Hannele Aalto

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

Department of Bacteriology and Immunology
Department of Medicine
University of Helsinki
Helsinki, Finland

ACADEMIC DISSERTATION
Helsinki University Biomedical Dissertations No. 93

To be presented, with the permission of the Medical Faculty of the University of Helsinki, for public examination in the lecture hall 2 of the Biomedicum Helsinki, on August 15th 2007, at 12 noon

Helsinki 2007

SUPERVISED BY
Annika Takala, MD, PhD
Department of Bacteriology and Immunology
University of Helsinki
Helsinki, Finland

Docent Heikki Repo
Department of Medicine and Department of Bacteriology and Immunology
University of Helsinki and
Department of Medicine
Helsinki University Central Hospital
Helsinki, Finland

REVIEWED BY
Docent Matti Ristola
Department of Medicine, Division of Infectious Diseases
Helsinki University Central Hospital
Helsinki, Finland

Docent Esa Rintala
Department of Infectious Diseases
Satakunta Central Hospital
Pori, Finland

TO BE DISCUSSED WITH
Docent Jarmo Oksi
Department of Medicine
Turku University Central Hospital
Turku, Finland

ISSN 1457-8433
ISBN 978-952-10-4079-5 (paperback)
ISBN 978-952-10-4080-1 (PDF)
(http://ethesis.helsinki.fi)
Yliopistopaino 2007

To my family

Contents

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:

I Aalto H, Takala A, Kautiainen H, Repo H. Laboratory markers of systemic inflammation as predictors of bloodstream infection in acutely ill patients admitted to hospital in medical emergency. Eur J Clin Microbiol Infect Dis (2004) 23:699-704

II Aalto H, Takala A, Kautiainen H, Siitonen S, Repo H. Combination of laboratory markers of systemic inflammation in diagnostics of hidden infection in acutely-ill patients with abnormal body temperature. Submitted.

III Aalto H, Takala A, Kautiainen H, Repo H. Peripheral blood phagocyte CD14 and CD11b expression on admission to hospital in relation to mortality among patients with community-acquired infection. Inflamm Res (2005) 54(10):428-34

IV Aalto H, Takala A, Kautiainen H, Siitonen S, Repo H. Monocyte CD14 and soluble CD14 in predicting the mortality of patients with severe community-acquired infection. Scand J Infect Dis (2007) 39(6):596-603

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

NFκB	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

3 ABSTRACT IN FINNISH

Sairaalan päivystysvastaanotolle lähetetyllä äkillisesti sairaalla potilaalla sairauden yleinen syy on infektio eli elimistöön joutuneen mikrobin aiheuttama oireinen sairaus. Infektio voi aiheuttaa elimistössä paikallisen tulehduspesäkkeen eli fokuksen tai se voi synnyttää yleistyneen tulehdusvasteen, jossa verenkiertoon vapautuu suuria määriä tulehduksen välittäjäaineita. Infektion aiheuttamaa yleistynyttä tulehdusvastetta kutsutaan sepsikseksi. Sepsiksen vastine puhekielessä on verenmyrkytys, jolla perinteisesti on tarkoitettu mikrobien esiintymistä veressä ja veriviljelyn positiivisuutta. Äkillisesti kotona sairastuneella potilaalla kyseessä on ns. avohoito- eli kotisyntyinen infektio. Useimmiten infektio ja fokus pystytään toteamaan sairaalaan tullessa ja antibioottilääkehoito parantaa taudin. Toisilla potilailla äkillisen sairauden syy ei selviä ensimmäisen vuorokauden aikana eikä piilevän infektion mahdollisuutta pystytä poissulkemaan. Erityisesti sepsiksen varmistuminen saattaa viivästyä, jos infektiofokusta ei tulovaiheessa löydy ja veriviljelyn tulosta joudutaan odottamaan useita päiviä. Infektiopotilaiden yleistila voi huonontua nopeasti ja johtaa verenkierron lamaantumiseen eli sokkiin, jopa kuolemaan. Näiden potilaiden tunnistaminen varhaisvaiheessa esimerkiksi verestä mitattavilla tulehduksen merkkiaineilla auttaisi tehostamaan hoitotoimenpiteitä sekä kohdentamaan uusia, kalliita tulehdusvasteeseen vaikuttavia lääkehoitoja.

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

Meilahden sairaalan eettinen toimikunta hyväksyi tutkimussuunnitelman. Tutkimusprojektia varten tutkittiin 1092 äkillisesti sairasta potilasta Helsingin yliopistollisen sairaalan Meilahden sisätautien päivystyspoliklinikalla vuosina 1997–98. Päivystävän lääkärin epäiltyä sepsistä potilaista otettiin päivystyspoliklinikalla veriviljelynäytteet, ja samalla otettiin rinnakkainen verinäyte tutkimusta varten. Näytteistä mitattiin vuorokauden sisällä virtaussytometrilla solusitoisia tulehduksen merkkiaineita, minkä jälkeen loppu plasmanäyte pakastettiin myöhempiä liukoisten merkkiaineiden mittauksia varten. Lopulliseen analyysiin otettiin 531 potilasta, joilla varmentui kotisyntyinen infektio tai oli vahva epäily siitä.

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

Piilevän kotisyntyisen infektion diagnostiikkaa voitiin parantaa tulehduksen merkkiaineilla. Parhaita merkkiaineita olivat PCT ja IL-6 mitattuna positiivisella uskottavuusosamäärällä (positive likelihood ratio). Mikään yksittäinen merkkiaine ei kuitenkaan löytänyt kaikkia potilaita, joilla oli piilevä infektio. Sen sijaan yhdistelmä sisältäen nopeasti reagoivan merkkiaineen (CD11b), hitaammin nousevan merkkiaineen (CRP) ja kudoslähtöisen merkkiaineen (IL-8) kykeni tunnistamaan kaikki infektiopotilaat. Tätä yhdistelmää käytettiin myös niiden potilaiden tarkasteluun, joilla infektiota ei löytynyt, mutta sitä ei voitu varmuudella poissulkeakaan. Näistä potilaista 86,5%:lla ainakin jokin yhdistelmän merkkiaineista ylitti raja-arvon vahvistaen infektion mahdollisuutta.

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

Tulehduksen merkkiaineet parantavat piilevien kotisyntyisten infektioiden diagnostiikkaa taudin alkuvaiheessa, mutta yksittäisiä potilaita tutkittaessa täytyy käyttää useiden merkkiaineiden yhdistelmää mieluummin kuin yksittäistä merkkiainetta. Sairaalaan tulovaiheessa matala solusitoisen lipopolysakkaridireseptorin (mCD14) määrä ennustaa kuolemaa kotisyntyistä infektiota sairastavalla potilaalla.

4 ABSTRACT

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-2Rα) 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.

5 INTRODUCTION

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_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 (H_2O_2), 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 H_2O_2 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 naive 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-Lewis^x 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].

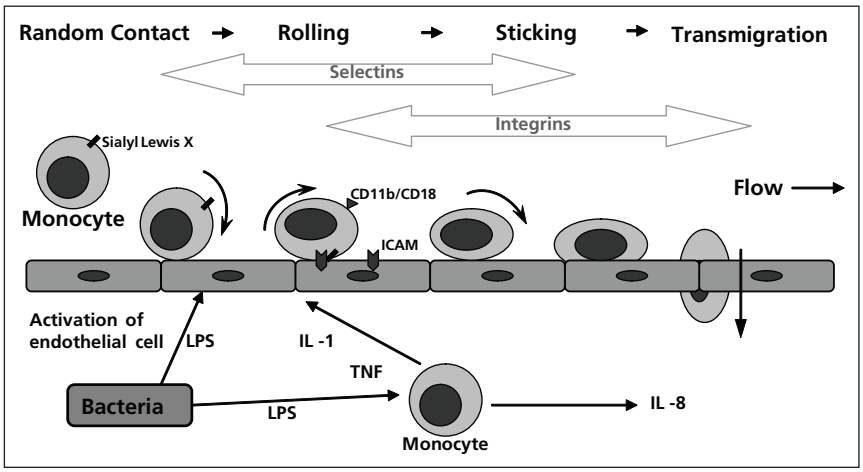

Figure 1. Monocytes circulating in the blood recognise peripheral venule walls near sites of inflammation and leave the bloodstream to migrate into the tissue toward the site of infection and inflammation. The initial interactions are mediated by adhesion molecules that first capture the monocyte from 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_aCO_2 < 32 mmHg (4.3 kPa); 4. white blood cell count > 12 x10^9/l, < 4 x10^9/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 [Yagupsky 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*, *Staphyloccus aureus*, and other *Staphylococcus* species, followed by *Streptococcus pneumoniae*; and in patients aged > 65 years were *Escherichia coli*, *Staphyloccus 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].

The measurement of serum levels of CRP is currently the most widely used means in evaluation of the acute phase response. Elevated levels are detectable in bacterial infections [Morley and Kushner 1982, Harbarth *et al.* 2001, Chan *et al.* 2004, Sierra *et al.* 2004, Simon *et al.* 2004]. In patients with suspected CAP, elevated CRP levels can differentiate between an infectious and a non-infectious cause of an illness [Castro-Guardiola *et al.* 2000, Almirall *et al.* 2004]. The novel acute phase protein, PCT, has been very specific [de Werra *et al.* 1997, Bossink *et al.* 1999, Harbarth *et al.* 2001] and even better than CRP [Selberg *et al.* 2000, Persson *et al.* 2004, Simon *et al.* 2004] in differentiating infection from sepsis.

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 $\geq$ 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 other soluble markers, elevated circulating levels of IL-6 [Hack *et al.* 1989, Damas *et al.* 1992, de Werra *et al.* 1997, Selberg *et al.* 2000, Harbarth *et al.* 2001] and IL-8 [Lin *et al.* 1994, Fujishima *et al.* 1996, Harbarth *et al.* 2001] have been detectable in patients with sepsis. Increased levels of sIL-2R have appeared in patients with CAP [Moussa *et al.* 1994], pancreatitis [Kylanpaa-Back *et al.* 2001b], and sepsis [Takala *et al.* 1999a]. High levels of sCD14 have appeared in patients with infection [Landmann *et al.* 1995, Burgmann *et al.* 1996, Wenisch *et al.* 1996, Carrillo *et al.* 2001].

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.*

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 [Gluck *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.

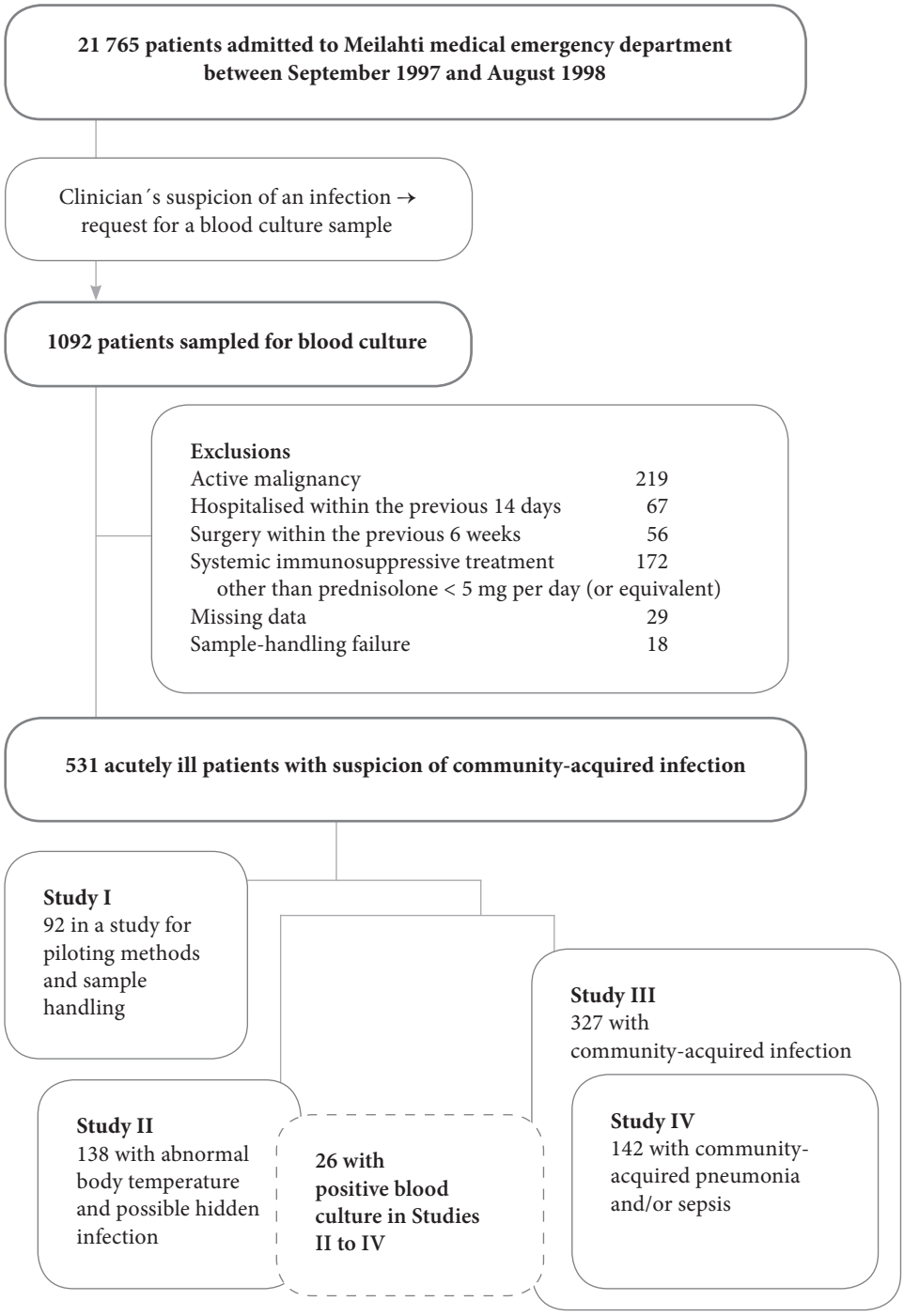

Figure 3. Patients, exclusion criteria, and allocation to Studies I to IV

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 IgG_{2b}, 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 5×10^3 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 $\chi 2$ (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^{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

Variable	Study			
	I	II	III	IV
Number of patients	92	138	327	142
Age, years, mean (range)	52 (18-88)	50 (16-93)	55 (16-94)	60 (16-94)
Sex, male/female, n	44 / 48	79 / 59	175 / 152	83 / 59
Length of stay, mean (range)	9 (1-40)	8 (1-46)	10 (1-69)	13 (1-69)
Mortality in 28 days, n (%)	3 (3.3)	4 (2.9)	11 (3.4)	10 (7.0)
Positive blood culture, n (%)	13 (14.1)	14 (10.1)	54 (16.5)	53 (37.3)

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)

Group I	Number of patients (positive for blood culture)	Group II	Number of patients (positive for blood culture)	Group III	Number of patients (positive for blood culture)
		With infectious focus*			
Pneumonia	20 (1)	Pneumonia	3	Exacerbation of asthma or COPD	3
UTI	13 (4)	Sepsis	1 (1)	Exacerbation of IBD	3
Erysipelas	10 (1)	Peritonsillitis	1 (1)	Suspected infection	2
Acute diarrhea	3	Viral meningitis	1	Epileptic convulsion	1
Acute encephalitis	2	Sinusitis	1 (1)	Exacerbation of vasculitis	1
Liver abscess	2 (2)	Acute bronchitis	1	Gastritis	1
Malaria	1			Mononucleosis	1
Paravertebral abscess	1 (1)	Without infectious focus†		Supraventricular tachycardia	1
Tonsillitis	1	Acute fever	4	**Total**	**13 (0)**
Skin infection	1 (1)	Acute MI	2		
Total	**54 (10)**	Common cold	2		
		Anemia	1		
		Temporal arteritis	1		
		Carcinoma of pancreas	1		
		Diabetic hypoglycaemia	1		
		Epileptic convulsion	1		
		Congestive heart failure	1		
		Henoch-Schönlein purpura	1		
		Subacute thyroiditis	1		
		Eosinophilic pleuritis	1		
		Total	**25 (3)**		

* 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)

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

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)

Variable	Group			p-value
	Control (N=23)	Possible-infection (N=89)	Infection (N=26)	
Demographic data:				
Male, n (%)	13 (56.5)	50 (56.2)	16 (61.5)	0.89
Age, years, mean (SD)	46 (19)	49 (22)	55 (19)	0.25
Mortality in 28 days, n (%)	0 (0.0)	2 (2.3)	2 (7.7)	0.28
Clinical data:				
Body temperature:				0.021
> 38°C, n (%)	20 (87.0)	88 (98.9)	24 (92.3)	
< 36°C, n (%)	3 (13.4)	1 (1.1)	2 (7.7)	
MAP, mmHg, mean (SD)	93 (19)	88 (15)	82 (18)	0.072
Heart rate, beats per minute, mean (SD)	104 (21)	95 (19)	101 (18)	0.11
Laboratory data:				
WBC count:				0.13
normal, 4-12 x10^9/l, n (%)	18 (78.3)	60 (67.4)	13 (50.0)	
high, > 12 x10^9/l, n (%)	5 (21.7)	20 (22.5)	11 (42.3)	
low, < 4 x10^9/l, n (%)	0 (0.0)	9 (10.1)	2 (7.7)	
Platelet count, x10^9/l, median (IQR)	250 (204, 344)	200 (144, 241)	180 (121, 256)	0.031

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)

Variable	Survivors (N=316)	Non-survivors (N=11)	p-value
Demographic data			
Male, n (%)	168 (53)	7 (64)	0.49
Age, years, mean (SD)	55 (21)	70 (17)	0.018
Clinical data:			
Infectious focus on admission, n (%)	290 (92)	9 (82)	0.24
Body temperature			0.41
36-38°C, n (%)	136 (44)	4 (40)	
> 38°C, n (%)	168 (54)	5 (50)	
< 36°C, n (%)	9 (3)	1(10)	
MAP, mmHg, mean (SD)	88 (16)	74 (22)	0.010
Heart rate, beats per minute, mean (SD)	93 (18)	113 (30)	0.001
Laboratory data:			
WBC count, x 10^9/l, median (IQR)	10.9 (7.3, 14.5)	11.8 (7.9, 19.8)	0.43
WBC count			0.25
normal, 4–12 x10^9/l, n (%)	177 (57)	5 (46)	
high, > 12 x10^9/l, n (%)	121 (39)	5 (46)	
low, < 4 x10^9/l, n (%)	13 (4)	1 (9)	
Serum CRP, mg/l, median (IQR)	82 (32, 158)	113 (65, 315)	0.028
Platelet count, x10^9/l, median (IQR)	219 (174, 265)	141 (83, 210)	0.048
CD11b			
PMN, x10^3 ABC, median (IQR)	32.8 (24.0, 44.9)	36.9 (26.6, 55.0)	0.16
MO, x10^3 ABC, median (IQR)	38.2 (28.7, 52.2)	43.8 (32.9, 58.0)	0.76
mCD14, x10^3 ABC, median (IQR)	84.9 (67.0, 108.5)	61.8 (40.6, 76.5)	0.022

CD, cluster of differentiation; PMN, neutrophil; MO, monocyte; other abbreviations as in Tables 4 and 5

Table 7. Characteristics of non-survived patients (Study III)

Patient	Sex	Age, years	Length of stay, days	Diagnosis	Co-existing disease	Microbe in blood culture
1	m	88	8	Cholecystitis	None	*Escherichia coli*
2	m	78	22	Gangrene	Atherosclerotic vascular disease	None
3	m	70	20	Meningitis	None	*Streptococcus pneumoniae*
4	m	72	8	Pneumonia	Alcoholism	None
5	f	88	7	Pneumonia	Acute myocardial infarction	None
6	m	86	3	Pneumonia	Congestive heart disease	None
7	f	50	10	Pneumonia	Amyotrophic lateral sclerosis	None
8	f	83	5	Pneumonia	Congestive heart disease	None
9	m	41	1	Pneumonia	Alcoholism	*Streptococcus pneumoniae*
10	m	51	11	Pneumonia, suspected encephalitis	Diabetes, alcoholism	None
11	f	63	2	Sepsis	None	*Staphylococcus aureus*

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)

	Study	
Marker of inflammation	**I**	**II**
Serum CRP, mg/l	≥ 125	≥ 57
Plasma PCT, µg/l	≥ 0.40	≥ 0.30
Plasma IL-6, ng/l	≥ 257	≥ 104
Plasma IL-8, ng/l	≥ 19	≥ 11.8
CD11b		
MO, $x10^3$ ABC	N/A	≥ 38.5
PMN, $x10^3$ ABC	N/A	≥ 29.6
Plasma sIL-2R, kU/l	≥ 2 400	N/A
Plasma sIL-2Rα, µg/l	N/A	≥ 1.63

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)

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

AUC^{ROC}, 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 $x10^9$/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

	Study I			Study II			
	Infection group	Combined possible-infection and control group	p-value*	Infection group	Possible-infection group	Control group	p-value†
Marker of inflammation	N=8	N=17		N=26	N=89	N=23	
Serum CRP, mg/l	87 (28, 196)	43 (18, 98)	0.06	75 (36, 143)	47 (22, 100)	22 (7, 92)	0.032
Plasma PCT, μg/l	0.81 (0.22, 5.40)	0.23 (0.17, 0.45)	0.23	0.80 (0.30, 3.00)	0.20 (0.10, 0.30)	0.10 (0.10, 0.20)	< 0.001
Plasma IL-6, ng/l	281 (46, 659)	53 (10, 86)	0.005	121 (55, 409)	26 (12, 66)	23 (5, 79)	< 0.001
Plasma IL-8, ng/l	13 (7, 135)	8 (< 5, 12)	0.069	20.3 (12.3, 66.2)	7.4 (0.0, 15.8)	6.3 (0.0, 10.8)	0.001
Plasma sIL-2R, kU/l	1418 (836, 4735)	852 (451, 1340)	0.046	N/A	N/A	N/A	
Plasma sIL-2Rα, μg/l	N/A	N/A		2.04 (1.63, 3.58)	1.25 (0.92, 2.03)	1.00 (0.63, 1.49)	< 0.001
CD11b							
MO, x10^3 ABC	N/A	N/A		53.5 (37.9, 68.1)	42.1 (33.1, 60.3)	26.2 (23.0, 37.8)	< 0.001
PMN, x10^3 ABC	N/A	N/A		40.8 (32.1, 60.1)	35.2 (26.6, 49.4)	26.5 (21.4, 39.9)	0.014

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

Values are expressed as median (interquartile range, IQR). For abbreviations see Table 8

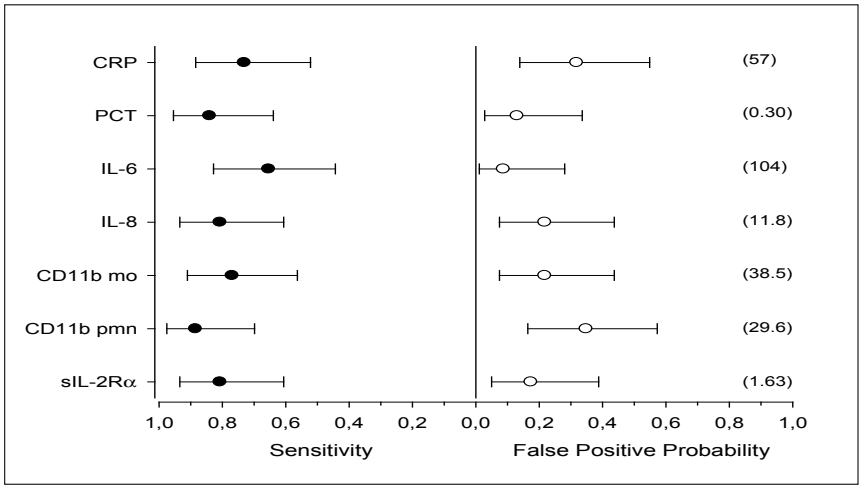

Figure 3. The sensitivity and false-positive probability of on-admission-measured inflammation markers in diagnosing community-acquired hidden infection in patients with abnormal body temperature (Study II)

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)

Patient group*	Number of patients (%) with markers below their cut-off levels			
	All markers	**PMN CD11b**	**IL-8**	**CRP**
Possible-infection group (N=89)	12 (13.5)	40 (44.9)	73 (82.0)	60 (67.4)
Fever/Hypothermia (n=40)	6 (15.0)	19 (47.5)	31 (77.5)	27 (57.5)
Fever with respiratory symptoms (n=35)	4 (11.4)	11 (31.4)	30 (85.7)	24 (68.6)
Fever with miscellaneous symptoms (n=14)	2 (14.3)	10 (71.4)	12 (85.7)	9 (64.3)
Control group (no infection) (N=23)	12 (52.2)	15 (65.2)	21 (91.3)	17 (73.9)
Infection group (N=26)	0 (0.0)	4 (15.4)	5 (19.2)	7 (26.9)

*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 mCD114 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)

Variable	Univariate*		Multivariate†	
	Relative mortality RR (95% CI)	p-value	Relative mortality RR (95% CI)	p-value
Male gender	1.75 (0.53-5.84)	0.36		
Age, years	1.04 (1.01-1.07)	0.021	1.05 (1.01-1.08)	0.016
Infectious focus on admission	0.47 (0.10-2.10)	0.32		
Body temperature		0.48		
36-38°C	Reference‡			
> 38°C	1.10 (0.31-3.93)			
< 36°C	3.49 (0.41-29.70)			
MAP, lowest tertile	5.41 (1.48-19.76)	0.011		
Heart rate, highest tertile	3.86 (1.17-12.80)	0.027		
WBC count		0.35		
4-12 $x10^9$/l	Reference‡			
> 12 $x10^9$/l	1.29 (0.39-4.30)			
< 4 $x10^9$/l	3.80 (0.45-32.33)			
Serum CRP, highest tertile	1.65 (0.80-3.39)	0.17		
Platelet count, lowest tertile	3.00 (0.73-12.04)	0.13		
PMN CD11b, highest tertile	1.65 (0.53-5.21)	0.39		
MO CD11b, highest tertile	2.39 (0.72-7.88)	0.15		
mCD14, lowest tertile	7.59 (1.66-34.76)	0.009	7.49 (1.63-34.33)	0.010

*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

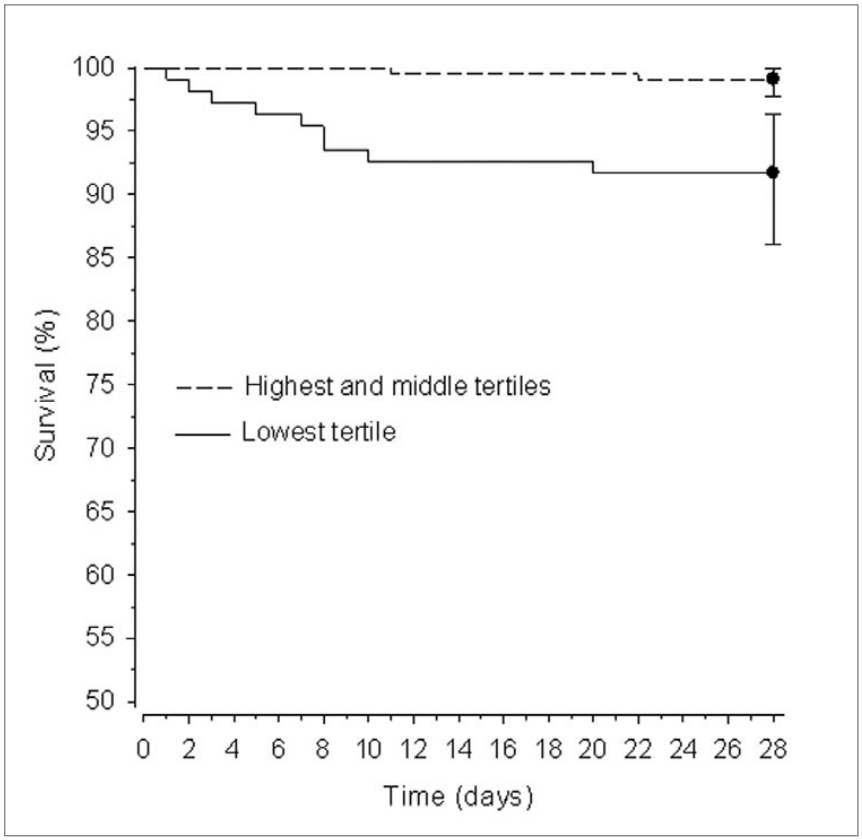

Figure 4. Kaplan-Meier curves for the lowest and the combined middle and highest tertiles of mCD14 expression
• denotes estimates of 28-day survival rate. 95% CI obtained by bias-corrected and accelerated bootstrapping (5000 replications)

10 DISCUSSION

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 lead 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)

12 ACKNOWLEDGMENTS

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.

I wish to express my sincere gratitude to:

Professor Seppo Meri, Head of the Department, for the possibility to use the working facilities at the Department of Bacteriology and Immunology, University of Helsinki, and for his participation in thesis committee meetings.

Docent Liisa-Maria Voipio-Pulkki, for her participation in thesis committee meetings. Docent Sanna Siitonen for providing the facilities for flow cytometry, and Maija-Leena Peltoperä, for measuring all the flow cytometry data. Hannu Kautiainen, biostatistician, for co-authorship and for his crucial role in the analysis of data.

Docent Heikki Repo, one supervisor of this thesis, for the opportunity to work with the unique and vast "sepsis" patient series, for sharing his vast and thorough knowledge of medicine and laboratory science, for his great support during the longer than planned time span of this project, and for giving me courage, patience, and perspective in science, life, and objectives.

Annika Takala, MD, PhD, the other supervisor of this thesis, as well, for the opportunity to work with the sepsis patient series, for her practical and useful lectures in science and in the practise of medicine, and for her advice in relationships, children, and life in general. It was a longer road than we expected, so thank you for your great attitude and patience.

Docents Esa Rintala and Matti Ristola for their prompt and constructive criticism of the manuscript. Carol Norris for author-editing the language. Docent Petteri Carlson, HUSLAB, and Ulla Shemeikka-Marttinen, Information Systems Manager, ICT and Medical Engineering/HUCH, for providing background information.

Eine Virolainen, for expert technical assistance, for being the warm heart of the laboratory, and for being the centre of this research "family" in which I

was allowed to grow up. I am sincerely grateful to all of you for your support during these eventful years.

Head of Department, Jouko Laurila, Maria Hospital, for the friendly atmosphere and support in my taking my first steps in clinical internal medicine and for his flexibility in organizing clinical work periods, together with the possibility to finance research work through the often quite interesting night shifts in Maria Hospital.

Research assistants: Päivi Systä, Sari Roos, Anu Meriö, for their huge effort in preparing the patient data files. Research group colleagues Minna Ilmakunnas, Antti Kuuliala, and Riikka and Arto Turunen, for nice meetings of our version of "Tulehdusklubi."

My dear friends and acquaintances in music and dance – for your company and caring sharing in never-to-be-forgotten journeys, projects, processes, and life in general.

My indefinable, limitless neofamily with all its unique branches full of my dear ones: Mother Kaisa and stepfather Pekka, brother Pekka & Petra, stepbrothers Ilmari and Waltteri y Maria, stepmother Pirkko, half-sister Johanna, and half-brothers Matti and Seppo, together with their families. I cannot imagine how anyone can grow into true tolerance, acceptance, and open-heartedness without having known you and shared a life with all of you! Mother Kaisa, for teaching me her practical common sense, and for subtly acting as the central care-taker and organiser of this neofamily. My brother Pekka especially for work in the beginning with patient data files and for always focusing on the moment at hand. My father, the late Juhani Aalto, for unfailing support and belief in me.

Helsinki Biomedical Graduate School, the Finnish Medical Foundation, and Helsinki University Central Hospital for their financial support.

Helsinki 21 July 2007

Hannele Aalto

13 REFERENCES

Adams NG. Diagnostic use of C-reactive protein in bacteraemic emergency department patients. Emerg Med Australas 17:371-375, 2005.

Almirall J, Bolibar I, Toran P, Pera G, Boquet X, Balanzo X, Sauca G, Community-Acquired Pneumonia Maresme Study Group. Contribution of C-reactive protein to the diagnosis and assessment of severity of community-acquired pneumonia. Chest 125:1335-1342, 2004.

Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 365:63-78, 2005.

Anonymous. HUSLAB Ohjekirja 1153 B-BaktVi Bakteeri, veriviljely, seulomaton näyte. 2006a. (In Finnish)

Anonymous. Veriviljelylöydökset ikäryhmittäin 1995-2005 in: Tartuntataudit Suomessa 2005. Kansanterveyslaitoksen Julkaisuja B, 2006b. (In Finnish)

Anonymous. Guidelines on selection of laboratory tests for monitoring the acute phase response. International Committee for Standardization in Haematology (expert panel on blood rheology). J Clin Pathol 41:1203-1212, 1988.

Antunes G, Evans SA, Lordan JL, Frew AJ. Systemic cytokine levels in community-acquired pneumonia and their association with disease severity. Eur Respir J 20:990-995, 2002.

Aouifi A, Piriou V, Bastien O, Blanc P, Bouvier H, Evans R, Celard M, Vandenesch F, Rousson R, Lehot JJ. Usefulness of procalcitonin for diagnosis of infection in cardiac surgical patients. Crit Care Med 28:3171-3176, 2000.

Aronson MD, Bor DH. Blood cultures. Ann Intern Med 106:246-253, 1987.

Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 341:515-518, 1993.

Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci (Lond) 94:557-572, 1998.

Bas S, Gauthier BR, Spenato U, Stingelin S, Gabay C. CD14 is an acute-phase protein. J Immunol 172:4470-4479, 2004.

Bazil V, Strominger JL. Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. J Immunol 147:1567-1574, 1991.

Beale R. Are we ready for MEDS in the ED? Crit Care Med 35:318-319, 2007.

Blondin C, Le Dur A, Cholley B, Caroff M, Haeffner-Cavaillon N. Lipopolysaccharide complexed with soluble CD14 binds to normal human monocytes. Eur J Immunol 27:3303-3309, 1997.

Bochner BS, Schleimer RP. Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. Immunol Rev 179:5-15, 2001.

Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 101:1644-1655, 1992.

Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 89:3503-3521, 1997.

Bossink AW, Groeneveld AB, Koffeman GI, Becker A. Prediction of shock in febrile medical patients with a clinical infection. Crit Care Med 29:25-31, 2001.

Bossink AW, Groeneveld AB, Thijs LG. Prediction of microbial infection and mortality in medical patients with fever: plasma procalcitonin, neutrophilic elastase-alpha1-antitrypsin, and lactoferrin compared with clinical variables. Clin Infect Dis 29:398-407, 1999.

Boussekey N, Leroy O, Georges H, Devos P, d'Escrivan T, Guery B. Diagnostic and prognostic values of admission procalcitonin levels in community-acquired pneumonia in an intensive care unit. Infection 33:257-263, 2005.

Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. Lancet 368:157-169, 2006.

Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. Clin Exp Immunol 118:235-241, 1999.

Bryan CS. Clinical implications of positive blood cultures. Clin Microbiol Rev 2:329-353, 1989.

Bufler P, Stiegler G, Schuchmann M, Hess S, Kruger C, Stelter F, Eckerskorn C, Schutt C, Engelmann H. Soluble lipopolysaccharide receptor (CD14) is released via two different mechanisms from human monocytes and CD14 transfectants. Eur J Immunol 25:604-610, 1995.

Burgmann H, Winkler S, Locker GJ, Presterl E, Laczika K, Staudinger T, Knapp S, Thalhammer F, Wenisch C, Zedwitz-Liebenstein K, Frass M, Graninger W. Increased serum concentration of soluble CD14 is a prognostic marker in gram-positive sepsis. Clin Immunol Immunopathol 80:307-310, 1996.

Calafat J, Kuijpers TW, Janssen H, Borregaard N, Verhoeven AJ, Roos D. Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b558 and the adhesion molecule CD11b/CD18. Blood 81:3122-3129, 1993.

Calandra T, Gerain J, Heumann D, Baumgartner JD, Glauser MP. High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. The Swiss-Dutch J5 Immunoglobulin Study Group. Am J Med 91:23-29, 1991.

Carrillo EH, Gordon L, Goode E, Davis E, Polk HC,Jr. Early elevation of soluble CD14 may help identify trauma patients at high risk for infection. J Trauma 50:810-816, 2001.

Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. Ann Intern Med 119:771-778, 1993.

Castell JV, Gomez-Lechon MJ, David M, Hirano T, Kishimoto T, Heinrich PC. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. FEBS Lett 232:347-350, 1988.

Castelli GP, Pognani C, Meisner M, Stuani A, Bellomi D, Sgarbi L. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. Crit Care 8:R234-42, 2004.

Castro-Guardiola A, Armengou-Arxe A, Viejo-Rodriguez A, Penarroja-Matutano G, Garcia-Bragado F. Differential diagnosis between community-acquired pneumonia and non-pneumonia diseases of the chest in the emergency ward. Eur J Intern Med 11:334-339, 2000.

Chan YL, Tseng CP, Tsay PK, Chang SS, Chiu TF, Chen JC. Procalcitonin as a marker of bacterial infection in the emergency department: an observational study. Crit Care 8:R12-20, 2004.

Chassagne P, Perol MB, Doucet J, Trivalle C, Menard JF, Manchon ND, Moynot Y, Humbert G, Bourreille J, Bercoff E. Is presentation of bacteremia in the elderly the same as in younger patients? Am J Med 100:65-70, 1996.

Chi CY, Wong WW, Fung CP, Yu KW, Liu CY. Epidemiology of community-acquired Staphylococcus aureus bacteremia. J Microbiol Immunol Infect 37:16-23, 2004.

Chirouze C, Schuhmacher H, Rabaud C, Gil H, Khayat N, Estavoyer JM, May T, Hoen B. Low serum procalcitonin level accurately predicts the absence of bacteremia in adult patients with acute fever. Clin Infect Dis 35:156-161, 2002.

Chishti AD, Shenton BK, Kirby JA, Baudouin SV. Neutrophil chemotaxis and receptor expression in clinical septic shock. Intensive Care Med 30:605-611, 2004.

Christ-Crain M, Jaccard-Stolz D, Bingisser R, Gencay MM, Huber PR, Tamm M, Muller B. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. Lancet 363:600-607, 2004.

Cinader B. Aging and the immune system. In Delves PJ, Roitt IM (eds). Encyclopedia of immunology, 2nd ed. Academic Press, Harcourt Brace & Company Publishers, 1999.

Claeys R, Vinken S, Spapen H, ver Elst K, Decochez K, Huyghens L, Gorus FK. Plasma procalcitonin and C-reactive protein in acute septic shock: clinical and biological correlates. Crit Care Med 30:757-762, 2002.

Clec'h C, Ferriere F, Karoubi P, Fosse JP, Cupa M, Hoang P, Cohen Y. Diagnostic and prognostic value of procalcitonin in patients with septic shock. Crit Care Med 32:1166-1169, 2004.

Cohen J. The immunopathogenesis of sepsis. Nature 420:885-891, 2002.

Cooney RN, Yumet G. Cytokine signaling in sepsis: redundancy, crosstalk, and regulatory mechanisms. Crit Care Med 30:262-263, 2002.

Damas P, Canivet JL, de Groote D, Vrindts Y, Albert A, Franchimont P, Lamy M. Sepsis and serum cytokine concentrations. Crit Care Med 25:405-412, 1997.

Damas P, Ledoux D, Nys M, Vrindts Y, De Groote D, Franchimont P, Lamy M. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Ann Surg 215:356-362, 1992.

Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, Bohuon C. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 79:1605-1608, 1994.

de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, Culpepper J, Dang W, Zurawski G, de Vries JE. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. J Immunol 151:6370-6381, 1993.

de Werra I, Jaccard C, Corradin SB, Chiolero R, Yersin B, Gallati H, Assicot M, Bohuon C, Baumgartner JD, Glauser MP, Heumann D. Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. Crit Care Med 25:607-613, 1997.

de Werra I, Zanetti G, Jaccard C, Chiolero R, Schaller MD, Yersin B, Glauser MP, Calandra T, Heumann D. CD14 expression on monocytes and TNF alpha production in patients with septic shock, cardiogenic shock or bacterial pneumonia. Swiss Med Wkly 131:35-40, 2001.

Dellinger RP, Bone RC. To SIRS with love. Crit Care Med 26:178-179, 1998.

Dempsey PW, Vaidya SA, Cheng G. The art of war: Innate and adaptive immune responses. Cell Mol Life Sci 60:2604-2621, 2003.

Dinarello CA. Proinflammatory cytokines. Chest 118:503-508, 2000.

Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med 3:678-681, 1997.

Dofferhoff AS, Bom VJ, de Vries-Hospers HG, van Ingen J, vd Meer J, Hazenberg BP, Mulder PO, Weits J. Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute-phase proteins during the treatment of severe sepsis in humans. Crit Care Med 20:185-192, 1992.

Duffield JS. The inflammatory macrophage: a story of Jekyll and Hyde. Clin Sci (Lond) 104:27-38, 2003.

Dziarski R, Viriyakosol S, Kirkland TN, Gupta D. Soluble CD14 enhances membrane CD14-mediated responses to peptidoglycan: structural requirements differ from those for responses to lipopolysaccharide. Infect Immun 68:5254-5260, 2000.

Ebnet K, Vestweber D. Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. Histochem Cell Biol 112:1-23, 1999.

Engel A, Steinbach G, Kern P, Kern WV. Diagnostic value of procalcitonin serum levels in neutropenic patients with fever: comparison with interleukin-8. Scand J Infect Dis 31:185-189, 1999.

Epstein D, Raveh D, Schlesinger Y, Rudensky B, Gottehrer NP, Yinnon AM. Adult patients with occult bacteremia discharged from the emergency department: epidemiological and clinical characteristics. Clin Infect Dis 32:559-565, 2001.

Ertel W, Krombach F, Kremer JP, Jarrar D, Thiele V, Eymann J, Muenzing S, Faist E, Messmer K, Schildberg FW. Mechanisms of cytokine cascade activation in patients with sepsis: normal cytokine transcription despite reduced CD14 receptor expression. Surgery 114:243-50; discussion 250-1, 1993.

Esel D, Doganay M, Alp E, Sumerkan B. Prospective evaluation of blood cultures in a Turkish university hospital: epidemiology, microbiology and patient outcome. Clin Microbiol Infect 9:1038-1044, 2003.

File TM. Community-acquired pneumonia. Lancet 362:1991-2001, 2003.

Fink MP. The prevention and treatment of sepsis: is interleukin-6 a drug target or a drug? Crit Care Med 34:919-921, 2006.

Fontanarosa PB, Kaeberlein FJ, Gerson LW, Thomson RB. Difficulty in predicting bacteremia in elderly emergency patients. Ann Emerg Med 21:842-848, 1992.

Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, Lamm W, Clark C, MacFarquhar J, Walton AL, Reller LB, Sexton DJ. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 137:791-797, 2002.

Fujihara M, Muroi M, Tanamoto K, Suzuki T, Azuma H, Ikeda H. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacol Ther 100:171-194, 2003.

Fujishima S, Sasaki J, Shinozawa Y, Takuma K, Kimura H, Suzuki M, Kanazawa M, Hori S, Aikawa N. Serum MIP-1 alpha and IL-8 in septic patients. Intensive Care Med 22:1169-1175, 1996.

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448-454, 1999.

Gaffen SL, Liu KD. Overview of interleukin-2 function, production and clinical applications. Cytokine 28:109-123, 2004.

Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. Am J Infect Control 16:128-140, 1988.

Geppert A, Steiner A, Delle-Karth G, Heinz G, Huber K. Usefulness of procalcitonin for diagnosing complicating sepsis in patients with cardiogenic shock. Intensive Care Med 29:1384-1389, 2003.

Geppert A, Steiner A, Zorn G, Delle-Karth G, Koreny M, Haumer M, Siostrzonek P, Huber K, Heinz G. Multiple organ failure in patients with cardiogenic shock is associated with high plasma levels of interleukin-6. Crit Care Med 30:1987-1994, 2002.

Gleckman R, Hibert D. Afebrile bacteremia. A phenomenon in geriatric patients. JAMA 248:1478-1481, 1982.

Gluck T, Silver J, Epstein M, Cao P, Farber B, Goyert SM. Parameters influencing membrane CD14 expression and soluble CD14 levels in sepsis. Eur J Med Res 6:351-358, 2001.

Glynn P, Coakley R, Kilgallen I, O'Neill S. Neutrophil CD11b and soluble ICAM-1 and E-selectin in community acquired pneumonia. Eur Respir J 13:1380-1385, 1999.

Groeneveld AB, Bossink AW, van Mierlo GJ, Hack CE. Circulating inflammatory mediators in patients with fever: predicting bloodstream infection. Clin Diagn Lab Immunol 8:1189-1195, 2001.

Guven H, Altintop L, Baydin A, Esen S, Aygun D, Hokelek M, Doganay Z, Bek Y. Diagnostic value of procalcitonin levels as an early indicator of sepsis. Am J Emerg Med 20:202-206, 2002.

Guyre PM, Munck A. Glucocorticoids. In Delves PJ, Roitt IM (eds). Encyclopedia of Immunology, 2nd edition. Academic Press, Harcourt Brace & Company Publishers, 1999.

Hack CE, De Groot ER, Felt-Bersma RJ, Nuijens JH, Strack Van Schijndel RJ, Eerenberg-Belmer AJ, Thijs LG, Aarden LA. Increased plasma levels of interleukin-6 in sepsis. Blood 74:1704-1710, 1989.

Harbarth S, Holeckova K, Froidevaux C, Pittet D, Ricou B, Grau GE, Vadas L, Pugin J, Geneva Sepsis Network. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 164:396-402, 2001.

Hardman JG, Limbird LE. Goodman & Gillman´s The pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division, New York, 2001.

Hasday JD, Fairchild KD, Shanholtz C. The role of fever in the infected host. Microbes Infect 2:1891-1904, 2000.

Hausfater P, Garric S, Ayed SB, Rosenheim M, Bernard M, Riou B. Usefulness of procalcitonin as a marker of systemic infection in emergency department patients: a prospective study. Clin Infect Dis 34:895-901, 2002.

Hedlund J, Hansson LO. Procalcitonin and C-reactive protein levels in community-acquired pneumonia: correlation with etiology and prognosis. Infection 28:68-73, 2000.

Heidenreich S, Schmidt M, August C, Cullen P, Rademaekers A, Pauels HG. Regulation of human monocyte apoptosis by the CD14 molecule. J Immunol 159:3178-3188, 1997.

Heinzelmann M, Mercer-Jones M, Cheadle WG, Polk HC,Jr. CD14 expression in injured patients correlates with outcome. Ann Surg 224:91-96, 1996.

Huang HH, Yan HC, Han CL, Yu FC, Kao WY, Chen WT. Association of in vitro oxidative stress, serum ferritin concentration and C-reactive protein in febrile emergency room patients. Clin Invest Med 28:48-54, 2005.

Igonin AA, Armstrong VW, Shipkova M, Lazareva NB, Kukes VG, Oellerich M. Circulating cytokines as markers of systemic inflammatory response in severe community-acquired pneumonia. Clin Biochem 37:204-209, 2004.

Ispahani P, Pearson NJ, Greenwood D. An analysis of community and hospital-acquired bacteraemia in a large teaching hospital in the United Kingdom. Q J Med 63:427-440, 1987.

Jacobs JW, Lund PK, Potts JT,Jr, Bell NH, Habener JF. Procalcitonin is a glycoprotein. J Biol Chem 256:2803-2807, 1981.

Jaimes F, Garces J, Cuervo J, Ramirez F, Ramirez J, Vargas A, Quintero C, Ochoa J, Tandioy F, Zapata L, Estrada J, Yepes M, Leal H. The systemic inflammatory response syndrome (SIRS) to identify infected patients in the emergency room. Intensive Care Med 29:1368-1371, 2003.

Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology The Immune System In Health And Disease. Garland Science, London, 2005.

Jones GR, Lowes JA. The systemic inflammatory response syndrome as a predictor of bacteraemia and outcome from sepsis. QJM 89:515-522, 1996.

Kitchens RL, Thompson PA, Viriyakosol S, O'Keefe GE, Munford RS. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. J Clin Invest 108:485-493, 2001.

Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 13:818-829, 1985.

Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest 128:3854-3862, 2005.

Kuikka A. Factors associated with the outcome of bacteremia during twenty years (1976-1996). Academic Dissertation, University of Helsinki,1999.

Kuuliala A, Takala A, Siitonen S, Leirisalo-Repo M, Repo H. Cellular and humoral markers of systemic inflammation in acute reactive arthritis and early rheumatoid arthritis. Scand J Rheumatol 33:13-18, 2004.

Kylanpaa-Back ML, Takala A, Kemppainen E, Puolakkainen P, Kautiainen H, Jansson SE, Haapiainen R, Repo H. Cellular markers of systemic inflammation and immune suppression in patients with organ failure due to severe acute pancreatitis. Scand J Gastroenterol 36:1100-1107, 2001a.

Kylanpaa-Back ML, Takala A, Kemppainen EA, Puolakkainen PA, Leppaniemi AK, Karonen SL, Orpana A, Haapiainen RK, Repo H. Procalcitonin, soluble interleukin-2 receptor, and soluble E-selectin in predicting the severity of acute pancreatitis. Crit Care Med 29:63-69, 2001b.

Landmann R, Knopf HP, Link S, Sansano S, Schumann R, Zimmerli W. Human monocyte CD14 is upregulated by lipopolysaccharide. Infect Immun 64:1762-1769, 1996.

Landmann R, Muller B, Zimmerli W. CD14, new aspects of ligand and signal diversity. Microbes Infect 2:295-304, 2000.

Landmann R, Zimmerli W, Sansano S, Link S, Hahn A, Glauser MP, Calandra T. Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. J Infect Dis 171:639-644, 1995.

Laudanna C, Kim JY, Constantin G, Butcher E. Rapid leukocyte integrin activation by chemokines. Immunol Rev 186:37-46, 2002.

Leibovici L, Konisberger H, Pitlik SD, Samra Z, Drucker M. Bacteremia and fungemia of unknown origin in adults. Clin Infect Dis 14:436-443, 1992.

Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G, SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 31:1250-1256, 2003.

Liao CH, Chen SY, Chang SC, Hsueh PR, Hung CC, Chen YC. Characteristics of community-acquired and health care-associated Staphylococcus aureus bacteremia in patients treated at the emergency department of a teaching hospital. Diagn Microbiol Infect Dis 53:85-92, 2005.

Lin KJ, Lin J, Hanasawa K, Tani T, Kodama M. Interleukin-8 as a predictor of the severity of bacteremia and infectious disease. Shock 14:95-100, 2000.

Lin RY, Astiz ME, Saxon JC, Rackow EC. Altered leukocyte immunophenotypes in septic shock. Studies of HLA-DR, CD11b, CD14, and IL-2R expression. Chest 104:847-853, 1993.

Lin RY, Astiz ME, Saxon JC, Saha DC, Rackow EC. Relationships between plasma cytokine concentrations and leukocyte functional antigen expression in patients with sepsis. Crit Care Med 22:1595-1602, 1994.

Macfarlane J, Holmes W, Gard P, Macfarlane R, Rose D, Weston V, Leinonen M, Saikku P, Myint S. Prospective study of the incidence, aetiology and outcome of adult lower respiratory tract illness in the community. Thorax 56:109-114, 2001.

Mandell GL, Bennett JE, Dolin R. Mandell, Douglas, and Bennett´s Principles and Practice of Infectious Diseases. Elsevier, Philadelphia, 2005.

March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G, Price V, Gillis S, Henney CS, Kronheim SR, Grabstein K. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature 315:641-647, 1985.

Martins GA, Da Gloria Da Costa Carvalho,M., Rocha Gattass C. Sepsis: a follow-up of cytokine production in different phases of septic patients. Int J Mol Med 11:585-591, 2003.

Matsukawa A, Yoshinaga M. Sequential generation of cytokines during the initiative phase of inflammation, with reference to neutrophils. Inflamm Res 47 Suppl 3:S137-44, 1998.

McCabe WR, Jackson GG. Gram-Negative Bacteremia I. Etiology and Ecology. Arch Intern Med 110:83-91, 1962.

Meisner M. Pathobiochemistry and clinical use of procalcitonin. Clin Chim Acta 323:17-29, 2002.

Mentula P, Kylanpaa ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H. Plasma anti-inflammatory cytokines and monocyte human leucocyte antigen-DR expression in patients with acute pancreatitis. Scand J Gastroenterol 39:178-187, 2004.

Mentula P, Kylanpaa-Back ML, Kemppainen E, Takala A, Jansson SE, Kautiainen H, Puolakkainen P, Haapiainen R, Repo H. Decreased HLA (human leucocyte antigen)-DR expression on peripheral blood monocytes predicts the development of organ failure in patients with acute pancreatitis. Clin Sci (Lond) 105:409-417, 2003.

Monneret G, Arpin M, Venet F, Maghni K, Debard AL, Pachot A, Lepape A, Bienvenu J. Calcitonin gene related peptide and N-procalcitonin modulate CD11b upregulation in lipopolysaccharide activated monocytes and neutrophils. Intensive Care Med 29:923-928, 2003.

Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann N Y Acad Sci 389:406-418, 1982.

Moscovitz H, Shofer F, Mignott H, Behrman A, Kilpatrick L. Plasma cytokine determinations in emergency department patients as a predictor of bacteremia and infectious disease severity. Crit Care Med 22:1102-1107, 1994.

Moussa K, Michie HJ, Cree IA, McCafferty AC, Winter JH, Dhillon DP, Stephens S, Brown RA. Phagocyte function and cytokine production in community acquired pneumonia. Thorax 49:107-111, 1994.

Mylotte JM, Tayara A. Blood cultures: clinical aspects and controversies. Eur J Clin Microbiol Infect Dis 19:157-163, 2000.

Nijsten MW, Olinga P, The TH, de Vries EG, Koops HS, Groothuis GM, Limburg PC, ten Duis HJ, Moshage H, Hoekstra HJ, Bijzet J, Zwaveling JH. Procalcitonin behaves as a fast responding acute phase protein *in vivo* and *in vitro.* Crit Care Med 28:458-461, 2000.

Norrby-Teglund A, Pauksens K, Norgren M, Holm SE. Correlation between serum TNF alpha and IL6 levels and severity of group A streptococcal infections. Scand J Infect Dis 27:125-130, 1995.

Nupponen I, Andersson S, Jarvenpaa AL, Kautiainen H, Repo H. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics 108:E12, 2001.

Nuutila J, Hohenthal U, Laitinen I, Kotilainen P, Rajamaki A, Nikoskelainen J, Lilius EM. Quantitative analysis of complement receptors, CR1 (CD35) and CR3 (CD11b), on neutrophils improves distinction between bacterial and viral infections in febrile patients: comparison with standard clinical laboratory data. J Immunol Methods 315:191-201, 2006.

Oberhoffer M, Karzai W, Meier-Hellmann A, Bogel D, Fassbinder J, Reinhart K. Sensitivity and specificity of various markers of inflammation for the prediction of tumor necrosis factor-alpha and interleukin-6 in patients with sepsis. Crit Care Med 27:1814-1818, 1999.

Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 117:1162-1172, 2000.

Ortqvist A, Hedlund J, Wretlind B, Carlstrom A, Kalin M. Diagnostic and prognostic value of interleukin-6 and C-reactive protein in community-acquired pneumonia. Scand J Infect Dis 27:457-462, 1995.

Parkin J, Cohen B. An overview of the immune system. Lancet 357:1777-1789, 2001.

Pawelec G. Immunity and ageing in man. Exp Gerontol 41:1239-1242, 2006.

Peduzzi P, Shatney C, Sheagren J, Sprung C. Predictors of bacteremia and gram-negative bacteremia in patients with sepsis. The Veterans Affairs Systemic Sepsis Cooperative Study Group. Arch Intern Med 152:529-535, 1992.

Persson L, Engervall P, Magnuson A, Vikerfors T, Soderquist B, Hansson LO, Tidefelt U. Use of inflammatory markers for early detection of bacteraemia in patients with febrile neutropenia. Scand J Infect Dis 36:365-371, 2004.

Pettila V, Hynninen M, Takkunen O, Kuusela P, Valtonen M. Predictive value of procalcitonin and interleukin 6 in critically ill patients with suspected sepsis. Intensive Care Med 28:1220-1225, 2002.

Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest 103:565-575, 1993.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282:2085-2088, 1998.

Povoa P. C-reactive protein: a valuable marker of sepsis. Intensive Care Med 28:235-243, 2002.

Pugin J, Schurer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A 90:2744-2748, 1993.

Raaphorst J, Johan Groeneveld AB, Bossink AW, Erik Hack C. Early inhibition of activated fibrinolysis predicts microbial infection, shock and mortality in febrile medical patients. Thromb Haemost 86:543-549, 2001.

Rau BM, Kemppainen EA, Gumbs AA, Buchler MW, Wegscheider K, Bassi C, Puolakkainen PA, Beger HG. Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): a prospective international multicenter study. Ann Surg 245:745-754, 2007.

Remskar M, Horvat M, Hojker S, Noc M. Procalcitonin in patients with acute myocardial infarction. Wien Klin Wochenschr 114:205-210, 2002.

Reny JL, Vuagnat A, Ract C, Benoit MO, Safar M, Fagon JY. Diagnosis and follow-up of infections in intensive care patients: value of C-reactive protein compared with other clinical and biological variables. Crit Care Med 30:529-535, 2002.

Repo H, Harlan JM. Mechanisms and consequences of phagocyte adhesion to endothelium. Ann Med 31:156-165, 1999.

Reyes WJ, Brimioulle S, Vincent JL. Septic shock without documented infection: an uncommon entity with a high mortality. Intensive Care Med 25:1267-1270, 1999.

Rinder CS, Bonan JL, Rinder HM, Mathew J, Hines R, Smith BR. Cardiopulmonary bypass induces leukocyte-platelet adhesion. Blood 79:1201-1205, 1992.

Rintala EM, Aittoniemi J, Laine S, Nevalainen TJ, Nikoskelainen J. Early identification of bacteremia by biochemical markers of systemic inflammation. Scand J Clin Lab Invest 61:523-530, 2001.

Roitt I, Brostoff J, Male D. Immunology. Mosby, Edinburgh, 2002.

Rosenthal AJ, McMurtry CT, Sanders KM, Jacobs M, Thompson D, Adler RA. The soluble interleukin-2 receptor predicts mortality in older hospitalized men. J Am Geriatr Soc 45:1362-1364, 1997.

Roson B, Carratala J, Fernandez-Sabe N, Tubau F, Manresa F, Gudiol F. Causes and factors associated with early failure in hospitalized patients with community-acquired pneumonia. Arch Intern Med 164:502-508, 2004.

Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J Immunol 135:3172-3177, 1985.

Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. Ann Intern Med 113:619-627, 1990.

Ruiz M, Ewig S, Marcos MA, Martinez JA, Arancibia F, Mensa J, Torres A. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am J Respir Crit Care Med 160:397-405, 1999.

Russwurm S, Vickers J, Meier-Hellmann A, Spangenberg P, Bredle D, Reinhart K, Losche W. Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. Shock 17:263-268, 2002.

Selberg O, Hecker H, Martin M, Klos A, Bautsch W, Kohl J. Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein complement 3a, and interleukin-6. Crit Care Med 28:2793-2798, 2000.

Selby P, Hobbs S, Viner C, Jackson E, Jones A, Newell D, Calvert AH, McElwain T, Fearon K, Humphreys J. Tumour necrosis factor in man: clinical and biological observations. Br J Cancer 56:803-808, 1987.

Serke S, van Lessen A, Huhn D. Quantitative fluorescence flow cytometry: a comparison of the three techniques for direct and indirect immunofluorescence. Cytometry 33:179-187, 1998.

Shapiro MF, Greenfield S. The complete blood count and leukocyte differential count. An approach to their rational application. Ann Intern Med 106:65-74, 1987.

Shapiro N, Howell MD, Bates DW, Angus DC, Ngo L, Talmor D. The association of sepsis syndrome and organ dysfunction with mortality in emergency department patients with suspected infection. Ann Emerg Med 48:583-90, 590.e1, 2006.

Shapiro NI, Howell MD, Talmor D, Donnino M, Ngo L, Bates DW. Mortality in Emergency Department Sepsis (MEDS) score predicts 1-year mortality. Crit Care Med 35:192-198, 2007.

Shapiro NI, Wolfe RE, Moore RB, Smith E, Burdick E, Bates DW. Mortality in Emergency Department Sepsis (MEDS) score: A prospectively derived and validated clinical prediction rule. Crit Care Med 31:670-675, 2003.

Shorr AF, Tabak YP, Killian AD, Gupta V, Liu LZ, Kollef MH. Healthcare-associated bloodstream infection: A distinct entity? Insights from a large U.S. database. Crit Care Med 34:2588-2595, 2006.

Siegman-Igra Y, Fourer B, Orni-Wasserlauf R, Golan Y, Noy A, Schwartz D, Giladi M. Reappraisal of community-acquired bacteremia: a proposal of a new classification for the spectrum of acquisition of bacteremia. Clin Infect Dis 34:1431-1439, 2002.

Sierra R, Rello J, Bailen MA, Benitez E, Gordillo A, Leon C, Pedraza S. C-reactive protein used as an early indicator of infection in patients with systemic inflammatory response syndrome. Intensive Care Med 30:2038-2045, 2004.

Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 39:206-217, 2004.

Smith RP, Lipworth BJ, Cree IA, Spiers EM, Winter JH. C-reactive protein. A clinical marker in community-acquired pneumonia. Chest 108:1288-1291, 1995.

Soker M, Colpan L, Ece A, Devecioglu C, Haspolat K. Serum levels of IL-1 beta, sIL-2R, IL-6, IL-8, and TNF-alpha in febrile children with cancer and neutropenia. Med Oncol 18:51-57, 2001.

Stoiser B, Knapp S, Thalhammer F, Locker GJ, Kofler J, Hollenstein U, Staudinger T, Wilfing A, Frass M, Burgmann H. Time course of immunological markers in patients with the systemic inflammatory response syndrome: evaluation of sCD14, sVCAM-1, sELAM-1, MIP-1 alpha and TGF-beta 2. Eur J Clin Invest 28:672-678, 1998.

Stucker F, Herrmann F, Graf JD, Michel JP, Krause KH, Gavazzi G. Procalcitonin and infection in elderly patients. J Am Geriatr Soc 53:1392-1395, 2005.

Su GL, Dorko K, Strom SC, Nussler AK, Wang SC. CD14 expression and production by human hepatocytes. J Hepatol 31:435-442, 1999.

Sumegi A, Antal-Szalmas P, Aleksza M, Kovacs I, Sipka S, Zeher M, Kiss E, Szegedi G. Glucocorticosteroid therapy decreases CD14-expression and CD14-mediated LPS-binding and activation of monocytes in patients suffering from systemic lupus erythematosus. Clin Immunol 117:271-279, 2005.

Takala A, Jousela I, Jansson SE, Olkkola KT, Takkunen O, Orpana A, Karonen SL, Repo H. Markers of systemic inflammation predicting organ failure in community-acquired septic shock. Clin Sci (Lond) 97:529-538, 1999a.

Takala A, Jousela I, Olkkola KT, Jansson SE, Leirisalo-Repo M, Takkunen O, Repo H. Systemic inflammatory response syndrome without systemic inflammation in acutely ill patients admitted to hospital in a medical emergency. Clin Sci (Lond) 96:287-295, 1999b.

Takala A, Jousela I, Takkunen O, Kautiainen H, Jansson SE, Orpana A, Karonen SL, Repo H. A prospective study of inflammation markers in patients at risk of indirect acute lung injury. Shock 17:252-257, 2002a.

Takala A, Nupponen I, Kylanpaa-Back ML, Repo H. Markers of inflammation in sepsis. Ann Med 34:614-623, 2002b.

Takala AJ, Jousela IT, Takkunen OS, Jansson SE, Kyosola KT, Olkkola KT, Leirisalo-Repo M, Repo H. Time course of beta 2-integrin CD11b/CD18 (Mac-1, alpha M beta 2) upregulation on neutrophils and monocytes after coronary artery bypass grafting. CD11b upregulation after CABG surgery. Scand J Thorac Cardiovasc Surg 30:141-148, 1996.

Tang BM, Eslick GD, Craig JC, McLean AS. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 7:210-217, 2007.

Terregino CA, Lopez BL, Karras DJ, Killian AJ, Arnold GK. Endogenous mediators in emergency department patients with presumed sepsis: are levels associated with progression to severe sepsis and death? Ann Emerg Med 35:26-34, 2000.

Thijs LG, Hack CE. Time course of cytokine levels in sepsis. Intensive Care Med 21 Suppl 2:S258-63, 1995.

Todd RF,3rd, Arnaout MA, Rosin RE, Crowley CA, Peters WA, Babior BM. Subcellular localization of the large subunit of Mo1 (Mo1 alpha; formerly gp 110), a surface glycoprotein associated with neutrophil adhesion. J Clin Invest 74:1280-1290, 1984.

Tschaikowsky K, Hedwig-Geissing M, Schiele A, Bremer F, Schywalsky M, Schuttler J. Coincidence of pro- and anti-inflammatory responses in the early phase of severe sepsis: Longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonin, C-reactive protein, and changes in T-cell subsets in septic and postoperative patients. Crit Care Med 30:1015-1023, 2002.

Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 34:1996-2003, 2006.

Valles J, Rello J, Ochagavia A, Garnacho J, Alcala MA. Community-acquired bloodstream infection in critically ill adult patients: impact of shock and inappropriate antibiotic therapy on survival. Chest 123:1615-1624, 2003.

van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med 351:1849-1859, 2004.

van der Meer, J.W.M. & B.J. Kullberg. Defects in Host Defense Mechanisms. R. H. Rubin and L. S. Young(eds). In "Clinical Approach to Infection in the Compromised Host, 4th ed.". New York:Kluwer Academic/Plenum Publishers (2002).

van der Meer W, van Gelder W, de Keijzer R, Willems H. Does the band cell survive the 21st century? Eur J Haematol 76:251-254, 2006.

van Deventer SJ, Buller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 76:2520-2526, 1990.

van Gameren MM, Willemse PH, Mulder NH, Limburg PC, Groen HJ, Vellenga E, de Vries EG. Effects of recombinant human interleukin-6 in cancer patients: a phase I-II study. Blood 84:1434-1441, 1994.

van Langevelde P, Joop K, van Loon J, Frolich M, Groeneveld PH, Westendorp RG, van Dissel JT. Endotoxin, cytokines, and procalcitonin in febrile patients admitted to the hospital: identification of subjects at high risk of mortality. Clin Infect Dis 31:1343-1348, 2000.

Van Zee KJ, Fischer E, Hawes AS, Hebert CA, Terrell TG, Baker JB, Lowry SF, Moldawer LL. Effects of intravenous IL-8 administration in nonhuman primates. J Immunol 148:1746-1752, 1992.

Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 22:707-710, 1996.

Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR, Payen D, Sepsis Occurrence in Acutely Ill Patients Investigators. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 34:344-353, 2006.

Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 169:333-338, 1989.

Washington JA. An international multicenter study of blood culture practices. The International Collaborative Blood Culture Study Group. Eur J Clin Microbiol Infect Dis 11:1115-1128, 1992.

Weinstein MP, Murphy JR, Reller LB, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev Infect Dis 5:54-70, 1983a.

Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 5:35-53, 1983b.

Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, Reller LB. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 24:584-602, 1997.

Wenisch C, Wenisch H, Parschalk B, Vanijanonta S, Burgmann H, Exner M, Zedwitz-Liebenstein K, Thalhammer F, Georgopoulos A, Graninger W, Looareesuwan S. Elevated levels of soluble CD14 in serum of patients with acute Plasmodium falciparum malaria. Clin Exp Immunol 105:74-78, 1996.

Westfall PH, Young SS. Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment. John Wiley & Sons, Inc, New York, 1993.

Wigmore SJ, Fearon KC, Maingay JP, Lai PB, Ross JA. Interleukin-8 can mediate acute-phase protein production by isolated human hepatocytes. Am J Physiol 273:E720-6, 1997.

Wile MJ, Homer LD, Gaehler S, Phillips S, Millan J. Manual differential cell counts help predict bacterial infection. A multivariate analysis. Am J Clin Pathol 115:644-649, 2001.

Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest 80:617-653, 2000.

Wolk K, Docke WD, von Baehr V, Volk HD, Sabat R. Impaired antigen presentation by human monocytes during endotoxin tolerance. Blood 96:218-223, 2000.

Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249:1431-1433, 1990.

Wyllie DH, Bowler IC, Peto TE. Bacteraemia prediction in emergency medical admissions: role of C reactive protein. J Clin Pathol 58:352-356, 2005.

Xiao H, Siddiqui J, Remick DG. Mechanisms of mortality in early and late sepsis. Infect Immun 74:5227-5235, 2006.

Yagupsky P, Nolte FS. Quantitative aspects of septicemia. Clin Microbiol Rev 3:269-279, 1990.

Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. Annu Rev Immunol 22:405-429, 2004.

Yu B, Hailman E, Wright SD. Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. J Clin Invest 99:315-324, 1997.