septic shock) in 100 hematological patients (study III). Logistic regression, age, sex, and hematological malignancy (AML vs. ASCT) included in the multivariate analysis. Values are standardized for IL-10, PCT, IL-6 and CRP. See also *Figure 7*.

	Wald's test value	Odds ratio (95% CI)	*p*-value
Values on day 0			
IL-10	11.5	3.06 (1.60 – 5.85)	0.001
PCT	8.8	4.33 (1.65 – 11.39)	0.003
IL-6	8.3	2.90 (1.41 – 5.97)	0.004
CRP	6.5	1.87 (1.16 – 3.03)	0.011
Maximal values from day 0 to day 3			
PCT	12.8	3.20 (1.69 – 6.07)	<0.001
IL-6	10.8	3.32 (1.62 – 6.78)	0.001
IL-10	10.8	2.58 (1.47 – 4.54)	0.001
CRP	7.9	2.11 (1.25 – 3.53)	0.005

Table 5. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of early levels of interleukin-6 (IL-6), interleukin-10 (IL-10), procalcitonin (PCT), and C-reactive protein (CRP) for a complicated course of neutropenic fever in 100 hematological patients (study III). The values represent maximal plasma values of each variable from day 0 to day 1 from the onset of fever. Best cut-offs were determined by the Youden's indices based on receiver operating characteristic curve analyses.

Cut-off	Sensitivity	Specificity	PPV[a]	NPV[b]	Youden's index[c]
		Bacteremia or septic shock (n = 21)			
IL-6 ≥ 330 ng/L	0.43	0.95	0.69	0.86	0.38
IL-10 ≥ 37 ng/L	0.71	0.82	0.52	0.92	0.54
PCT ≥ 0.13 μg/	0.95	0.53	0.36	0.98	0.48
CRP ≥ 84 mg/L	0.62	0.63	0.31	0.86	0.25
		Gram-negative bacteremia or septic shock (n = 10)			
IL-6 ≥ 330 ng/L	0.60	0.92	0.46	0.95	0.52
IL-10 ≥ 37 ng/L	1.00	0.79	0.34	1.00	0.79
PCT ≥ 0.13 μg/L	1.00	0.48	0.18	1.00	0.48
CRP ≥ 84 mg/L	0.80	0.62	0.19	0.96	0.42

[a] Positive predictive value.
[b] Negative predictive value.
[c] Youden's index = [sensitivity − (1 − specificity)].

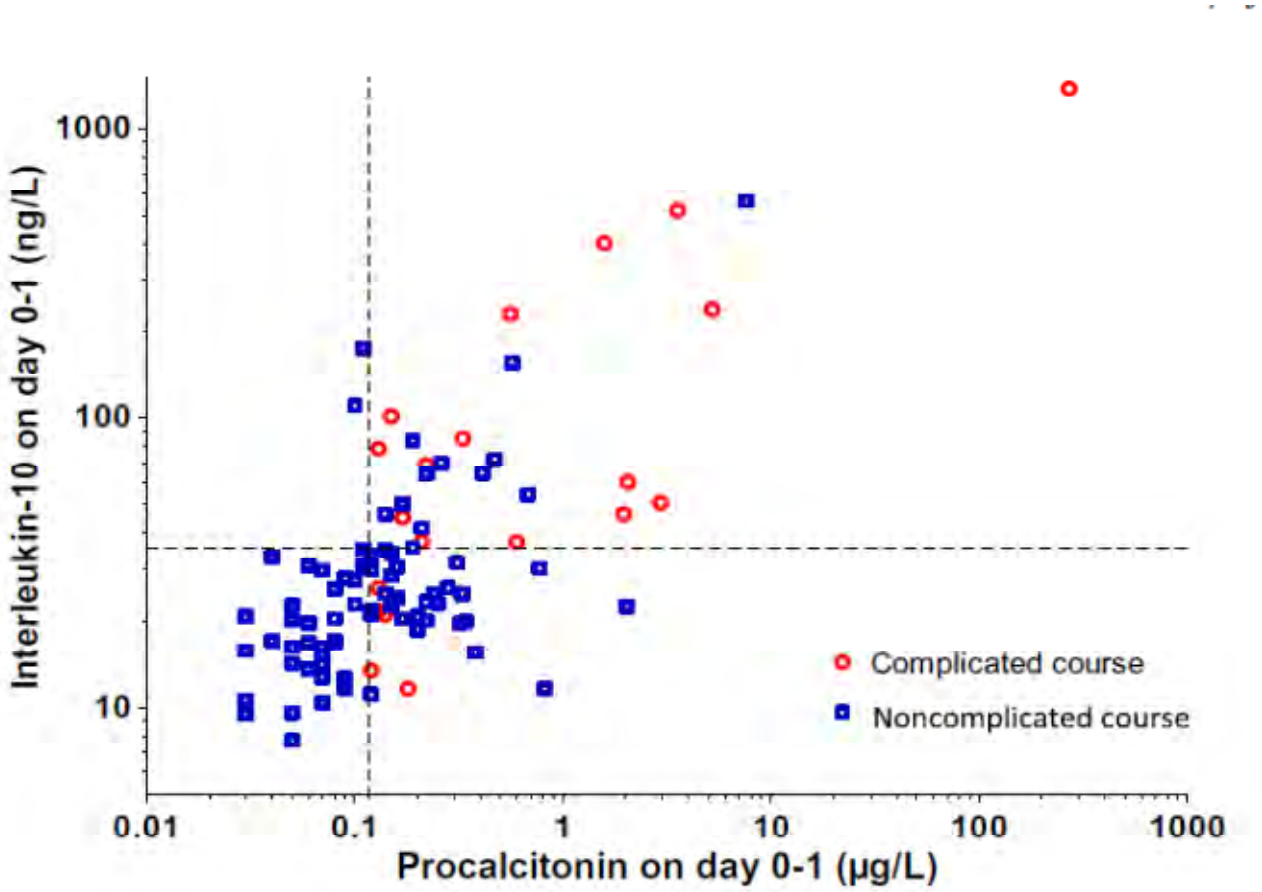

Figure 8. The maximal concentrations of procalcitonin (x-axis) and interleukin-10 (y-axis) during days 0–1 used as a combination improved prognostic value. The proportion of true positive (upper right quadrant) out of all test positive results increases. Complicated episodes of neutropenic fever defined as bacteremia or septic shock are marked with red circles and non-complicated episodes with blue squares. (III)

5.2.6 SuPAR

The suPAR levels on day 1 and day 3 and the maximal suPAR (day 0 – day 3) predicted complications defined as gram-negative bacteremia or septic shock (p=0.002). SuPAR predicted a complicated course of neutropenic fever also in the repeated measures analysis. SuPAR did not, however, predict gram-positive bacteremia or any bacteremia. Levels of AML patients and ASCT recipients did not differ. Increases in suPAR levels did not predict complications of neutropenic fever (Study IV).

In the ROC curve analysis day 1 suPAR predicted complications (gram-negative bacteremia and/or septic shock), but in comparison to IL-10 and PCT the area under the curve (AUC) for suPAR was smaller (*Fig. 9*). Maximal suPAR predicted the combined endpoint of gram-negative bacteremia, septic shock, or 90-day mortality. The optimal cut-off level based on Youden´s index for suPAR was 3.8–4.0 µg/L (sensitivity 66.7%, specificity 73.9%, PPV 20.7%, NPV 95.6%) (Study IV).

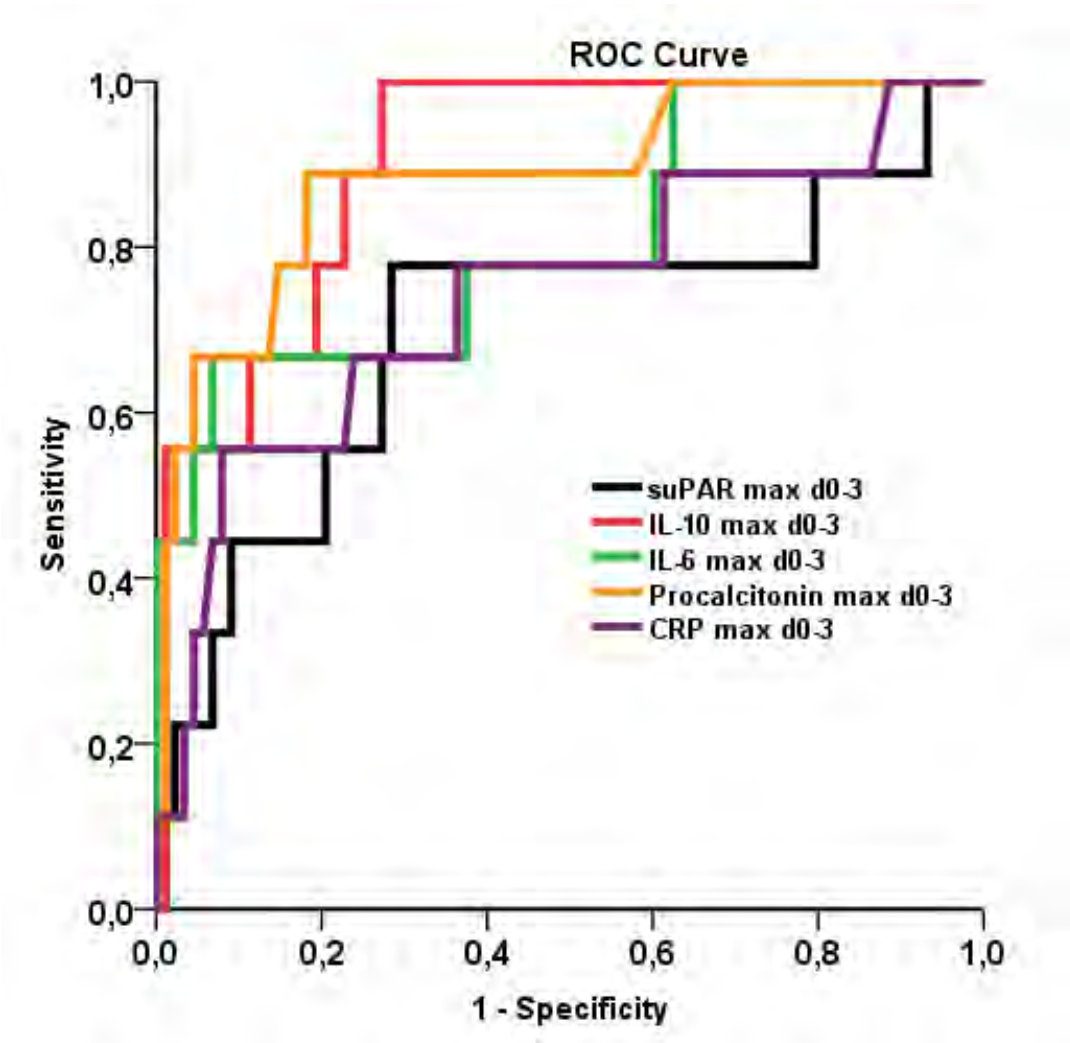

Figure 9. The receiver operating characteristic curve for suPAR on days 0-3 in predicting gram-negative bacteremia or septic shock compared with the other biomarkers (IL-10, IL-6, PCT, and CRP). IL, interleukin; PCT, procalcitonin; CRP, C-reactive protein.

5.3 CORRELATIONS BETWEEN THE BIOMARKERS

PTX3 correlated with CRP on the same day and following day from day 0 to day 3, with all *p*-values <0.001 (Study I). IL-6, IL-10, PCT and CRP correlated with each other on day 0, and the correlation was stronger between CRP and IL-6 ($r = 0.649$, $p < 0.001$) than between CRP and IL-10 ($r = 0.358$, $p < 0.001$). Moreover, the correlation was stronger between PCT and IL-10 ($r = 0.471$, $p < 0.001$) than between PCT and IL-6 ($r = 0.406$, $p < 0.001$) (Study III). There was no correlation between between suPAR and CRP, IL-6 or IL-10, but the correlation was close to significant between suPAR and PCT on day 0 (Study IV).

5.4 KINETICS OF THE BIOMARKERS

The level of CRP continued to increase until day 2. The medians were 35 mg/L, 77 mg/L, 101 mg/L, and 97 mg/L for days 0, 1, 2, and 3, respectively. The maximal CRP was achieved on day 0 in 7%, on day 1 in 21%, on day 2 in 40%, and on day 3 in 32% of the patients (Study I).

PTX3 concentration increased from the beginning of the fever until day 2, dropping slightly on day 3 with medians of 9.0 μg/L, 11.8 μg/L, 15.1 μg/L, and 12.5 μg/L, on days 0, 1, 2, and 3, respectively. Only the rise from day 0 to day 1 was statistically significant. Compared to CRP levels maximal PTX3 levels were reached a little earlier (Study I) (*Table 6*).

Table 6. Percentage of subjects reaching the peak pentraxin 3 (PTX3) and C-reactive protein (CRP) levels by day from the onset of neutropenic fever ($p = 0.035$ for the difference by the chi-square test for trend).

Peak at	PTX3	CRP
Day 0	14 %	7 %
Day 1	31 %	21 %
Day 2	28 %	40 %
Day 3	27 %	32 %

The kinetics of IL-6, IL-10, and PCT are shown in *Fig. 10* and in study III (supplementary table). For IL-6 the median was highest on day 1 in the groups of gram-negative bacteremia, gram-positive bacteremia, and in patients without bacteremia or septic shock, the group with septic shock had highest median IL-6 on day 0. The median IL-10 peaked on day 0 in the group of gram-negative bacteremia. The medians differed only a little in the other goups.

The median PCT values increased modestly in the group without bacteremia or septic shock and only moderately in the group of gram-positive bacteremia, with peaks on days 2 and 3, respectively. In patients with gram-negative bacteremia the median PCT rose on day 1 (0.6 μg/L) and reached its peak on day 3. In septic shock the median PCT was high throughout the study period from day 0 to day 3, 1.7 μg/L, 2.5 μg/L, 2.6 μg/L, and 2.3 μg/L, respectively (Study III). SuPAR levels were very stable, and alterations in daily levels were marginal (Study IV).

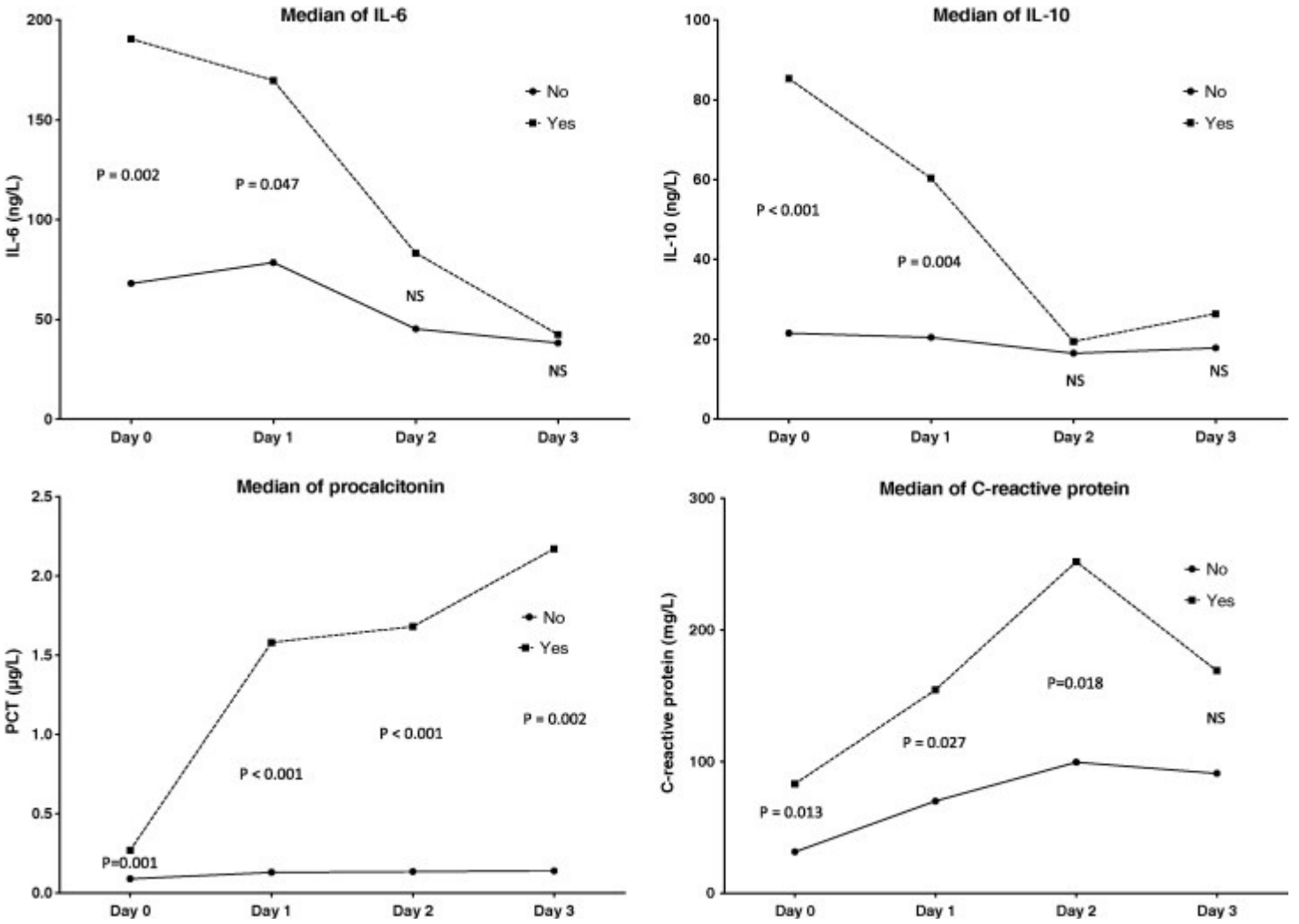

Figure 10. Kinetics of the biomarkers IL-6, IL-10, PCT, and CRP on days 0 – 3. Medians and significant *p*-values for gram-negative bacteremia or septic shock vs. no are shown for each day (study III). *P*-values stand for testing null hypothesis that distributions are equal between the groups. IL, interleukin; PCT, procalcitonin; CRP, C-reactive protein.

6 Discussion

6.1 MAIN FINDINGS (I-IV)

The present studies encompassed the phase of early neutropenic fever in 103 patients with AML or ASCT, focusing on the diagnostic and prognostic value of PCT, PTX3, IL-6, IL-10, and suPAR, comparing them with CRP. Special attention was paid to the kinetics of the biomarkers and their roles in the management of patients with neutropenic fever.

Nineteen percent of the patients had bacteremia, 13% gram-positive and 6% gram-negative. The overall infection-related mortality was only 3% during the first febrile period. PCT measured at the start of the fever predicted major complications of neutropenic fever, gram-negative bacteremia and septic shock throughout the study period (Study III). PTX3 predicted bacteremia and septic shock better than CRP, but only if the dissimilar cutoffs depending on the underlying malignancy were taken into account. (Studies I, II). Early IL-6 and IL-10 levels were clearly associated with complications with their levels then decreasing within few days (Study III). Furthermore, we observed that combining PCT and IL 10 could slightly improve the early detection of complications (Study III). SuPAR on day 1 and its peak value were also associated with complications of neutropenic fever (Study IV). CRP rose slightly more slowly than PTX3 (Study 1). As a predictive biomarker it was slightly inferior to PCT, IL-6 and IL-10 (Study III).

6.2 POPULATION AND METHODS (I-IV)

The setting of this study gave some advantages when compared with studies conducted, e.g., in emergency departments (ED) or intensive care units (ICU). All of our carefully monitored patients were at the specialized hospital ward already during the neutropenic period before the onset of fever and were immediately assessed when the fever rose. The first study samples were taken at the onset of neutropenic fever, which is one of the strengths of this study. The patients were thoroughly examined daily and essential parameters were frequently measured and documented already days before the febrile episode. Thus it was possible to have the blood samples taken at the very beginning of the fever compared with much later sampling in ED and ICU settings. The study population was quite homogenous, and most were otherwise healthy adults. The study was prospective and endpoints were concrete and clearly determined (any or specified bacteremia, septic shock, death, and their combinations). The ward staff was highly competent and experienced in managing neutropenic patients with evolving infections and possible complications, making episodes comparable in terms of management.

However, the setting and the study population had intrinsic shortcomings. It is not entirely clear what are the effects of hematological malignancies, intensive chemotherapy, and neutropenia to the secretion and levels of biomarkers. From this perspective, measurement of the baseline levels could facilitate later interpretation of these biomarkers. The patients were frequently given medication affecting the rise of fever like paracetamol and non-steroidal anti-inflammatory drugs. Furthermore, the threshold of 38.0°C for fever is somewhat artificial, and the infectious processes have most likely advanced before that in many cases.

CRP was collected and analyzed as a routine procedure at the hospital. The study samples were collected at the same time and were frozen for later analysis. Commercial analysis kits were used for all experimental biomarkers making results reproducible and

reliable. All samples for each single biomarker were analyzed together and, if several runs were needed, between-run variations were minor. It is, however, not thoroughly clear how these biomarkers are distributed and eliminated, e.g., which proportion is bound to carrier proteins or seen free in the circulation, and what are the determinants of the binding rate, neutralization, and clearance. The origin, induction, distribution, elimination, and half-life of, e.g. PTX3 and suPAR are still partly unclear. Revealing the best time of sampling for distinct biomarkers was a central goal of this study.

6.3 COURSE OF NEUTROPENIC FEVER (I-IV)

The incidence of bacteremia in our hematological patients with neutropenic fever was less than 20%, low compared to incidence reported in previous studies in ASCT recipients (109), and especially in AML patients (89, 109, 116). The proportions of gram-negative and gram-positive pathogens found in blood cultures were in line with most recent studies in neutropenic fever (101, 109, 116), with a dominance of gram-positive bacteria. The most common isolated pathogen was *Staphylococcus epidermidis*. There were no invasive fungal infections detected in this population, likely because of common use of antifungal profylaxis and empirical antifungal therapy.

The incidence of septic shock was 5%, mortality in septic shock was 40%, and early infection-related mortality was 3%, low or similar in comparison with most previous studies in adult patients with neutropenic fever (52, 97, 101, 109, 116). The number of deaths (3%) was low, probably because of the intensive monitoring, empiric broad-spectrum antibiotics, antibiotic prophylaxis in NHL patients receiving ASCT, and early supportive care of good quality. The low mortality decreased the statistical power for estimating predictive value of these biomarkers and, therefore mortality was not used as an endpoint with the exception of study IV.

By chance, there was an exceptional influenza pandemic (H1N1 2009) taking place during the study period, which caused an unfortunate death in one of our ASCT patients due to respiratory failure. The pathophysiological events leading to this specific death were probably basically very different from, e.g., the classical gram-negative sepsis and septic shock. This might have had an impact on the results from statistical analysis of the biomarkers studied here.

6.4 C-REACTIVE PROTEIN AND PENTRAXIN 3 (I AND II)

C-reactive protein was measured in all neutropenic patients with fever and it was considered as a gold standard in the assessment of the actual study biomarkers. Early CRP did not predict bacteremia and the levels did not differ in survivors and non-survivors. However, CRP was associated with development of septic shock and with increasing severity of complications in patients with neutropenic fever. These findings are parallel with most previous studies (134, 136, 138, 139) and the current understanding of the physiological role of CRP in inflammatory activity (22, 30) and indicate a moderate value for CRP in the management of neutropenic fever.

In study I PTX3 predicted complications of neutropenic fever, defined as bacteremia or septic shock, already at the onset of fever. PTX3 levels were also associated with increasing severity of complications and the concentrations were especially high in non-survivors. These findings are in line with the existing data, including also Finnish data, on PTX3 as a predictor of bacteremia, septic shock, and lethal outcome in non-neutropenic patients with sepsis (10, 148-151). Previous data on neutropenic fever are very scarce, but are in accordance with our results (13).

When the kinetics of PTX3 was explored, PTX3 correlated with CRP levels on the same day and the next day and rose to its peak earlier than CRP. In this analysis PTX3 was not superior to CRP in predicting complications of neutropenic fever.

PTX3 levels were higher in NHL patients with ASCT than in AML patients, and the optimal cut-offs for predicting complications differed significantly between these groups. Neutropenia was longer in AML patients, but deeper in patients with NHL and ASCT, possibly explaining part of the ten-fold difference in concentrations. On the other hand, the concentrations of CRP and the number of patients with high PCT levels did not differ between these groups, suggesting that PTX3 is basically different from these more traditional sepsis biomarkers. Glucocorticoid treatment as a part of lymphoma treatment and as a probable cause of elevated PTX3 by some studies (10, 12), could partly explain different PTX3 levels in patients with NHL and AML, though there is usually a significant period of time between the intensive lymphoma treatment and the following episode of neutropenic fever. Anyway, when the effect of the malignancy was taken into account PTX3 was superior to CRP in predicting complications of neutropenic fever, which is a new and interesting finding.

In summary, PTX3 predicts complications of neutropenic fever and rises earlier than CRP, probably because of its distinct biology (12, 147). Compared to CRP PTX3 was considered to be of limited diagnostic value, but better and faster in predicting mortality in critically ill patients in a recent review (220). The drawback in using PTX3 as a biomarker of neutropenic fever is the dependence of PTX3 on the underlying hematological malignancy and that PTX3 is expensive and still an experimental biomarker, not readily available at all times. In forthcoming studies the dependence of PTX3 to the background malignancy should be taken into account.

6.5 PROCALCITONIN, IL-6 AND IL-10 (III)

In this study PCT ultimately performed the best of all the biomarkers. It had a sustained ability to detect complications of neutropenic fever at all time-points (d0-d3). At the onset of fever PCT was the best indicator of developing septic shock compared to all other studied biomarkers. Early and maximal PCT also had a good diagnostic value for bacteremia, especially gram-negative bacteremia. Similar results have been reported from previous studies in patients with neutropenic fever (105, 111, 131, 134, 135, 137, 138, 169-172, 221). The sensitivity and stability of PCT make it attractive for clinicians, but requires a different interpretation. PCT is not just “a faster CRP” rising in any infection or other inflammatory condition but differentiates possibly the most severe conditions. There is enough evidence for the recommendation to wider use of PCT (159) also in hematological patients with neutropenic fever.

IL-10 rose rapidly and early levels predicted well bacteremia, especially gram-negative bacteremia, and complications like septic shock. These results are parallel with previous data in patients with neutropenic fever (143, 193, 204, 205). Use of PCT and IL-10 in combination seemed promising in detecting more complicated cases than either biomarker alone, which is a new finding. The concentrations of IL-10 decreased rapidly after the first days. IL-10 should be measured very early after the first signs of infection making its use feasible in patients who are already at hospital.

Early IL-6 levels predicted bacteremia, especially gram-negative bacteremia, and were associated with complications as has been seen in most previous studies in patients with neutropenic fever (135, 137, 138, 142, 171, 190, 192, 193). IL-6 performed best on days 0 and 1, but also maximal IL-6 value was associated with complications. The peak level of IL-6 was reached at the onset of fever in the group with septic shock and the next morning in

the other groups. The concentrations of IL-6 and IL-10 also decreased rapidly which is in line with previous clinical studies and the biology of these early cytokines (41, 45, 193).

Strong correlations existed between IL-6 and CRP on day 0, which is consistent with known induction of secretion of CRP by IL-6. Interestingly, a correlation was also found between PCT and IL-10 on day 0. This has not been found before and may be explained by a similar stimulus inducing secretion of both molecules from several sources.

The cut-off levels obtained in this study were similar to those found in previous studies for IL-6, IL-10, and CRP (138, 190, 205), but not for PCT (111, 135, 138, 167, 170). This could result from early sampling in our study as we caught the rise of cytokines close to their peak, while the secretion of acute phase proteins was just at its beginning (*Fig.* 11).

In addition to PCT, IL-6 and IL-10 demonstrated capability as early biomarkers for detecting complications of neutropenic fever.

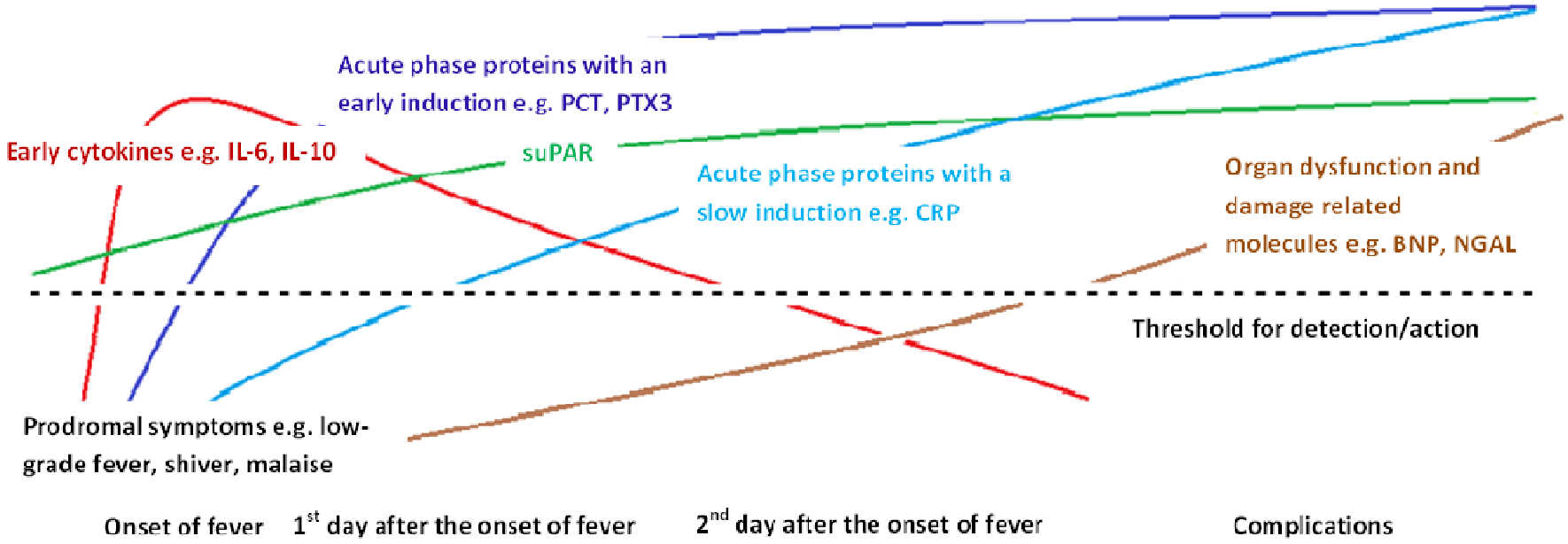

Figure 11. Kinetics of the archetypes of biomarkers and soluble urokinase-type plasminogen activator receptor (*suPAR*) in early neutropenic fever caused by a bacteremic infection, a schematic view. *IL*, interleukin; *PCT*, procalcitonin; *PTX3*, pentraxin 3; *CRP*, C-reactive protein; *BNP*, B-type natriuretic peptide; *NGAL*, neutrophil gelatinase-associated lipocalin.

6.6 SUPAR (IV)

This study found an association between suPAR and the risk of gram-negative bacteremia, septic shock and 90-day mortality in hematological patients with neutropenic fever. The diagnostic potential of suPAR compared with CRP, IL-6, IL-10, and PCT was inferior. This observation is consistent with most previous studies in non-neutropenic patients where suPAR has been better in prognostic than diagnostic use in sepsis (16, 158, 206, 209, 210, 213). The existing data in neutropenic fever is scarce and somewhat conflicting (17, 18).

Clear correlations with other biomarkers were not found, although the correlation was close to significant with PCT. There was no difference in suPAR levels between AML patients and those with ASCT. The best cut-off level for detecting complications was found to be lower than in previous studies in non-neutropenic (16, 206) and neutropenic febrile patients (17). Within-subject alterations in suPAR levels had no significant association with complications. SuPAR seems of limited value in the diagnostics of acute conditions. However, in a previous study with patients with neutropenic fever the concentrations of suPAR started to rise before fever was established (17), possibly explaining our findings and indicating need for different timing of samples than we had.

Though questionable in regard to its diagnostic potential, suPAR could have rather unique prognostic capacity in hematological patients as in a variety of other conditions. This may reflect predisposition to complications and increased risk of death probably associated with chronic low-grade inflammation (14). Nonetheless, more studies are needed to establish the role of suPAR.

6.7 KINETICS OF THE BIOMARKERS (I-IV)

This project provided a rare opportunity for observing the kinetics of early markers of inflammation right at the beginning of the neutropenic fever. The setting is the single most important advantage of our study series making timely sampling possible and biomarker levels between subjects comparable. Taking into account the distinct kinetics of various biomarkers is essential in the interpretation of biomarker levels. Biomarkers can be roughly divided into early cytokines, e.g., IL-6 and IL-10, early induced acute phase proteins, e.g., PCT and PTX3, slowly-induced acute phase proteins, e.g., CRP, and then organ damage or dysfunction-related molecules like creatinine and troponin. SuPAR does not seem to fit into any of these categories (4, 158) (*Fig. 11*).

Early cytokines perform best at the very early onset of infectious symptoms, or even during prodromal symptoms like low-grade fever, shiver, or malaise. Based on our study findings PCT rises slower in comparison to IL-6 and IL-10 and functions best during the first three days from the onset of fever, however less well right at the onset of fever than next day. PTX3, in turn, is faster than the related molecule CRP. APPs reach the peak level later than cytokines, obviously because the latter is induced by the former. Among APPs, the difference in kinetics of CRP compared to that of PCT or PTX3 probably follows from the source of the molecule; CRP is almost entirely produced by the limited capacity of liver, while the other two molecules are produced throughout the body by several types of cells if the stimulus is intense enough. Our findings in that aspect are also in line with previous observations (4, 158).

7 Future Perspectives

"It is far more important to know what person the disease has than what disease the person has."

- *Hippocrates (c. 460 BC – 370 BC)*

Biomarkers definitely have a significant role in the management of patients with neutropenic fever. Microbiological identification of the pathogen allows only partial prediction of the course of the illness, while biomarkers give unique information about the processes taking place in the host.

The outcome of sepsis is influenced by numerous factors, including age, co-morbidities, hematological malignancy, genetic polymorphisms of the immune system, the pathogen, localization of the infection, phase and nature of the host reaction, and the treatment (222). It seems hard to find a single biomarker to fulfill the needs and expectations of a clinician in variable situations, although PCT performs rather well in infectious episodes (158, 159).

It is not clear why PCT, and interleukins 6 and 10, are so scarcely used in Finland in the management of hematological patients with neutropenic fever. High cost and limited availability are possible explanations. Another explanation is lack of experience, which grows only through use.

Most likely the best results are obtained when the patients are individually profiled and a selection of biomarkers is used in a planned manner in right time frames (223). Effective early risk-stratification and assessment of response to treatment using biomarkers could enable targeted measures of supportive care and more sparing use of antibiotics. This would hopefully reduce mortality of hematological patients needing intensive chemotherapy and diminish problems related to antibiotic resistance.

The physiological alterations in early infections, if detected before the rise of fever, could revolutionize sepsis diagnosis among inpatients and could even provide tools to be used in an outpatient setting (224, 225). Methods based on, e.g., heart rate, muscle activity, and breathing could reveal evolving problems even before the onset of fever and could enable early initiation of empirical treatment. Such monitoring of a hematological neutropenic patient would be a convenient and inexpensive way to improve care.

MicroRNA (miRNA) is an interesting class of molecules that is involved in post-transcriptional regulation of gene expression. Preliminary data have been published concerning the role of miRNA as an early inflammatory stimulus in sepsis, involved in NF-κB activation after TLR-mediated pathogen recognition. Several miRNA have been identified that could be used as diagnostic biomarkers of sepsis (226, 227), but no studies have been conducted in hematological patients with neutropenic fever.

The detection of pathogens in blood with rapid polymerase chain reaction (PCR) methods has been studied also in patients with neutropenic fever, with promising results (228-230). Instant detection of the infecting agent provides an obvious advantage for initiating adequate and timely treatment. This approach is already used in some centers, though in limited clinical situations.

7.1 COMBINED METHODS

In patients with a hematological malignancy and neutropenic fever a method combining MASCC (Multinational Association of Supportive Care in Cancer) score and CRP was able

to identify those at high or low risk of death within 30 days (231). In another study Kofoed and colleagues used a six-marker test (including PCT, CRP, and suPAR) to discriminate those with bacterial infection among patients with SIRS, and achieved improved diagnostic accuracy over any single marker (232). Also Park and colleagues developed a multi-marker model for risk stratification for neutropenic fever in patients with hematological diseases and reported promising results (233).

A method combining several different aspects in severe sepsis, the PIRO staging system (234), is an attempt to estimate total risk. It is composed of predisposing factors (P), the nature of the infection (I), the host response (R), and indicators of organ dysfunction (O). These include, e.g., genetic polymorphisms, pre-existing conditions, microbiological and clinical features of the infection, measurement of immune response, and signs of evolving complications.

7.2 ROLE OF GENETICS

Genetic profiling covering fundamental immune mechanisms like pathogen recognition, cytokine production and their receptors, and complement and coagulation systems could provide interesting background for understanding of the factors leading to adverse outcomes in individuals with a specific pathogen and treatment (235). Some of the genetic factors predisposing to infectious complications have already been identified (179).

Genetic variability has been reported in the HLA-system and in the production of molecules used or studied as biomarkers, including TNF, CRP, PTX3, lipopolysaccharide binding-protein, IL-6, and IL-10 (22, 30, 42, 45, 180, 236, 237). Some genetic variants have also been associated with susceptibility to specific pathogens, with higher or lower risk of developing complications (72, 147, 237-242).

Testing the feasibility of genetic profiling in clinical practice would require large studies that are challenging to carry out. Genetic studies would, however, be especially useful for tailored prophylactic measures targeted to selected individuals, e.g., to patients with hematological malignancies and planned intensive chemotherapy. Advances in understanding of the genome shifts medicine towards individualized, tailored patient management.

8 Conclusions

1. PTX3 was an early biomarker for bacteremia and evolving septic shock in patients with neutropenic fever. The concentrations of PTX3 were elevated already at the onset of fever. PTX3 was especially high in the non-survivors (I).

2. PTX3 concentrations were dependent on the underlying malignancy. Significantly higher cut-off values were observed in ASCT recipients with NHL than in AML patients (II).

3. PCT was a powerful biomarker in neutropenic fever, with an early rise and persisting diagnostic value for bacteremia and septic shock from the onset of fever throughout the whole study period (III). IL-10 and IL-6 were early predictors of complications of neutropenic fever, but their concentrations decreased rapidly. IL-10 combined with PCT could slightly improve diagnostic accuracy (III).

4. SuPAR levels were associated with complications of neutropenic fever, but performed inferior to the other studied biomarkers (IV).

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Matti Vänskä

Biomarkers of Sepsis in Neutropenic Hematological Patients

Neutropenic period after intensive chemotherapy for hematological malignancies is often complicated with sepsis and severe infections are the leading cause of treatment-related mortality in these patients. New tools are needed to reduce sepsis mortality in hematological patients with neutropenia. Biomarkers may be helpful in the evaluation of sepsis revealing excessive host response, evolving organ dysfunction, and microbial etiology. We studied early levels of C-reactive protein, procalcitonin, interleukin-6, interleukin-10, pentraxin 3, and soluble urokinase-type plasminogen activator receptor using serial measurements in adult hematological patients with neutropenic fever to analyze their usefulness as diagnostic and prognostic tools.

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