MARKERS OF SYSTEMIC INFLAMMATION
IN DIAGNOSTICS AND
IN PREDICTION OF OUTCOME OF
COMMUNITY-ACQUIRED INFECTION

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To my family
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1 LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals:


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2 ABBREVIATIONS

ABC  antibody-binding capacity
ACCP  the American College of Chest Physicians
APP  acute phase protein
AUC  area under curve
BSI  bloodstream infection
CAI  community-acquired infection
CAP  community-acquired pneumonia
CD  cluster of differentiation
CI  confidence interval
COPD  chronic obstructive pulmonary disease
CR  complement receptor
CRP  C-reactive protein
ED  emergency department
ELISA  enzyme-linked immunosorbent assay
FACS  fluorescence-activated cell sorter
FITC  fluorescein isothiocyanate
GPI  glycosylphosphatidylinositol
HLA  human leukocyte antigen
HUCH  Helsinki University Central Hospital
HUSLAB  the laboratories of the hospital district of Helsinki and Uusimaa
IBD  inflammatory bowel disease
ICAM  intercellular adhesion molecule
IFN  interferon
IL  interleukin
IQR  interquartile ratio
LFA  leukocyte functional antigen
LPS  lipopolysaccharide
LBP  LPS-binding protein
LR+  positive likelihood ratio
LRTi  lower respiratory tract infection
MAP  mean arterial pressure
MEDS  Mortality in Emergency Department score
MHC  major histocompatibility complex
MI  myocardial infarction
MO  monocyte
N/A  not available
NADPH  nicotinamide adenine dinucleotide phosphate
NFkB  nuclear factor kappa B
NPV  negative predictive value
PAF  platelet-activating factor
PAMP  pathogen-associated molecular pattern
PCT  procalcitonin
PMN  polymorphonuclear leukocyte
PPV  positive predictive value
PRR  pattern-recognition receptor
QSC  Quantum Simply Cellular®
SCCM  the Society of Critical Care Medicine
mCD14  membrane CD14
sCD14  soluble CD14
sIL-2R  soluble interleukin-2 receptor
SIRS  systemic inflammatory response syndrome
TLR  Toll-like receptor
TNF  tumour necrosis factor
UTI  urinary tract infection
WBC  white blood cell

Tämän väitöskirjan tarkoituksena oli selvittää verestä mitattavien, pääasiassa synnynnäiseen immuniteettiin (engl. innate immunity) kuuluvien tulehdusen merkkaaineiden käyttökoefisiutta sairaalan päivystysvastaanotolle tulevilla potilailla. Erityisesti tutkittiin tulehdusen merkkaaineiden käyttökoefisiutta positiivisen veriviljelyn ennustamisessa ja piilevän kotisyntyisen infektion toteamisessa. Lisäksi selvitettiin, onko merkkaaineista taudit alkuvaiheessa hyötyä kotisyntyistä infektioita sairastavien potilaiden kuolleisuuden ennustamisessa.

Veriviljelyn positiivisen tuloksen ennustaminen tulehduksen merkkiaineilla mukaan lukien C-reaktiivinen proteiini (CRP), prokalsitoniini (PCT), interleukiini (IL)-6, IL-8 ja liuokainen IL-2 reseptori (sIL-2R) ei onnistunut paremmin kuin pelkillä kliinisillä mittareilla. Kliinisinä ennustekijöinä käytettiin kuumetta, infektiofokusta, verenpainetta (MAP) ja pulssia.


Kotisyntyistä infektiota sairastavien potilaaiden 28-päivän kuolleisuus oli matala, 3,4%. Monimuuttuja-analyysissä korkea ikä ja monosyyttien solusitoisen lipopolysakkaridireseptorin eli CD14-molekyyn halainen määrä ennustivat kuolleisuutta taudin alkuvaiheessa. CD14-reseptoria esiintyy myös liuokaisessa muodossa (sCD14), mutta korkea sCD14 pitoisuus ei ollut kuoleman suhteen ennusteellinen. Korkeampi kuolleisuus todettiin potilailla, joilla oli suotamaisesti matala solusitoisen CD14-reseptorin määrä ja korkea sCD14-pitoisuus.

Tulehduksen merkkiaineet parantavat piilevien kotisyntyisten infektioiden diagnostiikkaa taudin alkuvaiheessa, mutta yksittäisiä potilaata tutkittaessa täytyy käyttää useiden merkkiaineiden yhdistelmää mieluummin kuin yksittäistä merkkiainetta. Sairaalaan tulovaiheessa matala solusitoisen lipopolysakkaridireseptorin (mCD14) määrä ennustaa kuolemana kotisyntyistä infektiota sairastavalla potilaalla.
Sepsis is associated with a systemic inflammatory response. It is characterised by an early proinflammatory response and followed by a state of immunosuppression. In order to improve the outcome of patients with infection and sepsis, novel therapies that influence the systemic inflammatory response are being developed and utilised. Thus, an accurate and early diagnosis of infection and evaluation of immune state are crucial. In this thesis, various markers of systemic inflammation were studied with respect to enhancing the diagnostics of infection and of predicting outcome in patients with suspected community-acquired infection.

A total of 1092 acutely ill patients admitted to a university hospital medical emergency department were evaluated, and 531 patients with a suspicion of community-acquired infection were included for the analysis. Markers of systemic inflammation were determined from a blood sample obtained simultaneously with a blood culture sample on admission to hospital. Levels of phagocyte CD11b/CD18 and CD14 expression were measured by whole blood flow cytometry. Concentrations of soluble CD14, interleukin (IL)-8, and soluble IL-2 receptor α (sIL-2Ra) were determined by ELISA, those of sIL-2R, IL-6, and IL-8 by a chemiluminescent immunoassay, that of procalcitonin by immunoluminometric assay, and that of C-reactive protein by immunoturbidimetric assay. Clinical data were collected retrospectively from the medical records.

No marker of systemic inflammation, neither CRP, PCT, IL-6, IL-8, nor sIL-2R predicted bacteraemia better than did the clinical signs of infection, i.e., the presence of infectious focus or fever or both. IL-6 and PCT had the highest positive likelihood ratios to identify patients with hidden community-acquired infection. However, the use of a single marker failed to detect all patients with infection. A combination of markers including a fast-responding reactant (CD11b expression), a later-peaking reactant (CRP), and a reactant originating from inflamed tissues (IL-8) detected all patients with infection. The majority of patients (86.5%) with possible but not verified infection showed levels exceeding at least one cut-off limit of combination, supporting the view that infection was the cause of their acute illness.

The 28-day mortality of patients with community-acquired infection was low (3.4%). On admission to hospital, the low expression of cell-associated lipopolysaccharide receptor CD14 (mCD14) was predictive for 28-day mortality. In the patients with severe forms of community-acquired infection, namely pneumonia and sepsis, high levels of soluble CD14 alone
did not predict mortality, but a high sCD14 level measured simultaneously with a low mCD14 raised the possibility of poor prognosis.

In conclusion, to further enhance the diagnostics of hidden community-acquired infection, a combination of inflammatory markers is useful. The 28-day mortality is associated with low levels of mCD14 expression at an early phase of the disease.
Community-acquired infection (CAI) can affect everyone. It is contracted during normal activities of daily life, and only the most serious forms require evaluation and subsequent treatment at a tertiary care hospital emergency department (ED). Infectious focus is usually evident on referral to hospital. Classical signs of inflammation, namely rubor (erythema), tumour (edema), calor (heat), dolor (pain), and functio laesa (disturbed function) aid in clinical diagnosis of infection. The innate immune response aroused at an early stage of infection is the essential part of the host defence against invading organisms. Systemic inflammation is accompanied by a large number of changes in humoral and cell-associated functions of innate immunity. Although the main reason for these changes is the defence and adaptation of the host, an exaggerated or inappropriately suppressed systemic inflammatory response may lead to severe disturbance of organs or even death. The critical question is how to measure the severity of the innate immune response triggered by community-acquired infection and how to identify the patients at risk for poor outcome. An ideal marker of sepsis should allow an early diagnosis, help to differentiate infectious from non-infectious causes of systemic inflammation, and be informative as to the course and prognosis of the condition in question. The search for a single “magic” marker with high sensitivity and specificity for infection and with the ability to accurately predict outcome has encountered numerous setbacks [Cooney and Yumet 2002, Takala et al. 2002b, Beale 2007, Tang et al. 2007].

Attending clinicians in an ED often encounter an acutely ill patient with fever. Infection is one of the most common disorders underlying fever, and its detection and diagnostics have greatly improved. The diagnosis of sepsis, a serious consequence of the body’s failed control over a local infectious focus and the microbes involved, is traditionally based on blood cultures. The father of microbiology, Louis Pasteur, showed for the first time that bacteria were present in the blood from patients with puerperal sepsis. Robert Koch and his assistant Julius Richard Petri laid the foundation for the techniques of culturing bacteria. Koch underlined the interconnection between laboratory results and clinical illness. Still, despite serious acute septic-like illness, blood cultures often remain negative for many reasons, and the patient’s poor outcome results not from spreading of the microbe(s) as such but mostly from an exaggerated or inappropriately suppressed systemic inflammatory response. For this reason, the focus of sepsis research has been extended to the systemic inflammatory response.
This thesis comprises a uniquely large series of acutely ill patients with a suspicion of CAI in a tertiary care hospital ED. The aim of these studies was, upon admission to an ED, to enhance early diagnostics of CAI, and to predict outcome among patients with CAI.
6 REVIEW OF THE LITERATURE

6.1 INFLAMMATION

Inflammation is the host's response to tissue injury produced by mechanical, chemical, or microbial stimuli. Any immune response involves, firstly, the recognition of the pathogen or other foreign material, and secondly, its elimination [Roitt et al. 2002]. Immune responses are classically divided into two types based on the speed and specificity of the reaction, namely innate and adaptive responses [Dempsey et al. 2003]. Innate immunity provides an immediate host defence (neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins). It is rapid and occurs to the same extent independently to frequent encounters with the same infectious agent. The adaptive response consists, among other things, of antigen-specific reactions through T-lymphocyte immunity involving CD4-positive T-helper (T\textsubscript{H} cells) and CD8-positive cytotoxic T cells, and of antibody formation by B lymphocytes. The adaptive response is precise but takes several days or weeks to develop, and it has a memory [Parkin and Cohen 2001].

The sensing of invading micro-organisms by innate immune cells is considered to involve pattern recognition. Microbial pathogens are characterised by specific arrangements of key molecules called pathogen-associated molecular patterns (PAMPs). Because PAMPs are structures vital for the pathogen's function, they have altered little throughout evolutionary time. They include structures such as lipoproteins, lipopolysaccharides (LPS) of gram-negative bacteria, peptidoglycans of gram-negative and gram-positive bacteria, and viral envelope glycoproteins. The PAMPs are recognised by pattern recognition receptors (PRRs) expressed by the cells of the innate immune system. PRRs are present on many types of innate immune cells and comprise several families such as Toll-like receptors (TLR), CD14, formyl peptide receptors, and complement receptors [Dempsey et al. 2003]. Of these, monocyte CD14 is a receptor for bacterial lipopolysaccharide (LPS). LPS binds to CD14 with the assistance of a LPS-binding protein (LBP). This may lead to appropriate activation of a cluster of receptors and eventually to the synthesis of inflammatory mediators. The essential part of this activation pathway is the family of evolutionarily conserved transmembrane receptors, Toll-like receptors (TLRs). Of these, TLR-4 signals the presence of LPS after LPS has connected to it with the help of LBP. TLR-4 then activates the
transcription factor NFκB, which in turn activates genes encoding proteins involved in defence against infection [Wright et al. 1990, Poltorak et al. 1998], reviewed in Fujihara et al. [2003].

### 6.1.1 Inflammatory cells

All the cellular elements of the blood derive ultimately from the same cells—the pluripotent haematopoietic stem cells in the bone marrow. These give rise to stem cells of more limited potential, which are the immediate progenitors of, for instance, the two main categories of white blood cells, the myeloid and the common lymphoid progenitors. The myeloid progenitor is the precursor of the granulocytes (neutrophils, eosinophils, basophils), macrophages, dendritic cells, and mast cells of the innate immune system, whereas the common lymphoid progenitor gives rise to the lymphocytes and to the natural killer cells [Janeway et al. 2005]. Eosinophils, basophils, and mast cells are, for example, responsible for the defence against parasitic infections and are involved in allergic reactions [Bochner and Schleimer 2001]. Natural killer cells recognise abnormal cells such as those infected with a virus—thus inducing apoptosis [Yokoyama et al. 2004].

The cells involved in the acute inflammatory response are phagocytes (monocytes, macrophages, polymorphonuclear neutrophils) and lymphocytes. Phagocytic cells bind to micro-organisms, internalise them, and then kill them. Upon phagocytosis, they produce a variety of other toxic products that help kill the engulfed micro-organism. The most important of these are nitric oxide, the superoxide anion, and hydrogen peroxide (H₂O₂), all of which are directly toxic to bacteria. Superoxide is generated by a multicomponent, membrane-associated NADPH oxidase in a process known as the respiratory burst because it is accompanied by a transient increase in oxygen consumption. Ultimately, superoxide is converted into H₂O₂ by the enzyme superoxide dismutase. Macrophages can ingest pathogens and produce the respiratory burst immediately when encountering an infecting micro-organism, and this can be sufficient to prevent an infection from becoming established [Janeway et al. 2005].

#### 6.1.1.1 Monocytes and macrophages

Monocytes circulating in the blood are relatively inactive but upon migration into the tissues differentiate continuously into active phagocytosing macrophages. The majority of circulating monocytes express membrane-bound CD14 (mCD14), an LPS receptor which mediates monocyte activation via TLR-4 [Wright et al. 1990, Poltorak et al. 1998]. Two soluble forms of CD14 (sCD14) are constitutively generated: one through liberation from glycosylphosphatidylinositol (GPI) anchoring, and the other by proteolytic cleavage by a serine protease [Bufler et al. 1995]. Expression of mCD14 and release of sCD14 are regulated by cytokines and bacteria. Interleukin-4 (IL-4) and IL-10 reduce levels of mCD14 and sCD14, whereas interferon-γ (IFNγ),
tumour necrosis factor (TNF), and bacterial ligands cause their upregulation (reviewed in Landmann et al. [2000]). Low mCD14 levels occur in patients with sepsis [de Werra et al. 2001], but the importance of the downregulation of mCD14 is unknown [Bazil and Strominger 1991, Ertel et al. 1993].

The phenotypic form taken by a macrophage depends on the environmental factors present in the tissue [Duffield 2003]. Macrophages exist in especially large numbers in connective tissue, in the submucosal layer of the gastrointestinal tract, in the lung (in both the interstitium and the alveoli), along certain blood vessels in the liver (Kupffer cells), and in the spleen where they remove senescent blood cells. The cytokines secreted by macrophages in response to pathogens are a structurally diverse group of molecules that include IL-1β, IL-6, IL-12, TNFα, and the chemokine IL-8 (also called CXCL8). In addition to cytokine production and phagocytosis, macrophages and closely related dendritic cells are highly efficient in presenting antigens to CD4-positive T cells via class II major histocompatibility (MHC) antigen complex, such as the human leukocyte antigen, HLA-DR. In patients with sepsis, a decrease in HLA-DR expression [Docke et al. 1997] leads to impaired antigen presentation capacity which suppresses helper T-cell activation [Wolk et al. 2000]. Decreased HLA-DR expression is associated with adverse outcomes including septic complications and increased mortality [Tschaikowsky et al. 2002, Mentula et al. 2003].

6.1.1.2 Neutrophils

Polymorphonuclear neutrophils (PMN) are the most numerous leukocytes in the blood but are not present in normal, healthy tissues. Cytokines produced by phagocytes upon the activation of the innate immune system induce leukocytosis, which mainly is due to an increase in circulating neutrophils. These neutrophils derive from two sources: from the bone marrow where they are produced, and from the sites in blood vessels where they are attached loosely to endothelial cells. Each neutrophil has a multilobed nucleus and contains granules and secretory vesicles [Borregaard and Cowland 1997]. Peroxidase-positive (azurophilic or primary) granules carry myeloperoxidase; azurophilic granules are particularly active in the digestion of phagocytosed material. The peroxidase-negative granules are classed as specific (secondary) and gelatinase (tertiary) granules. This classification is based on their relative content of lactoferrin and gelatinase. Specific granules play important roles in initiating the inflammatory response. Additionally, there are secretory vesicles which are important reservoirs of membrane proteins such as CD11b/CD18. These membrane proteins, upon activation, become incorporated into the plasma membrane of neutrophils [Todd et al. 1984, Witko-Sarsat et al. 2000].

Neutrophils have surface receptors for formyl peptides, which are derived from and are specific to bacterial metabolism, and for complement-derived C5a. CD11b/CD18 receptors mediate neutrophil binding to the bacterial
surface opsonised with complement, i.e., iC3b molecules on the bacterial cell wall. In addition to complement components, the microbes are opsonised with antibodies. Neutrophils have Fcγ receptors (FcγIII receptor or CD16 and FcγII receptor or CD32), which bind to the Fc-portion (the tail) of the antibody molecule on the bacterial cell wall [Brown et al. 2006].

6.1.1.3 Lymphocytes
Lymphocytes are responsible for the specific recognition of pathogens and initiation of adaptive immune responses. The characteristic of adaptive immunity is the use of antigen-specific receptors on T and B cells to drive targeted effector responses. B and T lymphocytes develop from progenitor cells within the bone marrow; B cells remain within the marrow for the duration of their development, but T cells migrate to the thymus at an early stage as thymocytes [Parkin and Cohen 2001]. For naïve T cells to be activated by antigen, the antigen must be bound to an MHC molecule on an antigen-presenting cell that also expresses co-stimulatory molecules.

The differentiation of naïve CD4+ T cells into different subclasses of effector T cells is influenced by cytokines elicited by the pathogen. Many pathogens, especially intracellular bacteria and viruses, activate dendritic cells and natural killer cells to produce IL-12 and IFNγ, which then cause proliferating CD4+ T cells to differentiate into T_{H1} cells. IL-4, which can be produced by various cells, is produced in response to parasitic worms and other pathogens and acts on proliferating CD4+ T cells to cause them to become T_{H2} cells. The two subsets of CD4+ T cells–T_{H1} and T_{H2}–have very different functions: T_{H2} cells are the most effective activators of B cells, especially in primary responses, whereas T_{H1} cells are crucial for activating macrophages and are also involved in directing the production of certain antibody isotypes [Dempsey et al. 2003].

6.1.2 Phagocyte-endothelial cell interaction
The recruitment of activated phagocytes to sites of infection is one of the most important functions of innate immunity. Recruitment occurs as part of the inflammatory response and is mediated by cell-adhesion molecules induced on the surface of the local blood vessel endothelium (Fig. 1).

Three families of adhesion molecules are important for leukocyte recruitment. The selectins are membrane glycoproteins with a distal lectin-like domain that binds specific carbohydrate groups. Three types of selectins comprise one on endothelial cells (E-selectin), one on leucocytes (L-selectin), and one on platelets (P-selectin). E-selectin is induced on activated endothelium. Selectins initiate endothelium–leukocyte interactions (rolling; Fig. 1) by binding to the fucosylated oligosaccharide ligands on leukocytes passing by. The subsequent tighter adhesion is due to the binding of intercellular adhesion molecules (ICAMs) on the endothelium to
heterodimeric proteins of the integrin family on leukocytes. The leukocyte integrins important for extravasation are leukocyte functional antigen-1 (LFA-1, also known as CD11a/CD18) and CR3 (complement receptor type 3, also known as CD11b/CD18 or Mac-1), and they both bind to ICAM-1. Strong adhesion between leukocytes and endothelial cells is promoted by the induction of ICAM-1 on inflamed endothelium and the activation of a conformational change in LFA-1 and CD11b/CD18 that occurs in the response to chemokines, among other leukocyte-activating agents [Repo and Harlan 1999].

Activation of endothelium is driven by interactions with macrophage cytokines, particularly TNFα, which induce the rapid externalisation of granules called Weibel–Palade bodies in the endothelial cells. These granules contain preformed P-selectin, which is thus expressed within minutes on the surface of local endothelial cells after the production of TNFα by macrophages. Shortly after the appearance of P-selectin on the cell surface, mRNA encoding E-selectin is synthesised, and within 2 hours, the endothelial cells are expressing mainly E-selectin. Both these proteins interact with the sulfated-sialyl-Lewisx that is present on the surface of neutrophils.

Resting endothelium carries low levels of ICAM-2, apparently in all vascular beds. This may be used by circulating monocytes to navigate out of the vessels and into their tissue sites. This monocyte migration happens continuously and essentially ubiquitously. However, upon exposure to TNFα, local expression of ICAM-1 is strongly induced on the endothelium of small vessels near or within the infectious focus. ICAM-1 in turn binds to LFA-1 or CD11b/CD18 on circulating monocytes and polymorphonuclear leukocytes, in particular neutrophils [Ebnet and Vestweber 1999].
Monocytes circulating in the blood recognise peripheral venule walls near sites of inflammation and leave the bloodstream to migrate into the tissue toward the site of infection and inflammation. The initial interactions are mediated by adhesion molecules that first capture the monocyte from the bloodstream and cause it to adhere to the vascular endothelium. Chemokines bound to the vascular endothelium then signal the monocyte to migrate across the endothelium into the underlying tissue. The monocyte, now differentiating into a macrophage, continues to migrate, under the influence of chemokines released during inflammatory responses, toward the site of infection (adopted from Janeway et al. [2005]).

6.1.3 Local inflammation

Inflammation plays three essential roles in combating infection. The first is to deliver additional effector molecules and cells to sites of infection, to augment the killing of invading micro-organisms by the front-line macrophages. The second is to provide a kind of physical barrier in the form of microvascular coagulation to prevent the spread of the infection in the bloodstream. The third is to promote the repair of injured tissue [Janeway et al. 2005].

Inflammation has three main components: an increased blood supply to the area, bringing leucocytes and serum molecules to the affected site; an increased capillary permeability allowing exudation of the serum proteins (antibody, complement, kininogens) required to control the infection. These two processes account for the heat, redness, and swelling. Finally, an increase in leukocyte migration into the tissue, together with the release of bradykinins and prostaglandins, accounts for the pain. Neutrophils are the first cells entering the sites of acute inflammation caused by infection, but from the first days onwards, mononuclear phagocytes and activated lymphocytes start to arrive. The outcome of an acute reaction depends on whether the antigen or the infectious agent is cleared. The infectious agent can be destroyed by neutralisation with specific antibodies or complement (antibodies bind to a bare particle and prevent it from infecting cells, or
they introduce it to a phagocyte), phagocytosis (the micro-organism is internalised and degraded in a phagosome), or cytotoxic reactions (contents of cytoplasmic granules are secreted to resist the micro-organisms of the infected cell) [Roitt et al. 2002]. Four major plasma enzyme systems play an important role in haemostasis and control of inflammation. These are the clotting system, the fibrinolytic (plasmin) system, the kinin system, and the complement system. The loss of local control or an overly activated response may result in an exaggerated systemic response.

### 6.1.4 Soluble mediators of inflammation

An inflammatory response is induced by a variety of inflammatory mediators released as a consequence of the recognition of pathogens by macrophages. These inflammatory mediators include prostaglandins, leukotrienes, and platelet-activating factor, all of which are rapidly produced by macrophages through enzymatic pathways that degrade membrane phospholipids. Their actions are followed by those of the chemokines and cytokines that are synthesised and secreted by macrophages in response to pathogens. Another way in which pathogen recognition rapidly triggers an inflammatory response is through activation of the complement cascade, which includes facilitation of phagocytosis and generation of potent cleavage products such as C5a. C5a is engaged in the increase in vascular permeability and induction of the expression of some adhesion molecules and also acts as a powerful chemoattractant for neutrophils and monocytes. C5a also activates phagocytes and local mast cells, which are in turn stimulated to release their granules containing the small inflammatory molecule histamine and the cytokine TNFα.

**Cytokines**

Cytokines are small proteins (approximately 25 kDa) that are released by various cells, usually in response to an activating stimulus, and they induce responses through binding to specific receptors. They can act in an autocrine manner, affecting the behaviour of the cell that releases the cytokine; and act in a paracrine manner, affecting the behaviour of adjacent cells. Some cytokines are even sufficiently stable to act in an endocrine manner, affecting the behaviour of distant cells. The two major structural families of cytokines are the haematopoietin family, which includes growth hormones and also many interleukins with roles in both adaptive and innate immunity; and the TNF family, which functions in both innate and adaptive immunity and includes many members that are membrane-bound. Cytokines with chemoattractant activity are called chemokines, those that cause differentiation and proliferation of stem cells are called colony-stimulating factors, and those that interfere with viral replication are called interferons. The cytokines have been divided into pro- and anti-inflammatory depending on their principal actions, but since the cytokines
act as a network with various feedback systems, the overall effect depends on the context and possibly also on local cytokine concentration [Dinarello 2000, Opal and DePalo 2000].

TNFα is the primary mediator of sepsis and is derived mainly from activated macrophages and dendritic cells. It induces changes in vascular endothelium (expression of cell-adhesion molecules, loosening of cell–cell junctions with increased fluid loss, and induction of local blood clotting). TNFα is an inducer of local inflammatory response. TNFα also plays a role in stimulating the migration of dendritic cells from their sites in peripheral tissues to the lymph nodes and in their maturation into antigen-presenting cells. Its systemic release causes vasodilatation, which leads to a drop in blood pressure, increased vascular permeability leading to a drop in plasma volume, and eventually to shock. According to Selby et al., the administration of recombinant human TNFα was found to result in rigors, fever, and tachycardia within 20 minutes to 2 hours after the beginning of infusion, with hypotension in a dose-dependent manner following 6 to 12 hours after TNFα. Leukocytosis, elevated serum creatinine kinase levels and increased CRP concentration were also induced. The half-life of TNFα was extremely short, only 17 minutes [Selby et al. 1987]. The other cytokine involved in the pathogenesis of septic shock is IL-1, which acts synergistically with TNFα. Of the two forms of IL-1, α and β [March et al. 1985], only IL-1β has been detected in plasma of patients with sepsis [Casey et al. 1993]. The short elimination time of TNFα and methodological problems in the determination of IL-1β hamper their use in clinical studies [Thijs and Hack 1995].

**Interleukin-6**

IL-6 is produced in response to IL-1β by macrophages, dendritic and glial cells, skeletal muscle cells, adipocytes, endothelial and intestinal epithelial cells. Locally, it induces lymphocyte activation and increased antibody production. Together with TNFα and IL-1β, it induces the production of acute phase proteins in the liver and induces fever, which favours effective host responses in many ways. IL-6 has both pro- and anti-inflammatory effects [Fink 2006]. According to van Gameren et al., intravenous administration of recombinant human IL-6 to cancer patients induces fever, chills, leukocytosis, and anaemia and increased serum C-reactive protein (CRP)- and amyloid A levels [van Gameren et al. 1994]. High levels of circulating IL-6 appear in experimental human endotoxaemia [van Deventer et al. 1990] and in sepsis patients [Damas et al. 1992].

**Interleukin-8**

IL-8 (recently renamed, being a member of the chemoattractant family, as CXCL8) is a chemoattractant for neutrophils. All the chemokines are related in amino acid sequence, and their receptors are all integral membrane...
proteins containing seven membrane-spanning helices. Chemokines function mainly as chemoattractants for leukocytes: recruiting monocytes, neutrophils, and other effector cells from the blood to sites of infection, for example, by regulating adhesive responses of immune cells [Laudanna et al. 2002]. IL-8 can be produced by many different cell types. It mobilises, activates, and degranulates neutrophils and also induces angiogenesis. In an in vitro experiment, IL-8 could mediate acute-phase protein production by human hepatocytes [Wigmore et al. 1997]. Elevated levels of IL-8 have been detected in sepsis patients [Lin et al. 1994, Fujishima et al. 1996].

**Soluble IL-2Rα**

IL-2 is made by T cells, some B cells, and dendritic cells. It is required for the proliferation of CD8+ T (cytotoxic) cells [Gaffen and Liu 2004]. The IL-2 receptor (IL-2R) is composed of three subunits, α, β, and γc. A soluble form of IL-2Rα is released upon cell activation, denoting activation of T-lymphocytes [Rubin et al. 1985, Rubin and Nelson 1990]. Elevated levels of sIL-2Rα have been detected in patients with sepsis [Takala et al. 1999a].

### 6.1.5 Systemic inflammation

Systemic inflammation is characterised by the activation of inflammatory cells, of the coagulation system, and of the complement system, all occurring in the circulation. As the noxious stimulus is being resolved, proinflammatory mediators are produced, and anti-inflammatory mediators control the inflammatory response. However, in some cases, no homeostasis is achieved. The effects of inflammatory mediators become destructive, and the systemic inflammatory response may proceed to hypotension and circulatory collapse, and to the development of injury in distant organs [Cohen 2002, Annane et al. 2005]. A recent hypothesis postulates that in the case of sepsis, i.e., infection with systemic inflammation, the phases of enhanced inflammation can alternate with periods of immune suppression [Xiao et al. 2006].

Corticosteroid drugs, powerful anti-inflammatory agents, are pharmacological derivatives of members of the glucocorticoid family of steroid hormones. Cortisol acts through intracellular cortisol-binding receptors expressed in almost every cell of the body. These receptors regulate the transcription of specific genes, either by direct binding to hormone-response elements in the promoters of various genes, or by regulating gene expression through interaction with other transcription factors, such as NF-κB. The gene interference then leads to effects on inflammatory processes, which include cessation of the production of inflammatory mediators, including cytokines, prostaglandins, and nitric oxide; the inhibition of inflammatory cell migration to sites of inflammation by inhibition of the expression of adhesion molecules; and an increase in the death of leucocytes and lymphocytes by apoptosis [Barnes 1998, Guyre and Munck 1999].
6.1.6 Acute phase response

The acute-phase response includes a large number of behavioural, physiological, biochemical, and nutritional changes. Biochemical changes include changes in the concentrations of many plasma proteins, known as the acute-phase proteins. An acute-phase protein (APP) has been defined as a plasma protein whose concentration increases (positive APPs) or decreases (negative APPs) by at least 25% as a response to inflammation. The proteins whose production is induced by cytokines in the liver include C-reactive protein (CRP), [Gabay and Kushner 1999], procalcitonin (PCT) [Nijsten et al. 2000], and sCD14 [Bas et al. 2004].

C-reactive protein

IL-6 induces CRP synthesis in the liver [Castell et al. 1988, Wigmore et al. 1997]. CRP, a member of the pentraxin protein family, binds to the phosphocholine portion of certain bacterial and fungal cell-wall lipopolysaccharides [Povoa 2002]. CRP is able to opsonise bacteria, thus activating the complement cascade. CRP is currently the most widely used laboratory test for the evaluation of the acute-phase response. After the insult eliciting systemic inflammation, CRP levels start to rise in 6 to 10 hours and peak within 24 to 48 hours [Anonymous 1988]. Among patients with CRP concentrations above 100 mg/l, 80 to 85% have a bacterial infection [Morley and Kushner 1982].

Procalcitonin

PCT, a 14 kDa propeptide of calcitonin, is normally produced in the C-cells of the thyroid gland [Jacobs et al. 1981]. Normally, only a very few PCT molecules are released into the circulation, and plasma PCT levels in healthy humans are approximately 5 to 50 ng/l. PCT has an intermediate half-life of approximately 22 to 33 h in serum. PCT is a novel marker of infection [Assicot et al. 1993, Meisner 2002]. During an inflammatory response, PCT has been shown to originate from hepatocytes [Nijsten et al. 2000]. After the administration of endotoxins to healthy volunteers, plasma PCT level began to rise after 4 hours, peaked at 6 h, and remained near its peak level for up to 24 h [Dandona et al. 1994]. Whether PCT has anti- or proinflammatory effects remains unanswered [Monneret et al. 2003].

Soluble CD14

Monocyte mCD14, the receptor for the LPS-LBP complex, promotes intracellular signalling via TLR-4, which induces NFκB activation. The cleavage product of mCD14 exists in soluble form (sCD14) within the circulation. Soluble CD14 also binds bacterial structures [Blondin et al. 1997]. Many cells, among them epithelial and endothelial cells, express no mCD14. The activation of these cells by microbial structures involves sCD14 molecules [Pugin et al. 1993]. Soluble CD14 may enhance an mCD14-positive
cell-response to bacterial structures [Dziarski et al. 2000] and contribute to elimination and detoxification of bacterial endotoxins [Yu et al. 1997], also by transferring cell-bound LPS to plasma lipoproteins [Kitchens et al. 2001]. In addition to those functions, it is thought to be an APP, due to its cytokine-induced production by hepatocytes [Su et al. 1999, Bas et al. 2004]. Increased levels of sCD14 occur in patients with infection [Landmann et al. 1995, Burgmann et al. 1996, Wenisch et al. 1996, Carrillo et al. 2001].

6.2 COMMUNITY-ACQUIRED INFECTION

Community-acquired infection is defined as the absence of circumstances and predisposing factors defining a nosocomial infection. The exact definition varies among studies. Usually the criteria include the absence of any hospitalisation within the previous 2 weeks, for example, [Valles et al. 2003], surgery, or major trauma. Nosocomial, i.e., hospital-acquired infection, is defined as an infection for which no evidence exists that the infection was present or incubating at the time of hospital admission. The influence of previous hospitalisation is defined case-by-case. Each infection is to be assessed individually for evidence that links it to hospitalisation [Garner et al. 1988].

As a third category, the concept of health-care-associated infection has been proposed; this applies to the elderly living in care homes, nursing homes, and rehabilitation centres, and to patients receiving dialysis or chemotherapy, and it is reported to have similarities with nosocomial infections in terms of frequency of various comorbid situations, source of infection, pathogens and their susceptibility patterns, and mortality rate at follow-up due to the usage of catheters and other body boundary-breaking devices [Friedman et al. 2002, Kollef et al. 2005, Shorr et al. 2006].

Clinical criteria of systemic inflammation

To improve the diagnostics of infection, clinical criteria of systemic inflammation were developed [Bone et al. 1992] (Table 1) and later re-evaluated [Levy et al. 2003] by the 2001 International Sepsis Definitions Conference. In the re-evaluation process, the exact definition of sepsis was still recognised as unattainable; therefore, to enhance the sensitivity of the concept, the meeting focused on listing all the possible indicators of sepsis. Furthermore, presence of infection was included as verified or suspected.
Table 1. Definitions for infection, systemic inflammatory response syndrome, and sepsis according to the ACCP/SCCM Consensus Conference [Bone et al. 1992]

**Infection** = a pathologic process caused by the invasion of normally sterile tissue of fluid or the body cavity by pathogenic or potentially pathogenic microorganisms.

**Systemic inflammatory response syndrome (SIRS)** = the systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more of the following conditions: 1. temperature > 38°C or < 36°C; 2. heart rate > 90/min; 3. respiratory rate > 20 breaths/min or P<sub>CO<sub>2</sub> < 32 mmHg (4.3 kPa); 4. white blood cell count > 12 x10<sup>9</sup>/l, < 4 x10<sup>9</sup>/l or >10% immature band forms.

**Sepsis** = systemic response, SIRS, to infection

**Severe sepsis** = sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status.

**Septic shock** = sepsis-induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status.

**Sepsis-induced hypotension** = a systolic blood pressure < 90 mmHg or a reduction of ≥ 40 mmHg from baseline, in the absence of other causes of hypotension.

**Multiple organ dysfunction syndrome (MODS)** = presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

**Bloodstream infection (BSI)** is traditionally defined as an acute illness due to an infection, in which the pathogen(s) spread to the circulation, either transiently or more prevalently, and thus can be confirmed in the laboratory by culture of pathogens from the blood sample [Weinstein et al. 1983a, 1983b]. BSI accompanied by a systemic inflammatory response is defined as sepsis. Primary BSI is a situation in which the pathogen isolated from blood culture is unrelated to an infectious focus elsewhere in the body, whereas in a secondary BSI, a pathogen isolated from blood culture is associated with an infection at another site [Garner et al. 1988]. However, it is possible for a patient to have severe sepsis with altered organ function such as disorientation, hypoxemia, metabolic acidosis, and oliguria without the presence of bacteria in any blood culture sample. This situation was earlier referred to as blood culture-negative sepsis.
Respiratory infections

Of the CAIs in an emergency department (ED), are respiratory tract infections are the most common. Differentiating pneumonia from other lower respiratory tract infections relies on radiological findings which may not always be present on admission. In the case of (suspected) pneumonia, identification of the causative agent is mainly based on blood cultures. Community-acquired pneumonia (CAP) is defined by a new infiltrate in the on-admission chest x-ray together with compatible clinical features supporting the diagnosis of pneumonia, including symptoms of lower respiratory tract infection (LRTi), and fever [File 2003].

LRTi is defined as an acute illness presenting with cough and at least one other symptom such as sputum production, dyspnoea, wheeze, chest discomfort/pain, without any alternative explanation (sinusitis, pharyngitis, or a new presentation of asthma) [Macfarlane et al. 2001]. Without the new infiltrate on chest x-ray, these symptoms are non-specific, and beside LRTi, may also be present in patients with upper respiratory tract infections, with chronic bronchitis; and with non-infectious diseases such as reactive airways disease, atelectasis, congestive heart failure, vasculitis, pulmonary embolism, or malignant disease.

6.3 INFECTION DIAGNOSTICS

6.3.1 Abnormal body temperature

Elevation of body temperature is caused mainly by TNFα, IL-1β, and IL-6. These are termed endogenous pyrogens because they cause fever and derive from an endogenous source rather than from bacterial components such as LPS, which is an exogenous pyrogen. These cytokines act on the hypothalamus, altering the body’s temperature regulation, and on muscle and fat cells, altering energy mobilisation to elevate body temperature. At elevated temperatures, bacterial and viral replication decrease, and the adaptive immune response is enhanced. Host cells are also protected from the deleterious effects of TNFα at elevated temperatures (reviewed by Hasday et al. [2000]).

Abnormal body temperature, i.e., fever or hypothermia, is a major clinical finding associated with systemic inflammation and is one of the criteria of SIRS. In patients with infection, abnormal body temperature and chills are common. Elderly patients in particular may, however, develop infection and BSI without fever [Gleckman and Hibert 1982, Fontanarosa et al. 1992], and those signs and symptoms considered typical for infection appear irregularly [Chassagne et al. 1996]. A BSI without fever has been associated with poor prognosis [Ispahani et al. 1987; Weinstein et al. 1983a, 1997].
Abnormal body temperature is included in the definition of systemic inflammatory response syndrome (SIRS, Table 1). As the re-evaluation conference concluded, the SIRS criteria had proved to be sensitive but not specific to infection [Jaimes et al. 2003, Levy et al. 2003]. The presence of SIRS criteria alone was not prognostic for mortality [Stoiser et al. 1998], not even in ED patients with suspected infection [Shapiro et al. 2006]. Beside infection, SIRS is associated with major trauma, burns, pancreatitis, haemorrhagic shock, and exogenous administration of cytokines. SIRS may, however, even occur in patients without any measurable systemic inflammation [Takala et al. 1999b]. Dr. Bone, the inventor of the concept, underlined the importance of the context in which SIRS is used, never encouraging its use as a stand-alone definition [Dellinger and Bone 1998].

6.3.2 Blood culture

Blood culture is an essential laboratory examination when severe infection is suspected. Sometimes, for example, in acute illness causing generalised symptoms, it may also be used to rule out systemic infection. No general recommendation exists as to the frequency at which blood culture samples should be drawn, but a common practise is that the proportion of samples revealing growth should be approximately 10% of the total number of samples drawn [Aronson and Bor 1987, Washington 1992, Mylotte and Tayara 2000]. At Helsinki University Central Hospital (HUCH) the annual number of blood cultures has been rising (in 2001, 7761 samples vs. 2006 with 9125). Since 2001 the percentage of positive blood cultures has ranged from 6.6 to 8.2% (personal communication, Head of Department, Docent Petteri Carlson, HUSLAB/Bacteriology).

According to the blood culture guidelines of the Laboratories of the Hospital District of Helsinki and Uusimaa (HUSLAB), a blood sample of 10 ml is drawn for the aerobic and anaerobic culturing media. The skin is carefully decontaminated at the site of sampling in order to avoid false positive results. Normally, the sampling procedure is repeated after 30 minutes. In suspected endocarditis the number of samples is double. A positive result is reported as soon as any bottle reveals growth. This usually takes at least 1 or 2 days. The negative result is reported 6 days after sampling, if no slowly growing specimen is suspected [Anonymous 2006a].

A positive blood culture is not always clinically significant, since contamination may occur, or the positive result may represent the transient and self-limited presence of micro-organisms in the blood. On the other hand, significant pathogens may be leaking from the site of infection into the circulation only periodically and may therefore be harder to detect (false negative result). The blood culture may incorrectly appear negative also if (intravenous) antimicrobial therapy has been administered before sampling. The clinical significance of the positive blood culture result depends on the pathogen, on the number and type of positive blood culture bottles compared
with the total number of bottles, and on the whole clinical picture: the patient’s clinical history, physical findings, body temperature at the time of blood culture, results from cultures of specimens from other sites, imaging results, histopathologic findings, and clinical course and response to therapy [Yagupsyk and Nolte 1990].

In general, a BSI verified by a positive blood culture is a sign of poor prognosis [Bryan 1989] and predisposes patients to vascular hypotension and shock [Bossink et al. 2001] with high mortality rates. Bacteraemia of unknown origin has been independently associated with fatal outcome [Leibovici et al. 1992, Reyes et al. 1999]. In the European SOAP study in multivariate analysis, a bloodstream infection had an OR of 1.7 (95% CI 1.2-2.4) for ICU mortality of sepsis patients and was an independent risk factor for death [Vincent et al. 2006]. However, for an occult bacteraemia verified in patients already discharged from the ED, delayed initiation of antimicrobial chemotherapy did not affect outcome [Epstein et al. 2001].

The incidence and the total number of deaths due to BSIs has been increasing [Weinstein et al. 1983a, 1983b, 1997], particularly in older people, in hospitalised patients, and in patients treated in intensive care units [Kuikka 1999]. In the 1990s, in one report half of all BSI episodes confirmed by positive blood culture were nosocomial, with a quarter having no recognised source [Weinstein et al. 1997]. The incidence of the causative organism has changed over the years; in the 1930s, the most common cause of bacteraemia was Streptococcus pneumoniae; in the 1950s, Staphylococcus aureus, and by the 1960s, gram-negative rods, while in the 1980s, gram-positive cocci led in the statistics [Kuikka 1999]. According to the statistics of the National Public Health Institute of Finland, in all reported blood cultures during the last 10 years, the most common species found in patients aged 15 to 64 years were Escherichia coli, Staphylococcus aureus, and other Staphylococcus species, followed by Streptococcus pneumoniae; and in patients aged > 65 years were Escherichia coli, Staphylococcus aureus and other Staphylococcus species followed by Klebsiella species [Anonymous 2006b].

6.3.3 Markers of systemic inflammation

In acutely ill patients, differentiating between infection and other causes of acute illness relies on the “whole picture,” i.e., the results of clinical and laboratory examinations including markers of systemic inflammation. One of the first indicators is the rise in the number of circulating leukocytes (leukocytosis) largely due to the demargination of neutrophils adhered to the endothelium of the blood vessels, for instance in the lungs, and also due to the mobilisation of bone marrow neutrophils [Mandell et al. 2005]. An exaggerated response to an infection may result in a vigorous consumption of leukocytes due to transmigration to the tissues and may therefore lead to leukopenia. Leukopenia [Valles et al. 2003], leukocytosis [Gleckman and Hibert 1982, Bossink et al. 1999], or, in general, an abnormal leukocyte
(white blood cell, WBC) count [Oberhoffer et al. 1999, Guven et al. 2002] indicates the possibility of bacterial infection underlying the acute systemic inflammatory response. Leukocytosis can also occur after tissue damage, in acute gout, or in acute myocardial infarction [Remskar et al. 2002]. In such cases, microscopy of a blood smear could help: a rise in the number of circulating young neutrophils, i.e., the band forms a proportion > 10%, is an indicator of bacterial infection and therefore is included in the SIRS criteria [Bone et al. 1992, Levy et al. 2003]. Metabolic changes detectable in severe sepsis include increased blood lactate concentration, which seems to result, among other reasons, from limited tissue oxygenation and from increased glycolysis. Lactate measurement in an ED is not useful, due to technical problems [Mandell et al. 2005].


PCT has been useful in detecting infectious complications during postoperative periods [Aouifi et al. 2000] and cardiogenic shock [Geppert et al. 2003, Clec’h et al. 2004]. PCT has aided in separating patients with atypical CAP from those with bacterial CAP [Hedlund and Hansson 2000]. In one ED setting, a PCT level as high as 0.6 μg/l was the most accurate for diagnosing bacterial CAI [Chan et al. 2004]. In patients with LRTi, antimicrobial therapy has been successfully reduced on the basis of PCT results [Christ-Crain et al. 2004]. Still, in elderly patients the levels of PCT ≥ 0.5 μg/l had only limited ability to distinguish between those with or without infection [Stucker et al. 2005]. In patients with organ dysfunction, both high CRP and high PCT may be associated with infection [Castelli et al. 2004].


Of the cellular markers, phagocyte CD11b expression levels peak within hours after the insult that elicits systemic inflammation. Neutrophil CD11b has been upregulated in CAP [Glynn et al. 1999] and sepsis [Chishti et al. 2001].
2004], and aids in differentiating viral from bacterial infections as a part of a “clinical infection score” [Nuutila et al. 2006]. Both neutrophil and monocyte CD11b expression level are increased in patients with sepsis [Russwurm et al. 2002]. Despite its being a very sensitive and quickly responding marker of systemic inflammation, phagocyte CD11b has not been specific for infection [Takala et al. 1996]. Trauma patients developing infection have shown a decrease in mCD14 expression level [Heinzelmann et al. 1996]. Patients with sepsis have been reported to present with decreased expression of mCD14 [de Werra et al. 2001], together with an increased level of sCD14 [Gluck et al. 2001].

6.3.4 Prediction of the positive blood culture

Patients with a positive blood culture, i.e., bacteraemia, represent quite a heterogeneous population. Many of them have a clinically significant infection, i.e., BSI, whereas in many the clinical significance of bacteraemia is not so clear. False-negative cultures are hidden among the patients thought of as controls or the patients labelled as having a “possible infection.” A positive blood culture is a practical and clear endpoint and has been the gold standard for infection diagnostics research. Recognition of patients with BSI on admission to hospital is not feasible by any single clinical or laboratory variable [Peduzzi et al. 1992] or the clinical criteria of SIRS [Jones and Lowes 1996, Bossink et al. 1999]. Many markers of systemic inflammation have been reported to be associated with later verified bacteraemia. Of the acute phase proteins, PCT, unlike CRP, predicted bacteraemia in an ED setting [Guven et al. 2002, Chan et al. 2004] and in patients with fever [Bossink et al. 1999]. PCT has been able to differentiate among bacteraemic, non-bacteraemic bacterial, and viral infections [Rintala et al. 2001] and was higher in post-operative patients with bacteraemia [Aouifi et al. 2000]. CRP is reported to have only limited diagnostic utility for the detection of bacteraemia [Adams 2005]. Of the other soluble markers, high levels of circulating IL-6 [Groeneveld et al. 2001] and IL-8 have been reported to predict bacteraemia [Lin et al. 2000]. In neutropenic patients with fever, PCT and IL-8 [Engel et al. 1999] and in neutropenic children with cancer presenting with fever, IL-6, IL-8, and sIL-2R have predicted bacteraemia similarly [Soker et al. 2001]. In another study PCT, CRP, IL-6, and IL-8 had comparable high negative predictive values (NPV), and comparable but low positive predictive values (PPV) for bacteraemia [Persson et al. 2004].

The soluble receptor sCD14 [Burgmann et al. 1996], or the cell-associated receptors phagocyte CD11b [Kuuliala et al. 2004] and mCD14 [Ertel et al. 1993] have not been so intensively studied concerning specifically the prediction of bacteraemia. Many studies concerning prediction of bacteraemia with markers of systemic inflammation have involved patients with cytopenia; thus it has been impossible to determine the density of cellular markers.
6.4 OUTCOME OF PATIENTS WITH INFECTION

6.4.1 Clinical predictors of outcome

Of patients with CAI, only a small percentage require evaluation and possible treatment at a tertiary care hospital, and even among these patients, a poor outcome is quite rare [Esel et al. 2003, Shapiro et al. 2006]. Poor prognosis is associated with several predisposing factors, of which the most important are age and the underlying disease and its severity [Kuikka 1999, van Langevelde et al. 2000]. Severity of the underlying disease has been characterised by evaluation of patients according to their estimated life expectancy. Four categories of disease severity were originally proposed by McCabe and Jackson, who described the underlying disease as rapidly fatal, ultimately fatal (within 4 years), or not fatal; or there even may be no underlying illness [McCabe and Jackson 1962]. Application of this division in a Finnish study of bacteraemic patients showed that mortality increases along with severity of the underlying disease [Kuikka 1999].

In patients with serious infections, a factor documented in several studies to be associated with poor prognosis is advanced age [Ruiz et al. 1999, van Langevelde et al. 2000, van de Beek et al. 2004, Roson et al. 2004]. In the elderly, decline in the quality of the first line of defence (i.e., atrophy and dryness of the skin and mucous membranes), reduced vitality, and increased risk for trauma, together with retardation of the repair process, should probably be regarded as the major causes of increased susceptibility to infections [van der Meer and Kullberg 2002]. Certain changes in the inflammatory response of the elderly include impaired production of proinflammatory cytokines in response to LPS stimulation [Bruunsgaard et al. 1999], but the clinical significance of many findings remains unknown [Cinader 1999, Pawelec 2006].

Several models have been developed for intensive care patients to score the severity of illness and predict their risk of death, including the Acute Physiology and Chronic Health Evaluation (APACHE II) [Knaus et al. 1985] and the Sepsis-related Organ Failure Assessment (SOFA) [Vincent et al. 1996]. Most of these scoring systems have, however, been developed and validated only for intensive care units and therefore cannot be applied to the ED setting. APACHE II can be utilised for the first 24 h, since after that time, many of the variables are influenced by the treatment. Additionally, the different factors in the scoring systems can be interpreted differently with regard to predicting outcome. For example, when a patient is deeply unconscious but otherwise in a stable condition, prognosis is often poorer than the total score would imply.

Shapiro et al. [Shapiro et al. 2003] developed the first prediction guidelines for the ED setting: the Mortality in Emergency Department Sepsis (MEDS)
score. In the derivation set of that study, independent multivariate predictors for death were terminal illness, tachypnea or hypoxia, septic shock, platelet count <150 000/l, band proportion > 5%, age > 65 yrs, LRTi, nursing home residence, and altered mental status. Points were assigned to each predictor in relation to the odds ratio given by multivariate analysis, allowing a MEDS score to be calculated. The resulting scores were grouped into five risk groups from very low to very high. When applied to the validation set, this score successfully stratified the patients into groups at increasing risk of death. Originally it was used for predicting 28-day mortality, but a recent report confirmed its ability also for predicting 1-year mortality [Shapiro et al. 2007].

6.4.2 Laboratory predictors of outcome

Leukopenia, i.e., diminished WBC count, is associated with a greater susceptibility to severe infections and with a poor prognosis [Valles et al. 2003]. However, in one study of critically ill patients with suspected sepsis, WBC count had no prognostic value [Pettila et al. 2002]. CRP does not predict mortality [Smith et al. 1995] or organ failure [Takala et al. 1999a] when measured on admission, but is informative in follow-up [Smith et al. 1995, Takala et al. 1999a, Reny et al. 2002]. In addition, a constantly high or rising level of PCT during follow-up indicates adverse events and poor outcome, as reported in febrile patients [van Langevelde et al. 2000], in patients with CAP [Boussekey et al. 2005], in patients with severe acute pancreatitis [Rau et al. 2007], and in patients with septic shock [Clec’h et al. 2004]; whereas a decreasing level indicates higher probability of survival [Claeys et al. 2002].

High circulating levels of IL-6 are consistently associated with poor outcome in patients with fever [van Langevelde et al. 2000], bacterial infections [Norrby-Teglund et al. 1995], sepsis [Hack et al. 1989, Calandra et al. 1991, Dofferhoff et al. 1992, Casey et al. 1993, Pinsky et al. 1993, Damas et al. 1997, Martins et al. 2003], and cardiogenic shock [Geppert et al. 2002]. On the basis of their results, Martins et al. [Martins et al. 2003] also concluded that levels of IL-6 are not influenced by administration of antimicrobial therapy. A study of patients with CAP showed high on-admission levels of IL-6 to be associated with mortality, with the highest among patients with CAP caused by pneumococcus [Ortqvist et al. 1995]. High circulating levels of IL-8 have predicted mortality in patients with sepsis [Damas et al. 1997]. In an ED setting, high circulating levels of IL-8 as well as APACHE II scores have predicted mortality of patients with SIRS better than did IL-6 or age [Lin et al. 2000]. In CAP, both IL-6 and IL-8 have been associated with severity of illness [Igonin et al. 2004]. Soluble IL-2R has not been studied with regard to its utility in predicting mortality in patients with infection, but high levels of sIL-2R have been reported to predict all-cause mortality in hospitalised elderly men [Rosenthal et al. 1997].
High levels of phagocyte CD11b expression have been associated with organ failure in patients with sepsis [Takala et al. 1999a]. Glück et al. found that mCD14 failed to predict mortality of patients with sepsis, whereas a high level of sCD14 was related to 28-day survival in patients with a severe form of the disease [Glück et al. 2001]. Other studies report that high sCD14 levels may predict mortality in patients with sepsis [Landmann et al. 1995, Burgmann et al. 1996]. In patients with trauma, persistently low mCD14 has been associated with secondary infections and death [Heinzelmann et al. 1996].
7 AIMS OF THE STUDY

The overall purpose of this study was to investigate various markers of systemic inflammation in patients with community-acquired infection, and their value in the diagnostics of infection and in prediction of mortality.

The specific aims were as follows:

1. To investigate whether the value of markers of systemic inflammation (CRP, PCT, IL-6, IL-8, or sIL-2R) predict a positive blood culture result in acutely ill patients with a suspicion of community-acquired bloodstream infection (I)

2. To investigate whether any single marker of systemic inflammation (CRP, PCT, IL-6, IL-8, sIL-2R, or phagocyte CD11b) or a combination of markers can identify patients with a hidden infectious focus (II)

3. To evaluate whether on-admission-measured markers of systemic inflammation (CRP, phagocyte CD11b, or monocyte CD14) can predict 28-day mortality of patients with community-acquired infection (III)

4. To explore the interrelationship between monocyte CD14 and soluble CD14 and their combined predictive value for 28-day mortality in patients with community-acquired pneumonia or blood culture-positive sepsis or both (IV)
8 MATERIAL AND METHODS

8.1 PATIENTS

A total of 1092 acutely ill patients (age 16 years or over) admitted during an 11-month period between September 1997 and August 1998 to the medical emergency department (ED) of the Meilahti Hospital, Helsinki University Central Hospital (HUCH), were evaluated. The trigger for enrolment was the clinician’s suspicion of infection and request for a blood culture. Laboratory assays were done in the Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki; in the Departments of Clinical Chemistry and the laboratory of Women’s Hospital, HUCH; and in the laboratory of the Central Finland’s Central Hospital, Jyväskylä. The institutional review board of HUCH approved the study protocol.

The medical history, clinical findings, diagnosis of hospitalisation, and data on 28-day survival were collected retrospectively from the medical records and National Population Register Centre of Finland. On admission, clinical parameters collected (when available) were peak pulse rate, and nadir mean arterial pressure (MAP, mmHg), respiratory rate, and body temperature. Acute onset fever (I) was defined as a body temperature > 38°C, and acute abnormal body temperature (II) as body temperature > 38°C or < 36°C on admission or within 48 hours before admission.

Because the focus was on those patients with a suspicion of CAI and on the enhancing diagnostics of CAIs by means of markers of systemic inflammation, exclusion criteria were developed accordingly. Fig. 2 shows the patients, exclusion criteria, and patient allocation to the studies. Patients with active malignancies were excluded due to the possible immune-inflammatory changes accompanying malignant processes. All patients on any systemic immunosuppressive treatment other than prednisolone < 5 mg per day (or equivalent) were excluded, due to the possible effect on levels of inflammation markers. The effect of other corticosteroids can be estimated with the equivalent dose of prednisone [Hardman and Limbird 2001]. Cytotoxic drugs can cause immunosuppression by interfering with DNA synthesis and thus killing cells that are dividing. Next, patients who had undergone surgery within the previous 6 weeks and patients hospitalised within the previous 14 days were excluded, due to the possibility of nosocomial infection. Lastly, patients with insufficient data on the hospitalisation period or 28-day survival or whose sample handling had failed were also excluded.
In **Study I**, recruitment and sample handling were tested in acutely ill patients. The analysis comprised 92 acutely ill patients who were allocated into 3 groups according to their clinical features on admission: Group I comprised 54 patients with infectious focus verified on admission, Group II 25 patients with acute onset fever without infectious focus on admission, and Group III, 13 patients with neither fever nor infectious focus. Groups II and III were further divided into 2 subgroups (A and B), to denote patients whose infectious focus was and was not verified within the 3-day follow-up.

**Study II** included 138 patients with an abnormal body temperature: < 36°C or > 38°C on admission or within 48 hours before hospital referral, but without infectious focus verified on admission. According to the cumulative information during a follow-up lasting up to 3 days, of the 138, 26 had a verified CAI (infection group), 23 had a non-infectious cause of acute illness (control group), and 89 had neither an infectious focus nor any other reason verified, but the presence of hidden infection could not be excluded (possible-infection group).

**Study III** included 327 patients with a verified CAI with an infectious focus or positive blood culture on admission or within the 3-day follow-up after admission.

**Study IV** included 142 patients who had, on admission or within the 3-day follow-up, CAP (99 patients, 10 positive for blood culture) or positive blood culture without pneumonia (43).

**Blood samples from healthy volunteers**
Parallel to the patient series, healthy volunteers’ blood samples were collected from among laboratory research personnel. This group included 59 persons without clinical signs of infection, from whom 83 blood samples were drawn between January and August 1998 and processed in a similar manner as the patient samples.
21,765 patients admitted to Meilahti medical emergency department between September 1997 and August 1998

Clinician’s suspicion of an infection → request for a blood culture sample

1092 patients sampled for blood culture

Exclusions
- Active malignancy: 219
- Hospitalised within the previous 14 days: 67
- Surgery within the previous 6 weeks: 56
- Systemic immunosuppressive treatment other than prednisolone < 5 mg per day (or equivalent): 172
- Missing data: 29
- Sample-handling failure: 18

531 acutely ill patients with suspicion of community-acquired infection

Study I
92 in a study for piloting methods and sample handling

Study II
138 with abnormal body temperature and possible hidden infection

26 with positive blood culture in Studies II to IV

Study III
327 with community-acquired infection

Study IV
142 with community-acquired pneumonia and/or sepsis

Figure 3. Patients, exclusion criteria, and allocation to Studies I to IV
8.2 CLINICAL DIAGNOSIS OF THE INFECTIOUS FOCI

The infectious foci were defined as pneumonia, evidenced by respiratory symptoms associated with an acute infiltration in the chest radiograph; urinary tract infection, evidenced by compatible findings in urinalysis; central nervous system infection, evidenced by findings in cerebrospinal fluid; abscesses, evidenced by findings in ultrasound examination or computerised tomography; sinusitis, evidenced by radiological findings and purulent discharge in sinus puncture; malaria, evidenced by the presence of malarial plasmodia in the microscopic examination of the blood; spondylitis, evidenced by compatible findings in radiographs; cholecystitis, cholangitis, or endocarditis, evidenced by compatible findings in ultrasound examination; hepatitis A or nephropatia epidemica (a form of haemorrhagic fever caused by Puumala Hantavirus), evidenced by compatible findings in serologic tests; and skin infection, tonsillitis, acute bronchitis, or acute-onset diarrhoea on the basis of the clinical picture. Lastly, an isolated positive blood culture was considered as an infectious focus, with the exception of blood cultures containing *Staphylococcus epidermidis*, *Micrococcus*, and *Pseudomonas fluorescens*, which were considered contaminated if only one bottle revealed growth, and clinical findings during follow-up were incompatible with sepsis; additionally, in the case of *Staphylococcus epidermidis*, if no indwelling vascular catheters were in use [Weinstein et al. 1983b].

8.3 BLOOD SAMPLING

Simultaneously with sampling for blood culture, an additional blood sample was collected into a pyrogen-free acid/citrate/dextrose anticoagulated tube (Venoject; Terumo Europe, Leuven, Belgium). In order to minimise phagocyte activation *ex vivo*, each tube was immediately pressed into thawing ice (0°C) and kept cooled until cell labelling. After the extraction of whole blood aliquots for the cell labelling, plasma was separated by centrifugation at 4°C and stored in aliquots at -70°C until use. Repeated freeze-thaw cycles were avoided as far as possible.

8.4 DETERMINATION OF CELLULAR MARKERS CD11b AND CD14

8.4.1 Cell labelling

Monoclonal antibodies were as follows: CD11b- phytoerythrin PE (mouse anti-CD11b IgG1, clone 2LPM19c, Dako A/S, Glostrup, Denmark), and CD14-fluorescein-isothiocyanate (FITC) (mouse anti-CD14 IgG2b, clone
MφP9, Becton Dickinson, San Jose, CA, USA). Blood samples were processed for flow cytometry daily. Preliminary experiments verified that staining properties of the cells were virtually unaffected by 24-h storage of the blood samples at 0°C (data not shown). Of the whole blood, 25 μl aliquots were double-labelled by the addition of saturating amounts of the mAbs on the basis of leukocyte count. Cells were washed by the addition of 1:10 diluted ice-cold FACS lysing solution (Becton Dickinson) and collected by centrifugation at 4°C to lyse any remaining red cells. This procedure was repeated with the FACS lysing solution at room temperature. The pellets were resuspended in 400 μl of ice-cold saline supplemented with formaldehyde (final concentration 0.5%), and the samples were kept at 0°C until analysis by flow cytometer. Next, 50 μl of microbeads (Quantum Simply Cellular®, QSC, Flow Cytometry Standards Corporation, San Juan, PR, USA) were stained and processed in a similar manner. The QSC preparation comprises a mixture of four highly uniform microbead populations of the same size which have varying capacities to bind mouse monoclonal IgG antibodies, plus a blank population [Serke et al. 1998].

8.4.2 Flow cytometry
A FACSort flow cytometer (Becton Dickinson) and CellQuest software were used for acquisition and analysis of the data. A data set of 5 x 10^3 neutrophils identified on the basis of their light-scattering properties was acquired. In order to determine CD11b fluorescence of these neutrophils, a histogram of red fluorescence was produced, and the arbitrary units (channel number) were transformed into antibody-binding capacity (ABC) units by analysis of similarly stained QSC microbeads. A regression curve of channel number against ABC was constructed by use of Quickal, the calibration software provided with each lot of QSC microbeads. A data set of 10^3 CD14-positive events with light-scattering properties compatible with those of monocytes was acquired, and an analysis of CD11b fluorescence of these cells was conducted as above. In addition, to determine the CD14 fluorescence as ABC units, a histogram of green fluorescence was analysed against similarly stained QSC microbeads.

The median level of mCD14 in samples from healthy volunteers was 105 536 ABC (interquartile range (IQR) 91 087, 117 965), of neutrophil CD11b 24 170 ABC (17 748, 26 295), and of monocyte CD11b 27 411 ABC (21 919, 31 087).
8.5 DETERMINATION OF SOLUBLE MEDIATORS

8.5.1 Interleukin-6
IL-6 was measured by a chemiluminescent immunoassay system (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA) with a detection limit of 5 ng/l.

8.5.2 Interleukin-8
IL-8 was measured by a chemiluminescent immunoassay system (Immulite®, Diagnostic Products Corporation) with a detection limit of 5 ng/l (I) and Quantikine® Human IL-8 Immunoassay (R&D Systems Inc., Minneapolis, MN, USA) with a minimum detectable dose of 3.5 ng/l (II).

8.5.3 C-reactive protein
Serum concentrations of CRP were determined by an immunoturbidimetric assay with a detection limit of 5 mg/l.

8.5.4 Procalcitonin
Plasma samples for PCT were analysed by an immunoluminometric assay (LUMItest PCT®, B.R.A.H.M.S. Diagnostica, Berlin, Germany), with a detection limit of 0.01 μg/l and a functional assay sensitivity of 0.30 μg/l.

8.5.5 Soluble receptors
Soluble IL-2R (I) was measured by a chemiluminescent immunoassay system (Immulite®, Diagnostic Products Corporation) with a detection limit of 10 kU/l. Soluble CD14 and sIL-2rα (II) were measured by Quantikine® Human sCD14 and IL-2rα Immunoassay (R&D Systems Inc.) with a minimum detectable dose of 125 pg/ml and 10 ng/l, respectively. Diagnostic Products Corporation was unable to provide any tool to compare Immulite sIL-2R measurements to R&D Systems Quantikine® sIL-2Rα measurements.

8.6 DATA ANALYSIS

The results are expressed as mean or median with SD or range and 95% confidence intervals (95% CI). Variables with non-normal or ordinal values are expressed by median and interquartile range (IQR). Measures with a discrete distribution are expressed as counts (%). Variables with normal distribution are expressed by mean and standard deviation (SD). The normality of variables was evaluated by Shapiro-Wilk statistics (III). Comparisons between multiple groups were performed with the Kruskal-Wallis test (I, II) and the permutation test (I). Comparison between two groups was done by t-test (II, III), Mann-Whitney U-test (III), or permutation
test with a Monte Carlo p-value (III, IV). In addition, measures with a discrete distribution were analysed by χ² (II, III) or Fisher's exact test (III). Linearity in inflammation markers between groups was analysed by rank-based linearity contrast with bootstrap-based multiplicity-adjusted p-values [Westfall and Young 1993].

Correlations were estimated by Spearman's correlation coefficient method (IV). The predictive value of measured markers was evaluated with the area under the receiver operating characteristic curve (AUC\textsubscript{ROC}) and bias-corrected bootstrap CI (I). In Study II, the inflammation marker level with the highest accuracy (sum of sensitivity and specificity) was determined from a receiver operating characteristic curve, the respective area under the curve (AUC) was calculated, and 95% CI was obtained by bias-corrected bootstrapping (50 000 replications). The cut-off level was chosen according to the combination of best possible sensitivity and specificity.

Survival time was analysed by univariate and multivariate (forward stepwise procedure) proportional hazard regression models, called Cox's regression models (III). The Kaplan-Meier curve illustrated information on the cumulative proportions of survival. Difference between groups was tested by Log-rank test (Monte Carlo p-value) (III) or by a permutation type Log-rank test (IV); 95% CI of survival rate was obtained by bias-corrected and accelerated bootstrapping.
9 RESULTS

9.1 PATIENTS

A total of 531 patients were included in the final analysis of Studies I to IV (Table 2).

Table 2. Characteristics of all patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Number of patients</td>
<td>92</td>
</tr>
<tr>
<td>Age, years, mean (range)</td>
<td>52 (18-88)</td>
</tr>
<tr>
<td>Sex, male/female, n</td>
<td>44/48</td>
</tr>
<tr>
<td>Length of stay, mean (range)</td>
<td>9 (1-40)</td>
</tr>
<tr>
<td>Mortality in 28 days, n (%)</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td>Positive blood culture, n (%)</td>
<td>13 (14.1)</td>
</tr>
</tbody>
</table>

In Study I, the analysis comprised 92 acutely ill patients allocated into 3 groups according to their clinical features on admission: Group I comprised 54 patients with infectious focus, Group II, 25 patients with acute onset of fever without infectious focus on admission, and Group III, 13 patients with neither fever nor infectious focus on admission. As a preliminary setting, Groups II and III were to be further divided into 2 subgroups (A and B), to denote patients in whom infectious focus was verified, and was not, within a 3-day follow-up. However, in Group III no infectious focus emerged during the 3-day follow-up. So the final subgroup analysis consisted of the infection group and the combined possible-infection and control group (Groups IIA and IIB, respectively, in Study I). For diagnoses see Table 3. A total of 13 patients, 10 in Group I and 3 in Group II, had positive blood cultures (see Table 4 for species and numbers of cases). In 21 patients, antimicrobial therapy had been started before sampling for blood culture, and of these patients, 4 had a positive blood culture.
Table 3. Diagnoses of patients presenting with infectious focus (Group I), with fever without infectious focus on admission (Group II), or with neither fever nor infectious focus (Group III) (Study I)

<table>
<thead>
<tr>
<th>Group I</th>
<th>Number of patients (positive for blood culture)</th>
<th>Group II</th>
<th>Number of patients (positive for blood culture)</th>
<th>Group III</th>
<th>Number of patients (positive for blood culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With infectious focus*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>20 (1)</td>
<td>Pneumonia</td>
<td>3</td>
<td>Exacerbation of asthma or COPD</td>
<td>3</td>
</tr>
<tr>
<td>UTI</td>
<td>13 (4)</td>
<td>Sepsis</td>
<td>1 (1)</td>
<td>Exacerbation of IBD</td>
<td>3</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>10 (1)</td>
<td>Peritonsillitis</td>
<td>1 (1)</td>
<td>Suspected infection</td>
<td>2</td>
</tr>
<tr>
<td>Acute diarrhea</td>
<td>3</td>
<td>Viral meningitis</td>
<td>1</td>
<td>Epileptic convulsion</td>
<td>1</td>
</tr>
<tr>
<td>Acute encephalitis</td>
<td>2</td>
<td>Sinusitis</td>
<td>1 (1)</td>
<td>Exacerbation of vasculitis</td>
<td>1</td>
</tr>
<tr>
<td>Liver abscess</td>
<td>2 (2)</td>
<td>Acute bronchitis</td>
<td>1</td>
<td>Gastritis</td>
<td>1</td>
</tr>
<tr>
<td>Malaria</td>
<td>1</td>
<td>Without infectious focus†</td>
<td></td>
<td>Mononucleosis</td>
<td>1</td>
</tr>
<tr>
<td>Paravertebral abscess</td>
<td>1 (1)</td>
<td>Acute fever</td>
<td>4</td>
<td>Supraventricular tachycardia</td>
<td>1</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>1</td>
<td>Acute MI</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin infection</td>
<td>1 (1)</td>
<td>Common cold</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54 (10)</td>
<td>Anemia</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temporal arteritis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinoma of pancreas</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic hypoglycaemia</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epileptic convulsion</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Congestive heart failure</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Henoch-Schönlein purpura</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subacute thyroiditis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophilic pleuritis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25 (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* denotes patients in whom an infectious focus was verified with 3 days of admission
† denotes patients with no infectious focus verified within 3 days of admission
COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; MI, myocardial infraction; UTI, urinary tract infection
Table 4. On-admission levels of markers of systemic inflammation in patients with a positive blood culture (Study I)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Microbe in blood culture</th>
<th>Serum CRP, mg/l</th>
<th>Plasma PCT, μg/l</th>
<th>Plasma IL-6, ng/l</th>
<th>Plasma IL-8, ng/l</th>
<th>Plasma sIL-2R, kU/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>32</td>
<td>0.1</td>
<td>16</td>
<td>6</td>
<td>210</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>126</td>
<td>1.3</td>
<td>79</td>
<td>47</td>
<td>1124</td>
</tr>
<tr>
<td>3</td>
<td><em>Escherichia coli</em></td>
<td>171</td>
<td>6.7</td>
<td>50</td>
<td>11</td>
<td>885</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>228</td>
<td>3.4</td>
<td>2000</td>
<td>174</td>
<td>5900</td>
</tr>
<tr>
<td>5</td>
<td><em>Escherichia coli</em></td>
<td>251</td>
<td>0.8</td>
<td>688</td>
<td>39</td>
<td>822</td>
</tr>
<tr>
<td>6</td>
<td><em>Escherichia coli</em></td>
<td>271</td>
<td>14.1</td>
<td>385</td>
<td>28</td>
<td>2964</td>
</tr>
<tr>
<td>7</td>
<td><em>Staphylococcus aureus</em></td>
<td>199</td>
<td>2.3</td>
<td>376</td>
<td>105</td>
<td>3972</td>
</tr>
<tr>
<td>8</td>
<td><em>Staphylococcus aureus</em></td>
<td>211</td>
<td>0.5</td>
<td>35</td>
<td>&lt; 5</td>
<td>683</td>
</tr>
<tr>
<td>9</td>
<td><em>Bacteroides fragilis</em></td>
<td>275</td>
<td>0.4</td>
<td>62</td>
<td>6</td>
<td>5127</td>
</tr>
<tr>
<td>10</td>
<td>β-hemolytic <em>Streptococcus</em> B</td>
<td>12</td>
<td>4.5</td>
<td>1052</td>
<td>20</td>
<td>495</td>
</tr>
<tr>
<td>11</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>284</td>
<td>1.3</td>
<td>531</td>
<td>12</td>
<td>5034</td>
</tr>
<tr>
<td>12</td>
<td><em>Fusobacterium necrophorum</em> and <em>Streptococcus milleri</em></td>
<td>204</td>
<td>335</td>
<td>4040</td>
<td>165</td>
<td>6437</td>
</tr>
<tr>
<td>13</td>
<td><em>Proteus mirabilis</em> and viridans –group <em>Streptococcus</em></td>
<td>160</td>
<td>126</td>
<td>297</td>
<td>&lt; 20</td>
<td>2512</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; PCT, procalcitonin; IL, interleukin; sIL-2R, soluble interleukin 2 receptor
Study II included 138 patients presenting with abnormal body temperature: < 36°C or > 38°C on admission or within 48 hours before hospital referral, but without infectious focus verified on admission. Of these 138, 26 patients had a CAI with verified focus (infection group) within a follow-up of up to 3 days (Table 5). Their diagnoses were: pneumonia (n=6), abscess (3), skin infection (2), nephropatia epidemica (2), and a single case each of urinary tract infection, endocarditis, cholangitis, and hepatitis A. In 23 patients, a non-infectious acute illness was found in concert with no clinical suspicion of infection during follow-up (control group). The diagnoses in this group were congestive heart failure (n=5), epileptic convulsion (4), thromboembolic disease (3), drug intoxication (2), reactive arthritis (2), and a single case each of diabetes, gout, multiple sclerosis, nitrofurantoin-induced lung disease, pleural pain, pneumothorax, and suspected sarcoidosis. Finally, 89 patients showed neither infectious focus nor other cause, yet the presence of hidden infection could not be excluded (possible-infection group). These patients were categorised into three subgroups: patients with fever or hypothermia but without other clinical symptoms (n=40), with fever with various respiratory symptoms (35), and with fever with miscellaneous symptoms (14). In 22 patients (5 in the infection group, 14 in the possible infection group, and 3 in the control group) antimicrobial therapy had been started before sampling for blood culture; in 2 of these patients the blood culture was positive.
Table 5. Demographic, clinical, and laboratory data (Study II)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (N=23)</td>
<td>Possible-infection (N=89)</td>
</tr>
<tr>
<td>Demographic data:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>13 (56.5)</td>
<td>50 (56.2)</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>46 (19)</td>
<td>49 (22)</td>
</tr>
<tr>
<td>Mortality in 28 days, n (%)</td>
<td>0 (0.0)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Clinical data:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body temperature:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 38°C, n (%)</td>
<td>20 (87.0)</td>
<td>88 (98.9)</td>
</tr>
<tr>
<td>&lt; 36°C, n (%)</td>
<td>3 (13.4)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>MAP, mmHg, mean (SD)</td>
<td>93 (19)</td>
<td>88 (15)</td>
</tr>
<tr>
<td>Heart rate, beats per minute, mean (SD)</td>
<td>104 (21)</td>
<td>95 (19)</td>
</tr>
<tr>
<td>Laboratory data:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal, 4-12 x10^9/l, n (%)</td>
<td>18 (78.3)</td>
<td>60 (67.4)</td>
</tr>
<tr>
<td>high, &gt; 12 x10^9/l, n (%)</td>
<td>5 (21.7)</td>
<td>20 (22.5)</td>
</tr>
<tr>
<td>low, &lt; 4 x10^9/l, n (%)</td>
<td>0 (0.0)</td>
<td>9 (10.1)</td>
</tr>
<tr>
<td>Platelet count, x10^9/l, median (IQR)</td>
<td>250 (204, 344)</td>
<td>200 (144, 241)</td>
</tr>
</tbody>
</table>

SD, standard deviation; MAP, mean arterial pressure; WBC, white blood cell; IQR, interquartile range
Study III comprised 327 acutely ill patients with a community-acquired infection verified on admission or during a follow-up of up to 3 days (Table 6). The 28-day mortality rate was low, 3.4% (11 patients). The most frequent types of infection were pneumonia (n=195), skin infection (81), and urinary tract infection (67). A total of 54 patients (16.5%) had blood cultures positive for Escherichia coli (n=15), Streptococcus pneumoniae (9), Staphylococcus aureus (5), β-haemolytic group G Streptococcus (4), Staphylococcus epidermidis (3), Klebsiella oxytoca (2), Streptococcus pyogenes (2), viridans group Streptococcus (4), and one each for Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella paratyphi B, Serratia liquefacien, Streptococcus agalactiae (B), Streptococcus intermedius, for both Escherichia coli and viridans group Streptococcus, and for both Staphylococcus epidermidis and viridans group Streptococcus. Nine patients had sepsis with positive blood culture but with no clinically detectable sign of infectious focus. Of the 11 patients who died, 7 had pneumonia and 4 a positive blood culture (Table 7). A total of 56 patients had received antimicrobial therapy before sampling for blood culture, and of these patients, 7 had a positive blood culture.

Emerging from the patient material in Study III, in Study IV the focus was narrowed to acutely ill patients with the most common and severe CAIs, namely to 142 patients who had either pneumonia (99 patients, 10 positive for blood culture) or positive blood culture without pneumonia (43).
Table 6. Demographic, clinical, and laboratory data (Study III)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (N=316)</th>
<th>Non-survivors (N=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>168 (53)</td>
<td>7 (64)</td>
<td>0.49</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>55 (21)</td>
<td>70 (17)</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Clinical data:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious focus on admission, n (%)</td>
<td>290 (92)</td>
<td>9 (82)</td>
<td>0.24</td>
</tr>
<tr>
<td>Body temperature</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>36-38°C, n (%)</td>
<td>136 (44)</td>
<td>4 (40)</td>
<td></td>
</tr>
<tr>
<td>&gt; 38°C, n (%)</td>
<td>168 (54)</td>
<td>5 (50)</td>
<td></td>
</tr>
<tr>
<td>&lt; 36°C, n (%)</td>
<td>9 (3)</td>
<td>1(10)</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg, mean (SD)</td>
<td>88 (16)</td>
<td>74 (22)</td>
<td>0.010</td>
</tr>
<tr>
<td>Heart rate, beats per minute, mean (SD)</td>
<td>93 (18)</td>
<td>113 (30)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Laboratory data:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count, x 10⁹/l, median (IQR)</td>
<td>10.9 (7.3, 14.5)</td>
<td>11.8 (7.9, 19.8)</td>
<td>0.43</td>
</tr>
<tr>
<td>WBC count</td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>normal, 4–12 x10⁹/l, n (%)</td>
<td>177 (57)</td>
<td>5 (46)</td>
<td></td>
</tr>
<tr>
<td>high, &gt; 12 x10⁹/l, n (%)</td>
<td>121 (39)</td>
<td>5 (46)</td>
<td></td>
</tr>
<tr>
<td>low, &lt; 4 x10⁹/l, n (%)</td>
<td>13 (4)</td>
<td>1 (9)</td>
<td></td>
</tr>
<tr>
<td>Serum CRP, mg/l, median (IQR)</td>
<td>82 (32, 158)</td>
<td>113 (65, 315)</td>
<td>0.028</td>
</tr>
<tr>
<td>Platelet count, x10⁹/l, median (IQR)</td>
<td>219 (174, 265)</td>
<td>141 (83, 210)</td>
<td>0.048</td>
</tr>
<tr>
<td>CD11b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN, x10⁴ ABC, median (IQR)</td>
<td>32.8 (24.0, 44.9)</td>
<td>36.9 (26.6, 55.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>MO, x10⁴ ABC, median (IQR)</td>
<td>38.2 (28.7, 52.2)</td>
<td>43.8 (32.9, 58.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>mCD14, x10⁴ ABC, median (IQR)</td>
<td>84.9 (67.0, 108.5)</td>
<td>61.8 (40.6, 76.5)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

CD, cluster of differentiation; PMN, neutrophil; MO, monocyte; other abbreviations as in Tables 4 and 5
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, years</th>
<th>Length of stay, days</th>
<th>Diagnosis</th>
<th>Co-existing disease</th>
<th>Microbe in blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>88</td>
<td>8</td>
<td>Cholecystitis</td>
<td>None</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>78</td>
<td>22</td>
<td>Gangrene</td>
<td>Atherosclerotic vascular disease</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>70</td>
<td>20</td>
<td>Meningitis</td>
<td>None</td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>72</td>
<td>8</td>
<td>Pneumonia</td>
<td>Alcoholism</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td>88</td>
<td>7</td>
<td>Pneumonia</td>
<td>Acute myocardial infarction</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>86</td>
<td>3</td>
<td>Pneumonia</td>
<td>Congestive heart disease</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>f</td>
<td>50</td>
<td>10</td>
<td>Pneumonia</td>
<td>Amyotrophic lateral sclerosis</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>f</td>
<td>83</td>
<td>5</td>
<td>Pneumonia</td>
<td>Congestive heart disease</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>41</td>
<td>1</td>
<td>Pneumonia</td>
<td>Alcoholism</td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>10</td>
<td>m</td>
<td>51</td>
<td>11</td>
<td>Pneumonia, suspected encephalitis</td>
<td>Diabetes, alcoholism</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>f</td>
<td>63</td>
<td>2</td>
<td>Sepsis</td>
<td>None</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
</tbody>
</table>
9.2 ENHANCING DIAGNOSTICS OF INFECTION

In Studies I and II various laboratory markers of systemic inflammation were evaluated in order to improve the prediction of positive blood culture and diagnostics of hidden community-acquired infection on admission. Cut-off levels for markers of inflammation were developed according to the presence or absence of infectious focus (II), or a positive blood culture (I) (Table 8).

Table 8. Cut-off levels for positive blood culture (I) and hidden community-acquired infection (II)

<table>
<thead>
<tr>
<th>Marker of inflammation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Serum CRP, mg/l</td>
<td>≥ 125</td>
</tr>
<tr>
<td>Plasma PCT, μg/l</td>
<td>≥ 0.40</td>
</tr>
<tr>
<td>Plasma IL-6, ng/l</td>
<td>≥ 257</td>
</tr>
<tr>
<td>Plasma IL-8, ng/l</td>
<td>≥ 19</td>
</tr>
<tr>
<td>CD11b MO, x10³ ABC</td>
<td>N/A</td>
</tr>
<tr>
<td>CD11b PMN, x10³ ABC</td>
<td>N/A</td>
</tr>
<tr>
<td>Plasma sIL-2R, kU/l</td>
<td>≥ 2 400</td>
</tr>
<tr>
<td>Plasma sIL-2Ra, μg/l</td>
<td>N/A</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; PCT, procalcitonin; IL, interleukin; CD, cluster of differentiation; MO, monocyte; PMN, neutrophil; ABC, antibody-binding capacity; N/A, not available; sIL-2R, soluble interleukin 2 receptor

9.2.1 Bloodstream infection (I)

In Study I, a total of 13 patients had positive blood cultures, 10 among patients who presented with an infectious focus (Group I) and 3 among patients with fever in the absence of an infectious focus on admission (Group II). Table 4 shows the levels of CRP, PCT, and cytokines of the 13 patients with positive blood cultures on admission.

According to clinical findings and the number of positive blood cultures, the presence of an infectious focus with fever had a sensitivity of 100% (95% CI, 75 to 100), a specificity of 16% (95%CI, 9 to 26), a PPV of 16% (95%CI, 9 to 26), and a NPV of 100% (95%CI, 75 to 100). On the other hand, evaluation of the diagnostic accuracy of the laboratory markers of inflammation measured (CRP, PCT, IL-6, IL-8, and sIL-2R) according to their respective cut-off levels, showed their range of PPVs to be 33 to 44% and of NPVs 92 to 98% (Table 9).
Table 9. Prediction of blood culture-positive infection by markers of systemic inflammation (Study I)

<table>
<thead>
<tr>
<th>Marker of inflammation</th>
<th>AUC&lt;sub&gt;ROC&lt;/sub&gt; (95% CI)</th>
<th>Best cut-off level</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>Positive Predictive value % (95% CI)</th>
<th>Negative Predictive value % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRP, mg/l</td>
<td>0.85 (0.63-0.96)</td>
<td>≥ 125</td>
<td>85 (55-98)</td>
<td>81 (71-89)</td>
<td>42 (23-63)</td>
<td>97 (89-100)</td>
</tr>
<tr>
<td>Plasma PCT, μg/l</td>
<td>0.85 (0.62-0.94)</td>
<td>≥ 0.40</td>
<td>92 (64-100)</td>
<td>70 (58-79)</td>
<td>33 (19-51)</td>
<td>98 (90-100)</td>
</tr>
<tr>
<td>Plasma IL-6, ng/l</td>
<td>0.77 (0.61-0.89)</td>
<td>≥ 257</td>
<td>62 (32-86)</td>
<td>87 (78-94)</td>
<td>44 (22-69)</td>
<td>93 (85-98)</td>
</tr>
<tr>
<td>Plasma IL-8, ng/l</td>
<td>0.75 (0.56-0.88)</td>
<td>≥ 19</td>
<td>58 (28-84)</td>
<td>83 (73-91)</td>
<td>35 (15-59)</td>
<td>93 (84-98)</td>
</tr>
<tr>
<td>Plasma sIL-2R, kU/l</td>
<td>0.69 (0.47-0.85)</td>
<td>≥ 2 400</td>
<td>54 (25-81)</td>
<td>87 (78-94)</td>
<td>41 (18-67)</td>
<td>92 (83-97)</td>
</tr>
</tbody>
</table>

AUC<sub>ROC</sub>, area under the receiver operating characteristic curve; CI, confidence interval; other abbreviations as in Table 8
9.2.2 Hidden infection (I, II)

In Study I, in addition to predicting positive blood culture, the patients in Groups II and III were further evaluated according to the results of the 3-day follow-up. In eight Group II patients, an infection was subsequently verified, but in Group III no patient proved to have an infection as the cause of fever. Thus, in the subgroup analysis of Group II, the levels of IL-6 and sIL-2R were significantly higher on admission in patients with fever due to infectious focus (infection group IIA) than were the respective levels in patients with fever only (combined possible-infection and control group IIB) (p = 0.005 and p = 0.046, respectively; Table 10). Between these groups, CRP, PCT, and IL-8 did not differ significantly. The three patients with a positive blood culture in the infection group (IIA) had the highest CRP levels (see also Figure 1A in Study I).

The small size of Group II in Study I did not allow any further statistical analysis of the diagnostic accuracy of the inflammation markers. The promising differences between those subgroups prompted us to conduct a larger study including acutely ill patients with abnormal body temperature but without any infectious focus on admission. In Study II, the combined possible-infection and control group was further divided into a possible-infection group and a control group. Beside the soluble markers of inflammation measured in Study I (CRP, PCT, IL-6, IL-8, and sIL-2R), Study II introduced a cell-bound marker of systemic inflammation: phagocyte CD11b expression level. All the on-admission levels of these inflammation markers differed significantly between the patient groups (Table 10). Of the standard laboratory variables, the WBC count fell within the reference limits in half of the patients with a hidden infection, and > 12 x10⁹/l in 5 (21.7%) of the patients in the control group (Table 5). In further analysis, IL-6 and PCT had the highest LR+ for infection (7.52 and 6.44) (Figure 3; see also Table 4 in Study II), but at the same time, two patients in the infection group, despite a clear infectious focus, presented with IL-6 and PCT levels below their respective cut-off levels (for cut-off levels see Table 8). On the other hand, all the patients in the infection group were detected with the combination of neutrophil CD11b, IL-8, and CRP. When this combination was applied to the possible-infection group, all markers fell below their respective cut-off levels in 13.5% of the patients (for post hoc clinical analysis of the possible-infection group, see Table 11).
Table 10. Comparison of on-admission inflammation markers in patients with fever (I) / abnormal body temperature (II), but no verifiable infectious focus on admission. Within a 3-day follow-up, the infection was verified (infection group), was possible but not verifiable (possible-infection group), or the underlying cause of the acute illness was other than infection (control group). The latter two groups were combined in Study I and separate in Study II.

<table>
<thead>
<tr>
<th>Marker of inflammation</th>
<th>Study I</th>
<th>Study II</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection group</td>
<td>Combined possible-infection and control group</td>
<td>p-value*</td>
</tr>
<tr>
<td>Serum CRP, mg/l</td>
<td>N=8</td>
<td>N=17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87 (28, 196)</td>
<td>43 (18, 98)</td>
<td>0.06</td>
</tr>
<tr>
<td>Plasma PCT, μg/l</td>
<td>0.81 (0.22, 5.40)</td>
<td>0.23 (0.17, 0.45)</td>
<td>0.23</td>
</tr>
<tr>
<td>Plasma IL-6, ng/l</td>
<td>281 (46, 659)</td>
<td>53 (10, 86)</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma IL-8, ng/l</td>
<td>13 (7, 135)</td>
<td>8 (&lt; 5, 12)</td>
<td>0.069</td>
</tr>
<tr>
<td>Plasma sIL-2R, kU/l</td>
<td>1418 (836, 4735)</td>
<td>852 (451, 1340)</td>
<td>0.046</td>
</tr>
<tr>
<td>Plasma sIL-2Rα, μg/l</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>CD11b</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>MO, x10⁷ ABC</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>PMN, x10⁷ ABC</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* Permutation test
† Bootstrap multiplicity-adjusted rank-based linearity contrast.

Values are expressed as median (interquartile range, IQR). For abbreviations see Table 8.
Figure 3. The sensitivity and false-positive probability of on-admission-measured inflammation markers in diagnosing community-acquired hidden infection in patients with abnormal body temperature (Study II)

Values are expressed with 95% CI. In parentheses, the respective cut-off levels; see Table 8
For abbreviations see Table 8
Table 11. *Post hoc* clinical analysis using cut-off points calculated for CRP, PMN CD11b, and IL-8 (Study II)

<table>
<thead>
<tr>
<th>Patient group*</th>
<th>Number of patients (%) with markers below their cut-off levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All markers</td>
</tr>
<tr>
<td>Possible-infection group (N=89)</td>
<td>12 (13.5)</td>
</tr>
<tr>
<td>Fever/Hypothermia (n=40)</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Fever with respiratory symptoms (n=35)</td>
<td>4 (11.4)</td>
</tr>
<tr>
<td>Fever with miscellaneous symptoms (n=14)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Control group (no infection) (N=23)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>Infection group (N=26)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

*For definition of patient groups, see p.46. Possible-infection group was divided into subgroups according to clinical findings

For cut-offs and abbreviations see Table 8
9.3 PREDICTION OF 28-DAY MORTALITY (III, IV)

In Study III, the on-admission expression levels of phagocyte CD11b and mCD14 along with demographic, clinical, and basic laboratory variables were evaluated in predicting 28-day mortality of acutely ill patients with CAI. Compared to survivors, non-survivors were significantly older, had a lower mCD14 expression level, lower MAP, higher heart rate, higher CRP level, and lower platelet count. The groups did not differ significantly in terms of gender, body temperature, WBC count, or phagocyte CD11b expression level.

Further statistical analyses revealed that in univariate analysis, age, lowest tertile of MAP, highest tertile of heart rate, and lowest tertile of mCD14 expression level each had a significant predictive value (Table 12). Secondly, in multivariate analysis, age and lowest tertile of mCD14 expression level remained predictive for 28-day mortality (Figure 4).

After the result concerning the predictive value of mCD14, a question arose as to the value of the simultaneous measurement of sCD14. In Study IV, the focus was narrowed to the most common and severe CAIs, namely pneumonia and sepsis, and evaluation of the combined value of mCD14 and sCD14 followed. As a result, no significant correlation appeared between mCD14 and sCD14 (see also Figure 2 in Study III). CRP was related to sCD14 ($r = 0.29$, 95% CI 0.13 to 0.45), but not to mCD14 ($r = -0.10$, 95% CI -0.27 to 0.09). The 28-day survival rate was 83.4% (95% CI 70.3 to 94.2). The non-survivors had significantly lower mCD14 expression levels than did survivors, whereas sCD14 levels did not differ between groups. Patients in the lowest tertile of mCD14 were 9.79 times (95% CI 1.31 to > 50, $p = 0.006$) more likely to die than were patients in the combined middle and highest tertiles. Survival rates in the highest and in the combined middle and lowest tertiles of sCD14 levels were comparable. After stratification by sCD14, patients in the lowest tertile of mCD14 were 14.4 times (95% CI 1.90 to 39.44) more likely to die than were patients in the combined middle and highest tertiles.
Table 12. Predictive values of demographic, clinical, and laboratory parameters for 28-day mortality in univariate and multivariate analysis (Study III)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate* Relative mortality</th>
<th>p-value</th>
<th>Multivariate† Relative mortality</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td></td>
<td>RR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>1.75 (0.53-5.84)</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>1.04 (1.01-1.07)</td>
<td>0.021</td>
<td>1.05 (1.01-1.08)</td>
<td>0.016</td>
</tr>
<tr>
<td>Infectious focus on admission</td>
<td>0.47 (0.10-2.10)</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body temperature</td>
<td></td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-38°C</td>
<td>Reference‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 38°C</td>
<td>1.10 (0.31-3.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 36°C</td>
<td>3.49 (0.41-29.70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, lowest tertile</td>
<td>5.41 (1.48-19.76)</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, highest tertile</td>
<td>3.86 (1.17-12.80)</td>
<td>0.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count</td>
<td></td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-12 x10⁹/l</td>
<td>Reference‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 12 x10⁹/l</td>
<td>1.29 (0.39-4.30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 x10⁹/l</td>
<td>3.80 (0.45-32.33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum CRP, highest tertile</td>
<td>1.65 (0.80-3.39)</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count, lowest tertile</td>
<td>3.00 (0.73-12.04)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN CD11b, highest tertile</td>
<td>1.65 (0.53-5.21)</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MO CD11b, highest tertile</td>
<td>2.39 (0.72-7.88)</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCD14, lowest tertile</td>
<td>7.59 (1.66-34.76)</td>
<td>0.009</td>
<td>7.49 (1.63-34.33)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*All figures age- and sex-adjusted. † Using a forward stepwise procedure. Only entered variables shown. ‡ Denominator of risk ratios

RR, risk ratio; MAP, mean arterial pressure; mCD14, membrane CD14; for other abbreviations see Tables 8 and 9
Figure 4. Kaplan-Meier curves for the lowest and the combined middle and highest tertiles of mCD14 expression
• denotes estimates of 28-day survival rate. 95% CI obtained by bias-corrected and accelerated bootstrapping (5000 replications)
The present study is based on an analysis of 531 patients with pure community-acquired infection (CAI). The markers of systemic inflammation in emergency department (ED) settings have frequently been studied in series including patients with nosocomial infections as well as in patients with CAI [Terregino et al. 2000, Guven et al. 2002, Hausfater et al. 2002, Adams 2005, Huang et al. 2005, Wyllie et al. 2005]. Data in some recent reports have, however, supported the more detailed classification of patients' illnesses as community-acquired, health-care-associated or nosocomial [Siegman-Igra et al. 2002]. In patients with CAI, the microbiology and outcome differ from those in patients with health-care-associated and nosocomial infection [Friedman et al. 2002, Chi et al. 2004, Kollef et al. 2005, Liao et al. 2005]. This difference may also involve the markers of systemic inflammation. The homogeneity of the triggering external and internal environment is the strength and unique feature of this study.

10.1 ENHANCING DIAGNOSTICS OF INFECTION

The diagnostics of infection on admission still rely mostly on indirect methods (radiology, urinalysis, other semi-quantitative quick tests of soluble markers) and the patient's clinical appearance, since the results of various cultures are available at the earliest on the following day. Similarities in their clinical presentation between viral and bacterial infections result in an extensive use of antimicrobial therapy. Besides the general signs of acute illness (fever, fatigue, loss of appetite) together with the characteristic clinical signs of local bacterial infection, other indicators of bacterial infection include various markers of systemic inflammation.

When infection is suspected, leukocyte count is routinely checked to discover any abnormality. The increase in proportion of immature neutrophils, known as band forms, might be more specific for infection than is total count of leukocytes [Wile et al. 2001]. The band proportion is determined manually [van der Meer et al. 2006] and is often unavailable, for example, in Helsinki, outside office hours. Whether the band proportion gives additional information concerning the diagnosis of infection is controversial [Shapiro and Greenfield 1987].
10.1.1 Bloodstream infection (I)

A positive blood culture is not always clinically significant, and also false-negative results represent a significant clinical problem. Although patients with a positive blood culture, i.e., bacteraemia, represent quite a heterogeneous population, a positive blood culture is a practical and clear endpoint and has been the gold standard for infection diagnostics research. Therefore, for screening acutely ill patients, a marker predicting with sensitivity and accuracy microbial growth in blood culture would prove extremely useful.

In Study I, each of the 13 patients with bloodstream infection (BSI) had a detectable infectious focus, or if no obvious focus could be verified, at least had fever. Thus, the absence of fever or of an infectious focus had a high negative predictive value (NPV, 100%), but a very low positive predictive value (PPV, 16%) for BSI, in accordance with previous reports [Groeneveld et al. 2001]. Like the clinical markers, the present cut-off levels of CRP, PCT, IL-6, IL-8, and sIL-2R had high NPVs (92 to 98%) and low PPVs (33 to 44%), indicating that the markers of systemic inflammation did not improve the sensitivity at which we have detected patients with BSI, and this is also supported by other studies [Moscovitz et al. 1994, Engel et al. 1999, Persson et al. 2004].

Our set of laboratory markers for the detection of BSI was developed to measure both early (PCT, IL-6, IL-8) and advanced (CRP, sIL-2R) immune inflammatory reactions [Takala et al. 2002b]. In Finland, the most widely used marker of infection and BSI is CRP. Its present cut-off level of 125 mg/l to rule out BSI (Study I) showed power equal to that of newer markers in an ED setting. Numerous other illnesses affect the level of CRP, often increasing it, CRP not being a specific marker for BSI [Adams 2005]. When examined at an early stage of BSI, CRP is not associated with patient outcome [Takala et al. 1999a].

PCT has been studied extensively in patients with hospital-acquired infection but studied less as a marker of community-acquired BSI. PCT is useful in the early phase of bacteraemia [Rintala et al. 2001]. In a study of community-acquired bacteraemia, the optimal cut-off level of 0.4 μg/l for PCT was associated with a NPV of 98.8% [Chirouze et al. 2002]. In the present study, the respective values were strikingly similar, 0.4 μg/l and 98%, but the PPVs in both studies were low: 25% and 33%, which strongly limits the use of PCT in clinical medicine. Besides PCT, when compared to CRP, the use of IL-6 [Moscovitz et al. 1994] or IL-8 [Lin et al. 2000] provided little impact in predicting BSI. In sum, these markers performed better in ruling out than in ruling in the possibility of BSI.
10.1.2 Hidden infection (I, II)
In patients presenting with an infectious focus, the initial working diagnosis and the initiation of appropriate antimicrobial therapy are easy. In contrast, the diagnostics is difficult in patients presenting with only fever or hypothermia but without an evident infectious focus on admission. The clinical follow-up and further investigation of these patients may finally verify or exclude the infection as the underlying cause of acute illness. Study I revealed that on-admission levels of IL-6 and sIL-2R were higher, and those of IL-8 and CRP tended to be higher in patients with infection than in those without.

In Study II these markers of systemic inflammation were used to screen a larger population of patients without infectious focus on admission. Of the single markers, IL-6 and PCT had the highest positive likelihood ratio (LR+) for detecting CAI, a finding supported by other studies that include patients with infection [Terregino et al. 2000, Chan et al. 2004, Uzzan et al. 2006]. However, in Study II, IL-6 and PCT failed to detect 2 patients with clinically significant pneumonia. That all the patients with infection could be detected with a combination of markers including CRP, CD11b, and IL-8 supports the idea that inflammation is a dynamic process involving sequential activation of the innate immune system [Waage et al. 1989, Cooney and Yumet 2002]. Identifying all patients with systemic inflammation therefore requires combined markers covering all the stages of systemic inflammation. An increase in CD11b expression requiring no time-consuming de novo protein synthesis [Calafat et al. 1993] is an early marker of systemic inflammation [Rinder et al. 1992]. Sequential generation of cytokines also occurs within hours in response to an insult [Waage et al. 1989, Matsukawa and Yoshinaga 1998], and increased cytokine levels co-occur with onset of fever. CRP levels peak later, 24 to 48 hours after onset of fever [Anonymous 1988, Rintala et al. 2001]. IL-8 guides neutrophil accumulation into tissues [Van Zee et al. 1992] and therefore may indicate a hidden tissue infection. Neutrophil CD11b, a sensitive marker of systemic inflammation [Takala et al. 2002a] but nonspecific for infection [Takala et al. 1999b], correlates positively with circulating IL-8 levels, and both markers facilitate the diagnosis of sepsis in neonates [Nupponen et al. 2001]. CD11b expression levels may, however, remain normal even in patients with blood culture-positive sepsis [Kuuliala et al. 2004]. The other markers evaluated in Study II all serve as markers of systemic inflammation: PCT [Guven et al. 2002, Hausfater et al. 2002, Chan et al. 2004], IL-6 [Moscovitz et al. 1994, Ortqvist et al. 1995, Antunes et al. 2002], and sIL-2R [Kylanpaa-Back et al. 2001b], a fact also demonstrated in Study II.

Clinical studies of inflammatory markers have included patients with an evident infectious focus and excluded patients with a possible infection [Groeneveld et al. 2001, Raaphorst et al. 2001, Chan et al. 2004, Nuutila et al. 2006]. The latter group is important because it comprises those patients not necessarily benefiting from antimicrobial therapy. Study II included
a *post hoc* clinical analysis of the possible-infection group. The possible-infection group proved very large, 89 (64.5%) of the 138 patients. Of these 89, 12 (13.5%) displayed the combined markers below the marker cut-off levels, and 9 of them received empiric antimicrobial therapy. Obviously, in these patients without immunosuppression or any other similar predisposing factor, antimicrobial therapy could have been withheld or discontinued safely. On the other hand, in 12 patients (13.5%), the combined markers were all above their respective cut-off levels, and all these patients were appropriately treated with antimicrobial drugs. Among the other 65 patients (a large majority of the possible-infection group) 1 or 2 of the markers exceeded the cut-off level(s); thus, in these patients the combined markers did not add to clinical decision-making.

### 10.2 PREDICTION OF 28-DAY MORTALITY (III, IV)

In the present study, 28-day mortality of those patients with a CAI was low (3.4%), and the number of non-survivors small (n=11), but in accordance with earlier data [Esel et al. 2003, Shapiro et al. 2006]. Thus, interpretation of outcome data must be limited to patients with CAI and be confirmed in larger populations. Our patients with CAI represented a heterogeneous population, including subgroups already with various prognostic factors on admission, for example, an elderly patient with a urinary tract infection (UTI) vs. a community-acquired pneumonia (CAP), influencing 28-day mortality. A laboratory marker for predicting the prognosis of CAI would still prove useful, since the status of any patient with any CAI may worsen quickly and may lead to shock and even to death. Such a marker might aid in intensifying treatment early enough and in better targeting the expensive new immunomodulative treatments.

Study III showed that age and low monocyte membrane CD14 (mCD14) expression level, as determined on admission to hospital, were associated with poor prognosis among CAI patients. In patients with serious infections, age is associated with poor prognosis [Ruiz et al. 1999, van Langevelde et al. 2000, van de Beek et al. 2004, Roson et al. 2004]. To our knowledge, mCD14 expression level as a prognostic factor has not been studied in patients with CAI. In hospitalised patients, on-admission levels of mCD14 in those with sepsis were lower [Ertel et al. 1993, Lin et al. 1993, Gluck et al. 2001], and low levels correlated with severity of infection [de Werra et al. 2001], whereas persistently low levels correlated with fatal outcome [Gluck et al. 2001]. In patients with severe trauma [Heinzelmann et al. 1996] or severe acute pancreatitis [Kylanpaa-Back et al. 2001a]–both representing systemic inflammation in the absence of infection–on-admission mCD14 expression level was not associated with clinical outcome. These findings
thus suggest that low mCD14 expression on admission may indeed predict mortality in pure CAI.

An important question is whether the clinical outcome of those patients with low mCD14 expression might be improved. According to the current concept, systemic inflammation contributes to the development of dysfunction of vital organs which, in patients with sepsis, is the major cause of mortality (reviewed in Takala et al. [2002b]). At an early, proinflammatory stage of systemic inflammation, characterised by systemic release of TNF and other proinflammatory cytokines, interfering with the cytokines might prove beneficial. This early stage is, however, rapidly followed by an anti-inflammatory reaction which leads to immune suppression or even anergy characterised by depressed monocyte HLA-DR expression and TNF production capacity [Tschaikowsky et al. 2002, Mentula et al. 2004]. At this stage, depression of TNF production may be detrimental and immune stimulation beneficial [Docke et al. 1997]. Stimulated TNF production by monocytes expressing low mCD14 is reported to be decreased [de Werra et al. 2001], and this may indirectly suggest that low mCD14 reflects defective monocyte function. In vitro bacterial products have led to increased CD14 expression and survival of monocytes [Landmann et al. 1996], whereas anti-inflammatory cytokines [de Waal Malefyt et al. 1993] and also glucocorticoids [Sumegi et al. 2005] usually down-regulate CD14 expression. In vitro down-regulation of mCD14 expression has been sufficient to trigger monocyte apoptosis [Heidenreich et al. 1997]. Thus, our patients with low mCD14 levels may be candidates for immune-stimulatory therapy to alter the course of their systemic inflammation, but always on the basis of evaluation of immune–inflammatory status in a single patient.

In Study IV, low mCD14 levels, but not the circulating soluble CD14 (sCD14) levels, predicted 28-day mortality of the patients with CAP or community-acquired blood culture-positive sepsis or both. Our findings differ from the results of a study by Glück et al. [2001] indicating no correlation between mCD14 levels and 28-day mortality in patients with sepsis. In addition, Glück et al. [2001] found that a high sCD14 level was associated with survival by day 28. Study IV indicated that sCD14 levels did not correlate with clinical outcome. These discrepancies may be explained by differences in inclusion of patients, in sample handling, and in flow cytometric analysis.

In addition, in Study IV, mCD14 levels did not correlate with sCD14 levels. This is understandable in the context of the findings that besides being shed from monocytes [Bazil and Strominger 1991], sCD14 is expressed and secreted by human hepatocytes [Su et al. 1999]. Indeed, evidence has accumulated to show that sCD14 serves as an acute phase reactant in parallel with CRP, a classical acute phase protein [Bas et al. 2004]. This idea is supported by our finding that the patients’ sCD14 levels correlated
with their CRP levels. In addition, sCD14 may enhance mCD14-positive cells’ response to bacterial structures [Dziarski et al. 2000], and contribute to elimination and detoxification of bacterial endotoxins [Yu et al. 1997]. Furthermore, despite the lack of correlation between sCD14 and mCD14, high sCD14 levels appeared to strengthen the ability of mCD14 to predict 28-day mortality.

Of the other markers of inflammation, in patients with sepsis, an increased level of phagocyte CD11b/CD18 expression at an early stage of the disease has been associated with the development of organ failure and also has been a suitable early marker for screening systemic inflammation in acutely ill patients [Takala et al. 2002b]. In patients with septic shock, neutrophil CD11b/CD18 expression level has been correlated with severity of disease [Chishti et al. 2004]. However, in the present study, phagocyte CD11b/CD18 expression level was not associated with mortality. This may be due to the milder nature of CAI when compared to the conditions suffered by ICU patients [Takala et al. 1999a]. The differences between these studies may also lie in their differing end-points (organ failure vs. mortality). CRP, an acute phase protein, is widely acknowledged as an infection marker, but without any prognostic value at the early stage of disease [Smith et al. 1995, Takala et al. 1999a]. The present study, therefore, although being significantly higher in non-survivors than in survivors, showed that on-admission CRP levels did not predict poor outcome.

In sum, the results suggest that mCD14 expression could serve as a prognostic marker in patients with CAI, but this finding should be verified in a larger population with a greater number of non-survivors.
11 CONCLUSIONS

1. Markers of systemic inflammation (CRP, PCT, IL-6, IL-8, or sIL-2R) were unable to predict community-acquired BSI (I)

2. In patients presenting with abnormal body temperature, all the markers of systemic inflammation (CRP, PCT, IL-6, IL-8, sIL-2R, or phagocyte CD11b) were significantly higher in patients with a hidden CAI than in patients without such infection. IL-6 and PCT had the highest LR+ for diagnosing infection, but they detected 24 of 26 (92.3%) of the patients with infection. A combination of CD11b, CRP, and IL-8 detected all patients with infection. Evaluation of patients with a possible infection requires a combination of inflammatory markers (II)

3. In patients with CAI, advanced age and low on-admission mCD14 expression level were associated with 28-day mortality (III)

4. In patients with severe CAI, high levels of sCD14 alone were not predictive of 28-day mortality. However, a high sCD14 level in combination with a low mCD14 level enhanced the prognostic value of a low mCD14 level (IV)
The studies included in this thesis were carried out at the Department of Bacteriology and Immunology and the Department of Medicine, University of Helsinki, from 1997 to 2006.

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