SARI HÄMÄLÄINEN

Severe Sepsis in Neutropenic Haematological Patients

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Severe Sepsis in Neutropenic Haematological Patients

Doctoral dissertation

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ABSTRACT

Neutropenic fever and septic infections are common in patients with acute myeloid leukaemia (AML) and in autologous stem cell transplant (ASCT) recipients. Severe sepsis is important cause of treatment-related morbidity and mortality in both patient groups. Readily available circulatory markers for severe sepsis could be useful.

This study evaluated epidemiology, microbiology and outcome of neutropenic fever and severe sepsis in haematological patients with special reference to the kinetics of C-reactive protein (CRP), vascular endothelial growth factor (VEGF) and amino-terminal pro-brain natriuretic peptide (NT-proBNP).

Retrospective series included 84 AML patients and 319 ASCT recipients. In the prospective series of 70 patients with the same diagnoses CRP, VEGF and NT-proBNP were determined at the beginning of the neutropenic fever and then daily.

In retrospective series, severe sepsis was found in 13% of AML patients and in 5% of ASCT recipients. In the prospective study, severe sepsis was found in 14% of febrile periods. Severe sepsis was associated with 27% mortality in AML patients and 53% in ASCT recipients. In the prospective study including careful supportive care, the mortality rate for severe sepsis was 15%.

Gram-positive microbes were more common in blood cultures than gram-negative ones. During 1996-2006 *Pseudomonas spp.* bacteraemia was found in 30% of neutropenic periods with severe sepsis in ASCT recipients.

VEGF concentrations were generally low. Less than 24 hours after the start of neutropenic fever the VEGF concentrations were higher in patients with severe sepsis than those without. Neither serial NT-proBNP nor CRP showed early predictive value for development of severe sepsis.

Despite of intensive interventions, mortality remained high in haematological patients who developed severe sepsis. Higher CRP values coincided with severe sepsis but could not predict it. VEGF was a more rapid indicator for severe sepsis than CRP. Neither serial NT-proBNP nor CRP showed early predictive value for development of severe sepsis.

Severe sepsis is frequent in neutropenic haematological patients and is associated with significant mortality. Careful patient monitoring and supportive care are needed to improve the outcome of severe sepsis in haematological patients.

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Pieni ja hento ote ihmisestä kiinni
Aivan sama tunne kuin koskettava tuuli
Pieni ja hento ote - siinä kaikki

Dave Lindholm
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Kuopio, December 2009.
ABBREVIATIONS

AML    acute myeloid leukaemia
ASCT   autologous stem cell transplantation
AUC    area under curve
BEAC   carmustine, etoposide, cytarabine and cyclophosphamide
BEAM   carmustine, etoposide, cytarabine and melphalan
BNP    brain natriuretic peptide
CD4    helper T-cell
CD8    cytotoxic T-lymphocyte
CI      confidence interval
CLL     chronic lymphocytic leukaemia
CRP    C-reactive protein
EBMT    the European Group of Blood and Marrow Transplantation
ESBL   extended-spectrum betalactamase
ELISA  enzyme-linked immunosorbent assay
HDT    high-dose chemotherapy
HL     Hodgkin lymphoma
IL     interleukin
ICU    intensive care unit
IDSA   Infectious Diseases Society of America
MM     multiple myeloma
MOF    multiorgan failure
MRSA   methicillin-resistant Staphylococcus aureus
NHL    non-Hodgkin lymphoma
NT-proBNP amino-terminal pro-brain natriuretic peptide
PaCO₂   arterial partial pressure of CO₂
PAMPs  pathogen-associated molecular patterns
PCR    polymerase chain reaction
PCT    procalcitonin
ROC    receiver operating characteristic curve
SIRS   systemic inflammatory response syndrome
SOFA   sepsis-related organ failure assessment
Th1    type-1 helper T-cell
Th2    type-2 helper T-cell
TLRs   toll-like receptors
SPSS   statistical package for the social sciences
S-TnT   serum cardiac troponin T
TNF-α   tumor necrosis factor-alpha
VEGF   vascular endothelial growth factor
VRE    vancomycin-resistant enterococci
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals.


IV. Hämäläinen S, Juutilainen A, Kuittinen T, Nousiainen T, Matinlauri I, Pulkki K, Koivula I, Jantunen E. Serum amino-terminal pro-brain natriuretic peptide in haematological patients with neutropenic fever: a prospective comparison with C-reactive protein [Submitted]
CONTENTS

1. INTRODUCTION ....................................................................................................................15

2. REVIEW OF THE LITERATURE .............................................................................................16

2.1. Infections in neutropenic haematological patients..........................................................15
  2.1.1. Bloodstream infections in patients with neutropenic sepsis ......................................17
  2.1.2. Infectious complications in patients with acute myeloid leukaemia ......................19
  2.1.3. Infectious complications in autologous stem cell transplant recipients ..............20

2.2. Sepsis and severe sepsis..............................................................................................21
  2.2.1. Pathophysiology of sepsis ..................................................................................23
  2.2.1.1. Innate immunity response ........................................................................23
  2.2.1.2. Adaptive immunity response ..................................................................24
  2.2.1.3. Complement system, coagulation and inflammation ..................................25
  2.2.1.4. Endothelium and inflammation .................................................................26
  2.2.1.5. Multiorgan failure ....................................................................................26
  2.2.2. Epidemiology of sepsis ...................................................................................27
  2.2.3. Microbiological aetiology of sepsis ....................................................................28

2.3. Markers for severe sepsis .............................................................................................29
  2.3.1. C-reactive protein .............................................................................................30
  2.3.2. Vascular endothelial growth factor ....................................................................33
  2.3.3. Amino-terminal pro-brain natriuretic peptide .......................................................34
  2.3.4. Other markers ...................................................................................................35
  2.3.4.1. Lactate .....................................................................................................35
  2.3.4.2. Procalcitonin ............................................................................................36
  2.3.4.3. Interleukins ...............................................................................................37

3. AIMS OF THE STUDY .............................................................
5.1.2. Microbiological findings and site of infection .......................................................... 55
5.1.3. Factors associated with severe sepsis ................................................................... 56
5.1.4. Serum C-reactive protein in relation to severe sepsis ........................................... 60

5.2. Serum vascular endothelial growth factor in patients with neutropenic fever: a comparison with C-reactive protein (III) ........................................................................ 60
5.2.1. Epidemiological features of severe sepsis ................................................................ 60
5.2.2. Microbiological findings and site of infection ......................................................... 60
5.2.3. C-reactive protein ............................................................................................... 61
5.2.4. Serum vascular endothelial growth factor ............................................................. 63

5.3. Serum amino-terminal pro-brain natriuretic peptide in patients with neutropenic fever: a comparison with C-reactive protein (IV) .................................................. 64
5.3.1. Epidemiological features of severe sepsis ............................................................ 64
5.3.2. Microbiological findings and site of infection ......................................................... 68
5.3.3. Factors associated with severe sepsis ................................................................... 68
5.3.4. C-reactive protein ............................................................................................... 68
5.3.5. Amino-terminal pro-brain natriuretic peptide ....................................................... 69
5.3.6. Association of amino-terminal pro-brain natriuretic peptide with fluid intake and previous cardiovascular disease ................................................................. 69

6. DISCUSSION ................................................................................................................... 75

6.1. Patients and methods .................................................................................................. 75

6.2. Epidemiological features and outcome of severe sepsis ............................................. 76
6.2.1. Patients with acute myeloid leukaemia ............................................................... 76
6.2.2. Autologous stem cell transplant recipients ......................................................... 77
6.2.3. Prospective study ............................................................................................... 78

6.3. Microbiological findings ............................................................................................ 78

6.4. Kinetics of C-reactive protein in patients with neutropenic fever (Studies I-II) .......... 80

6.5. Comparison of serum vascular endothelial growth factor and C-reactive protein in patients with neutropenic fever (Study III) ...................................................... 80

6.6. Comparison of serum amino-terminal pro-brain natriuretic peptide and C-reactive protein in patients with neutropenic fever (Study IV) ...................................................... 82

6.7. Study limitations ........................................................................................................ 83

6.8. Concluding remarks .................................................................................................. 84
6.8.1. Implications for further research .......................................................................... 85

7. CONCLUSIONS .............................................................................................................. 87

8. REFERENCES .................................................................................................................. 88

ORIGINAL PUBLICATIONS I – IV
1. INTRODUCTION

Treatment of haematological malignancies has improved during recent decades. This is due to modern effective drugs and regimens but also due to improvements in supportive care. About 40% of younger patients with acute myeloid leukaemia (AML) are cured with intensive chemotherapy (1, 2). Relatively high percentage of patients with relapsed non-Hodgkin lymphoma (NHL) (3) or Hodgkin lymphoma (HL) (4) are cured by high-dose therapy supported by autologous stem cell transplantation (ASCT).

Intensive chemotherapy has certain disadvantages. Chemotherapy induces breakdown of mucosal barriers, usually leading to high risk of neutropenic fever, which is associated with development of severe sepsis and high mortality. Thus, early initiation of antimicrobial therapy (5-7) and other supportive measures are vital in the management of haematological patients receiving intensive chemotherapy. In spite of some obvious advances, the need for improvement remains.

Several studies have evaluated the epidemiology and prognostic factors of septic patients admitted to intensive care units (ICU) (8-10). However, only a minority of these patients has had haematological malignancies. Therefore, the risk factors for severe sepsis and outcome of sepsis in these ICU-based studies are not necessarily applicable for patients with neutropenic sepsis. In fact, there are very limited data available on the incidence, risk factors and outcome of severe sepsis in neutropenic haematological patients receiving intensive chemotherapy.

C-reactive protein (CRP) has traditionally been used to evaluate the severity of infection and also the response to antimicrobial therapy in patients with febrile neutropenia, even though its predictive value has been controversial (11-14). Other markers of septic infections that are also used to predict outcome in the ICU, like serum vascular endothelial growth factor (VEGF) (15) or amino-terminal pro-brain natriuretic peptide (NT-proBNP) (16) have not been prospectively evaluated in a haematological ward setting. Even though marked improvements have been observed in the treatment of severe sepsis in ICUs (8), there are only limited data available on careful patient monitoring and vigilant supportive care in haematological wards of patients with severe sepsis.

The aim of this study was to evaluate the epidemiological features, microbiological aetiology and outcome of severe sepsis in neutropenic haematological patients. Early prognostic markers for the development of severe sepsis were also evaluated. These markers included VEGF and NT-proBNP, with special reference to the kinetics of CRP. In addition, the purpose of the prospective treatment protocol was to improve the clinical management of septic neutropenic patients in haematological ward.
2. REVIEW OF THE LITERATURE

2.1. Infections in neutropenic haematological patients

Unfortunately, as long as patients are neutropenic it is likely that some of them will develop infectious complications

GP Bodey (17)

The mechanisms and pathophysiology of sepsis in neutropenic patients is similar to that in patients without neutropenia but with the exception of pancytopenia. Neutropenia enables the infection to progress in a more insidious and aggressive way. The role of neutrophils in septic infections is controversial. They are thought to be essential for the eradication of pathogens but at the same time, excessive release of oxidants and proteases by neutrophils might be responsible for tissue injury (18). In studies carried out with granulocyte colony-stimulating factor in patients with pneumonia the markedly increased neutrophil count was not deleterious but did not have obvious clinical benefits, either (19). Neutropenic patients with septic infections have higher mortality rates emphasizing the protective role of an appropriate acute inflammatory response (20).

Infections have been recognised as complications of leukaemia for the first time as early as in 1845 (21). Until 1948, there was no specific treatment for acute leukaemia (22). When very little was to do for the natural course of the disease, there were no major interest to study or understand the possibility of infectious complications. Although neutropenia is a common consequence of acute leukaemia, its role in infection was not fully recognised until the early 1960´s (17).

Patients with haematological malignancies are immunocompromised because of both the underlying malignancy and the therapy employed to manage it. Therapeutic interventions such as corticosteroids, stem cell transplant and radiotherapy also produce deficiencies in host defenses. Normal human skin and mucosal colonisation change during chemotherapy and hospitalisation. Within all this, neutropenia acts as a common risk factor for severe bacterial infections. It is estimated that at least 30-60% of neutropenic patients develop an infection, of whom 13-37% develop bacteraemia (23-26). During the last three decades the overall mortality rate due to septic infections in neutropenic patients has decreased from 21% to 7% (27), but febrile neutropenia still remains a major reason for significant morbidity and mortality in this patient group (28). The depth and duration of neutropenia is directly related to incidence
of serious bacterial infections. The risk of severe infection is significantly greater at low neutrophil counts (absolute neutrophil count <0.1x10^9/l) (29).

Neutropenia alters the host’s inflammatory response and makes the infection difficult to detect because the classic signs and symptoms of infection are often missing (30, 31). The endogenous pyrogen (IL-1) is produced by mononuclear cells, not by granulocytes, and these mononuclear cells include also fixed-tissue macrophages that persist after chemotherapy. This explains the presence of fever despite the otherwise poor inflammatory response in neutropenic patients (32). Fever is the principal sign of infection, and it is often the only evidence of infection in neutropenic patients. In clinical practice, careful history of possible symptoms and physical examination to seek for subtle signs of infection remain essential. The prompt initiation of empirical antibiotics has been the most important advance in the treatment of febrile neutropenic patients (33) – prior to this policy the mortality from gram-negative infections was as high as 80% (29, 34). The importance of early antimicrobial treatment for febrile neutropenia was for the first time demonstrated in the study of Schimpff et al (35). Today the overall survival rate for febrile neutropenic patients is over 90%.

In Finland empirical antimicrobial treatment in febrile neutropenic patients includes usually a combination of a third generation cephalosporin and an aminoglycoside. If *Pseudomonas aeruginosa* bacteraemia is suspected, antimicrobial treatment should be initiated with antipseudomonal penicillin together with an aminoglycoside. Vancomycin is only rarely used empirically (36).

### 2.1.1. Bloodstream infections in patients with neutropenic sepsis

Over the last three decades, several studies have demonstrated a shift in the aetiology of bacteraemic infections from predominance of gram-negative rods to gram-positive cocci (37, 38). Prior to the availability of methicillin, penicillin-resistant *Staphylococcus aureus* was the main threat to neutropenic patients and mortality rate from *Staphylococcus aureus* infections exceeded 50%. Methicillin became available during the 1960s, curing most neutropenic infections caused by *Staphylococcus aureus* (17). During the subsequent two decades gram-negative bacteria emerged as the main causative agents (39). Infections with *Pseudomonas aeruginosa* were common and were associated with a high mortality rate (40).

By the end of the 1980s and lately during 1990s, gram-positive microbes re-emerged (41, 42). The most common infective agent was coagulase-negative staphylococci, mainly *Staphylococcus epidermidis*. The cause for this change has not been clearly identified. Possible factors responsible for this process include widespread use of indwelling central
venous catheters, a higher incidence of severe oropharyngeal mucositis and bowel damage with the use of more intensive chemotherapy regimens and more profound and prolonged neutropenia. In addition, use of quinolone-based prophylactic antibiotics suppressing aerobic gram-negative microbes colonizing the gastrointestinal tract but failing to suppress sufficiently gram-positive flora and use of other antibiotics with selective pressure may be potential factors. Furthermore, administration of histamine H₂ receptor blockers or proton-pump inhibitors may promote infections by reducing gastric pH and promoting overgrowth of oropharyngeal gram-positive flora (43-45).

Mucositis, which is a well-known consequence of cytotoxic chemotherapy (46), predisposes patients to infections arising from patient’s own flora – primarily of oropharyngeal and gastrointestinal origin. Mainly because of this, clinically important gram-positive microbes include also viridans group streptococci (e.g. *Streptococcus milleri* or *Streptococcus mitis*), which belong to the normal flora of the oropharynx and gastrointestinal tract. Among neutropenic patients both the morbidity and mortality to streptococcal bacteraemia remains high (6, 47). Other new gram-positive microorganism include species belonging to *Enterococci*, *Stomatococci*, *Leuconostoc*, *Lactobacillus* and *Corynebacterium* species – microbes that were traditionally considered contaminants in blood cultures.

There is also an interesting difference in the incidence of gram-negative bacteraemias between developed and developing countries (48-51). In developing countries, the amount of gram-negative bacteraemias remains high, probably due to the less frequent use of central lines and prophylactic antibiotics (52, 53). This distribution may nevertheless be reversing since in some western centers the gram-negative microbes are making a comeback (48, 52, 54). Even though the general incidence of gram-negative microbes as a cause of bacteraemic infections in developed countries has declined, they are nonetheless still a problem. This is primarily because of their traditional association with high mortality rates and also the alarming rise of multidrug-resistant strains (49, 55). In recent study the distribution of bacteraemias in neutropenic cancer patients was gram-positive in 57%, gram-negative in 34% and polymicrobial in 10% (56). The mortality rates were 5%, 18% and 13%, respectively. The most common causative agents of gram-negative rods are *Klebsiella* and *Enterobacter* species as well as *Stenotrophomonas maltophilia* and *Burkholderia cepacia*, not forgetting *Pseudomonas species* (44, 57).
2.1.2. Infectious complications in patients with acute myeloid leukaemia

In patients with acute myeloid leukaemia (AML) intensive chemotherapy regimens enable complete remission in 80-85% of patients below 65 years of age. About 40% of patients are cured with intensive consolidation courses (2, 58).

Infections are the major cause of morbidity and mortality in patients with AML. The treatment protocol of AML induces long lasting periods of neutropenia, which predisposes patients to recurring infections. These arise mainly during the first course of induction chemotherapy. This may cause delays in consolidation therapy and increase the risk of leukaemia relapse. Also consolidation courses are associated with long periods of neutropenia and the risk of fatal infections both in adults and children (59, 60).

The infectious mortality during AML treatment ranges between 5.5 -13% (60-64). The initial source of infection often remains unknown and therefore the first antimicrobial therapy choice is empirical. Therapy is directed primarily against gram-negative microbes. Like in other septic neutropenic infections, the spectrum of causative pathogens has changed over the past decades. Coverage of coagulase-negative staphylococci is not necessary for the initial antimicrobial therapy. Coverage of oropharyngeal streptococci is sufficient when combination therapy or monotherapy with highly active third-generation cephalosporins or carbapenems are used (43).

In recent studies the amount of bloodstream infections among neutropenic AML patients has varied between 34% and 38% (24, 59, 65). In the study of Madani (24) the frequency of isolation of gram-positive cocci was 74.2% and gram-negative bacilli 12.1%. The most common pathogens in the gram-positive group were coagulase-negative staphylococci (34.8%) followed by Streptococcus viridans (22.7%) and in the gram-negative group Klebsiella pneumoniae (3%) followed by Enterobacter cloacae (1.5%). Ciprofloxacin prophylaxis was used during the neutropenic period. In spite of this there were still gram-negative bacteraemias. The amount of resistant microbes was not discussed. In this study, the site of infection was identified in 81% of all febrile episodes. Mucositis (21.7%), pneumonia (13.2%) and central venous catheter infection (12.4%) were the most common sites of infection. Bloodstream infections (37.9%) were commonly associated with cellulitis, mucositis and central venous catheter infection.

In a study analysing the infections occurring in newly diagnosed AML patients under 65 years of age the amount of bloodstream infections was 34% (59). During induction phase 13% of neutropenic patients had bloodstream infection caused by gram-negative rods and 21% by gram-positive cocci. The case fatality rates for gram-negative and gram-positive bacteraemias
were 10% and 8%, respectively. When the potential age-related differences in nosocomial infections between younger (<60 years old) and older leukaemia patients with neutropenic fever were evaluated, no significant age-related differences were found in the overall incidence of infections, nosocomial pattern, or overall outcome(66).

2.1.3. Infectious complications in autologous stem cell transplant recipients

High-dose chemotherapy (HDT) supported by ASCT is a commonly applied treatment for haematological malignancies, mainly lymphomas and multiple myeloma (MM) (67, 68). Annually more than 15 000 procedures are performed in Europe (69). The procedure is considered to be relatively safe with a low incidence of severe complications and transplant-related mortality. However, approximately 1-5% of neutropenic periods after HDT are complicated by fatal infections (70-74).

After ASCT, there is an unavoidable period of neutropenia, where the risk for infections is high. The conditioning regimen causes neutropenia and defects in mucosal and cutaneous barriers. Neutropenic periods are shorter than in AML treatment, but the intensity of treatment in ASCT recipients is greater and the mucosal damage may thus become more serious. Hence, mucositis and indwelling central venous catheters commonly lead to bacterial and fungal bloodstream infections. During the first 4 weeks after haematopoietic stem cell transplant, bacterial infections and invasive fungal infections predominate. Between 30 and 100 days after transplant, impaired cellular immunity increases the risk of viral infections (75).

The percentage of bloodstream infections varies between 12% and 20% in ASCT recipients (72-74, 76). As in all other neutropenic bacteraemias, gram-positive microbes cause at least half of the bloodstream infections occurring after ASCT. In a recent study 60.3% of the positive blood cultures were gram-positive cocci and 28.9% gram-negative rods (70). The most common isolates in the gram-positive group were coagulase-negative staphylococci (43.4%) followed by Streptococcus viridans (5.7%) and in the gram-negative group Stenotrophomonas maltophilia (10.1%), followed by Acinetobacter baumannii (6.9%). Of the 314 study patients 12 (3.8%) died because of infectious complications – 6/12 patients because of gram-negative bacteraemia (Stenotrophomonas maltophilia, Acinetobacter baumannii and Pseudomonas aeruginosa). In that study norfloxacin was used as a prophylactic antibiotic. During the study period (1994-2005) Pseudomonas aeruginosa was resistant to ceftazidime and aminoglycosides in half of the cases and to fluoroquinolones in 25-50% of the cases. Fluoroquinolone resistance seemed to diminish during the study period. The patients who died due to infection had markedly longer median time of severe neutropenia (70).
2.2. Sepsis and severe sepsis

Inflammation is a reactive state of the organism against disturbances of homeostasis with the goal of healing and repair of the injured tissue

JB Cone (77)

In 1991 the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM) compiled the current definition of sepsis as a systemic inflammatory syndrome in response to infection. When this was associated with hypotension, hypoperfusion or acute organ dysfunction sepsis was considered to be severe (78, 79). Before these definitions the terms sepsis, septicemia, sepsis syndrome and bacteraemia were used without precise characterisation.

When the ACCP/SCCM consensus conference released these definitions they also proposed a new term to describe inflammatory processes that occur in parallel with systemic infection (78). Systemic inflammatory response syndrome (SIRS) has several clinical manifestations, including abnormalities in body temperature, respiratory and heart rate.

The definition of SIRS has been noted to be controversial and challenging for clinical use. American and European critical care societies reconsidered the definitions of ACCP/SCCM in 2001. The decision was that although the concepts based on SIRS were too sensitive and indefinite, these were useful as a baseline construction for the diagnosis of sepsis (79). Subsequently, these definitions have been used widely both in clinical practice and in research worldwide (Table 1).
Table 1. Criteria for the systemic inflammatory response syndrome, sepsis and severe sepsis according to the Consensus Conference of the ACCP/SCCM (79)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome.</td>
</tr>
<tr>
<td></td>
<td>The systemic inflammatory response is manifested by two or more of the following criteria:</td>
</tr>
<tr>
<td></td>
<td>Fever (body temperature &gt; 38°C) or hypothermia (&lt;36°C)</td>
</tr>
<tr>
<td></td>
<td>Tachycardia (heart rate &gt; 90 beats/min)</td>
</tr>
<tr>
<td></td>
<td>Tachypnea (&gt;20 breaths/min) or PaC02 &lt; 4.3 kPa</td>
</tr>
<tr>
<td></td>
<td>Leucocytosis or leucopenia (white blood cell count &gt; 12,000 or &lt; 4000/mm³ or &gt; 10% immature forms</td>
</tr>
<tr>
<td>SEPSIS</td>
<td>Presence of SIRS in response to infection. SIRS is manifested by two or more of the criteria mentioned above</td>
</tr>
<tr>
<td>SEVERE SEPSIS</td>
<td>Sepsis associated with organ dysfunction, hypoperfusion or hypotension. Organ dysfunction and hypoperfusion abnormalities may include, but are not limited to lactatic acidosis, oliguria or alteration in mental status</td>
</tr>
<tr>
<td></td>
<td>Septic hypotension is defined as a systolic blood pressure &lt; 90 mmHg or a decrease in systolic blood pressure by 40 mmHg or more from the baseline</td>
</tr>
</tbody>
</table>

Abbreviations: PaC02, arterial partial pressure of C0₂; kPa, kiloPascal.
2.2.1. Pathophysiology of sepsis

The microorganisms that seem to have it in for us...turn out...to be rather more like bystanders...It is our response to their presence that makes the disease. Our arsenal for fighting off bacteria are so powerful...that we are more in danger from them than the invaders Lewis Thomas (80)

The English word sepsis is derived from the Greek term σεπτικός for rotten or “to make putrid”. Sepsis, defined as the systemic host response to microorganisms in previously sterile tissue, is a syndrome related to severe infections. In its severe form, sepsis is characterised by end-organ dysfunction often far away from the primary site of infection.

The pathophysiology of sepsis is understood as a continuum. The normal response of host to infection is both to identify pathogen invasion and to start tissue repair. The immune response consists of innate immunity and adaptive immunity responses. The innate immunity response embodies front-line reaction towards invading pathogens. It includes recognition of microbial components, activation of local phagocytosis (e.g. neutrophils, eosinophils, monocytes and macrophages), complement system and coagulation as well as production of acute phase proteins. Adaptive immunity comprises responses of cell-mediated and humoral immunity. These phenomena progress in parallel and are contingent on each other. These both give rise to anti-inflammatory and proinflammatory responses (81). If this continuum is disturbed, a chain of events occurs in which the promotion and liberation of mediators leads inevitably to sepsis (82).

2.2.1.1. Innate immunity response

The natural physical barriers to host invasion are formed externally by the skin and internally by mucous membranes lining the gastrointestinal, genitourinary and respiratory tracts. These form a mechanical barrier towards invading pathogens in co-operation with local normal flora. In the hospital environment patients’ indwelling catheters, intravenous cannulas and urinary catheters must be noted as possible sources of infection (83). Sepsis may be caused by numerous invasive pathogens, including bacteria, yeasts, viruses and parasites.

The structural components of the microbe responsible for triggering the septic process are important not only for understanding the mechanisms of infection and inflammation, but also for identifying potential therapeutic targets. Endotoxin is a lipopolysaccharide present in the outer portion of gram-negative bacteria. Exposure to endotoxins, exotoxins produced by gram-
positive bacteria, or other types of microbial components triggers intracellular events in immune cells and the epithelium, endothelium and neuroendocrine system through microbial-associated molecular patterns (84).

The initiation of the response involves pattern of recognition receptors which recognise specific structures of microbes (85). Part of this family are toll-like receptors (TLRs), which are transmembrane proteins on the surface of immune cells. These are capable of sensing invading microbes. Microbes have unique cell-wall molecules known as pathogen-associated molecular patterns (PAMPs). PAMPs bind to TLRs on the surface of immune cells. This binding activates, in turn, intracellular signaling pathways. At the end of this pathway, proinflammatory cytokines are released. Also macrophages and monocytes participate in the secretion of proinflammatory cytokines. Neutrophils and endothelial cells are activated to produce adhesion molecules, which help to kill pathogens, but also cause damage to the endothelium (85). Macrophages release VEGF-like mediators, which increase vascular permeability and vascular proliferation, contributing to coagulation and inflammatory processes (86).

2.2.1.2. Adaptive immunity response

Following the initial host-microbe interaction there is a widespread activation of adaptive immune response, which coordinates defence response involving both humoral and cellular immune systems.

The **humoral immune response** is mediated by secreted antibodies (immunoglobulins) produced in the cells of B lymphocyte lineage (plasma cells). Secreted antibodies bind to antigens on the surfaces of invading microbes, marking them for destruction by components of innate immune system (87, 88). Pathogens are destroyed by phagocytic cells (e.g. neutrophils, macrophages or natural killer cells) with the help of complement activation or recognition by antibodies (89, 90).

**Cellular immunity** is an immune response mediated by T-cells. It involves activation of macrophages, natural killer cells, antigen-specific cytotoxic T-lymphocytes and the release of various cytokines in response to an antigen. Cytotoxic T-lymphocytes (CD8) are able to destroy cells infected by viruses or cells with intracellular bacteria. Helper T cells (CD4) secrete cytokines by differentiating into type 1 helper T-cells (Th1) and type 2 helper T-cells (Th2) (or other subsets). Th1 secretes pro-inflammatory cytokines and Th2 anti-inflammatory cytokines. Proinflammatory mediators (e.g. interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)) contribute to eradication of invading pathogens and anti-
inflammatory mediators (e.g. interleukin-4 (IL-4) and interleukin-10 (IL-10)) to control this response (91, 92). This proinflammatory response leads inevitably to damage of the host tissues, whereas the anti-inflammatory response causes leukocyte reprogramming and changes immune status (93). During this time sequence, circulatory abnormalities (e.g. intravascular volume depletion, peripheral vasodilatation and myocardial depression) lead to an imbalance between systemic oxygen delivery and demand.

2.2.1.3. Complement system, coagulation and inflammation

The complement system and coagulation are the major components of plasma cascades. They are closely related and are activated in a contiguous manner by common stimuli (e.g. infection, trauma). They both contribute to inflammation and mutually interact at several stages (94, 95).

Complement is not only a part of the innate immune system, but also an effector of antibody-mediated immunity. The major functions are the defence against pyogenic bacterial infections bridging innate and adaptive immunity, and clearance of immune complexes and products of inflammatory injury. Circulating components of the complement are activated via three pathways: 1) the classical pathway initiated by the binding of component C1q to antigen-antibody complex, 2) the lectin pathway initiated by binding of mannose-binding lectin to sugars present in the bacterial wall, and 3) an alternative pathway initiated after exposure to surface molecules of invading pathogens. At the end of these pathways several convertases (e.g. C3, C5) are released and they in turn facilitate the phagocytosis of opsonised pathogens by macrophages and neutrophils, act as inflammatory mediators and, further, participate in the lysis of bacterial cell membranes. The regulatory mechanisms of the complement system are sensitively balanced. The aim is to focus the activation of complement on the surface of invading pathogens, while on limiting deposition on normal cells and tissues (94, 96).

A delicate balance of pro- and anticoagulant factors maintains haemostasis. In normal conditions three anticoagulant pathways prevent systemic activation of coagulation: antithrombin, activated protein C and tissue factor pathway inhibitor. During inflammation-induced activation of coagulation all three functions are impaired; IL-6 release triggers tissue factor upregulation, initiating the coagulation cascade, and TNF-α mediates the suppression of natural anticoagulants. The latter can lead to disseminated intravascular coagulation, which has been thought to be central in the pathogenesis of multiorgan failure (MOF) in sepsis patients. Thus, sepsis is a chaotic environment associated with exacerbated coagulation, decreased anticoagulation and impaired fibrin removal (95, 97, 98).
Complement and coagulation cascades are intended to act locally. If they are activated systemically as a result of a failure of the relevant control mechanisms, the effect of this broad reaction can be irreversible.

2.2.1.4. Endothelium and inflammation

Vascular endothelial cells play an active role in the regulation of blood vessel tone, permeability, coagulation, angiogenesis and both leukocyte and platelet activation. Endothelial cells synthesise several pro- and anti-inflammatory molecules. They also synthesise proteins which increase vascular permeability to fluid and large molecules (e.g. antibodies and components of complement) (95, 99).

Several microbes can adhere to the endothelium, causing localised inflammation and recruitment of neutrophils and monocytes. Increased vascular permeability allows leukocyte migration into surrounding tissue as a response to chemotactic factors generated at the site of injury. Bacterial endotoxins or other foreign particles induce neutrophil activation and release of pro-inflammatory cytokines. Strong adhesion initiates the coagulation cascade (97, 99). The endothelium is one of the key organs involved in the pathogenesis of sepsis. Endothelial damage may result in global tissue hypoxia or shock (99). Worsening tissue hypoxia may ultimately lead to MOF and death.

2.2.1.5. Multiorgan failure

The ultimate cause of death in patients with severe sepsis is multiorgan failure. The exact mechanisms for MOF are unknown. Earlier observations indicate that the number of failing organs or organs with dysfunction correlates with mortality, with an increase of 20% for each additional organ failure (100, 101). In-hospital mortality with the failure of at least two organ is four-fold compared with patients who have only a single organ failure (102).

Mechanisms of multiorgan failure include widespread microvascular occlusion, development of tissue exudates that further compromise tissue oxygenation and disorders in microvascular homeostasis, which all result from elaboration of vasoactive substances (e.g. histamine). Cellular infiltrates (mainly neutrophils) damage tissue directly by releasing lysosomal enzymes. Proinflammatory cytokines (mainly TNF-α) mediate increased production of nitric oxide, causing further vascular instability. This may also contribute to the direct myocardial depression that occurs commonly in sepsis (82).
Ongoing tissue hypoxia acts as an indicator for progressive MOF. The extent of oxygen debt, i.e. the amount by which oxygen requirements exceed oxygen delivery, is related to the outcome of sepsis. Also impaired endocrine responses to sepsis may result due to cytokines and metabolic and ischaemic derangements in hypothalamic-pituitary axis and adrenal glands. Deficiencies in adrenal gland function and vasopressin production contribute to hypotension and eventual death (103, 104).

2.2.2. Epidemiology of sepsis

Despite extensive study and new therapeutic modalities, sepsis is still a challenge in clinical medicine. Severe sepsis and septic shock are both relatively common and represent an important cause of morbidity and mortality.

Both the incidence and mortality in severe sepsis varies in different studies. In Scandinavia, there are only a few nationwide studies, the most important of which are the Norwegian study in 1999 and the FINNSEPSIS study in 2004-2005. The national health database in Norway was retrospectively screened for cases of severe sepsis. The incidence of severe sepsis was 1.5/1000 of the population. Severe sepsis was diagnosed in 31.8% of sepsis patients, and the hospital mortality for severe sepsis was 27% (9). The FINNSEPSIS study was a prospective study in the ICUs of 21 hospitals in Finland. Severe sepsis was diagnosed in 470 of 4500 patients (10.5%) admitted to ICUs. The incidence of ICU-treated severe sepsis in Finland was 0.38/1000 of the adult population. The ICU mortality rate was 15.5% and the number of organ failures affected the mortality rate. In patients with a single organ failure the mortality was 11.5% but in patients with three organ failures mortality increased up to 34% (10).

In the EPISEPSIS study sepsis was prospectively evaluated among all admissions of 206 adult ICUs of public hospitals in France (8). The incidence of severe sepsis was 0.95/1000. Altogether 14.6% of patients had severe sepsis or septic shock. The in-hospital mortality rate was 35% at 30 days. This study was a reappraisal for an earlier study (105) in which 8.4% of patients were found to have severe sepsis or septic shock and 56% of them died at hospital. Although the admission rate of severe sepsis in French ICUs appears to have increased, hospital mortality has decreased suggesting improved management (105). In a large retrospective British study, the incidence of severe sepsis was 0.46-0.66/1000 of population. Of adult patients admitted to ICU, 27% met criteria for severe sepsis during the first 24 hours (in-hospital mortality 47%). In the multicentre prospective European SOAP-study the corresponding percentage was 25% (total in-hospital mortality 36%) (106). Authors found a considerable variation among the European countries, with a strong correlation between the
frequency of sepsis and the intensive care unit mortality rates. In two large retrospective epidemiological studies, the incidence of severe sepsis was 0.76/1000 of the population and in-hospital mortality 31% in Australia. In the United States, the incidence of severe sepsis was 3/1000 in population and overall hospital mortality rate 29% (100, 107).

With the exception of the FINNSEPSIS-study, the occurrence and mortality rates of severe sepsis seem to be rather similar. Most studies were made in ICUs and in adult population, considering all patient groups admitted to intensive care (surgical and non-surgical). The different classifications of intensive care units between countries should also be noted – ICU and lighter step-up/step-down units might have been classified together in these studies. The amount of malignancies among study patients varied between 4.6% and 11.6%, but the number of haematological patients with neutropenia was not mentioned (10, 108, 109).

An important finding in FINNSEPSIS study was that even though there were national guidelines for the treatment of severe sepsis in adults (110) the compliance to the guidelines was noted to be poor. This was also the case in the international setting (111, 112), even though relative simple therapeutic interventions might save a significant number of lives (113). It is well known that the time window for interventions in the evolution of sepsis is short. The transition from a mild to serious situation occurs during the critical “golden hours” when recognition and aggressive treatment provide maximal benefit in terms of outcome (114).

2.2.3. Microbiological aetiology of sepsis

Blood cultures have been positive in 30-60% of the patients (9, 10, 115) in ICU-based sepsis studies. In most studies the percentage of positive blood cultures was less than 60% - underlining the fact that severe sepsis must primarily be a clinical diagnosis.

Among the ICU studies including both community- and hospital-acquired infections the respiratory tract has been the most common site of infection, followed by the abdomen, urinary tract and skin or soft tissue infections (5, 8, 100, 109, 115, 116). In the recent FINNSEPSIS study (10) blood cultures were positive in 40% of patients with severe sepsis. Of these, the proportion of gram-positive bacteria was 59% and gram-negative bacteria 33%, respectively. The most common site of infection was the respiratory system (43%) followed by intra-abdominal (32%) and skin or soft tissue (10%) infections. In patients older than 65 years the urinary tract has been observed to be a common site of infection (117).

Since the late 1980s, gram-positive organisms have slowly replaced gram-negative bacteria as a cause of sepsis (116, 118). This was demonstrable in most studies during this decade (8, 107, 115) with some exceptions. In a large Chinese study (108) gram-negative microbes were
the most common isolates in blood cultures. This was also the situation in the study by Valles et al (119). In that study 339 patients were admitted to ICU with community-acquired bacteraemia (25% with sepsis, 20% with severe sepsis and 55% with septic shock). The most common pathogens were *Escherichia coli* (25%), *Streptococcus pneumoniae* (16%) and *Staphylococcus aureus* (14%).

It has been showed that initiation of effective antimicrobial therapy within the first hour following the onset of septic shock-related hypotension is associated with an 80% survival. Every additional hour of delay in the initiation of antimicrobial therapy decreased survival by an average of 7.6% (5). The importance of broad coverage in the initial treatment was illustrated by the poor prognosis of patients in whom the first line antibiotics were found to be ineffective in sensitivity assays (120).

At presentation both the source of infection and the microbial nature of the disease are often unknown. Antibiotic treatment should be therefore guided by symptoms and signs, bedside clinical findings and knowledge of local bacterial resistance. The epidemiological change of nosocomial bloodstream infections can be insidious, which was observed recently in Taiwan and the Republic of Korea (121-123). This was characterised by the notable predominance of gram-negative microbes with increasing antimicrobial resistance.

### 2.3. Markers for severe sepsis

In severe sepsis and other serious infections, the circulating levels of biomarkers depend on the origin and extent of the infection. In addition, microbes may induce a distinct response in various organs, resulting in a variable diversity of circulating biomarkers and mediators. It is obvious that any infection is far too complex to be reduced to a single cutoff of any biomarker. Nevertheless, the dynamics of biomarker levels have prognostic implications, and increasing levels are associated with an unfavourable outcome. On the contrary, decreasing levels suggest favourable outcome (124). Biomarkers may be valuable and helpful tools in the diagnostic dilemma of severe sepsis, especially in distinguishing severe sepsis from less severe forms of infections in early phase of the disease.

Various treatment strategies are effective in septic infections, but the disease should be diagnosed early to be effectively treated. Early diagnosis with an unknown microbiological aetiology, but early initiation of sepsis therapy is more effective than specific, but lately initiated sepsis therapy (125). This is especially true in neutropenic sepsis, where symptoms and clinical findings of infection may be obscure or even absent. Thus, early prognostic markers of bacterial infections are warranted in the treatment of neutropenic patients.
These markers should be released and regulated independently of neutrophil cell counts and activity of the underlying disease. An ideal prognostic marker should be also able to distinguish septic infections from several other causes of the systemic inflammatory response syndrome. It should also reflect the severity of the infection and distinguish periods with high risk of complications from those with low risk (126).

Leukocyte count and leukocyte differentiation are among the oldest markers of infection, but are useless in patients with severe neutropenia. CRP, procalcitonin (PCT) and proinflammatory cytokines (IL-6 and IL-8) are all useful markers of inflammation. In addition, VEGF, natriuretic peptides, lactate and procalcitonin have been a persistent interest of study. Among other potential future candidates are lactoferrin, neopterin and prostaglandins (126).

2.3.1. C-reactive protein

CRP is a member of the acute-phase reactants, because its level rises rapidly in response to inflammatory processes. CRP is produced by hepatocytes, predominantly under transcriptional control by IL-6 (Table 2). About 90% of healthy population has CRP concentrations < 3 mg/l (127). Synthesis after the inciting stimulus starts rapidly, and serum concentrations of CRP rise above 5 mg/l by about 6 hours. The peak values are achieved in around 48 hours. The sole determinant of circulating CRP concentration is the synthesis rate, and it reflects directly the intensity of the pathological process stimulating CRP production (128). When the stimulus terminates, the concentration of circulating CRP falls rapidly.

CRP production is part of the nonspecific acute-phase response to most forms of inflammation, infection and tissue damage. Persistent increases in CRP can also occur in chronic inflammatory disorders, including autoimmune diseases and malignancy. It has been shown that CRP has an important role in host defence by complement action, opsonization and by inducing phagocytosis (129, 130). CRP has been used clinically for monitoring infections and autoimmune disorders. CRP concentrations are most useful clinically when combined with full knowledge of all other clinical and pathological results (126, 131). Of note, in severe liver diseases CRP is generated at a markedly lower pace than in patients without liver pathology (132).

The use of CRP is common in many European countries, mainly because of its low costs and easy availability in everyday clinical practise. In many studies, CRP has usually been used as a comparator for other markers of inflammation, mainly because its well-known kinetics and broad use in various infectious diseases (133).
The time from suspicion to diagnosis in septic infections is critical because uncontrolled infections can rapidly lead to severe sepsis, with a mortality rate of 20-52% (81, 82, 141). In many recent sepsis studies, the initial slow kinetics of CRP has diminished its value as an early marker of septic infection. In the study by Rintala et al (142) CRP peaked 24h later than procalcitonin (PCT) in bacteraemic patients. The finding was similar in another study (143), where the PCT concentrations reacted more quickly than CRP to the severity of sepsis in ICU patients. The diagnostic utility of CRP and other inflammation markers was studied in cancer patients with suspected infection (144). Only PCT seemed to be a good marker to discriminate bacteraemic patients from other patients.

In studies with neutropenic patients, the results are in line with the studies in sepsis patients without known neutropenia. CRP identifies patients with infection, but when the time interval is short (<12 hours), predictive capacity of CRP declines significantly. When CRP kinetics during infections in leukaemia patients was evaluated, it was concluded that CRP-level over 100 mg/l was predictive for infection (145). Manian (12) found that an increase in CRP level of > 40 mg/l over 2 or 3 days may suggest infection. A CRP level > 200 mg/l for over 5 days during neutropenia was associated with a mortality rate of 50%. In another study CRP levels were significantly higher in bacteraemic haematological patients and in patients whose infection focus was identified than in those with fever of unknown origin (11). On the other hand, Yonemori and colleagues (13) found no predictive value for serial measurement of CRP in their study of 47 neutropenic patients. In these studies, CRP kinetics was not compared between periods with severe sepsis and those without.

Persson et al (14) studied prospectively the predictive value of systemic inflammation markers to determine the clinical course of febrile neutropenic patients. CRP did not differ at any time point between patients with and those without complications. Another study from the same group evaluated the ability of CRP and other inflammatory markers to predict bacteraemia during the first 48 hours of fever in neutropenic patients (146). During the first 10 hours, CRP had sensitivity of 42% and specificity of 76% for bacteraemia. The positive predictive value was 33%. CRP reached the highest levels after 20 to 30 hours of neutropenic fever in patients with bacteraemia. In another study there were no differences in early CRP concentrations between bacteraemic and non-bacteraemic neutropenic patients (147). The result was similar in the study of Sandri and colleagues (148), where PCT concentrations increased early only in bacteraemic patients with the highest levels at day +1 after the onset of fever. CRP reached its peak level also at day +1 after the onset of fever but could not distinguish bacteraemic patients from non-bacteraemic patients. Based on these studies, CRP is useless from a predictive point of view.
<table>
<thead>
<tr>
<th>Marker</th>
<th>Main source</th>
<th>T½</th>
<th>Nature</th>
<th>Major activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Liver (hepatocytes)</td>
<td>19 hours</td>
<td>Acute phase reactant</td>
<td>Ability to bind phosphocholine and recognise foreign pathogens&lt;br&gt;Activation of complement system&lt;br&gt;Induction of inflammatory cytokines and tissue factor in monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Both anti- and proinflammatory properties</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>Monocytes Parenchymal cells</td>
<td>25-30 hours</td>
<td>Precursor of calcitonin</td>
<td>Mediator in systemic infections, exact function unknown&lt;br&gt;Activation of lymphocytes and monocytes</td>
</tr>
<tr>
<td>IL-4</td>
<td>Helper T cells (Th2) B cells Mast cells Basophils Eosinophils Stromal cells</td>
<td>Few minutes (NA)</td>
<td>Anti-inflammatory</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Helper T cells (Th2) Monocytes Macrophages Endothelial cells</td>
<td>&lt; 60 minutes</td>
<td>Proinflammatory Anti-inflammatory</td>
<td>Activation of lymphocytes, differentiation of B-cells, stimulation of the production of acute-phase proteins&lt;br&gt;Cheomotaxis of neutrophils, basophils and T-cells</td>
</tr>
<tr>
<td>IL-8</td>
<td>T cells Monocytes Macrophages Endothelial cells</td>
<td>&lt; 10 hours</td>
<td>Proinflammatory Anti-inflammatory</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>Monocytes Lymphocytes Macrophages</td>
<td>Approximately 2 minutes</td>
<td>Anti-inflammatory</td>
<td>Inhibits production of proinflammatory cytokines&lt;br&gt;Regulates T- and B cell proliferation&lt;br&gt;Stimulates Th2-mediated immunity&lt;br&gt;Angiogenesis&lt;br&gt;Mediator of vascular permeability</td>
</tr>
<tr>
<td>VEGFs</td>
<td>Lymphocytes Macrophages Platelets Vascular smooth muscle cells Lung epithelium</td>
<td>Approximately 3 minutes</td>
<td>Hypoxia induced mediators</td>
<td></td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>Inactive metabolite of BNP, which in turn is secreted by cardiomyocytes</td>
<td>120 minutes</td>
<td>Inactive metabolite of BNP</td>
<td>Maintain cardiovascular homeostasis</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; IL, interleukine; VEGF, vascular endothelial growth factor; NT-pro-BNP, amino terminal pro-brain natriuretic peptide; BNP, brain natriuretic peptide; T½, half-life.
There are no prospective studies evaluating predictive capacity of CRP kinetics in relation to development of severe sepsis in haematological patients with neutropenic fever.

2.3.2. Vascular endothelial growth factor

Vascular endothelial growth factors (VEGFs) are important signaling proteins involved in both vasculogenesis (de novo formation of the embryonic circulatory system) and angiogenesis (growth of blood vessels from pre-existing vasculature). VEGF is one of the most potent factors regulating angiogenesis and microvascular permeability (149-151). VEGF production is induced in hypoxaemic cells. When a cell is deficient in oxygen, it produces hypoxia-inducible factor (HIF). HIF in turn stimulates the release of VEGF (152). The role of VEGF as a mediator of vascular permeability is important in severe infections. VEGF has been found to cause vasodilatation influenced by endothelial nitric oxidase synthase in patients with sepsis (153). In the study of Yano and colleagues (154), experimental septic infections were associated with time-dependent increase in circulating levels of VEGF. They also showed that peak VEGF concentration occurred during the first 24 hours after the onset of experimental sepsis, and the concentration of VEGF remained elevated for several days.

Two groups have reported that VEGF levels increase in patients with severe sepsis. Pickkers and colleagues (155) studied meningococcal septic shock in children as a prototype of gram-negative septic shock. VEGF plasma concentrations were measured during the first 48 hours and were highest in the presence of septic shock. VEGF concentrations at admission also correlated with the severity of infection. Van der Flier and colleagues (156) measured plasma VEGF levels in patients with severe sepsis in the adult ICU. They found that VEGF levels were significantly elevated in patients with severe sepsis compared with healthy controls. Moreover, VEGF levels in non-survivors were higher than in survivors. Increased VEGF levels at study entry also correlated with the severity of MOF during the course of disease. Karlsson and colleagues (15) evaluated serum VEGF values in ICU patients with severe sepsis in order to predict organ dysfunction and mortality. They found that VEGF values were elevated in patients with severe sepsis compared with healthy controls. Low circulating VEGF levels were associated with haematological and renal dysfunction, suggesting possible disturbed production of VEGF in severe sepsis. Furthermore, very low concentrations of VEGF were associated with more severe forms of organ dysfunction and mortality, possibly because of endothelial injury.

There are no previous prospective studies available on VEGF in patients with neutropenic fever. Kraft and colleagues (157) analysed the VEGF values of 212 patients with various
malignant tumours without known neutropenia and compared the results with the VEGF values of both healthy controls and patients with non-malignant disease. Elevated levels of VEGF were detectable in 0-20% of patients with localised cancer but in 11-69% of patients with metastatic cancer. VEGF levels in acute infection (non-severe sepsis) were elevated compared with levels in healthy individuals.

The pathogenesis of sepsis, including the production of VEGF, is congruent in patients with and without neutropenia. It has been shown that in cancer patients VEGF production correlated with platelet count after chemotherapy-induced thrombocytopenia (158). Furthermore, a peak in VEGF production followed platelet recovery. Several other studies have shown that circulating VEGF resides not only in platelets but also in granulocytes, mainly in neutrophils (159-162). This might influence VEGF production in patients with neutropenic fever.

2.3.3. Amino-terminal pro-brain natriuretic peptide

Brain natriuretic peptide (BNP) is a prohormone secreted by cardiomyocytes. Secretion is primarily a response to the increased myocardial wall stress and is aimed to maintain cardiovascular homeostasis through its natriuretic, diuretic and vasodilatory properties (163). NT-proBNP is an inactive metabolite of BNP. NT-proBNP has markedly longer plasma half-life and better stability than BNP, which makes it better applicable for clinical use (164) (Table 2).

Increased levels of both BNP and NT-proBNP have been identified as early markers of myocardial dysfunction and increased mortality in the ICU setting (165-167). Elevated levels of natriuretic peptides have been found to be markers of unfavourable prognosis in patients with severe sepsis and septic shock (16, 168, 169). Varpula and colleagues (138) found that the NT-proBNP values were frequently increased in severe sepsis and septic shock in patients admitted to the ICU. Of note, NT-proBNP values were higher in non-survivors than survivors. Also patients with less severe infections seem to have elevated levels of natriuretic peptides (170).

A correlation between increased plasma levels of BNP and IL-6 in patients with septic shock has been shown earlier and in recent studies both BNP and NT-pro-BNP secretion have been linked to general inflammation (171). Rudiger and colleagues (172) showed a correlation between NT-pro-BNP and CRP levels in a small group of haemodynamically unstable patients. NT-pro-BNP or BNP levels did not differ significantly between patients with acute cardiac failure and those with septic shock. The result was similar in a study by Shor and colleagues (173), in which BNP levels correlated positively with CRP in septic patients without systolic
myocardial dysfunction. In another study the focus was in cancer patients with multiple co-morbidities (174). The BNP-levels of these patients were elevated but without association with volume overload or left ventricular dysfunction. Nonetheless, there was a significant association with both sepsis and 30-day mortality in patients with markedly elevated BNP-values. Nikolaou and colleagues (170) showed that BNP levels were elevated also in the acute phase of community-acquired microbial infections without severe sepsis or septic shock.

There are no earlier studies of the kinetics or predictive use of NT-pro-BNP in patients with neutropenic fever.

2.3.4. Other markers

2.3.4.1. Lactate

Plasma lactate is a result of the balance between lactate production and consumption. Hyperlactataemia is typically present in patients with severe sepsis or septic shock. In these patients not only high lactate but also poor lactate clearance has been recognized as an early marker of mortality (175). Hyperlactatemia may be secondary to anaerobic metabolism due to tissue hypoperfusion. The prognostic value of raised serum lactate levels has been well established in patients with septic shock, especially if the high levels persist (176, 177). Serial lactate measurements have been considered to be better markers for mortality and organ failure than a single lactate determination (178). Evaluation of serum lactate levels is essential to identify tissue hypoperfusion in patients who are not yet hypotensive, but who are at high risk for septic shock.

The estimation of lactate levels in septic patients is not always straightforward. For example, elevated lactate levels may result from decreased clearance by the liver or lactate acidosis rather than from global hypoperfusion. In the study by Ramzi and colleagues (179) elevated lactate (>3 mmol/l) and low serum bicarbonate (<17 mmol/l) at the onset of bacteraemia were useful biomarkers in predicting septic shock and mortality in neutropenic patients. In a multivariate analysis, especially two variables, pulmonary infection and serum lactate >3 mmol/l, were associated strongly with septic shock.

There are only limited data available on the kinetics or prognostic usefulness of lactate in haematological patients with neutropenic fever. In a recent study elevated plasma lactate level at start of neutropenic fever was not common, and did not distinguish severe sepsis. In contrast, a high lactate level and impaired lactate decrease signified a fatal course of neutropenic fever (180).
2.3.4.2. Procalcitonin

Calcitonin and its precursor procalcitonin (PCT) were initially used as a serum marker for detection and follow-up of therapy for neuroendocrine tumours. Later on PCT levels were found to be increased in patients with severe systemic inflammation (e.g. trauma, systemic bacterial infection and sepsis). Levels of procalcitonin are undetectable in healthy individuals (181).

The production and biological function of PCT involves a complex and time-dependent mechanism. Significant production of PCT has been observed in adherent monocytes, but not in circulating leukocytes. Monocytes produce PTC only for a limited time. Parenchymal cells start to produce PTC after interaction with adherent monocytes. Local or systemic inflammations affecting the parenchyma and monocyte adhesion are the preconditions for PCT production. This explains also why PCT is induced by not only local or systemic inflammation, but also after tissue trauma (182). In neutropenic patients, monocytes and other leukocytes are absent, leaving tissue-based mechanisms of host defence (124). PCT elevates rapidly (within 2-4 hours) in severe forms of systemic inflammation or bacterial infections. It has been shown in many settings that PTC serum concentrations react more rapidly than CRP concentrations in sepsis patients (142, 147).

In Switzerland PCT has been used to guide antibiotic therapy in non-neutropenic community-acquired pneumonia with success (183). The results have been similar when decrease in serial PCT concentrations was used to predict favourable outcome of febrile episodes in neutropenic haematological patients (184). In recent review by Sakr and colleagues (185) PCT was shown to discriminate fever due to systemic forms of infection from fever of non-infectious origin. It had only a minimal role in discriminating gram-negative from gram-positive infections. Of note, PCT was not superior to interleukin-6 or CRP concentrations in outcome prediction in patients with febrile neutropenia. In a large meta-analysis the results were in line with the findings of Sakr and colleagues: although high PCT occured commonly in infection, it was also elevated in many non-infectious conditions (186). There were also observations of febrile septic patients with documented bacteraemia having PCT values within normal range.

Accordingly, PCT is not a specific indicator of either infection or sepsis and publications concerning its prognostic utility have been contradictory (187-189). The authors point out that the most commonly applied assay is not sensitive enough to detect potentially important mild elevations or trends and that clinical studies with a more sensitive PCT assay are needed (125, 183).
2.3.4.3. Interleukins

Interleukins 6 (IL-6) and 8 (IL-8) are proinflammatory cytokines, mainly produced by monocytes. They are an important part of the cytokine cascade, together with inhibitory cytokines (126). The kinetics of these cytokines are very fast (releasing within less than 1-2 hours), but concentrations may also decline within a short time (Table 2). IL-6 is a multifunctional cytokine that regulates B- and T-cell function and acute-phase response such as secretion of CRP. IL-8 is an inflammatory cytokine that mainly functions as a neutrophil chemo-attractant and activating factor (190).

Both IL-6 and IL-8 have been demonstrated to be reliable predictors of sepsis in patients with neutropenic fever (190). Engel and colleagues (191) showed that both IL-6 and IL-8 had predictive value in neutropenic patients with bacteraemia. Especially IL-8 might have predictive capacity in this respect.

Anti-inflammatory cytokines, mainly interleukin 4 (IL-4) and 10 (IL-10), are produced to down-regulate the systemic inflammatory response in sepsis. The value of anti-inflammatory cytokines in different studies is rather obscure. Loisa and colleagues (192) studied the anti-inflammatory response in the development of MOF in the ICU setting, but overproduction of IL-10 was not observed. In another study the simultaneous detection of 17 different cytokines demonstrated that both pro- and anti-inflammatory cytokines were significantly higher in patients with septic shock than in patients with severe sepsis (193). Thus, higher cytokine concentrations were associated with severity and evolution of organ dysfunction, but anti-inflammatory cytokines had no specific role in this setting. In adult patients the measurement of IL-10 was of limited value in predicting the outcome of febrile neutropenia (194).
3. **AIMS OF THE STUDY**

The aim of this study was to examine the nature of severe sepsis and possible predictive factors of severe sepsis in neutropenic haematological patients. The specific study questions were:

1. **What are the epidemiological features, microbiological aetiology and outcome of severe sepsis patients with AML and ASCT? (I, II)**

2. **Are the kinetics of CRP associated with severe sepsis in neutropenic patients with AML and ASCT? (I, II)**

3. **Does serum VEGF have predictive value in severe neutropenic sepsis compared with CRP? (III)**

4. **Does serum NT-proBNP have predictive value in severe neutropenic sepsis when compared with CRP? (IV)**
4. PATIENTS AND METHODS

4.1. Patients

4.1.1. Patients in retrospective studies (I-II)

4.1.1.1. Characteristics of patients with acute myeloid leukaemia (I)

The study included altogether 84 patients aged ≤70 years with AML treated with intensive chemotherapy in the Department of Medicine, Kuopio University Hospital in 1996-2005. There were 44 males and 40 females with a median age of 50 years at diagnosis (range 18-69 years).

4.1.1.2. Characteristics of autologous stem cell recipients (II)

The study population consisted of 319 adult patients who received ASCT at the Department of Medicine, Kuopio University Hospital in 1996-2006. There were 189 males and 130 females, with a median age of 55 years (range 16-73). The most common diagnosis was NHL (n=160, 50%) followed by MM (n=113, 35%). The patient and transplant characteristics are presented in Table 3.

4.1.2. Patients in prospective studies (III-IV)

From first of December 2006 to 30th of November 2008 adult patients (≤70 years of age) treated in the haematology ward of Kuopio University Hospital were enrolled in the prospective part of the study. Patients were eligible if they had either AML or received high-dose chemotherapy supported by ASCT and if they had neutropenic fever after chemotherapy. Study III included all eligible patients treated during the first 12 months (n=42) and study IV all eligible patients during a 24-month period (n=70). Patient characteristics and chemotherapy given for study III are presented in Table 4 and for study IV patients in Table 5.

In study III, only the first episode of neutropenic fever was included in the study. In study IV, fourteen patients with AML had more than one period of neutropenic fever after induction and consolidation chemotherapy. Thus, the number of periods with neutropenic fever was 94 in 70 patients.
Table 3. Characteristics of ASCT patients transplanted in 1996-2006

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>319</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>189 (59%)</td>
</tr>
<tr>
<td>Female</td>
<td>130 (41%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>55 (16-73)</td>
</tr>
<tr>
<td>&lt; 60 years</td>
<td>223 (70%)</td>
</tr>
<tr>
<td>≥ 60 years</td>
<td>96 (30%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>160 (50%)</td>
</tr>
<tr>
<td>MM</td>
<td>113 (35%)</td>
</tr>
<tr>
<td>HL</td>
<td>22 (7%)</td>
</tr>
<tr>
<td>CLL</td>
<td>8 (3%)</td>
</tr>
<tr>
<td>Others</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>High-dose regimen</td>
<td></td>
</tr>
<tr>
<td>BEAC</td>
<td>132 (41%)</td>
</tr>
<tr>
<td>BEAM</td>
<td>54 (17%)</td>
</tr>
<tr>
<td>HD-MEL</td>
<td>118 (37%)</td>
</tr>
<tr>
<td>Other</td>
<td>15 (5%)</td>
</tr>
<tr>
<td>Stem cell source</td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>317 (99%)</td>
</tr>
<tr>
<td>BM</td>
<td>2 (1%)</td>
</tr>
</tbody>
</table>

Abbreviations: ASCT, autologous stem cell transplant recipients; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; HL, Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia; PB, peripheral blood; BM, bone marrow; BEAC, carmustine, etoposide, cytarabine and cyclophosphamide; BEAM, carmustine, etoposide, cytarabine and melphalan; HD-MEL, high-dose melphalan.
Table 4. Characteristics of patients in study III.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>26 (62%)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (38%)</td>
</tr>
<tr>
<td>Age: median, range</td>
<td>57, 18-70</td>
</tr>
<tr>
<td>&gt; 60 years, number (%)</td>
<td>16 (38%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
</tr>
<tr>
<td>NHL</td>
</tr>
<tr>
<td>MM</td>
</tr>
<tr>
<td>HL</td>
</tr>
<tr>
<td>CLL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High-dose regimen, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEAC</td>
</tr>
<tr>
<td>BEAM</td>
</tr>
<tr>
<td>HD-MEL</td>
</tr>
<tr>
<td>IAT</td>
</tr>
<tr>
<td>HD-AraC-Ida</td>
</tr>
<tr>
<td>Mito-HD AraC</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukaemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; HL, Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia; BEAC, carmustine, etoposide, cytarabine and cyclophosphamide; BEAM: carmustine, etoposide, cytarabine and melphalan; HD-MEL, high-dose melphalan; IAT, idarubicin, cytarabine, thioguanine; HD-AraC-Ida, high-dose cytarabine, idarubicin; Mito-HD AraC, mitoxantrone, high-dose cytarabine.
**Table 5.** Characteristics of patients in study IV.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>70</td>
</tr>
<tr>
<td>Male</td>
<td>44 (63%)</td>
</tr>
<tr>
<td>Female</td>
<td>26 (37%)</td>
</tr>
<tr>
<td>Age: median, range</td>
<td>56, 18-70</td>
</tr>
<tr>
<td>≥ 60 years, number (%)</td>
<td>23 (33%)</td>
</tr>
<tr>
<td>Diagnosis, number (%)</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>19 (27%)</td>
</tr>
<tr>
<td>NHL</td>
<td>25 (36%)</td>
</tr>
<tr>
<td>MM</td>
<td>14 (20%)</td>
</tr>
<tr>
<td>HL</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Previous cardiovascular disease, number (%)</td>
<td>10 (14%)</td>
</tr>
<tr>
<td>Hypertension and coronary heart disease</td>
<td>2</td>
</tr>
<tr>
<td>Hypertension and chronic atrial fibrillation</td>
<td>2</td>
</tr>
<tr>
<td>Previous coronary by-pass</td>
<td>2</td>
</tr>
<tr>
<td>Cardiomyopathy (anthracycline)</td>
<td>2</td>
</tr>
<tr>
<td>Mixed type cardiomyopathy</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension and aortic valve sclerosis</td>
<td>1</td>
</tr>
<tr>
<td>High-dose regimen during first period of neutropenic fever, number (%)</td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td>20 (29%)</td>
</tr>
<tr>
<td>BEAC</td>
<td>15 (21%)</td>
</tr>
<tr>
<td>HD-MEL</td>
<td>16 (23%)</td>
</tr>
<tr>
<td>IAT</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>HD-AraC-Ida</td>
<td>6 (9%)</td>
</tr>
<tr>
<td>IdAraC-Ida</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Mito-HD AraC</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Prior anthracycline therapy, number (%)</td>
<td>60 (86%)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>41 (59%)</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>19 (27%)</td>
</tr>
</tbody>
</table>

Abbreviations: NHL, non-Hodgkin lymphoma; AML, acute myeloid leukaemia; MM, multiple myeloma; HL, Hodgkin lymphoma; BEAM, carmustine, etoposide, cytarabine and melphalan; BEAC, carmustine, etoposide, cytarabine and cyclophosphamide; HD-MEL, high-dose melphalan; IAT, idarubicin, cytarabine, thioguanine; HD-AraC-Ida, high-dose cytarabine, idarubicin; IdAraC-Ida, intermediate-dose cytarabine, idarubicin; Mito-HD AraC, mitoxantrone, high-dose cytarabine.
4.2. Methods

4.2.1. Definitions

Neutropenic fever was defined using the criteria from the Infectious Diseases Society of America (IDSA) for the definition of neutropenia and neutropenic fever (6). Neutropenia was defined as a neutrophil count of $<0.5 \times 10^9/l$, or a count of $<1 \times 10^9/l$ with a predicted decrease to $<0.5 \times 10^9/l$. Fever was defined as a single oral temperature of $\geq 38.3^\circ C$ or a temperature of $\geq 38.0^\circ C$ for $\geq 1$ h.

Sepsis was defined as a clinical syndrome in which a systemic inflammatory response was present with infection. Severe sepsis was present if sepsis was complicated by organ dysfunction, hypoperfusion or hypotension. The definition for septic hypotension included systolic arterial pressure $<90$ mmHg, a mean arterial pressure $<60$ mmHg or a reduction in systolic blood pressure of $>40$ mmHg from baseline despite adequate volume resuscitation, in the absence of other causes of hypotension (78, 79).

4.2.2. Clinical management and supportive care measures (I-IV)

In retrospective studies (I-II) the information of chemotherapy regimen used, number of days with neutropenia, number of febrile days ($>38^\circ C$) and blood culture findings were collected from individual patient charts for each febrile neutropenic period (Figure 1). Haematological wards are accustomed to taking care of critically ill patients and individual monitoring charts are familiar. These are also commonly used, which makes the collection of data much easier. In retrospective part of the study each patient’s temperature, blood pressure and heart rate were observed twice a day after chemotherapy by the nursing staff of the haematological ward as a part of the normal treatment routine. In case of neutropenic fever bed-side monitoring continued at a two or three hour interval. In case of severe sepsis, the monitoring was continuous. A potential localisation of infection was searched for during each neutropenic period. In addition, re-induction chemotherapy cycles and intensive consolidation cycles were taken into account in patients with AML.

In the prospective part of the study (studies III-IV) the clinical aim was to improve management of haematological patients with neutropenic fever with close monitoring and vigilant supportive care in the haematology ward setting. The start of prospective study was preceeded by careful education of the whole nursing staff.
Information from each neutropenic fever period

- Chemotherapy regimen used
- Number of days with febrile neutropenia
- Blood culture findings
  - CRP-values
    - Baseline CRP <48 h prior to the rise of neutropenic fever
    - CRP 2-3 days after the rise of neutropenic fever
- Signs or findings of local infection
  - Radiological findings
  - Urine culture findings
  - Stool culture findings
- Blood pressure
- Heart rate
- Breath rate

CRP-slope velocity

Fulfilment of criteria for severe sepsis?

YES

Neutropenic fever period with severe sepsis

NO

Neutropenic fever period without severe sepsis

Figure 1. Collection of information from neutropenic patients and fever periods in studies I (AML) and II (ASCT).
Each patient was examined daily for signs and possible sources of infections. Special attention was paid to features indicating the development of severe sepsis. When a patient became neutropenic, monitoring (body temperature, blood pressure, heart rate, breath rate and peripheral blood oxygen saturation) began bedside twice a day. From the beginning of neutropenic fever monitoring was either hourly or continual – depending on the individual patient situation (Table 6). Daily fluid intake (intravenous and per os) and urine output were registered every 12 hours during the first three days of neutropenic fever by haematological ward nursing staff. The patient’s weight was monitored once a day. The chemotherapy used, number of days with neutropenia (<0.5 x 10^9/l), number of febrile days (>38°C) and blood culture findings were also noted for each febrile neutropenic period (Figure 2). In case of fever during neutropenia, fluid resuscitation using saline infusion was used.

Indwelling central venous catheters were used during the study period in all AML patients. Central venous catheters were used in about 80% of ASCT recipients. Granulocyte colony-stimulating factor (filgrastim or peg-filgrastim) was routinely used in ASCT recipients, whereas only a minority of neutropenic AML patients received growth factors as a part of supportive care, on an individual basis. Red blood cell concentrates were used to keep haemoglobin >80 g/l, and platelet concentrates were given routinely if the morning platelet count was less than 20 x10^9/l.

4.2.2.1. Chemotherapy

During the retrospective study period, patients with AML were treated with intensive induction and consolidation chemotherapy courses according to the Finnish Leukaemia Group prospective protocols. The chemotherapy for AML is given in the haematological wards of university hospitals. The intensive induction course takes weeks, with long lasting neutropenia until the bone marrow recovers. After this AML patients may leave hospital for a few days before consolidation courses begin. Peripheral blood samples are monitored on a regular basis. When cytopenia or neutropenic fever developes, patients return to the hospital. The AML-92 protocol (2) (Table 7) was used for 65 patients and the AML-2003 protocol for 19 patients. In the AML-2003 trial, the induction course is according to randomisation idarubicin, low-dose cytarabine and thioguanine (IAT) or intermediate-dose cytosine arabinoside plus idarubicin.
<table>
<thead>
<tr>
<th>klo</th>
<th>RR</th>
<th>pulssi</th>
<th>Satur %</th>
<th>O2-lisä</th>
<th>Lpö</th>
<th>HUOM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.05</td>
<td>81/53</td>
<td>108</td>
<td>96%</td>
<td>2,5 l</td>
<td></td>
<td>Ringer myydessään</td>
</tr>
<tr>
<td>14.20</td>
<td>83/54</td>
<td>110</td>
<td>94%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.40</td>
<td>87/57</td>
<td>111</td>
<td>93%</td>
<td></td>
<td></td>
<td>Hasbergin lääkkeet</td>
</tr>
<tr>
<td>15.00</td>
<td>90/60</td>
<td>118</td>
<td>94%</td>
<td></td>
<td></td>
<td>Ringer myydessään</td>
</tr>
<tr>
<td>15.20</td>
<td>90/60</td>
<td>120</td>
<td>93%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.00</td>
<td>80/55</td>
<td>122</td>
<td>92%</td>
<td>3 l</td>
<td></td>
<td>38,5</td>
</tr>
<tr>
<td>17.15</td>
<td>82/53</td>
<td>120</td>
<td>93%</td>
<td></td>
<td></td>
<td></td>
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<td>17.30</td>
<td>90/60</td>
<td>123</td>
<td>82%</td>
<td>38,72</td>
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<td>Perpalgan 1 g</td>
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<tr>
<td>18.05</td>
<td>92/59</td>
<td>124</td>
<td>92%</td>
<td>38,1 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.05</td>
<td>92/59</td>
<td>121</td>
<td>92%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.20</td>
<td>92/61</td>
<td>118</td>
<td>92%</td>
<td>36,8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.30</td>
<td>107/60</td>
<td>120</td>
<td>92%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.40</td>
<td>112/65</td>
<td>133</td>
<td>91%</td>
<td>36,7</td>
<td></td>
<td>36,7 e horkkaan Panadol 500 p. o.</td>
</tr>
<tr>
<td>21.00</td>
<td>122/71</td>
<td>142</td>
<td>89%</td>
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</tr>
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<td>107/65</td>
<td>123</td>
<td>94%</td>
<td>38,1</td>
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</tr>
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<td>113</td>
<td>92%</td>
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<td></td>
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<td>113/61</td>
<td>113</td>
<td>92%</td>
<td>39,8</td>
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<td></td>
</tr>
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<td>116</td>
<td>93%</td>
<td>38,9</td>
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<td></td>
</tr>
<tr>
<td>24.30</td>
<td>110/64</td>
<td>118</td>
<td>92%</td>
<td>38,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.15</td>
<td>150/74</td>
<td>150</td>
<td>94-97%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 6.** Example of a follow-up chart used in haematological ward in patients with neutropenic fever.
Figure 2. Collection of information from neutropenic patients and fever periods in studies III (VEGF) and IV (NT-proBNP).
The second course is high-dose cytosine arabinoside plus idarubicin. Responding patients receive risk-adapted consolidation courses all including high-dose cytosine arabinoside with an anthracycline or mitoxantrone.

HDT supported by ASCT is given in university hospitals. Chemotherapy characteristics for studies II-IV are presented in Tables 3-5. Additionally, in study IV, sixty patients (86%) had a history of anthracycline treatment. Nineteen of these patients (27%) received idarubicin (median dose 36, range 24-60 mg/m$^2$) and 41 (59%) received doxorubicin or epirubicin (median dose 300, range 100-500 mg/m$^2$).

4.2.2.2. Antimicrobial therapy

In case of neutropenic fever, 2-3 blood culture sets were drawn from cubital veins at a one-hour interval by laboratory technicians. Empirical antibiotic treatment was started immediately after blood cultures were obtained. A combination of a broad-spectrum beta-lactam and an aminoglycoside was used. Antimicrobial treatment was changed when needed, according to the microbiological findings, radiological or clinical findings. If the fever persisted for 3-5 days, new blood cultures were drawn, and the initial antimicrobial therapy was re-considered. In case of mucosal symptoms during neutropenia, oral fluconazole (200 mg/d) was started. In case of persistent fever, amphotericin B deoxycholate was given as empirical antifungal therapy. More recently caspofungin has been used for empirical antifungal therapy. Vancomycin was considered only if there were signs of infection in central venous catheter or if simultaneous blood cultures revealed coagulase-negative staphylococci. No systematic antibacterial prophylaxis was used until January 2008, when oral ciprofloxacin (500mg b.i.d) was given to neutropenic NHL patients receiving ASCT as a part of clinical protocol.

General infection control measures (e.g. isolation policies, hospital hygiene including cleaning and hand-washing policies) remained constant during the whole study period.

4.2.3. Laboratory methods

In the retrospective part of the study (I-II) serum concentrations of C-reactive protein (CRP) were collected from the individual patient charts. CRP was routinely determined three times per week during neutropenia. During clinically significant infection, CRP measurements were performed at 1-2 day intervals.
Table 7. Chemotherapy protocol in the AML-92 study of the Finnish Leukaemia Group (2)

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Dosage</th>
<th>Administration</th>
<th>Days administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>First course (IAT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idarubicin</td>
<td>12 mg/m²</td>
<td>i.v., 10–15 min</td>
<td>1, 3, 5</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>50 mg/m²</td>
<td>i.v., bolus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>100 mg/m²</td>
<td>i.v., continuously</td>
<td>1(–7)–9</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>75 mg/m²</td>
<td>p.o., twice daily</td>
<td>1(–7)–9</td>
</tr>
<tr>
<td>Second course (HDAraC-Ida)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine</td>
<td>1500 mg (1.0 g)/m²</td>
<td>i.v., 3 h, twice daily</td>
<td>1–5</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>8 mg/m²</td>
<td>i.v., 30 min</td>
<td>6–8</td>
</tr>
<tr>
<td>Third course (MEA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>100 mg/m²</td>
<td>i.v., 1 h</td>
<td>1–4</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>1000 mg (0.5 g)/m²</td>
<td>i.v., 2 h, twice daily</td>
<td>1–4</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>12 mg (8 mg)/m²</td>
<td>i.v., 30 min</td>
<td>2–5</td>
</tr>
<tr>
<td>Fourth course (Amsa-HDAraC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsacrine</td>
<td>115 mg/m²</td>
<td>i.v., 2 h</td>
<td>1–5</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>3000 mg/m²</td>
<td>i.v., 3 h, twice daily</td>
<td>1–2</td>
</tr>
<tr>
<td>Fifth course (HDAraC-Ida)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine</td>
<td>1500 mg (1.0 g)/m²</td>
<td>i.v., 3 h, twice daily</td>
<td>1–5</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>8 mg/m²</td>
<td>i.v., 10–15 min</td>
<td>6–8</td>
</tr>
</tbody>
</table>

Dosage and days administered in parenthesis indicate patients ≥ 56 years old. Abbreviations: i.v., intravenously; p.o., orally.

For the prospective part of the study (III-IV), the first blood samples for the measurement of CRP, vascular endothelial growth factor (VEGF) and amino-terminal pro-brain natriuretic peptide (NT-proBNP) serum concentrations were drawn at the beginning of neutropenic fever concomitantly with samples taken for blood cultures. Further samples were collected next morning and then every 24 hours up to three or five days (Figure 3). Platelet and leukocyte counts were routinely examined daily throughout the neutropenic period.
Blood samples for VEGF and NT-proBNP were centrifuged (2200 G 10 minutes) by laboratory technicians. VEGF samples were then stored at -20°C for later analysis and NT-proBNP samples were analysed within 12 hours.

### 4.2.3.1. Blood cultures

In case of neutropenic fever (neutropenia and a single oral measurement of ≥38.3°C or >38°C for ≥ one hour) 2-3 blood cultures were drawn from cubital veins at 1 hour interval by laboratory technicians. Blood cultures were processed using the automated blood culture system Bactec 9240 (Becton Dickinson, Sparks, USA). Incubation period for both aerobic and anaerobic bottles were 7 days and for MYCO F / Lytic bottles 42 days.

A single positive blood culture was considered significant if the microbe was a clinically relevant cause of infection. Common skin contaminants (e.g. coagulase-negative staphylococci) were considered significant only if they were found in two consecutive blood cultures or if there was a concurrent skin or catheter infection.

### 4.2.3.2. Serum C-reactive protein

The concentration of serum CRP was measured with an immunoturbidometric method (Otsuji, 1982, Clin Chem) using a multichannel Hitachi 717 Automatic Analyzer (Hitachi Ltd, Tokyo, Japan) until the year 2000 and then with a Konelab60i Clinical Chemistry Analyzer (Lab systems CLD, Konelab, Helsinki, Finland). The reference value for serum CRP in healthy persons was <10 mg/l.

In the retrospective studies (I-II) also CRP slope velocity was analysed. For all febrile neutropenic periods following intensive chemotherapy, baseline CRP (< 48 h prior to rise of fever, $CRP_0$) and the CRP level 2-3 days after the onset of fever ($CRP_{2-3}$) was registered. Peak CRP and the time from the rise of fever to the highest CRP value of a given neutropenic period was also registered. CRP slope velocity was defined as the difference between $CRP_{2-3}$ and $CRP_0$, divided by the number of days and expressed as mg/l/d.

$$CRP_{2-3} - CRP_0 \over \text{Number of days (2 or 3)}$$

In the prospective studies (III-IV), CRP was measured at the start of neutropenic fever and then every morning during the study period (Figure 3).
Written informed consent from all patients
• with AML ≤ 70 years
• with HDT supported by ASCT

Neutropenic fever:
• neutrophil count < 0.5 x 10⁹/l and
• fever (single oral temperature ≥38.3° or a temperature ≥38.3° for ≥1 h)

No neutropenic fever

At the beginning of neutropenic fever
at the same time when blood cultures
and other clinically important samples were obtained
• study blood samples (d0)

Study blood samples were not collected

On the first morning after the beginning
of neutropenic fever
• study blood samples (d1)

On the second morning from the beginning
of neutropenic fever
• study blood samples (d2)

On the third morning from the beginning
of neutropenic fever
• study blood samples (d3)

On the fourth morning from the beginning
of neutropenic fever
• study blood samples (d4)

Figure 3. Prospective study protocol.
4.2.3.3. Serum vascular endothelial growth factor

Serum VEGF concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantikine®, R&D Systems; Minneapolis, MN), which recognises the soluble isoforms (VEGF$_{121}$ and VEGF$_{165}$). The same VEGF ELISA method was used and described in detail in a recent Finnish study called FINNSEPSIS (15). In brief, the optical density at 450 nm was measured with wavelength correction at 540 nm (Multiscan RC plate reader; Thermolabsystems, Helsinki, Finland) and serum VEGF concentration was determined with Genesis (Life Sciences, UK) computer software capable of generating a four parameter logistic curve fit.

The intra-assay coefficient variation (CV) for a control sample (mean concentration 345 pg/ml) was 5.7% ($n=10$) and for pooled serum (mean concentration 96 pg/ml) 6.5% ($n=10$). The inter-assay CV for two control samples (mean concentration 167 pg/ml and 1002 pg/ml) was 8.9% and 4.3% ($n=12$). For pooled serum the inter-assay CV was 8.5% ($n=12$). In the FINNSEPSIS study, the samples of 30 healthy adult controls were collected. The mean age of these controls was 36±7 years. The number of males and females was equal (M/F 15/15). The median concentration of VEGF in the control group was 260 (IQR 126-459, range 63-809) pg/ml, which was used as the control value in the FINNSEPSIS study (15).

4.2.3.4. Plasma amino-terminal pro-brain natriuretic peptide

Samples for the measurement of serum NT-proBNP were drawn by venipuncture into Li heparin-containing tubes from patients lying in the supine position. Plasma was separated and NT-proBNP was determined within 24 hours using an electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics) on a Cobas e 601 analyzer (Hitachi High Technology Co, Tokyo, Japan). The measuring range, defined by the lower detection limit and the maximum of the master curve provided by the manufacturer was 0.6-35 000 ng/l. The reference values were given according to age and gender of the patients: for males under 50 years of age 0-84 ng/l, 50-65 years of age 0-194 ng/l and over 65 years of age 0-229 ng/l. For females under 50 years of age 0-155 ng/l, 50-65 years of age 0-222 ng/l and over 65 years of age 0-352 ng/l.
4.2.4. Statistical methods

Statistical analysis was performed with SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). For the analysis of nominal data, the Chi-square test was employed. Continuous variables were expressed as medians with ranges (interquartile ranges or ranges from minimum to maximum). Categorical variables were expressed as absolute numbers (percentages).

The correlation between continuous variables was evaluated by the non-parametric Spearman’s correlation. For single measurements, differences between subgroups were evaluated by the Mann-Whitney U-test, and by Student’s t-test for log-transformed variables. In study III the time period from the beginning of neutropenic fever to achieve maximal VEGF concentration was compared to the respective time to achieve maximal CRP concentration using Wilcoxon signed ranks test. The Youden index (sensitivity + specificity –1) (195), a summary measure of receiver-operating-characteristics curve analysis, was used to evaluate the goodness of CRP vs. VEGF on the first days of neutropenic fever to predict the development of severe sepsis (a high Youden index indicates good test performance). Repeated measures ANOVA was used to assess the impact of severe vs. non-severe sepsis (defined as between-subjects factor) on the VEGF and CRP concentrations. The within-subjects factor was defined as the VEGF concentration on day 0, 1, 2, and 3. The receiver-operating characteristic (ROC) curve analysis was used to evaluate the predictive discrimination of VEGF and CRP for severe sepsis on day 0 and day 1 of neutropenic fever.

In the study IV a logarithmic transformation was used to correct the skewed distribution of NT-proBNP and CRP. After logarithmic transformation, a near-normal distribution was achieved. When patient-related variables were evaluated, only the first episode of neutropenic fever for each patient was included in the analyses. When fever period-related variables were evaluated, all fever periods were included. ANOVA for repeated measurements was used to evaluate possible differences between subgroups in NT-proBNP and CRP levels during days 0-3. A p-value < 0.05 was considered significant.

4.3. Approval of the Ethics Committee

The study was approved by the Ethic Committee of the Kuopio University Hospital. All study patients gave their written informed consent.
5. RESULTS

5.1. Retrospective studies (I-II)

5.1.1 Epidemiological features of severe sepsis

In study I, there were altogether 290 neutropenic periods in 84 AML patients (median 3, range 1-7). In 280 periods (97%) neutropenic fever was present. Severe sepsis was observed in 35 periods (13%) and in 13 periods (5% of all neutropenic fever periods) severe sepsis led to ICU admission (Table 8). In 17 periods (49%) the criteria for severe sepsis was hypotension despite of fluid resuscitation. In 11 periods (31%) the criteria for severe sepsis was septic shock and in 3 periods (8%) respiratory failure. In 3 periods (8%) septic shock developed into MOF. One patient (3%) died due to septic shock on the way to the hospital (Table 9).

The median time from the onset of the fever to the point where criteria for severe sepsis were met was 2 days (range 0-39). Thirteen patients were treated in the ICU due to severe sepsis, with a median stay of 3 days (1-42). Five patients (38%) recovered and eight patients died due to irreversible septic shock a median of 2 days (1-7) after admission to the ICU.

After the first induction course in study I, neutropenic fever was found in 80 out of 84 patients (95%). Severe sepsis was observed in 10 patients (13%) and 7 patients (8%) were admitted to the ICU. Four patients (5%) died after the first induction course due to infectious complications.

In study II there were 319 ASCT recipients with 265 (83%) febrile neutropenic periods. Because of the treatment protocol, only one period of neutropenic fever was included for each patient. Severe sepsis was observed in 17 patients (5%). In 5 patients (29%) the criteria for severe sepsis was hypotension despite fluid resuscitation. In 12 patients (71%) the criteria for severe sepsis was septic shock (Table 10). In nine occasions (3%) severe sepsis led to ICU admission.

The median time from the onset of fever to the point, where criteria for severe sepsis were met was 3 days (range 1-10). The median time to ICU admission was 7 days after the start of fever (range 2-25), and the median treatment time at ICU was 3 days (1-17). Seven patients (78%) died due to irreversible septic shock a median of 2.5 days (1-12) after the admission to ICU. Only two patients treated in the ICU (22%) recovered.

Severe sepsis was the most common severe early (<30 d) complication in ASCT recipients (17/20 patients, 85%). Other severe early complications included pneumonia (n=1), severe cardiotoxicity (n=1) and a combination of liver and cardiac failure (n=1).
Table 8. Studies I-IV

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Number of patients</td>
<td>84</td>
<td>319</td>
<td>42</td>
<td>70</td>
</tr>
<tr>
<td>with severe sepsis</td>
<td>30 (36%)</td>
<td>17 (5%)</td>
<td>5 (12%)</td>
<td>13 (19%)</td>
</tr>
<tr>
<td><strong>Number of neutropenic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fever periods</td>
<td>280</td>
<td>265</td>
<td>42</td>
<td>94</td>
</tr>
<tr>
<td>- Number of periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with severe sepsis</td>
<td>35 (13%)</td>
<td>17 (5%)</td>
<td>5 (12%)</td>
<td>13 (14%)</td>
</tr>
<tr>
<td><strong>Length of neutropenia,</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>days median (range)</td>
<td>23 (7-85)</td>
<td>11 (6-25)</td>
<td>9 (3-25)</td>
<td>12 (3-25)</td>
</tr>
<tr>
<td>- Periods with severe sepsis</td>
<td>24 (5-227)</td>
<td>9 (3-63)</td>
<td>10 (4-23)</td>
<td>10 (4-30)</td>
</tr>
<tr>
<td><strong>Mortality associated with severe sepsis</strong></td>
<td>9/84 (11%)</td>
<td>9/319 (2%)</td>
<td>1/42 (2%)</td>
<td>2/70 (3%)</td>
</tr>
<tr>
<td><strong>Case fatality rate for periods with severe sepsis</strong></td>
<td>9/35 (27%)</td>
<td>9/17 (53%)</td>
<td>1/5 (20%)</td>
<td>2/13 (15%)</td>
</tr>
</tbody>
</table>

In study I neutropenic septic infection was the cause of death in 9 patients. Thus mortality due to neutropenic sepsis was 11% (9/84). Neutropenic sepsis was suspected cause of death also in 2 other patients: one patient (number 27 in table 9) died due to a septic shock outside hospital (paramedic care) and one patient (number 26 in table 9) with refractory leukaemia died without ICU admission in the hematology ward. The case fatality rate for periods with severe sepsis was 27% (9/35). In study II neutropenic sepsis was the cause of death in 9 patients and mortality due to neutropenic sepsis was 2% (9/319). The case fatality rate for periods with severe sepsis was 53% (9/17). The mortality due to severe sepsis was higher in NHL patients when compared to other patient groups (6% vs. 0%, *p*=0.003).

5.1.2 Microbiological findings and site of infection

In study I clinically significant microbiological findings were found by blood cultures in 165 out of 280 episodes with neutropenic fever (59%) (Table 11). Gram-positive cocci were the most commonly identified causes of neutropenic fever (94 episodes, 57%), followed by gram-
negative rods (70 episodes, 43%). Only one fungal sepsis was observed during this time. In cases of severe sepsis, gram-negative rods were found in 15/35 of periods (43%).

In study II clinically significant microbiological growth in blood cultures was seen in 68 out of 265 periods with neutropenic fever (26%) (Table 11). Gram-positive cocci (44 episodes, 65%) were the most common identified cause of neutropenic fever, followed by gram-negative rods (23 episodes, 34%). Only one fungal bloodstream infection was found, concomitantly with gram-positive bacteraemia. In cases of severe sepsis, gram-positive cocci were found in 7 out of 17 periods (41%). Gram-negative rods were found in six periods (35%). *Pseudomonas aeruginosa* was the most common (5 episodes, 30%).

The lungs and upper respiratory airways were the most common site of infection (48 episodes, 17%) in study I. The number of catheter-related infections was 45 (16%) and skin (including perineal region) infections 17 (6%). In study II, the localisation of infection remained in most cases unknown. The percentage of catheter-related infections was 11%.

5.1.3. Factors associated with severe sepsis

In both studies, the neutropenic febrile periods were divided into those with and those without severe sepsis. In study I there were no differences in the median length of neutropenia, but in study II the median length of neutropenia was longer in ASCT recipients with severe sepsis (11 vs. 9, *p*=0.05).

In study I gram-negative bacteria were found more often in blood cultures (43% vs. 22%, *p*=0.03) and blood culture findings were more often positive (66% vs. 58%, *NS*) in periods with severe sepsis than those without. In study II severe sepsis was more common in patients with NHL than in other patients (9% vs. 3%, *NS*). Patients with severe sepsis were also older than those without (59 vs. 55, *p*=0.007). Blood culture findings were significantly more often positive (76% vs. 22%, *p*<0.001) and gram-negative bacteria were found more often (35% vs. 7%, *p*=0.001) among patients with severe sepsis than those without. Of note, *Pseudomonas aeruginosa* was found in 30% (n=5) of patients with severe sepsis and only in 1% (n=3) of those without severe sepsis (*p*<0.001).
Table 9. Description of AML patients (n=30) with periods of severe sepsis (n=35) in study I.

<table>
<thead>
<tr>
<th>Number</th>
<th>Gender/age</th>
<th>Treatment protocol</th>
<th>Conditioning regimen number</th>
<th>Criteria for severe sepsis</th>
<th>ICU</th>
<th>Blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recovered</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F/57</td>
<td>AML-92</td>
<td>IV</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Gemella morbillorum</em></td>
</tr>
<tr>
<td>2</td>
<td>M/54</td>
<td>AML-92</td>
<td>II</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>3</td>
<td>F/69</td>
<td>AML-92</td>
<td>V</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td>4</td>
<td>F/43</td>
<td>AML-92</td>
<td>IV</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>5</td>
<td>M/38</td>
<td>AML-92</td>
<td>I</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>IV</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>7</td>
<td>F/59</td>
<td>AML-92</td>
<td>I</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>8</td>
<td>F/57</td>
<td>AML-92</td>
<td>IV</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>9</td>
<td>M/62</td>
<td>AML-92</td>
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<td>Respiratory failure</td>
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<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>10</td>
<td>M/44</td>
<td>AML-92</td>
<td>III</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>11</td>
<td>M/66</td>
<td>AML-92</td>
<td>III</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>12</td>
<td>M/25</td>
<td>AML-92</td>
<td>I</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>13</td>
<td>M/38</td>
<td>AML-92</td>
<td>IV</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>14</td>
<td>M/57</td>
<td>AML-92</td>
<td>V</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>15</td>
<td>M/55</td>
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<td>I</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Enterococcus faecalis</em></td>
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<tr>
<td>16</td>
<td>F/46</td>
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<td><em>Culture negative</em></td>
</tr>
<tr>
<td>17</td>
<td>F/52</td>
<td>AML 2003</td>
<td>I</td>
<td>Respiratory failure</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>IV</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Escherichia coli</em></td>
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<tr>
<td>19</td>
<td>F/55</td>
<td>AML-92</td>
<td>III</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>20</td>
<td>F/39</td>
<td>AML-92</td>
<td>I</td>
<td>Hypotension</td>
<td>Yes</td>
<td><em>Culture negative</em></td>
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<tr>
<td>21</td>
<td>M/59</td>
<td>AML 2003</td>
<td>II</td>
<td>Hypotension</td>
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<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td>V</td>
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<td><em>Enterococcus faecium</em></td>
</tr>
<tr>
<td><strong>Died</strong></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>23</td>
<td>F/59</td>
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<td>Hypotension</td>
<td>No</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td>III</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
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<td>Septic shock</td>
<td>Yes</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>26</td>
<td>M/20*</td>
<td>AML-92</td>
<td>II</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Escherichia coli</em> Died on the way to the hospital</td>
</tr>
<tr>
<td>27</td>
<td>M/64*</td>
<td>AML-92</td>
<td>III</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>28</td>
<td>F/33</td>
<td>AML-92</td>
<td>I</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Stenotrophomonas maltophilia</em></td>
</tr>
<tr>
<td>29</td>
<td>M/38</td>
<td>AML-92</td>
<td>I</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>30</td>
<td>F/39</td>
<td>AML-92</td>
<td>II</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Streptococcus mitis</em></td>
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<td>31</td>
<td>M/58</td>
<td>AML 2003</td>
<td>III</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Culture negative</em></td>
</tr>
<tr>
<td>32</td>
<td>M/61</td>
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<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>33</td>
<td>M/58</td>
<td>AML-92</td>
<td>I</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Staphylococcus epidermidis</em></td>
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<tr>
<td>34</td>
<td>F/58</td>
<td>AML 2003</td>
<td>III</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
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<td>M/53</td>
<td>AML-92</td>
<td>VI</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

Abbreviations: ICU, intensive care unit; F, female; M, male; MOF, multiorgan failure.

* Patients with suspected neutropenic sepsis
<table>
<thead>
<tr>
<th>No</th>
<th>Gender</th>
<th>Age</th>
<th>Dg</th>
<th>Conditioning Regimen / Year of Treatment</th>
<th>Criteria for Severe Sepsis</th>
<th>ICU</th>
<th>Blood Culture Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>M/54</td>
<td>54</td>
<td>MM</td>
<td>HD-MEL/2005</td>
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<td>No</td>
<td>Negative</td>
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<td>MM</td>
<td>HD-MEL/2004</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>3.</td>
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<td>59</td>
<td>NHL</td>
<td>BEAC/2003</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>4.</td>
<td>M/61</td>
<td>61</td>
<td>MM</td>
<td>HD-MEL/2001</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>5.</td>
<td>M/64</td>
<td>64</td>
<td>NHL</td>
<td>BEAC/2003</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>6.</td>
<td>F/58</td>
<td>58</td>
<td>MM</td>
<td>HD-MEL/2005</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>7.</td>
<td>M/62</td>
<td>62</td>
<td>NHL</td>
<td>BEAC/2005</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Streptococcus viridans</em></td>
</tr>
<tr>
<td>8.</td>
<td>F/59</td>
<td>59</td>
<td>NHL</td>
<td>BEAC/2006</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Staphylococcus hominis</em></td>
</tr>
<tr>
<td>10.</td>
<td>M/67</td>
<td>67</td>
<td>NHL</td>
<td>BEAC/2005</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>11.</td>
<td>M/62</td>
<td>62</td>
<td>NHL</td>
<td>BEAM/2006</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Enterococcus faecium</em></td>
</tr>
<tr>
<td>12.</td>
<td>F/66</td>
<td>66</td>
<td>NHL</td>
<td>BEAC/2006</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>13.</td>
<td>M/50</td>
<td>50</td>
<td>NHL</td>
<td>BEAC/1996</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Streptococcus pneumoniae</em>, <em>Candida albicans</em></td>
</tr>
<tr>
<td>14.</td>
<td>M/40</td>
<td>40</td>
<td>NHL</td>
<td>BEAM1998</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>15.</td>
<td>M/64</td>
<td>64</td>
<td>NHL</td>
<td>BEAM/1999</td>
<td>Septic shock, MOF</td>
<td>Yes</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>16.</td>
<td>F/58</td>
<td>58</td>
<td>NHL</td>
<td>BEAM/2000</td>
<td>Septic shock</td>
<td>Yes</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>17.</td>
<td>M/59</td>
<td>59</td>
<td>NHL</td>
<td>BEAC/2003</td>
<td>Septic shock</td>
<td>No</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female; ICU, intensive care unit; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; BEAC, carmustine, etoposide, cytarabine and cyclophosphamide; BEAM, carmustine, etoposide, cytarabine and melphalan; HD-MEL, high-dose melphalan; MOF, multiorgan failure.
Table 11. Blood culture findings and site of infection in patients with neutropenic fever periods in retrospective studies (I and II).

<table>
<thead>
<tr>
<th></th>
<th>Study I N=84</th>
<th>Study II N=319</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of neutropenic fever periods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>137 (49%)</td>
<td>198 (75%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lungs and upper respiratory airways</td>
<td>48 (17%)</td>
<td>9 (3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Catheter-related infection</td>
<td>45 (16%)</td>
<td>29 (11%)</td>
<td>NS</td>
</tr>
<tr>
<td>Skin (including perineal region)</td>
<td>17 (6%)</td>
<td>2 (1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>33 (12%)</td>
<td>27 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Positive blood culture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>94 (57%)</td>
<td>44 (65%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>51 (31%)</td>
<td>28 (41%)</td>
<td>0.011</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>9 (5%)</td>
<td>5 (7%)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>9 (5%)</td>
<td>2 (3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Others</td>
<td>25 (15%)</td>
<td>9 (13%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Gram negative rods</td>
<td>70 (43%)</td>
<td>23 (34%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>19 (12%)</td>
<td>9 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5 (3%)</td>
<td>8 (12%)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>27 (16%)</td>
<td>4 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>19 (12%)</td>
<td>2 (3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungal</td>
<td>1 (0.6%)</td>
<td>1 (1%)</td>
<td>NS</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1 (0.6%)</td>
<td>1 (1%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Negative blood culture</strong></td>
<td>114 (41%)</td>
<td>198 (74%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
5.1.4. Serum C-reactive protein in relation to severe sepsis

In study I the median time from the onset of the fever to peak CRP value was 4 days (range 1-39). The median peak CRP value was higher in patients with severe sepsis (261 mg/l vs. 153 mg/l, \( p<0.001 \)). CRP_{2-3} was higher in patients with severe sepsis (190 mg/l vs. 96 mg/l, \( p<0.001 \)) than those without. In this study the CRP slope velocity was steeper in patients with severe sepsis (48 vs. 34 mg/l/d, \( p=0.02 \)) (Table 12).

In study II the median time from the onset of fever to the peak CRP value was 3 days (range 1-7). CRP_{2-3} was higher in patients with severe sepsis (148 mg/l vs. 90 mg/l, \( p<0.001 \)) as well as the median peak CRP value (217 mg/l vs. 111 mg/l, \( p<0.001 \)). The CRP slope velocity was again found to be steeper in patients with severe sepsis (54 vs. 30 mg/l/d, \( p=0.007 \)) (Table 12).

5.2. Serum vascular endothelial growth factor in patients with neutropenic fever: a comparison with C-reactive protein (III)

5.2.1. Epidemiological features of severe sepsis

All patients with severe sepsis had received ASCT for NHL. The median time after the onset of fever to the fulfillment of the criteria for severe sepsis was 1 day (range 1-3). The diagnostic criteria for severe sepsis were fulfilled in five out of 42 patients (12%) (Table 8): one of them had septic shock requiring vasopressor support in the ICU, one patient had septic respiratory failure requiring CPAP ventilation and three patients had fluid-responsive hypotension. One patient (2%) died due to septic shock in the ICU during the first day after admission (Table 13).

5.2.2. Microbiological findings and site of infection

Blood cultures remained negative in the majority of the patients (36/42, 85%). Positive findings in blood cultures included Staphylococcus epidermidis (n=3) and Staphylococcus haemolyticus, Enterobacter cloacae and Escherichia coli, one each. The lungs were the most common site of infection (n=10, 24%), followed by skin infections (including perineal region) (n=8, 19%). The percentage of catheter related infections was 10%. In 18 periods (43%), the site of infection remained unknown.
Table 12. Comparison of CRP in neutropenic fever periods with or without severe sepsis in studies I and II.

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With severe sepsis</td>
<td>Without severe sepsis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline CRP (mg/l)</td>
<td>17 (5-194)</td>
<td>24 (5-227)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/l) 2 to 3 days median (range)</td>
<td>190 (26-379)</td>
<td>96 (3-359)</td>
<td>&lt;0.001</td>
<td>148 (76-375)</td>
</tr>
<tr>
<td>Peak CRP (mg/l) median (range)</td>
<td>261 (57-563)</td>
<td>153 (22-492)</td>
<td>&lt;0.001</td>
<td>217 (76-545)</td>
</tr>
<tr>
<td>Time to peak CRP (d) median (range)</td>
<td>4 (1-39)</td>
<td>5 (1-59)</td>
<td>NS</td>
<td>3 (1-7)</td>
</tr>
<tr>
<td>CRP slope velocity (mg/l/day) median (range)</td>
<td>48 (8-167)</td>
<td>34 (0-162)</td>
<td>0.02</td>
<td>54 (4-120)</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein.

5.2.3. C-reactive protein

The median CRP concentration was 35 mg/l (range 5-253 mg/l) at the beginning of the fever (d0), 65 mg/l (range 11-234 mg/l) on day 1 (d1), 83 mg/l (range 7-257 mg/l) on day 2 (d2) and 82 mg/l (range 12-241 mg/l) on day 3 (d3) in the whole group of patients.

The peak concentration of CRP was achieved on d0 in 10%, on d1 in 19%, on d2 in 40% and on d3 in 31% of the patients. There were no statistically significant differences in CRP values on any day between the groups with severe and non-severe sepsis (Table 14). In the analysis of variance of repeated measures the overall difference in CRP between patients with and without severe sepsis was not statistically significant either (p=0.344).
Table 13. Patients with severe sepsis in studies III and IV.

<table>
<thead>
<tr>
<th>Number</th>
<th>Gender/age</th>
<th>Diagnosis</th>
<th>Conditioning regimen number</th>
<th>Criteria for severe sepsis</th>
<th>ICU</th>
<th>Blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recovered:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>F/70*</td>
<td>AML</td>
<td>II</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td>2.</td>
<td>F/53</td>
<td>AML</td>
<td>II</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>3.</td>
<td>M/63</td>
<td>AML</td>
<td>II</td>
<td>Respiratory failure</td>
<td>No</td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>4.</td>
<td>M/61</td>
<td>AML</td>
<td>II</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>5.</td>
<td>F/36</td>
<td>HL</td>
<td>After ASCT</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>6.</td>
<td>F/51</td>
<td>AML</td>
<td>II</td>
<td>Hypotension</td>
<td>No</td>
<td><em>Enterococcus faecium</em></td>
</tr>
<tr>
<td>7.</td>
<td>M/44*</td>
<td>NHL</td>
<td>After ASCT</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>8.</td>
<td>F/58*</td>
<td>NHL</td>
<td>After ASCT</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>9.</td>
<td>M/61*</td>
<td>NHL</td>
<td>After ASCT</td>
<td>Respiratory failure</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>10.</td>
<td>F/42</td>
<td>HL</td>
<td>After ASCT</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td>11.</td>
<td>F/60</td>
<td>NHL</td>
<td>After ASCT</td>
<td>Hypotension</td>
<td>No</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Died:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>M/54</td>
<td>AML</td>
<td>II</td>
<td>Septic shock</td>
<td>Yes</td>
<td>Negative</td>
</tr>
<tr>
<td>13.</td>
<td>M/55*</td>
<td>NHL</td>
<td>After ASCT</td>
<td>Septic shock</td>
<td>Yes</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviations: ICU, intensive care unit; F, female; M, male; AML, acute myeloid leukaemia; HL: Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; ASCT, auologous stem cell transplantation.

* included in both prospective studies (III and IV).
Table 14. Comparison of laboratory measures on days 0 to 3 from the beginning of the neutropenic fever of the study patients with severe sepsis and without severe sepsis. The data are expressed as medians (range).

<table>
<thead>
<tr>
<th></th>
<th>Patients with severe sepsis (N=5)</th>
<th>Patients without severe sepsis (N=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VEGF (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>77 (71 – 100)</td>
<td>52 (14 – 210)</td>
<td>0.061</td>
</tr>
<tr>
<td>d1</td>
<td>82 (71 – 102)</td>
<td>56 (9 – 163)</td>
<td>0.048</td>
</tr>
<tr>
<td>d2</td>
<td>54 (44 – 60)</td>
<td>34 (9 – 162)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>37 (27 – 54)</td>
<td>39 (9 – 123)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>CRP (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>23 (16 – 253)</td>
<td>36 (5 – 212)</td>
<td>NS</td>
</tr>
<tr>
<td>d1</td>
<td>65 (28 – 215)</td>
<td>64 (11 – 234)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>69 (57 – 86)</td>
<td>92 (7 – 257)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>70 (59 – 132)</td>
<td>85 (12 – 241)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Platelet count (10⁹/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>27 (13 – 121)</td>
<td>26 (8 – 83)</td>
<td>NS</td>
</tr>
<tr>
<td>d1</td>
<td>25 (20 – 144)</td>
<td>38 (9 – 100)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>62 (6 – 78)</td>
<td>35 (11 – 74)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>37 (20 – 62)</td>
<td>34 (11 – 69)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: VEGF, vascular endothelial growth factor; CRP, C-reactive protein; d, day.

5.2.4. Serum vascular endothelial growth factor

The median VEGF concentration at the beginning of the fever (d0) was 53 pg/ml (range 14-210 pg/ml), on d1 58 pg/ml (range 9-163 pg/ml), on d2 48 pg/ml (range 9-162 pg/ml) and on d3 39 pg/ml (range 9-123 pg/ml) in the whole group. On d1 the median VEGF concentration was higher in patients with severe sepsis than in patients with non-severe sepsis (82 pg/ml vs. 56 pg/ml, p=0.048) (Table 14). The same was seen on day 0 but the difference did not reach statistical significance (77 pg/ml vs. 52 pg/ml, p=0.061).

The peak concentration of VEGF was achieved on d0 in 45%, on d1 in 24%, on d2 in 14%, and on d3 in 17% of the patients (Figure 4). Time to achieve peak VEGF concentration was significantly shorter than time to achieve peak CRP concentration (mean 1.02 with S.E. 0.18
days vs. mean 1.93 days with S.E. 0.15 days, \( p=0.002 \). On d0 and d1 VEGF seems to
distinguish the groups with severe and without severe sepsis better than CRP (Table 14,
Figure 5). There was no significant correlation between VEGF and platelet levels at any time
point. There were no statistically significant correlations between CRP and VEGF levels,
either. In the analysis of variance of repeated measures the overall difference in VEGF
between patients with and without severe sepsis was not statistically significant \( p=0.310 \).

The advantage of CRP compared to VEGF in the prediction of severe sepsis was evaluated
based on receiver operating characteristics curve analysis (ROC) and the Youden index. In the
ROC curve analysis, the AUC for CRP was 0.56 (95% CI 0.40-0.72, \( p=0.480 \)) on day 0 and
0.49 (95% CI 0.33-0.64, \( p=0.880 \)) on day 1. AUC for VEGF on day 0 was 0.76 (95% CI 0.62-
0.90, \( p=0.060 \)) and 0.80 (0.67-0.94 \( p=0.048 \)) on day 1 (Figure 6). The predictive capacity of
VEGF was better for severe sepsis during the first 24 hours after the beginning of neutropenic
fever.

5.3. Serum amino-terminal pro-brain natriuretic peptide in patients with
neutropenic fever: a comparison with C-reactive protein (IV)

5.3.1. Epidemiological features of severe sepsis

There were altogether 70 patients with 94 episodes of neutropenic fever when all
neutropenic fever periods were included (fourteen patients with AML had more than one period
of neutropenic fever). The diagnostic criteria for severe sepsis were fulfilled in 13/94
neutropenic fever periods (14%) (Table 8). As it is shown in table 13 there were only one
neutropenic fever period with severe sepsis for each patient. In 9 patients (69%, 9/13) the
criteria for severe sepsis was hypotension despite of fluid resuscitation. In 2 patients (15%,
2/13) the criteria for severe sepsis was septic shock and in 2 patients (15%, 2/13) respiratory
failure. Two patients (15% of the patients with severe sepsis) died due to septic shock in
intensive care unit; one ASCT recipient one day after admission and one after second
laparotomy because of appendicitis while still neutropenic following the second induction
course for AML. The median time after the onset of fever to the point when the criteria for
severe sepsis were fulfilled was 1 day (range 1-7).
Figure 4. The day of the peak concentration of VEGF (the upper panel) and of CRP (the lower panel) in patients with neutropenic fever.
Figure 5. VEGF (pg/ml) (in the upper panel) and CRP (mg/l) (in the lower panel) in patients with and without severe sepsis on days 0 to 3 after the start of neutropenic fever. The cross lines indicate the mean values and the p-values significances for differences between patients with and without severe sepsis.
Figure 6. In the ROC curve analysis for VEGF, AUC was 0.80 (0.67-0.94), \( p=0.048 \) on day 1 to discriminate between severe and non-severe sepsis among neutropenic fever periods. The diagonal line (with dashes) represents results no better than chance.
5.3.2. Microbiological findings and site of infection

Blood cultures were positive in 22 (23%) of the periods with neutropenic fever. Positive findings included *Staphylococcus epidermidis* \((n=4)\), *Escherichia coli* \((n=3)\), *Klebsiella pneumoniae* \((n=2)\), *Enterobacter cloacae* \((n=2)\), *Rothia mucilaginosa* \((n=2)\) and *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus mitis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Morganella morgani*, one each.

In about half of the neutropenic febrile periods the site of infection remained unknown. The lungs and upper respiratory airways were the most common known sites of infection (19 episodes, 20%), followed by skin infections (including the perineal region) (16 episodes, 16%) and catheter-related infections (8 episodes, 9%).

5.3.3. Factors associated with severe sepsis

The median age of patients in the subgroups with and without severe sepsis was similar. The median length of neutropenia was 10 days (range 3-30) without statistically significant difference between periods with and without severe sepsis.

The frequency of severe sepsis was similar in patients with and without previous cardiovascular disease. The incidence of severe sepsis was 14% (6/43) in AML patients and 14% (7/51) in ASCT recipients of all episodes of neutropenic fever.

5.3.4. C-reactive protein

The peak CRP level was achieved on d2 (Figure 7). The median CRP was 35 (17-61) mg/l on d0, 79 (38-79) mg/l on d1, 109 (56-109) mg/l on d2, 90 (54-160) mg/l on d3, and 84 (44-141) mg/l.

There were no differences between the subgroups with and without severe sepsis in the levels of CRP on d0 to d2 but the difference was found in CRP values between the subgroups on d3 (147 mg/l vs. 86 mg/l, \(p=0.052\)) and on d4 (179 mg/l vs. 79 mg/l, \(p=0.015\)) (Table 15, Figure 8). Even though in the repeated measures ANOVA from day 0 to 3 no difference was found in the levels of CRP \((p=0.454)\) between subgroups, the differences of CRP were significant between d0-d1 and between d1-d2 with \(p\)-values of < 0.001 and < 0.001, respectively.
5.3.5. Amino-terminal pro-brain natriuretic peptide

The peak median NT-proBNP concentration was achieved on d4. The median NT-proBNP was 127 (57-393) ng/l on d0, 236 (78-752) ng/l on d1, 318 (126-734) ng/l on d2, 351 (134-1023) ng/l on d3, and 542 (194-1385) ng/l on d4 (Figure 7).

There were no statistically significant differences between the subgroups with and without severe sepsis in the level of NT-proBNP at any time point (Table 15, Figure 8). After excluding subjects with previous cardiovascular disease, the findings remained similar. In the repeated measures ANOVA from day 0 to 3 no difference was found in the levels of NT-proBNP ($p=0.474$) between subgroups with and without severe sepsis, but the differences between d0-d1 and between d1-d2 NT-proBNP levels were significant ($p=0.001$ and 0.011, respectively).

There was no correlation between NT-proBNP and the level of the same day CRP on the first three days of neutropenic fever. However, NT-proBNP on d3 correlated slightly with CRP on d4 ($r=0.24, p=0.050$), and NT-proBNP on d4 correlated with CRP on d3 ($r=0.35, p=0.010$) and on d4 ($r=0.33, p=0.015$).

5.3.6. Association of amino-terminal pro-brain natriuretic peptide with fluid intake and previous cardiovascular disease

There was a slight negative correlation between NT-proBNP and the level of the fluid intake on the same day for d0 and d1 of neutropenic fever (d0: $r=-0.24, p=0.038$; d1: $r=-0.25, p=0.021$). Day 0 fluid intake correlated negatively with d4 NT-proBNP ($r=-0.35, p=0.020$).

The levels of NT-proBNP were higher in the subgroup of patients with previous cardiovascular disease than those without throughout the course of the neutropenic fever (Table 16, Figure 9). In the repeated measures ANOVA from d0 to d3 a significant difference was found in the levels of NT-proBNP ($p<0.001$) between subgroups with and without previous cardiovascular disease.
Table 15. Comparison of the laboratory values and fluid intake from the beginning of the neutropenic fever (n=94). The data are expressed as medians (range).

<table>
<thead>
<tr>
<th></th>
<th>Severe sepsis (N=13)</th>
<th>Without severe sepsis (N=81)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (ng/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>53 (18-11376)</td>
<td>137 (19-25529)</td>
<td>NS</td>
</tr>
<tr>
<td>d1</td>
<td>85 (17-2501)</td>
<td>273 (22-15331)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>177 (50-3020)</td>
<td>321 (34-9016)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>253 (50-3107)</td>
<td>352 (18-11162)</td>
<td>NS</td>
</tr>
<tr>
<td>d4</td>
<td>768 (50-3681)</td>
<td>521 (15-24368)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>39 (16-253)</td>
<td>34 (5-239)</td>
<td>NS</td>
</tr>
<tr>
<td>d1</td>
<td>72 (28-218)</td>
<td>80 (9-272)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>88 (57-251)</td>
<td>110 (7-333)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>147 (59-340)</td>
<td>86 (7-320)</td>
<td>0.052</td>
</tr>
<tr>
<td>d4</td>
<td>179 (44-452)</td>
<td>79 (8-239)</td>
<td>0.015</td>
</tr>
<tr>
<td>Fluid intake (l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d1</td>
<td>4.5 (3.0-6.8)</td>
<td>3.9 (2.0-7.1)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>4.0 (2.0-8.5)</td>
<td>4.4 (1.2-11.4)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>4.6 (2.0-5.3)</td>
<td>4.5 (2.0-8.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: NT-proBNP, amino-terminal pro-brain natriuretic peptide; CRP, C-reactive protein; d, day.
Figure 7. The median concentrations with interquartile ranges of NT-proBNP (solid line) and CRP levels (dotted line) on days 0-4 from the onset of neutropenic fever.
Figure 8. The median concentrations with interquartile ranges of NT-proBNP and CRP on days 0-4 from the onset of neutropenic fever in periods with (dotted lines) and without (solid lines) severe sepsis.
Table 16. Comparison of the laboratory values and fluid intake on days 0 to 4 from the beginning of the neutropenic fever for all neutropenic fever periods in patients with and without previous cardiovascular disease. The data are expressed as medians (range).

<table>
<thead>
<tr>
<th></th>
<th>Previous cardiovascular disease (N=16)</th>
<th>Without previous cardiovascular disease (N=78)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (ng/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>868 (22-25529)</td>
<td>100 (18-11376)</td>
<td>0.001</td>
</tr>
<tr>
<td>d1</td>
<td>1096 (46-15331)</td>
<td>214 (17-6957)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d2</td>
<td>1908 (37-9016)</td>
<td>264 (34-6929)</td>
<td>0.001</td>
</tr>
<tr>
<td>d3</td>
<td>2072 (146-7145)</td>
<td>243 (18-11162)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d4</td>
<td>1915 (204-7107)</td>
<td>260 (15-24368)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>24 (5-194)</td>
<td>37 (5-253)</td>
<td>NS</td>
</tr>
<tr>
<td>d1</td>
<td>47 (16-249)</td>
<td>85 (9-272)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>113 (12-264)</td>
<td>102 (7-333)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>134 (8-241)</td>
<td>85 (7-340)</td>
<td>NS</td>
</tr>
<tr>
<td>d4</td>
<td>103 (10-220)</td>
<td>79 (8-452)</td>
<td>NS</td>
</tr>
<tr>
<td>Fluid intake (l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d1</td>
<td>3.2 (2.0-6.1)</td>
<td>4.0 (2.0-7.1)</td>
<td>NS</td>
</tr>
<tr>
<td>d2</td>
<td>3.7 (1.2-6.2)</td>
<td>4.5 (2.0-11.4)</td>
<td>NS</td>
</tr>
<tr>
<td>d3</td>
<td>3.8 (2.0-5.3)</td>
<td>4.6 (2.0-8.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: NT-proBNP, amino-terminal pro-brain natriuretic peptide; CRP, C-reactive protein.
Figure 9. The median concentrations with interquartile ranges of NT-proBNP and CRP on days 0-4 from the onset of fever in neutropenic fever periods in patients with (dotted lines) and without (solid lines) previous cardiovascular disease.
6 DISCUSSION

6.1 Patients and methods

The present series of studies are based on two retrospective studies (I-II) and a prospective still ongoing study (III-IV). The intention of the study was to evaluate the various aspects of severe sepsis in haematological patients, especially in patients with a high risk of neutropenic fever. Therefore, only patients with AML and those receiving ASCT were included. Chemotherapy regimens used in the treatment of AML cause a markedly longer period of neutropenia than HDT supported by ASCT (Table 8). The intensity of the HDT in ASCT recipients, on the other hand, causes more severe mucosal damage in the patients. These patient groups present different sides of high-risk neutropenic fever and were analysed separately in the retrospective part of the present series. For prospective study, these patient groups were combined because interest was focused on clinical management of haematological patients with severe sepsis.

The definitions for neutropenia, neutropenic fever and severe sepsis were based on internationally used definition criteria of both the Infectious Diseases Society of America (6) and 2001 International Sepsis Definitions Conference (79). Even though the definition of SIRS and severe sepsis have been available for almost two decades (78), these have not been systematically applied in haematological patients receiving intensive therapy. The definition of SIRS includes leukocytosis or leucopenia, fever, tachypnea and tachycardia (Table 1), and two of these criteria must be manifested to fulfill the criteria of SIRS. All of the study patients met at least two of these other criteria than leucopenia. Thus, leucopenia is a consequence of the treatment, and this has also been taken into account in this study.

Despite the criteria for neutropenic fever and severe sepsis are definitive, the backgrounds for both entities are often heterogeneous. The treatment strategies for septic infections in haematological wards may differ, but the amount of supportive care remains crucial. Vigorous volume resuscitation as used in the prospective study might thus prevent or shorten the duration of severe sepsis - with obvious clinical benefits. Severe sepsis that responds quickly to supportive therapy may be different from the situation where hypotension responds more slowly and eventually necessitates ICU admission.

Based on retrospective studies it was evident that hard endpoints (ICU admission, death) were rather infrequent in this patient cohort. Because of this, severe sepsis was used as an endpoint in prospective studies and as a model to evaluate prognostic utility of various
laboratory markers. Therefore, the findings of the present studies do not as such apply to the ICU setting although the kinetics of various markers are likely to be the same.

During the study period, the empirical antimicrobial treatment practice and hospital infection control measures (e.g. isolation policies and hospital hygiene, including cleaning and hand-washing policies) remained constant. Systematic antibiotic prophylaxis was not used, except during the last study year in NHL patients receiving ASCT. In the prospective studies, each patient was daily carefully monitored for clinical signs and possible sources of infections. Special attention was paid to features indicating the development of severe sepsis. In addition, supportive care was provided including aggressive volume resuscitation when needed.

In regard to laboratory methods, serum CRP measurements have been routine for more than two decades, and the method is standard. Also serum NT-proBNP measurements have been in clinical use in ISLAB (Eastern Finland Laboratory Centre, Kuopio, Finland) for several years. NT-proBNP was preferred to BNP because of its markedly longer plasma life and better stability (164). VEGF was determined from serum samples instead of plasma. This was because it was the only method used by the laboratory (ISLAB) and because reference values were available only for serum VEGF based on the FINNSEPSIS study.

6.2. Epidemiological features and outcome of severe sepsis

The available data of incidence of severe sepsis in neutropenic patients is limited. One reason for this might be that most of the studies regarding incidence of severe sepsis or septic shock are made in patients treated at ICUs. The policy among admission practice of patients with malignant diseases to ICUs varies between centres (196, 197). In present studies the admission of patients with neutropenia and severe sepsis to ICU has had a relatively low threshold. The admission criteria to ICU may have had slight changes during the study period, and this naturally may influence the outcome of patients.

6.2.1. Patients with acute myeloid leukaemia

In AML patients, the criteria for severe sepsis were fulfilled in 13% of periods with neutropenic fever after intensive induction or consolidation courses. Nine out of 84 AML patients (11%) died due to severe sepsis. This underlines the importance of severe sepsis as a cause of death in AML patients treated with modern intensive chemotherapy. In other studies, the infection mortality rate has ranged between 5-13% (2, 60-64). In elderly patients, the risks may be even higher (198).
Even though the induction course may be the most dangerous in relation to severe or fatal infections, also the consolidation courses are associated with high risk of neutropenic fever (2, 62). Therefore, it is obvious that all measures aiming to decrease mortality due to severe sepsis might improve overall outcome of AML patients. Unfortunately, in the present study only 38% of patients treated in ICU recovered. At least in some patients the late referral to ICU might be a possible reason for this. During the study period (1996-2005) the practises of admission to ICU have changed: life-sustaining treatments are begun outside ICUs in different step-up/ step-down units. Moreover, the threshold admission to the ICU has become lower for patients with malignancies and severe co-morbidities. In AML patients, the goal of the intensive treatment is curative, and early ICU admission in severe sepsis may improve prognosis. Close clinical monitoring of these patients is therefore mandatory in the haematology ward.

6.2.2. Autologous stem cell transplant recipients

In ASCT recipients the incidence of severe sepsis was much lower (5%) than in AML patients, but patients receiving a transplant for NHL were at a higher risk than the rest of ASCT recipients. In earlier studies the percentage of septic bloodstream infections in ASCT recipients has varied between 12% and 20% (72-74, 76).

The case fatality rate in patients with severe sepsis was, however, high (9/17, 53%) and all patients who died due to severe sepsis after ASCT had NHL. Only 18% of patients admitted to ICU recovered. This figure is clearly inferior than in some ICU- based studies of haematological patients (199), but the reason for admission may effect ICU outcome in an important way (e.g. septic hypotension in young neutropenic patients vs. neutropenic septic shock in patients with co-morbidities).

The mortality for severe sepsis was 6% in patients with NHL. This is a high figure compared with other series where an early mortality of 2-4% has been observed (70-72, 74). One potential reason for the high mortality rate in this study may be the relatively high number of elderly NHL patients receiving ASCT. In an EBMT (European Group of Blood and Marrow Transplantation) study, the risk of early treatment-related mortality in older NHL patients (>60 years of age) was significantly higher (4.4%) than in younger patients (2.8%) (200). Other reasons for higher mortality of NHL patients due to severe sepsis might be the combination of longer neutropenia together with the known cardiotoxicity of prior anthracycline treatment (201). Therefore, a prospective treatment protocol for NHL patients receiving ASCT was initiated in January 2008. This includes oral ciprofloxacin prophylaxis during neutropenia,
cardiac pre-evaluation with radio-cardiography, ACE-inhibitor treatment in patients with decreased left ventricular ejection fraction and use of BEAM chemotherapy instead of earlier BEAC. The latter is known to be associated with early cardiotoxicity (201).

6.2.3. Prospective study

In the prospective series the incidence of severe sepsis was 14% without a difference between AML patients and ASCT recipients. Even though the incidence of severe sepsis was not lower than in retrospective series, the case fatality rate for periods with severe sepsis seemed to be lower (15%). There are many possible reasons for this. Improved supportive care during the prospective treatment protocol and thus possibly earlier detection of severe sepsis may be important factors. This includes starting effective treatment by the trained nursing personnel earlier. However the number of periods with severe sepsis is still relatively low in the prospective series and more experience is needed before drawing firm conclusions. Haematological patients themselves may today be better informed and aware of the risk of septic infections during chemotherapy. This in turn helps them to recognise symptoms of septic infections and contact hospitals earlier than other patients with community-acquired sepsis.

6.3. Microbiological findings

In general, the incidence of microbiologically documented bloodstream infections has been between 7.7% and 19% in patients with neutropenic fever (23, 25, 26). In the present studies, the percentage varied between 23 and 59%. In AML patients (study I) the amount of bloodstream infections was 59%, which is higher than in earlier studies (24, 66).

There are some obvious reasons for the high percentage of bloodstream infections in AML patients. The duration of neutropenia in AML patients during intensive induction and consolidation chemotherapy is markedly longer than in any other treatment protocol for malignancies. During the long-lasting treatment period, the use of long-term indwelling catheters (up to six months) is common. This predisposes patients to catheter-related infections. In addition, intensive chemotherapy itself causes mucosal damage. This in turn increases the risk of infections caused by microbes from the normal oro-pharyngeal and gastrointestinal flora. Long-lasting febrile neutropenia increases the number of blood cultures taken and hence, also the number of positive culture results.
As expected, gram-positive microbes caused the majority of bloodstream infections. This finding is in accord with other recent studies (24, 37, 74). The proportion of gram-negative microbes in blood cultures was high (9-25%) and especially as a cause of severe sepsis (35-41%). The presumable reason for this is the lack of prophylactic antibiotics in present series – the situation is similar in other centres where antibiotic prophylaxis has not been used (202-204).

Among gram-positive microbes causing bloodstream infections the most common finding was *Staphylococcus epidermidis*, which is in line with earlier observations (59, 205, 206). The amount of bloodstream infections arising from central venous catheters varied between 9% and 16%, with the highest percentage in AML patients. The plausible explanation for this was the long-term use of indwelling catheters in this patient group. The lungs and upper respiratory airways were the most common known localisation of infection in febrile neutropenic patients, as observed in other studies (66, 70).

*Candida albicans* was found in ≤1% of febrile neutropenic periods in both AML patients and ASCT recipients. The apparent reason for the small amount of fungaemia was the use of fluconazole prophylaxis during prolonged neutropenia and mucosal symptoms (207). In the Finnish nationwide series the risk of invasive Candida-infections was also <1% among 1188 ASCT recipients (208). In addition, the lack of systematic antibacterial prophylaxis may have had an impact.

In contrast to some other recent series (38, 209), there were not a single case of multiresistant bacteria like MRSA, VRE, extended-spectrum β-lactamase (ESBL) -producing gram-negative rods or multi-drug resistant gram-negative microbes. The explanation for this might be our treatment practice in Finland – antibacterial prophylaxis is not commonly used. Furthermore, empirical use of vancomycin has been restricted during the whole study period and the overall use of wide spectrum antibiotics has been under control.

In the beginning of 2008 the use of oral ciprofloxacin was started for NHL patients receiving ASCT. This was mainly because of the fatal *Pseudomonas aeruginosa*-bloodstream infections in NHL patients during years 1996-2006 (study II). The mortality with *Pseudomonas aeruginosa* bloodstream infections was high and this finding is in line with earlier studies (210-213). The present prophylaxis practice is experimental and is part of a prospective clinical protocol. Although preliminary, the early impression seems to be promising, because there has not been a single case of severe sepsis among fourteen consecutive patients treated within this protocol (214). The amount of patients who received ciprofloxacin prophylaxis during the present study time was low and probably does not have a major influence on the study findings.
6.4. Kinetics of serum C-reactive protein in patients with neutropenic fever (Studies I-II)

In the retrospective studies (I-II) the median time from the onset of neutropenic fever to the point where criteria for severe sepsis were met was 2 (study I) and 3 days (study II). CRP concentrations elevated in parallel with this but the peak CRP was achieved later than the criteria for severe sepsis. Neither CRP slope velocity nor CRP on days 2-3 reliably distinguish periods with severe sepsis from those without in advance. In the study of Persson and colleagues (14) the CRP concentrations on days 2 and 3 of febrile neutropenia were lower in those patients who did not develop complications (e.g. pneumonia). This is in line with our findings.

Although several parameters of CRP kinetics were statistically associated with severe sepsis, in most instances CRP parameters evaluated coincided or even followed the diagnosis of severe sepsis. This was observed also in earlier studies both in patients with febrile neutropenia (146) and those without (142, 143). CRP identifies patients with infection but when the time interval is short, the predictive capacity of CRP declines markedly. This is mostly explained by the biochemistry of CRP – the peak values are reached 48 hours after the beginning of secretion independently of the reason for secretion (128).

6.5. Comparison of serum vascular endothelial growth factor and C-reactive protein in patients with neutropenic fever (Study III)

The peak serum VEGF level was reached during the first 24 hours and the peak CRP values on day 2 after the onset of neutropenic fever in patients with severe sepsis. Serum VEGF levels were continuously low, but slightly higher in patients who developed severe sepsis than in those who did not. The difference reached statistical significance for VEGF after the first 24 hours but there were no statistically significant differences in CRP values on any day between the groups with severe and non-severe sepsis.

The serum VEGF values on d0 in study III were notably lower than in patients with severe sepsis in FINNSEPSIS study (median 423 pg/ml) or in healthy controls (median 260 pg/ml) (Figure 10) (15). However, during the first 24 hours the VEGF levels in neutropenic patients with severe sepsis were higher than in those without. This latter finding is in line with earlier studies (215-217), although those studies included patients without known neutropenia or thrombocytopenia.
Figure 10. The median concentrations with interquartile ranges of VEGF (mg/ml) on days 0 and 3 in subjects with neutropenic sepsis (n=42) (solid line) and in septic patients participating in the FINNSEPSIS-study (dotted line) (n=242) in 24 multidisciplinary intensive care units in Finland\textsuperscript{(15)} The comparison is shown with the kind permission of the FINNSEPSIS Study Group.

An apparent reason for the low VEGF values observed in this study may be thrombocytopenia and neutropenia in all patients. The close association of serum VEGF and platelet count was first considered when changes in VEGF levels were found to mirror changes in platelet counts during chemotherapy for breast cancer (158). Platelets tend to accumulate VEGF, acting as a storage for circulating VEGF both in healthy subjects and in cancer patients (218, 219). The result was similar in the study of Benoy and colleagues (220). Several studies have also shown that circulating VEGF resides not only in platelets, but also in granulocytes, mainly in neutrophils (159-161). The study patients all had severe neutropenia during the study period. Low values also in patients with severe sepsis support the theory that platelets and granulocytes are the major source of VEGF. The early increase in VEGF levels may be due to liberation of VEGF from endothelial stores caused by endothelial injury associated with sepsis.
In patients with neutropenic fever, there are no previous studies available on the kinetics and prognostic utility of serum VEGF. In the present study, it was demonstrated that also thrombocytopenic and neutropenic patients are capable of producing VEGF during fever. Although haematological patients had low VEGF concentrations during sepsis, the VEGF concentrations in severely septic patients were higher than in other febrile patients, especially on the first days after the start of fever. This implies that VEGF is a more rapid indicator than CRP in haematological patients with neutropenic fever, which deserves further study.

6.6. Comparison of serum amino-terminal pro-brain natriuretic peptide and C-reactive protein in patients with neutropenic fever (Study IV)

There were no statistically significant differences in the level of NT-proBNP between patients with and without severe sepsis at any time point analysed. CRP concentrations did not show any predictive utility either; the median level of CRP was significantly higher in the subgroup with severe sepsis only on day 3 and day 4. Taking into account that the median time to the fulfilment of criteria for severe sepsis was only one day in this prospective study, it is obvious that these markers are unsuitable for clinical use to predict severe sepsis.

Previous myocardial infarction, acute coronary syndrome, valvular disease, ventricular hypertrophy and cardiomyopathy are known cardiological reasons for increased values of natriuretic peptides. Non-cardiac factors include age, female gender, renal failure, glucocorticoid use and acute pulmonary embolism (138, 221, 222). In the present study, patients with a history of previous cardiovascular disease had significantly higher levels of NT-proBNP than those without, even though none of the study patients had clinically severe cardiovascular disease. Significant release of NT-proBNP continued during the entire study period. Of note, the amount of daily fluid intake did not show any significant association with NT-proBNP levels.

There are some previous studies showing a significant association with sepsis and ICU mortality in patients with markedly elevated BNP- and NT-proBNP values (16, 138, 168, 169). Nikolau and his colleagues showed that BNP levels were elevated also in the acute phase of community-acquired microbial infections without severe sepsis or septic shock (170). It was therefore reasonable to assume that natriuretic peptides would serve as prognostic markers also in the haematology ward setting. This was, however, not the case in the present study where NT-proBNP was used.

Neither serial NT-proBNP nor CRP showed any early predictive value in neutropenic periods with severe sepsis compared to those without. NT-proBNP seemed to reflect cardiac distress
more than the inflammatory response in this patient population. It might thus serve as a useful adjunct to optimise management of patients with previous cardiovascular disease during sepsis.

6.7. Study limitations

Because of the retrospective nature, studies I-II have several unavoidable limitations. The criteria for severe sepsis were well applicable for all study subjects, although the retrospective classification of patients with severe sepsis was challenging. All periods regarded to represent severe sepsis were confirmed by consultation between the clinical haematologist (EJ) and infectious disease specialist (SH), but uncertainties remain in patients who were not treated in the ICU.

CRP values on the first day of the fever and particularly 12-24 hours from the onset of fever were often missing. For this reason the values 2 or 3 days after the onset of fever were used. Further, in patients with clinical signs of severe infection CRP values were evaluated apparently more often than febrile neutropenic patients without these signs. The observers were not blinded to CRP values when evaluating febrile neutropenic periods.

In studies I and IV all febrile neutropenic periods in study patients were included. This is common practice in haematological studies, but it also causes a situation where the same patient is analysed repeatedly during new periods of neutropenic fever. It is unknown how inflammatory markers behave in repeating periods of febrile neutropenia in the same individual. However, in present series only four patients (study I) (Table 9) had more than one episode of severe sepsis. As with these patients and with all other study patients, it was impossible to predict the febrile period that will be complicated with severe sepsis. For this reason, all periods with neutropenic fever are included.

The site of infection remained unknown in most of the febrile neutropenic periods. Neutropenia alters the host’s inflammatory response and makes the infection difficult to detect because the classic signs and symptoms of infection are usually missing. This is mainly because of an almost total lack of neutrophils. In this respect traditional laboratory methods are also useless. Fever is often the only sign of infection in accordance with criteria of severe sepsis (e.g. hypotension). Sometimes medications like corticosteroids can obscure especially fever. Only patients with both neutropenia and fever (≥38.3°C or ≥38.0°C for ≥1 h) were included into the present studies. It is possible that there have been also patients with neutropenic infection but without fever. In septic infections, however, this is unlikely. Patients
are closely monitored during the treatment period. Even without fever, the other signs of severe infection (e.g. hypotension, dyspnoea) would have been noted.

Study III was the first prospective study published evaluating VEGF kinetics in cytopenic haematological patients. The number of patients was relatively small. Consequently, the number of patients who had severe sepsis was also small. The number of endpoints (severe sepsis, septic shock or death) was low in both prospective studies, but in line with the incidences observed in retrospective studies.

Sepsis-related Organ Failure Assessment (SOFA) or another comparable method would have been useful in patient monitoring. Classification of neutropenic patients with SOFA in haematological wards has some inevitable limitations. Patients have a low number of platelets because of chemotherapy even in the absence of infection, and platelet count is one of the main criteria for SOFA. A more important reason for not using SOFA is that usage of invasive monitoring is not routine outside ICUs.

More frequent early measurements (e.g. between 2-6 hour intervals after the beginning of fever) of both NT-proBNP and CRP might have been more informative in prospective studies. However, such a frequent sampling may not be applicable in clinical practice outside intensive care units. Considering the kinetics of these measures, it is also unlikely that more frequent sampling would have led to different conclusions.

6.8. Concluding remarks

The frequency of severe sepsis was lowest among ASCT recipients (study II) (5%) and highest in study IV (14%), where both AML patients and ASCT recipients were prospectively included. The outcome of severe sepsis was rather poor in the retrospective studies with a mortality of 11% in AML patients and 2% in ASCT recipients during intensive therapy. The case fatality rate for periods with severe sepsis were 27% and 53%, respectively. In the prospective study with close patient monitoring and supportive care, the mortality during intensive therapy was 3% and the case fatality rate for periods with severe sepsis 15%. Although the incidence of severe sepsis seems to remain the same, the outcome seems to have been better in the prospective part of the study.

The microbial aetiology of severe sepsis in neutropenic patients did not change during the study period (1995-2008). Gram-positive microbes were the predominant findings in blood cultures, which is well in line with other studies (37, 38, 223). Because antibacterial prophylaxis was not used, the proportion of gram-negative microbes in bloodstream infections was considerable compared with studies where prophylactic antibiotics were used. The incidence
of *Pseudomonas aeruginosa* was nevertheless alarming, and changed the policy concerning prophylactic antimicrobial use in patients with greatest risk of fatal septic infections. During the study period, not a single strain of MRSA, VRE or ESBL were found, which is an important finding concerning the hospital hygiene policies in our hospital.

CRP was one of the infection markers chosen for this study protocol. It is easily available and familiar as a reference marker for sepsis. Other chosen markers were compared with CRP. As demonstrated in the earlier studies (14, 142, 146), CRP was slow to change in the early phase (< 24 hours) of severe sepsis. The situation was similar with the NT-proBNP (study IV). The peak values came far too late for predictive purposes in patients with neutropenic sepsis.

The results with serum VEGF showed some promise. Study III was the first study published evaluating VEGF kinetics in haematological patients with neutropenic fever. It was demonstrated that also thrombocytopenic and neutropenic patients can produce VEGF during fever. Although the VEGF concentrations were in general low, the VEGF concentrations in severely septic patients were higher than in other febrile patients. The difference was obvious during the first critical hours from the start of neutropenic fever. This implies that VEGF might be a rapid indicator for severe sepsis in haematological patients with neutropenic fever. The problem with VEGF is the current lack of an appropriate methodology in clinical laboratories that can provide results within 12-24 hours. The definition of practical cut-off values with evaluation of positive and negative predictive capacity in neutropenic and thrombocytopenic patients is also needed.

6.8.1. Implications for further research

The major focus of this series of studies was on febrile neutropenic patients treated in a haematological ward. In these circumstances, the conditions and equipment are inferior to those in ICUs. Furthermore, the possibilities for respiratory and circulatory support are limited.

In retrospective studies only 38% of the AML patients and 22% of the ASCT recipients admitted to the ICU recovered. This contrasts with the results of the FINNSEPSIS study (10), where 86% of the patients admitted for sepsis to ICU recovered. In recent years, intensive care treatment with step-down units has become more available, and admission criteria have probably changed. In addition, the treatment strategies in haematological wards have changed. Although the supportive care in haematological ward has been rather efficient also in the near past, the need for improvement is obvious. The possibilities in the ward are, however,
limited and a flexible consultation system is essential to get these patients early into step-up-step-down units or the ICU when needed.

The question of antibiotic prophylaxis in neutropenic haematological patients needs to be reconsidered. Based on the findings in study II a new prospective protocol was started in the beginning of January 2008. One of its main principles is to determine the benefits and possible disadvantages of fluoroquinolone prophylaxis in high-risk ASCT recipients. Mucositis caused by intensive chemotherapy is in closely associated with this – if the incidence of mucositis could be reduced (224, 225), the amount of neutropenic infections and of course, septic infections would diminish. There are interesting possibilities for the development of locally administered nonpharmacological measures and pharmacotherapeutics to restore mucosal barriers in the future (226).

Readily available early circulatory markers for severe sepsis could be helpful in clinical practice, especially in haematological wards. Severe sepsis proceeds rapidly. Commonly used laboratory methods recognise this phenomenon too late and too slowly. Serum VEGF might be one option for further research. At present, the lack of an appropriate methodology applicable in clinical laboratories to get the result within 12-24 hours and the lack of practical cut-off values with known positive and negative prediction capacity are problems. Many other early markers are of potential interest in relation to the pathogenesis of sepsis in neutropenic haematological patients as well as polymerase chain reaction (PCR) based assays for fast microbial recognition (135, 227).

While continuing the search for clinically useful laboratory markers, it is of utmost importance that febrile neutropenic patients be carefully monitored for vital functions with optimised supportive care. In haematological wards, severe sepsis is still primarily a clinical diagnosis, and the experience of caregiving physicians is vitally important. Close co-operation with colleagues in ICUs and other supportive care units is crucial in the treatment of patients with severe neutropenic sepsis.
7. CONCLUSION

I Severe sepsis was observed in 13 % of periods with neutropenic fever in AML patients and 5% of periods in ASCT recipients. Gram-negative rods were more commonly found in blood cultures of periods with severe sepsis in both patient groups. In ASCT recipients, 30% of blood stream infections were caused by *Pseudomonas sp*. The case fatality rate of severe sepsis was 28 % in patients with AML and 53 % in ASCT recipients.

II Peak CRP values were higher and CRP slope velocity steeper in periods with severe sepsis in both patient groups. CRP kinetic merely coincided than preceded the development of severe sepsis.

III VEGF concentrations were in general low but < 24 hours after the start of neutropenic fever VEGF levels were higher in patients with severe sepsis. VEGF seems to be a more rapid indicator of severe sepsis than CRP in haematological patients with neutropenic fever.

IV Neither serial NT-proBNP nor CRP showed any early predictive value for the development of severe sepsis in haematological patients with neutropenic fever. NT-proBNP values were significantly higher in patients with previous cardiovascular disease.
8. REFERENCES


97


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