Serum Amyloid A Protein (SAA) in Healthy and Infected Individuals

ANDERS LANNERGÅRD
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Abstract


Serum amyloid A protein (SAA) is an acute phase protein that has recently gained increasing interest as a potential marker for disease and treatment monitoring. We investigated SAA and CRP levels in (a) patients with various common infectious diseases (n=98), (b) patients with pyelonephritis (n=37) versus patients with cystitis (n=32), (c) healthy individuals of varying ages (n=231), (d) very immature newborn infants with or without nosocomial infections (NIs) (n=72) and (c) patients with bacterial infections treated with cefuroxime (n=81).

SAA significantly correlated with CRP in viral as well as in bacterial infections (for the total group: r=0.757, p<0.0001) and showed a systemic inflammatory response in 90% of the patients with cystitis as compared with 23% for CRP. Equally high efficiencies (0.96 and 0.94 for SAA and CRP, respectively) were observed in discriminating between pyelonephritis and cystitis. SAA and high sensitive (hs) CRP were lower in umbilical cords (p<0.0001) and higher in elderly adults (p<0.0001-0.03) than in the other age groups; higher in immature newborn infants than in term infants; and higher in the NI group than in the non-NI group. Interindividual variabilities of the time course of the biomarkers SAA and CRP were considerable. Because of the smoothed distribution of SAA and CRP (i.e. elevations were both essentially unchanged during the first 3 days of cefuroxime treatment), these markers were not useful when deciding parenteral-oral switch of therapy, which occurred within this time period in most cases.

SAA is a sensitive systemic marker in cystitis. SAA and hsCRP in umbilical cord blood are close to the detection limit and increase with age. They increase in relation to NI in very immature newborn infants and might therefore be used in diagnosis and monitoring. Finally, SAA and CRP in adults with bacterial infections could not predict an early parenteral-oral switch of antimicrobial therapy.

Keywords: acute phase proteins, adult, Administration Oral, aminoglycosides, amyloidosis, antibiotics, bacterial infections, body temperature, C-reactive protein, cefuroxime, cystitis, cytokines, elderly, infant, interleukin-6, newborn, pyelonephritis, serum amyloid A protein, virus diseases

Anders Lannergård, Department of Medical Sciences, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden

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List of papers

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


III **Lannergård A, Friman G, Ewald U, Larsson A.** Serum amyloid A protein (SAA) and high sensitive C-reactive protein (hsCRP) in healthy newborn infants, and healthy young through elderly adults. In press, Acta Peadiatrica.

IV **Lannergård A, Larsson A, Friman G, Ewald U.** Human serum amyloid A (SAA) and high sensitive C-reactive protein (hsCRP) in very immature newborn infants with nosocomial infections. Submitted.

V  **Viberg A, Lannergård A, Cars O, Karlsson MO, Sandström M, Larsson A.** The time course of the biomarkers temperature, serum amyloid A protein (SAA), C-reactive protein (CRP) and interleukin- (IL) 6 during treatment with cefuroxime in patients with bacterial infections. In manuscript.

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>adenocorticotrope hormone</td>
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<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
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<td>APC</td>
<td>antigen-presenting cell</td>
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<td>APP</td>
<td>acute-phase protein</td>
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<td>APR</td>
<td>acute-phase response</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>C/EBPβ</td>
<td>Cytosine-Cytosine-Adenine-Adenine Thymine -enhancer binding protein β</td>
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<tr>
<td>CCAAT</td>
<td>Cytosine-Cytosine-Adenine-Adenine Thymine</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
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<tr>
<td>FOO</td>
<td>fever of other origin</td>
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<tr>
<td>gp</td>
<td>glycoprotein</td>
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<tr>
<td>GVHD</td>
<td>graft-versus-host disease</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenocortical</td>
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<td>IL-1</td>
<td>interleukin-1</td>
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<tr>
<td>IL-10</td>
<td>interleukin-10</td>
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<tr>
<td>IL-6</td>
<td>interleukin-6</td>
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<tr>
<td>I-κβ</td>
<td>Inhibitory-κβ</td>
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<tr>
<td>LBP</td>
<td>lipoprotein-binding protein</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharides</td>
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<tr>
<td>MAC</td>
<td>membrane attack complex</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>NF</td>
<td>Nuclear Factor</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NI</td>
<td>nosocomial infection</td>
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<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
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<td>nNI</td>
<td>not proven but documented nosocomial infection</td>
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<tr>
<td>nnNI</td>
<td>neither proven nor documented nosocomial infection</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>pNI</td>
<td>proven nosocomial infection</td>
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<tr>
<td>POS</td>
<td>parenteral-oral switch</td>
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<tr>
<td>SAF</td>
<td>SAA-activating factor</td>
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<tr>
<td>SAP</td>
<td>serum amyloid P-component</td>
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<td>SARS</td>
<td>severe acute respiratory syndrome</td>
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<td>SAS</td>
<td>SAA-activating sequence</td>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
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<tr>
<td>VLDL</td>
<td>very low density lipoprotein</td>
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<td>YY</td>
<td>Yin-Yang</td>
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Introduction

Microbes in both the internal and external environment live in harmony with the host until the delicate balance is broken. Internally, in open cavities and on skin, we are colonized by bacteria and viruses. Every “open” part of the body carries a unique composition of microbes, whereas “closed” parts normally are sterile. Externally, there are uncountable possibilities for transmission of contagious microbes of different pathogenicity. Because of the enormous number of infected people, human immunodeficiency virus (HIV) and tuberculosis are outstanding world-wide examples of this transmission process. Immunodeficient patients and post-operative patients are more prone to acquire infections by microbes that normally are harmless to healthy individuals. In order to be infectious, a pathogen has to break through at least three levels of barriers in the host: (a) the surface enzyme- and mucus-containing epithelium surrounding the body cavities and the keratinized skin surface, (b) the innate (natural) and (c) the acquired (adaptive) immune system (1). When infected, the magnitude of the reaction is related to the virulence and dose of the microbe. The symptoms are known to vary among individuals (2, 3).

The inflammatory immune events in the body can be divided into the innate (natural) responses and the acquired (adaptive) responses. From an evolutionary standpoint, the innate system is the oldest and reacts with the same strength, depending on the nature of the agent and the amount of dose, for each external or internal agent, stress or trauma (1). On stimuli, the macrophages and liver cells mount a cascade of cytokines, complement components, coagulation peptides and acute-phase proteins (APPs) intended for defense and tissue repair. These cascades will not develop an immunological memory (1). The acquired system, however, adapts to foreign agents and improves in strength for each exposure to a given antigen. The adaptation is stored in the immunological memory, which later responds with a fast and potent immune response upon the next exposure to similar antigens (4). This memory is used in vaccination schemes, which, however, is of limited use in neonates because of the immaturity of the immune system and possibly because of negative effects from maternal antibodies that are transferred through the umbilical cord and breast milk (5). The T-cell receptors recognize peptides from microbes presented on the major histocompatibility (MHC) molecule on antigen-presenting cells (APCs). The following steps include activation of B cells that lead to the production of humoral antibod-
ies taking part in the opsonization and elimination of foreign agents and ac-
tivation of cells represented in the cell-mediated defense system (1).

Clinicians have made decisions for hundreds and thousands of years on
how to treat patients. To their help they have had several tools: from the
beginning, only the sense of tumor, heat, pain, smell and color, but nowa-
days there are a broad array of laboratory and radiological tests. In the labo-
ratory inflammation can be monitored by measuring APPs as part of the
acute-phase response (APR). More specifically, viruses, bacterium, fungi
and parasites can be identified by indirect serology tests that measure anti-
bodies excreted from the plasma cells in the acquired immune system. Cul-
tures, electron microscopy and polymerase chain reaction (PCR) methods
may also directly identify microbes (6,7). In addition, the innate immune
system may reflect high levels of toxic products (8) and physical trauma (9).
Different degrees of inflammatory reaction are also shown in atherosclerosis
with or without tissue infarction, diabetes mellitus, amyloidosis, connective
tissue diseases and malignancy (10,11,12,13,14,15,16,17,18,19,20).

The acute-phase response

After challenge with microbes, several steps in the host defense can be
identified. During the incubation period, (a) the APCs (i.e. macrophages,
monocytes, endothelial, dendritic cells and fibroblasts) are up-regulated to
produce cytokines (21,22), (b) the hepatocytes increase the uptake of amino
acids for synthesis of proteins with high priority for the host defense and (c)
hormone-producing cells increase the synthesis and secretion of adenocorti-
cotrope hormone (ACTH), glucocorticoids and thyroxine (23).

During acute illness, physiological body reactions occur (e.g., fever, shiv-
ering, headache, anorexia, nausea, vomiting and diarrhea) as a response to
the inflammatory event. Endotoxins from gram-negative and exotoxins from
gram-positive killed bacteria (24), that now invade and overwhelm the im-
mune defense, bind lipoprotein-binding protein (LBP) to antigen receptors
(i.e. CD14) (8), or for exotoxins, in a similar way on APCs that, together
with toll-like receptors, cause the release of proinflammatory cytokines (25).
Further, mediators from the oxidative burst reaction influence macrophages
to synthesize the cytokines interleukin (IL)-1 and tumor necrosis factor
(TNF)-α, which potentiate the release of IL-6 from APCs (26,27,28). Cyto-
kine influence alerts the hormonal hypothalamic-pituitary-adrenocortical
(HPA) axis to increase the production of corticosteroids and thyroxine on
increasing demand (29,30). The cytokines, moreover, enhance the prolifera-
tion of stem cells that results in new generations of white blood cells in the
host defense (31,32). Muscle protein degradation following APR results in
an increasing pool of amino acids for the increased synthesis in the liver of
APPs and other important proteins and enzymes in extreme situations. The
increased hepatic uptake of iron gives rise to a secondary anemia. The carrier proteins - albumin, transferrin and transthyretin synthesis - decrease in favor of APPs. The impaired glucose tolerance turns the endocrine system to increased glucagon and insulin production, resulting in catecholamine release and mobilization of fatty acids. Fatty acid mobilization is inversely related to the plasma insulin concentration (33). A negative nitrogen balance, which lasts for several weeks during the convalescence period, is a result of the anorexia and muscle catabolism during the acute illness (34,23,35,36) (Figure 1).

Figure 1. Schematic illustration over the proinflammatory and inflammatory events in the liver, the antigen-presenting cells and B and T cells, indicating release of common inflammatory/defense peptides, with the human physiological reactions and catabolism included during the acute and convalescence phases

The counterbalance of immune response

The down-regulation of the APR is not fully understood, but as the immunological forces or antimicrobial therapy or both eliminate the invading microorganisms, the strength of the inflammatory response will be diminished and consequently abolished. The complicated networks both within and between hormonal and inflammatory protein pathways and immune
cells, of which several have both up- and down-regulating properties, have made attempts to find simple key explanations difficult. A selective pathway may result in effective therapeutic solutions. Suggested cytokines responsible for the down-regulating mechanisms are IL-6, IL-10 and IL-1 receptor antagonist (37,38,39) and among hormones glucocorticoids seem to be important but only sparsely evidenced (30).

The biological pathway for hepatic immune response

During infections, cell surface structures of microbes, such as lipopolysaccharides (LPS) in gram-negative bacteria and teichoic acids in gram-positive bacteria and mannans in fungi, initiate the proinflammatory response in APCs. These cells are spread throughout the whole body and comprise a broad spectrum of cells, including macrophages, endothelial cells, epithelial cells, smooth muscle cells, mesangial cells, chondrocytes, synoviocytes, osteoblasts, osteoclasts, fibroblasts, dendritic cells, glial cells and liver Kupffer cells (31,21). Antigens from bacteria alone or together with LBP stimulate receptors (i.e. CD14) that, through toll-like receptors on the cell surface, are responsible for the down-stream intracellular events in the cytoplasma and nucleus of APCs (40).

Figure 2. Schematic illustration over the intracellular events in the antigen-presenting cells when stimulated from endotoxin lipoprotein-binding protein complex through CD14.
Stimulation not only causes the synthesis and release of IL-6, IL-1 and TNF-α to the circulation but also the MHC-peptides mediate signals to B and T cells stimulating the humoral and cell-mediated defense (8). Among the circulating proinflammatory cytokines, IL-6 is the major cytokine. In concert with other cytokines (27,26,28,41,42) stimulation on the hepatocytes causes the APR in the liver (43,44,42,45,32,46) (Figures 2, 3).

Figure 3. Schematic illustration over the proinflammatory and inflammatory events during the acute-phase response

The IL-6-receptor, comprising an α-subunit (glycoprotein (gp) 80) and the co-reacting β-subunit (glycoprotein (gp) 130), which is the signal transducing peptide, are located on the cell surface of hepatocytes (47,31,48,49). Transmembrane signals evoked by cytokines binding to the receptors enhance the phosphorylating processes of tyrosine kinases (31) that activate cytoplasmatic nuclear factors (i.e. Nuclear Factor (NF)-κβ, SAA-activating sequence (SAS), SAA-activating factor (SAF) and Cytosine-Cytosine-Adenine-Adenine Thymine (CCAAT)-enhancer binding protein β (C/EBPβ)) (50,51,52,53). Alone or in co-operation, they affect intranuclear promotor loci on DNA leading to the transcription of APP mRNA (Messenger RNA) (50,54,47,55) that constitutes the reading frame for protein synthesis in the cytoplasmatic Golgi apparatus. During the unstimulated phase in healthy individuals, a repressor nuclear factor, Yin-Yang (YY)-1, is suggested to
control the promotor loci for APPs on DNA. YY-1 is replaced during the activation by nuclear factors (56). The NF-κβ is bound in an inactive complex in the cytoplasm together with the Inhibitory (I)-κβ that is degraded during stimuli from cytokine receptors on cell surface, causing NF-κβ to exert its effect on DNA (54,57). In hepatocytes (Hep 3B cells) SAA mRNA accumulation is more pronounced than CRP mRNA accumulation, probably because of post-transcriptional mechanisms (58,59). The liver synthesis leads to increasing levels of the APPs SAA and CRP in serum up to 100-1000-fold during infectious diseases in humans (50,22) (Figure 4).

![Figure 4. Schematic illustration over the intracellular events in the hepatocytes during stimulation from proinflammatory cytokines, resulting in increased synthesis of serum amyloid A protein.](image)

**Serum amyloid A protein (SAA)**

Human acute-phase SAA, which is a α-globulin belonging to the apolipoprotein family, is encoded by genes in chromosome 11 and consists of four isotypes with molecular weights of 12 kDa each (54,60,50,61,62). SAA1 and SAA2 are the two biologically active proteins while SAA3 is a pseudoisotype without a known function, and SAA4 (C-SAA) is a constitutive form associated with both “normal” and “acute phase” high-density lipoprotein (HDL), in similar amounts. SAA is mainly produced by liver cells, but ex-
trahepatic sources such as macrophage cell lines and endothelial and smooth muscle cells as well as adipocytes have been documented (54,50,62). In the peak acute phase the normal content of SAA in HDL increases to 30-80% of the apolipoproteins, whereas other apolipoprotein such as apoA-1 decreases (63,64). Moreover, the HDL particle becomes larger and has reduced content of phospholipid and increased content of triglyceride (65). SAA1 is expressed as five genotypes (SAA1.1-5) and SAA2 as two genotypes (SAA2.1-2) (former nomenclature: α, β, γ, δ and α, β, respectively) (54). SAA1 and SAA1.1-3 are evenly occurring in Japanese people, whereas SAA1.1 is dominantly occurring in Caucasian people; SAA2 and SAA2.1 are the dominating genotypes in all races (60).

The zenith of measurable SAA in serum is shown to be 36-48 h after intramuscular administration of the steroid pyrogen etiocholanolone (66) and 3 days after challenge with influenza virus in healthy individuals (67). SAA content in very low density lipoprotein (VLDL) and HDL has its peak 36-48 h after onset of myocardial infarction in humans (68). mRNA appears abundantly 2 days after stimulation of human hepatocytes with IL-1β and IL-6 (28). The disappearance time of SAA is mainly a consequence of the degradation and seems to be longer in acute-phase serum (69) and for SAA1.5 genotype (70). The estimated half-life in mice is in the range of 60-80 min (71,72). In hepatocytes (Hep 3B cells) mRNA disappearance time was 8.5 h after washing the pre-incubated cells to remove stimulating cytokines. However, a substantial on-going transcription was seen, which is possibly due to a lack of required gene products for the mRNA degradation process (59).

The functions of SAA are still not fully understood. Because of the strong association to the HDL particle, the most probable function in acute inflammation is related to the lipid metabolism, both locally in injury sites and systemically (73). SAA facilitates the transport of cholesterol from damaged tissue to the liver, as well as the reverse direction for the regeneration processes. SAA-rich HDL has high affinity to the increased amount of HDL receptors on macrophages during the acute phase. A possible role in the reparative processes in atherosclerosis has been proposed (64). On the other hand, SAA changes in HDL during the acute phase may be proatherogenic (74). In rheumatoid arthritis chronically elevated SAA, which causes induction of proteases, may have a role in joint destruction. (75). Several other functions of SAA have also been proposed: (a) inhibition of antibody production, platelet aggregation, fever and oxidative burst reaction, (b) induction of proteases (i.e. collagenase, stromelysin and matrix metalloproteinas) and (c) a chemotactic function (54,76). On stimuli from SAA, human neutrophils are found to release TNF-α, IL-1β and IL-8 in cell cultures indicating a (iv) regulatory role (77). On the other hand, high levels of SAA in mouse serum suppress the anti–sheep red cell response of lymphocytes, indicating an immunosuppressive role of SAA (17).
Amyloidosis

Amyloidosis is a disease that may be local or systemic. It is characterized by fibrillar formation that can be demonstrated with Congo red staining of biopsies or by electron microscopy. Primary amyloidosis is not associated with other diseases and is shown to depose light chain peptides. Hereditary amyloidosis is a heterogeneous group in which different proteins or fractions of them can be found (i.e. transthyretin, actin, cystatin C, amyloid β protein, apolipoprotein A-I, β2-microglobulin, prion protein and islet amyloid polypeptide). Serum amyloid P-component (SAP) is found in all types of amyloid deposits (78). After cleavage by proteases in acidic conditions from the precursor SAA (79), secondary amyloidosis develops from chronic inflammatory diseases with deposition of the N-terminal fragment of SAA (protein AA) (80), (54,81,82,78). Primary and secondary amyloidosis are shown to have higher levels of SAA than hereditary, but secondary amyloidosis has higher levels than primary (17). In secondary amyloidosis, renal impairment is the most common manifestation of the disease (78). The amyloid enhancing protein (AEP), complexed AA protein and SAP attach to the glomeruli basal membranes to form amyloid fibrils (57). Several rheumatic inflammatory diseases predispose for amyloid formation (57) and the most common underlying disease is found to be rheumatoid arthritis followed by recurrent pulmonary infections, Crohn’s disease, tuberculosis, osteomyelitis, Hodgkin’s disease and Familial Mediterranean fever (20). It has been shown that the genotype SAA1.1 is associated to Familial Mediterranean fever with renal deposits of amyloid (83). Therapeutic strategies to reduce the load of amyloid fibril precursor proteins (i.e. SAA) are found to have favorable outcome in long-term perspectives (84,85). Because of the reflection of disease activity, SAA is proposed to be a more valuable biomarker for the assessment of inflammatory joint disease as compared with CRP and erythrocyte sedimentation rate (ESR) (86). SAA seems to be a sensitive biomarker, and together with clinical data, is useful in the management of giant-cell arteritis and polymyalgia rheumatica (87). Furthermore, as compared with the inactive form in sarcoidosis SAA is elevated in inflammatory active disease (88).

SAA in infectious and other non-infectious diseases.

High SAA levels are shown in immunocompetent and neutropenic patients with bacterial infections (i.e. pneumonia, endocarditis, sepsis, erysipelas, salmonellosis, septic arthritis, pyelonephritis and meningitis) (19,89) (90,91); pneumocystis carinii (Pneumocystis jiroveci) pneumonia in AIDS (92) and in cystic fibrosis (CF) patients with exacerbation of P. aeruginosa pulmonary infection (93,94). In viral infections SAA is usually increased to a lesser extent than in bacterial infections (95,96), but in chronic hepatitis B and C SAA is shown to have a similar range as a control group (Miwata et
al). Additionally, SAA is serially investigated in patients with Plasmodium falciparum malaria infection (97) and recently it was studied in severe acute respiratory syndrome (SARS) (98). Furthermore, SAA levels are increased in renal and liver graft rejection (99) (100,101,102) and in acute graft-versus-host disease (GVHD) in bone marrow transplant recipients (103). Post-operatively, SAA is shown to increase during the first 24 h (104), as well as after trauma (the majority of cases were patients with fractures) (105). The SAA level is increased in such other non-infectious disorders as Kawasaki disease (95,106) pregnancy and labor (107), myocardial infarction (16) and neoplastic diseases (108,17).

C-reactive protein (CRP)

In 1930, Tillet and Francis demonstrated that the peptide fraction C in S pneumoniae from patients with pneumonia precipitated with the APP CRP (109). In 1939, Löfström described the non-specific capsular reaction whereby an extensive analysis using that method on different infections and myocardial infarction was performed by Hedlund et al (1961) (110). CRP, which belongs to the pentraxin superfamily, is encoded by chromosome 1 and consists of five subunits with a total molecular weight of 118 kDa. The source of production is the liver cells and in electrophoresis it appears in the β-globulin fraction. CRP exerts its biological activity through similar biological activities as immunoglobulins, namely, precipitation, agglutination and complement activation (22). The Ca²⁺ depending ligand binding to phosphocholin is the principle way of its general host defense (111). Phosphocholins are constituents of all cell membranes and are found in the cell membranes of S. pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Neisseria meningitidis, Neisseria gonorrhoeae, Proteus morganii and Aspergillus fumingatus (111). The CRP-ligand complex interacts with the classical complement system, which causes the production of opsonins and membrane attack complexes (MACs). Furthermore, complement mediated mechanisms solubilize CRP-bound chromatin from damaged cells (112). It has also been proposed that CRP itself has opsonin-binding and immune-modulating properties. Macrophages and neutrophils phagocytize CRP-bound proteins and cells (111,22). After cholecystectomy, CRP levels increase in serum 8 h post-operatively (113) and after cardiopulmonary bypass grafting the zenith of CRP levels is reached 48 h post-operatively (114) (115). The CRP half-life of 19 h is similar in healthy individuals and in patients with inflammatory rheumatic disorders and infections (116) and in neonates treated for a bacterial infection (117).

High levels of CRP are seen in most conditions with an acute inflammatory component, such as infectious diseases and inflammatory and tissue injury diseases (i.e. neoplasia, trauma, myocardial infarction)
New, sensitive methods have attracted considerable attention, especially because little elevation can predict coronary heart disease and stroke and diabetes mellitus, which are associated with proinflammatory activity (11,10,122,123).

Interleukin-6 (IL-6)

IL-6 is encoded by chromosome 7 and the protein, which comprises 211 amino acids with a molecular weight of 22 to 29 kD, is produced by a diversity of cells (21). The proposed functions of IL-6 are pleiotropic: (a) promoting the synthesis of immunoglobulins, (b) induction of the hepatic APR, (c) regulating the adaptive responses in hematopoiesis, (d) participation in bone mass regulation, (e) regulation of fever and (f) regulation of glucocorticoid synthesis (31,32,47). LPSs and endotoxins, as well as physical forces, activate the APC through the membrane bound receptor CD14 and the transmembrane protein kinase C. The increasing level of IL-6 reaches its zenith after 4 h post-surgery in coronary bypass grafting (CBP) in some studies (124,125) but other studies have shown zenith levels at 24 h after CBP (126) and after major abdominal surgery (127). The disappearance from circulation in mouse is biphasic and has a rapid phase with a half time at 3 min and a slower phase with a half time of 55 min (21).

The body temperature

Body temperature is one of the oldest used observations in inflammatory and infectious diseases, where the “crisis” was an important zenith signaling a lowering of the thermoregulation set point because of decreasing levels of bacterial products (i.e. endotoxins) and cytokines. The circadian thermoregulation rhythm in healthy individuals shows the lowest temperature early in the morning (i.e. about 06.00) (128). The usual range of normal body temperature is between 36.5°C and 37.5°C. In infectious diseases, pyrogens and cytokines from APCs up-regulate the set point for body temperature. Because of the stimulation of increasing circulating pyrogens and cytokines (129,130,131), the up-regulation of the set point is mediated through synthesis of prostaglandin E₂ in the tissue adjacent to the preoptic area of the anterior hypothalamus where the thermo-regulation centre is located. To reach the new set point of body temperature, efferent neuron signals cause peripheral vasoconstriction, piloerection and shivering that, in turn, increase the core body temperature to reach the new set point. When the core body temperature reaches above the new set point, warm sensitive neurons in the preoptic area of hypothalamus increase their firing rate, which causes new efferent signals leading to increased sweating and peripheral vasodilatation.
and thereby peripheral heat loss. The thermoregulation center strives for a temperature between 0.6°C below and above the set point, which is accomplished by equilibration through the mentioned peripheral mechanisms. At diminished influence from acute-phase reaction, the set point decreases toward the normal and consequently, normalizing the core body temperature. (131). The gold standard for temperature measuring is by rectal thermometer but this is being successively replaced by more simple tympanic measurements. A systematic literature review reveals that data for the lower range of normal body temperature differ between methods of measurements, gender and physiological aging. Future studies to examine these areas are proposed (132).

The aim of the studies

There still remain several questions about the use and usefulness of biomarkers in the clinical situation. Aging individuals (who normally consume more antibiotics than other age groups) and newborns often receive antibiotics on vague signs of infection. Despite a favorable clinical course of treated patients with bacterial infections, biomarkers are frequently used. How should we interpret the test results? Do we use the tests in a cost-effective manner? For instance, do the test results lead to optimal timing of iv. vs oral antimicrobial therapy? Arguments for studies on biomarkers in infectious diseases may be summarized as follows: (a) immunodeficiency that is due to malnutrition, aging, pregnancy, genetic defects, HIV or cytotoxic drugs or immunotherapy in cancer, organ transplantation and rheumatic diseases causes ineffective defense mechanisms. That, in turn, may be associated with atypical symptoms and signs making the clinical presentation difficult to interpret; (b) incomplete maturation of hepatic function and defensive immune system in newborns, especially premature infants, may have an impact on symptoms and signs of sepsis, which may cause an increased risk of fatal infections. New caring techniques increase the number of such patients making it important to find new, more sensitive tools to discover untreated and monitor treated infections; (c) surgical implant techniques give rise to new problems with low-virulent infections not easily monitored with conventional laboratory markers; (d) biomarkers need to respond more rapidly guiding the clinician’s decision of when to do safe parenteral-oral switch of therapy; (e) costs for in-hospital ward may be reduced if biomarkers can guide safe therapy in an outpatient setting; and (f) a good biomarker should aid in the interpretation of the antibiotic effects in clinical trials.

In this dissertation SAA was investigated in (1) common bacterial and viral infections at admission to a department of infectious diseases, (2) urinary tract infections (UTIs) with different invasitivity, (3) very immature newborn infants with nosocomial infections; (4) healthy individuals from new-
born to the elderly and (5) the time course during the initial intravenous anti-
biotic treatment of invasive bacterial infections. The biomarkers CRP, IL-6, 
body temperature and TNF-α were used for comparison.
Patients and methods

The studies were conducted at the Departments of Infectious Diseases, Neonatology, Women’s Diseases and Nephrology at the University hospital and Svartbäckens Health Center in Uppsala, Sweden and were approved by the ethics committee of the Medical Faculty at Uppsala University (I-V) and the Swedish Medical Products Agency (V). Written informed consent was obtained in paper III for newborns (parents’ consent) and papers IV-V.

Patients

In paper I, 98 consecutive patients, admitted to an infectious disease clinic in the acute phase of various viral and bacterial infections, were enrolled in the study. Blood for serum SAA and CRP determination was obtained on admission. The duration of illness from the onset of symptoms to the time of sampling was not evaluated. In paper II, 69 consecutive patients, 37 with acute pyelonephritis and 32 with acute cystitis, were included in the study. The acute pyelonephritis patients were admitted to an infectious disease clinic and the acute cystitis patients were examined at a primary health care center. Pregnant women were not included. In paper III, 231 blinded serum samples from healthy individuals and umbilical cords were collected from 1996 to 2001 and stored frozen. The population was divided into four groups: umbilical cord (n = 35), newborns (n = 35), young adults (n = 48), middle-aged adults (n = 28) and elderly adults (n=85). In paper IV, stored serum samples were used from very immature newborn infants (<32 weeks gestation and postnatal age ≥72 h to exclude early onset sepsis) that remained after routine surveillance of theophyllamine concentrations during 2000-2003. In total, 224 samples from 72 newborns were available for analysis. Of the 224 serum samples, 79 were associated to nosocomial infections (NIs). The NI samples were classified to proven (pNI, n=42), not proven but documented (nNI, n=12) or neither proven nor documented (nnNI, n=25). A temporal analysis was done on 55 of the available 224 samples in the NI group that were related to the calendar day for the initiation of microbial therapy. In paper V, patients were included with symptoms and signs indicating bacterial infection believed to be susceptible to treatment with cefuroxime or cefuroxime in combination with tobramycin. The duration of illness was defined as the patient’s estimated feeling of infection.
symptoms. Patients were excluded from the study if they were on hemodi-
alysis, if they had chronic inflammatory diseases (defined as treatment with
prednisolon more than 10 mg daily) or if they were on previous treatment
with intravenously antibiotics within the past 2 weeks before the start of the
study. In addition, patients were excluded if they were believed to be on
other antibiotic treatment than that postulated in the inclusion criteria. Pa-
tients whose intravenous treatment was stopped or changed to oral therapy
within 3 days were defined as early parenteral-oral switch (POS) and the
other patients were defined as late POS. Time to POS for each patient was
the number of days from inclusion. Duration of illness was defined as short
< 24 h and long ≥ 24 h from the patient’s estimated onset of fever. Totally,
81 patients (cefuroxime treated patients, n=62; cefuroxime and tobramycin
treated patients, n=19) were included. The diagnoses were sepsis (6%),
pneumonia (22%), bronchitis (11%), pyelonephritis (27%), skin and soft
tissue infections (22%) and fever of other origin (FOO) (11%).

Blood sample collection and handling

In papers I-II, blood was obtained in vacutainer tubes without additive
(367609, Becton Dickinson, Rutherford, NJ, USA) and centrifuged at 1300
xg for 10 min. The serum was stored at −20°C until analyzed. In papers III-
V, blood specimens from children were obtained in tubes without additives
by venapuncture (365950, Becton Dickinson, Rutherford, NJ, USA) or capill-
ary technique (Sarstedt Micorette CB300 Nr: 16.440.300). If at least 50 µL
serum remained after theophyllamine analyses, the serum was stored. Blood
from adults was collected in vacutainer tubes without additives by venipunc-
ture (367609, Becton Dickinson, Rutherford, NJ, USA). The tubes were
centrifugated at 10 000 xg for 3 min (children specimens) or 1300 xg for 10
min (adult specimens) in accordance with the local procedures at the labora-
tory. The serum was stored at −70°C until analyzed. Samples were with-
drawn before first dose of cefuroxime and at most five more time points
from 1 to 72 h after the start of treatment according to a flexible sparse data
sampling schedule.

SAA, CRP, IL-6 and TNF-α assays

In papers I-II, analysis of CRP was performed by turbidimetry on a Hi-
tachi 717 (Roche Diagnostics, Mannheim, Germany) and SAA was meas-
ured by an ELISA method (Cytoscreen™ Human SAA ELISA kit, Bio-
Source International, Camarillo, CA, USA). Human IL-6 and human TNF-α
(R&D Systems, Minneapolis, MN, USA) were measured by ELISA. Detect-
ion limits for SAA, CRP and TNF-α were set to 5 mg/L, 10 mg/L, 5 ng/L,
respectively, according to information from the manufacturers; the detection limit for IL-6 was set to 2.65 ng/L based on a study on healthy controls (11). The total analytical imprecision of the IL-6 method was less than 7%. The reference interval for SAA ELISA was verified locally at the Department of Clinical Chemistry by analysis of SAA levels in sera from 40 healthy blood donors that all were below 5 mg/L. In papers III-IV, SAA and CRP measurements were performed with latex enhanced reagent (Dade Behring Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). The total analytical coefficient of variation of the SAA method was 5.9% at 12.8 mg/L and 3.2% at 81.7 mg/L, and for the hsCRP method it was 1.4% at both 1.2 mg/L and 5.5 mg/L. The detection limit for SAA was 0.76 mg/L and 0.16 mg/L for hsCRP (Manufacturer’s brochure). There is no international standard for SAA. Thus, results obtained by using methods from different manufacturers cannot be easily compared. In paper V, analysis of CRP was performed by turbidimetry on an Advia 1650 (Bayer HealthCare Diagnostics, Tarrytown, NY, USA). The total analytical imprecision of the CRP method was less than 9% at 35 mg/L. SAA and IL-6 measurements were performed by the same methods as in papers III-IV. Body temperature was measured by rectal thermometer. The measurement was done twice daily, at least 4 h after administration of antipyretic drugs.

Statistical methods

Statview® for Windows NT, 1999 (SAS Institute, Cary, NC, USA) was used in the statistical calculations (papers I-IV). A p-value of <0.05 was considered statistically significant. In paper I, the data were analyzed by the simple linear regression model and the Spearman Rank Correlation test. In paper II, the non-parametric Mann-Whitney U test, Fischer’s exact test and the Spearman rank correlation test were used. The efficiency of the tests was defined by frequency of tests higher than the cut-off level in patients with pyelonephritis summed with frequency of tests lower than the cut-off level in patients with cystitis divided by all tested patients. In paper III, to determine differences in variables between age groups the Kruskal-Wallis non-parametric test for independent samples was computed. The simple linear regression model and the Spearman Rank Correlation test were used for correlation analysis of the data and the Mann Whitney U test to establish whether there were differences between age groups. In paper IV, the Mann Whitney U test was used to test the differences between continuous two group comparisons. The Chi Square test was used to test differences between groups expressed with nominal values. The Kruskal-Wallis test was used to test differences between continuous three group comparisons. Because of unexpected high values in some cases (outliers), trimmed (α=0.05) mean values were used. Values lower than the detection limit (for SAA 0.76 mg/L
and for hsCRP 0.16 mg/L) were set to detection limit. In paper V, the graphics and statistics were performed using S-PLUS® 6.1 for Windows (Insightful Corp., Seattle, WA, USA). The smoothes in the plots are locally weighted linear regression curves as implemented in the S-PLUS function loess. To describe the pharmacokinetics of cefuroxime the samples were also analyzed regarding the concentration of cefuroxime. These results are presented elsewhere (Viberg et al., submitted). ANOVA was used when comparing the different values at baseline. Because of the sample schedule, it was not possible to calculate whether there were differences in levels at other time points than baseline.
Main results

In paper I, correlation studies of SAA levels to those of CRP in sera from 98 patients admitted to an infectious disease clinic because of viral and bacterial infections (Table 1) revealed that SAA levels correlated significantly with CRP levels \( (r^2=0.757, p<0.0001) \) for the entire studied population. Furthermore, positive correlations in both viral and bacterial infections were obtained \( (r^2=0.572, p<0.0001; r^2=0.666, p <0.0001, \text{respectively}) \). Positive correlations were also obtained when the values were compared in accordance with a CRP level higher or lower than 100 mg/L \( (r^2=0.689, p=0.0002; \text{CRP}>100; r^2=0.397, p<0.0001; \text{CRP}<100) \) (Figure 5).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>SAA (^1) mg/L</th>
<th>CRP (^2) mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (+SD3)</td>
<td>Median (min-max)</td>
</tr>
<tr>
<td><strong>Viral infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>9</td>
<td>92.8 (+46.2)</td>
<td>95 (13-222)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>5</td>
<td>71.4 (+2.3)</td>
<td>73 (68-73)</td>
</tr>
<tr>
<td>Cytomegalovirus infection</td>
<td>7</td>
<td>291.3 (+297.2)</td>
<td>147 (87-783)</td>
</tr>
<tr>
<td>Varicellae-zoster</td>
<td>12</td>
<td>299.3 (+302.6)</td>
<td>235.5 (11-1105)</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>8</td>
<td>363.4 (+287.8)</td>
<td>264.5 (145-1001)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>11</td>
<td>924.2 (+594.4)</td>
<td>980 (59-1620)</td>
</tr>
<tr>
<td><strong>Bacterial infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial pneumonia</td>
<td>11</td>
<td>2508.0 (+1065.6)</td>
<td>2600 (618-4260)</td>
</tr>
<tr>
<td>Streptococcal pharyngitis</td>
<td>6</td>
<td>2106.2 (+2175.1)</td>
<td>1170.5 (465-5850)</td>
</tr>
<tr>
<td>Bacterial sepsis</td>
<td>19</td>
<td>1635.7 (+757.5)</td>
<td>1560 (557-3536)</td>
</tr>
<tr>
<td>Severe bacterial sepsis</td>
<td>10</td>
<td>2535.2 (+2546.6)</td>
<td>1483 (268-6262)</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)C-reactive protein; \(^2\)serum amyloid A protein; \(^3\)standard deviation

Table 1. Mean and median SAA and CRP levels (mg/L) in serum in the acute phase of different viral and bacterial infections. CRP levels below 10 mg/L are noted <10.
SAA and CRP were higher in bacterial infections than in viral infections (ps<0.0001), for instance bacterial tonsillitis when compared with infectious mononucleosis (ps<0.05, 0.01) and bacterial pneumonia when compared with influenza A (ps<0.001, 0.01). In paper II, SAA showed a systemic inflammatory response in cystitis in 90% of the patients as compared with 23, 42 and 0% for CRP, IL-6 and TNF-α, respectively (Table 2).
Table 2. Median and range values for SAA, CRP, IL-6 and TNF-α in patients with pyelonephritis and uncomplicated cystitis.

<table>
<thead>
<tr>
<th></th>
<th>Pyelonephritis</th>
<th>Cystitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median Range n</td>
<td>Median Range n</td>
</tr>
<tr>
<td>SAA mg/L</td>
<td>1952 188-9498</td>
<td>20 &lt;5-193</td>
</tr>
<tr>
<td>CRP mg/L</td>
<td>152 32-474</td>
<td>37 &lt;10</td>
</tr>
<tr>
<td>IL-6 ng/L</td>
<td>51.6 19.4-474.0</td>
<td>33 2.65</td>
</tr>
<tr>
<td>TNF-α ng/L</td>
<td>6.5 &lt;5-14.4</td>
<td>21 &lt;5 all&lt;5</td>
</tr>
</tbody>
</table>

*Mann Whitney U test. CRP = C-reactive protein, IL-6 = Interleukin-6, SAA = Serum amyloid A protein, TNF-α = Tumor necrosis factor alpha

In paper III, serum levels of both SAA and hsCRP were lower in umbilical cords than in the newborns and young, middle-aged and elderly adults (p<0.0001). The SAA and hsCRP levels were comparable in the newborns, young and middle-aged adults, but higher in elderly adults (ps<0.0001-0.03). SAA (r²=0.159, p<0.0001) (Figure 6) and hsCRP (r²=0.059, p<0.0001) were positively correlated with age and to each other (r²=0.385, p<0.0001).

Figure 6 Levels (mg/L) of SAA (A) and hsCRP (B) in umbilical cords and in the age groups newborns (3-7 days of age), young adults (20-42-year-olds); middle-aged adults (45-63-year-olds) and elderly adults (65-72-year-olds).

In paper IV, information on documented NIs was matched to levels of SAA and hsCRP in 224 serum samples from 72 immature infants. As a control group, 35 healthy term newborn infants were chosen. Of the 224 serum samples, 79 were associated to NIs. The NI samples were classified to proven (pNI, n=42), not proven but documented (nNI, n=12) or neither proven nor documented (nnNI, n=25). Trimmed mean (α=0.05) levels for SAA and hsCRP in immature newborn infants were higher than in control term infants (1.74, 2.67 mg/L vs. 0.78, 0.16 mg/L; p=0.01 and <0.0001, respectively), higher in the NI group than in the non-NI group (5.14, 5.74...
mg/L vs. 1.03, 1.18 mg/L; p<0.01 and <0.0001; respectively) and higher in the pNI group than in the nnNI group (13.10, 6.48 mg/L vs. 1.12, 3.47 mg/L; both ps<0.05, respectively). SAA and hsCRP increased before institution of antimicrobial therapy (median 0.76, 0.28 to 4.90, 7.30; p=0.0635, p<0.05, respectively) and decreased after the third antimicrobial therapy day (median 2.99, 9.34 to 0.76, 4.92, both ps<0.05, respectively) (Figure 7).

Figure 7. The levels of SAA and hsCRP in very immature newborn infants in (A) NI and non-NI, (B) pNI, nNI and non-NI and (C) temporally associated to the onset of antimicrobial therapy. The box plots cover the 10th, 25th, 50th (median), 75th and 90th percentiles.

In paper V, body temperature, SAA, CRP and IL-6 were studied in patients with bacterial infections during the first 3 days of cefuroxime treatment. The interindividual variability of the time course within each of the biomarkers was considerable. The baseline values of body temperature,
SAA, CRP or IL-6 did not differ statistically between the different bacterial diagnoses. The body temperature of the patients declined during the first 24 h but no substantial change was observed thereafter (Figure 8).

There was a trend indicating that temperature drop was larger in the groups of patients who had early POS as compared with the patients who had late POS (Figure 9). However, when calculating a linear slope of the temperature drop during the first 24 h of treatment, there was no difference when comparing early with late POS. The duration of illness had no impact on the time course of body temperature (Figure 9).

The smoothed time courses of SAA and CRP were similar and had an increasing trend up to approximately 24 h. After 24 h, the levels of SAA and CRP declined (Figure 8). Moreover, there were no differences in the change of levels between the groups of patients who had early POS as compared with the patients who had late POS. Additionally, in comparison with patients with a long illness duration, patients with a short duration of illness had increasing levels during the first 24 hours (Figures 10 and 11).
Figure 9. Body temperature (°C) during the initial antimicrobial treatment of patients with bacterial infections divided into early and late parenteral-oral switch (POS) and duration of illness before inclusion.

Figure 10. SAA (mg/L) during the initial antimicrobial treatment of patients with bacterial infections divided into early and late parenteral-oral switch (POS) and duration of illness before inclusion.
Figure 11. CRP (mg/L) during the initial antimicrobial treatment of patients with bacterial infections divided into early and late parenteral-oral switch (POS) and duration of illness before inclusion.

Figure 12. IL-6 (ng/L) during the initial antimicrobial treatment of patients with bacterial infections divided into early and late parenteral-oral switch (POS) and duration of illness before inclusion.
For IL-6, there was a trend of decreasing levels during the first 24 h but data showed great interindividual variability (Figure 8). No differences were observed when comparing short with long illness duration or early with late POS (Figure 12). The levels tend to decrease more for patients with sepsis (n=4) than for patients given other diagnoses (data not shown).
Discussion

We have found significant positive correlations between SAA and CRP in acute infectious diseases. Furthermore, similar correlation coefficients were observed in viral and bacterial infections. The correlation, however, was weaker in the low range of CRP levels (CRP<100 mg/L) than at higher CRP levels. Correlations between SAA and CRP levels in serum have previously been found to be positive in patients with viral infections, rheumatoid arthritis and osteoarthritis (133,134,95). In the present study both SAA and CRP could significantly discriminate between bacterial and viral infections, though the precision varied with the infecting organism and the cut-off levels chosen. Moreover, in the healthy state, we found an age-related variation with the lowest levels for both biomarkers in the umbilical cords and the highest levels in individuals above 65 years of age. Levels in newborns, young adults and middle-aged adults were comparable for both biomarkers, except that the hsCRP level in the newborn group was slightly higher than that in the middle-aged group (p=0.04). These results, which were obtained using modern methods, are essentially consistent with studies that have used older methodology (113,135,136,137). Furthermore, there was a positive correlation between SAA and hsCRP in the levels below 15 and 10 mg/L, respectively. Previously, we have reported a positive correlation between CRP and SAA at CRP levels above 10 mg/L (138).

SAA and CRP have equal sensitivity, specificity and efficiency in the discrimination of pyelonephritis and cystitis in these representative patients with community-acquired UTI. IL-6 and TNF-α were comparable with each other but indicated lower sensitivity as compared with SAA and CRP. In cystitis only SAA of the studied biomarkers indicated systemic inflammatory reaction in the majority of cases. Healthy blood donors served as controls. IL-6, which is the major proinflammatory cytokine eliciting the acute-phase reaction in the liver (50), has been shown to be elevated in serum and urine in patients with pyelonephritis (139). The cytokine response varies with the bacterial dose and properties, i.e. p-fimbriated E. coli or E. coli-producing hemolysin (140), as well as with the immunity of the host. The urinary IL-6 level in cystitis is significantly elevated as compared with that in normals (141). In our study 90% (28/31) of the women with cystitis had a serum SAA level above 5 mg/L, which was the detection limit for the test. Moreover, a positive correlation between serum IL-6 and SAA levels in cystitis was observed (p=0.0007, r²=0.157). The local inflammatory reaction in the
urinary tract in cystitis probably elicits a local production of SAA or a systemic proinflammatory cytokine response strong enough to stimulate the liver processes producing SAA. SAA might be a valuable adjunct for diagnosing uncomplicated cystitis, and especially in the elderly in whom specific symptoms and signs are often lacking (142). However, because difficulties exist in differentiating cystitis from asymptomatic bacteriuria, comparative studies of these conditions are first needed.

To determine the levels of SAA and hsCRP we report results on healthy individuals in different age groups, including umbilical cords. In umbilical cords and newborns (median 3 days) the median SAA levels were 0.76 and 1.52 mg/L, respectively and the median hsCRP levels 0.16 and 1.20 mg/L, respectively.

In very immature newborn infants with NIs the trimmed mean levels of SAA (6.39 mg/L) and hsCRP (5.74 mg/L) were slightly elevated as compared with the SAA (1.03 mg/L) and hsCRP (1.18 mg/L) levels in the non-NI group. Furthermore, the pNI group had higher levels of SAA and hsCRP (13.10 and 6.48 mg/L) than the nnNI group, indicating an inflammatory response comparable with a “normal” level in older age groups (10 mg/L or less). Therefore, these new sensitive methods may offer a possibility to detect early or low inflammatory response in a clinical setting in very immature newborn infants. The reason for the higher hsCRP level in the nnNI group than in the non-NI group cannot easily be explained. This difference may be that the prematurity of liver function is different for different liver-associated proteins, where immature lipid metabolism causes changes in SAA concentrations, which is an apolipoprotein in the HDL. In this early report on SAA and hsCRP in very immature newborn infants we show that the levels of SAA and hsCRP are possible to detect and are higher when compared with healthy term babies. The hitherto used method for CRP in clinical practice is limited to values beyond 10 mg/L, and even at this range, the method often has a high coefficient of variation (CV). These assays are therefore of limited use in newborn infants. For example, in very preterm newborn infants with a gestational age less than 30 weeks only 58% showed a CRP value above 10 mg/L in culture-proven neonatal bacterial infection (50). In a laboratory study on 410 CRP results only 8-11% of newborns at 24-31 weeks gestational age reached a CRP response greater than 60 mg/L (143). It is known empirically that APPs often give poor information about inflammatory events in this group of immature patients. HsCRP but not SAA showed a significant increase before onset of antimicrobial therapy through zenith (at calendar day 2-3) followed by decreasing levels after calendar day 4 as a response to therapy. These findings may be interpreted to mean that (a) very immature newborn infants react immunologically on NIs, (b) the acute-phase reactants precede the clinical decision of the physician to institute antimicrobial therapy on clinical signs and (c) a therapeutic effect can be measured as a lowering of the SAA and hsCRP levels. In summary, SAA
and hsCRP appear to be promising new tools for detection of infectious events, even in very immature newborn infants. We suggest that because these new methods use very small amounts of blood and can easily and readily be analyzed immediately, a prospective study should be performed to evaluate the usefulness in a Neonatal Intensive Care Unit (NICU).

IL-6, which is derived from stimulated APCs such as monocytes, is one of the most important cytokines promoting the acute-phase reaction (26,50). Together with CRP, IL-6 is proposed to be a sensitive biomarker for early establishment of neonatal infection (144). Median IL-6 levels have been found to be low in cord blood from healthy newborns. In one study the IL-6 level was 0 pg/mL (range 0-3400 pg/mL, detection limit for the test 5 pg/mL) (145) and in another it was 4.75 pg/mL (1.0-35.1, detection limit for the test 1pg/mL). In one of the studies the median CRP was 0 mg/L (0-30, cut-off limit for the test 15 mg/L) and the mean CRP was 0.76 mg/L (SD + 1.2, cut-off limit for the test 10 mg/L) (146). No transplacental transmission of cytokines or APPs have been found (146,107,147). Because of the very low proinflammatory signal in cord blood, it was anticipated that this group would show the lowest levels of SAA and hsCRP in our study. However, after a few days of life, the levels increased to those of adults, which may be due to the response of the immature immune system and liver function to the delivery trauma. To understand the dynamics of proinflammatory cytokines and the APR in newborns extended studies with consecutive sampling from healthy newborns after both vaginal delivery and caesarean section are needed.

The present elderly group was recruited from a health-screening program, including ostensibly healthy 70-year-old individuals. Yet, in an inquiry the patients reported co-morbidity, such as coronary heart disease and diabetes mellitus, both of which are associated with proinflammatory activity (11) (147,10,148). Body mass index (BMI) was reported normal in the group. It would seem likely that subclinical or clinically evident age-related diseases, such as atherosclerosis and diabetes mellitus, as well as degenerative processes associated with aging and smoking, all contribute to the increased proinflammatory level (149), including slight elevation of SAA and hsCRP levels in the elderly age group. In our study the majority of SAA and hsCRP recordings of 10-15 mg/L and 5-10 mg/L, respectively, including the excluded extreme values, were made in sera from the elderly group, i.e. individuals aged 65 years and above. This finding is similar to that of studies showing progressively increasing levels of SAA in individuals over 70 years old (17,137). Thus, in elderly patients with infectious and other inflammatory conditions we suggest decision levels for SAA and hsCRP with our method of 15 mg/L and 10 mg/L, respectively. In newborns and young and middle-aged adults the lower decision levels for SAA (10 mg/L) and hsCRP (5 mg/L) are suggested, although occasional outliers are to be expected in these age groups as well.
It is more costly to treat patients in a hospital ward than as outpatients. It is thus desirable to have biomarkers that can predict early treatment response, permitting decision at an early stage whether the treatment will require hospitalization or whether it could be continued outside the hospital. Another application for biomarkers concerns evaluations of antibiotic effects in clinical studies. Such studies are extremely costly and may be difficult to interpret. However, a good biomarker could aid in the interpretation of the study results. In Sweden, CRP is also used as a point-of-care test (POCT). Both SAA and CRP can be analyzed by nephelometry or turbidimetry with low reagent costs and utilizing instrumentation that most routine laboratories already have. From the time the sample has reached our laboratory, the turnaround time is normally less than 45 min for SAA and CRP results and the instruments used are available at all hours. IL-6 was traditionally batch-analyzed, as in our study, utilizing microtiter plates. Recently, IL-6 has been applied on the Immulite 2000 (DPC), which is a random-access instrument. Consequently, IL-6 could also be made available with short turnaround times at many hospitals and therefore SAA, CRP and IL-6 could be suitable in a clinical setting.

We report the time course of the biomarkers (body temperature, SAA, CRP and IL-6) in relation to the patient’s onset of illness before admission and to POS. The study group was made up of frequently occurring patients acutely admitted to an infectious disease clinic in which bacterial infections requiring intravenous antibiotic treatment were suspected. During the first 24 h, regardless of the duration time of illness, we found a temperature decrease that could be interpreted as a positive treatment effect. During the second and third days, body temperature did not change. There was no difference in the reduction of body temperature between patients with early and late POS. Although the study design aimed to eliminate the effect of anti-pyretic drugs on temperature, we cannot unequivocally exclude the possibility that this, as well as fluid replacement, had an impact on temperature decrease.

The SAA and CRP temporal courses had striking similarity. A short duration of illness was associated with increasing levels during the first 24 h after the start of treatment. An increased level would have had an impact on the total treatment duration, but that association was not found. After 24 h of treatment, the levels decreased in a similar way regardless of duration of illness. The similarity between SAA and CRP suggests that the former does not add any information compared with the established CRP in the early evaluation of patients with acute bacterial infections. No correlation (positive or negative) between age and the studied biomarkers (body temperature, SAA, CRP and IL-6) before the initiation of antibiotic treatment was found. Despite a suggested impaired immune function in the elderly, a hypothesized weaker APR was not observed. The patient body temperature is recorded bed-side, which provides short test result turnaround times and the procedure is well-established in the clinical setting. To our knowledge, there is limited
information on the time course of body temperature during infectious diseases. After 24 h the smoothed distribution of body temperature was unchanged, which is why its use as a biomarker is questioned. However, the initial drop in temperature tended to be larger in early than late POS. Possibly, body temperature influenced the clinician when deciding early POS. None of the studied serum biomarkers showed a substantial decline within 24 h. Thus, none of them was useful for monitoring the therapeutic response within that period. Thereafter, there was a decline but neither SAA nor IL-6 contributed with more information than CRP. Increasing values of SAA and CRP during the first 24 h were not associated with late POS. Because of the APR process, it was expected that the patients with short duration of illness would show increasing SAA and CRP levels during the first 24 h. Although CRP showed a decline between day 1 and day 3, the difference in levels was not correlated to early POS. It seems likely that the signs and symptoms of improvement, including body temperature, were guiding the clinician’s decision for POS. To be helpful in the early evaluation of patients with acute bacterial infections a biomarker needs to respond more rapidly.
In conclusion, we found a positive correlation between SAA and CRP in acute infectious diseases. The correlation is weaker in the low range of CRP levels (CRP<100 mg/L) than at higher CRP levels. The sensitivity and specificity for the tests are equal. SAA and CRP discriminate between bacterial and viral infections. SAA and hsCRP were determined with modern biochemical methods in varying age groups of healthy individuals. We conclude that these APPs exist at barely detectable levels in umbilical cord blood, then increase and persist at essentially similar low levels from the neonatal period to age 65 years, beyond which the levels increase with advancing age. This knowledge is useful when interpreting results from patients, including patients in the neonatal period and patients with low-virulent infections such as infections in prostheses. SAA is a sensitive biomarker for the detection of systemic inflammation in uncomplicated cystitis, as well as in the discrimination of pyelonephritis and cystitis. SAA and CRP have equal sensitivity, specificity and efficiency. The systemic inflammatory response in asymptomatic bacteriuria is still to be elucidated. SAA and hsCRP in NIs in immature newborn infants are potential candidates aiding the diagnostic and monitoring process. Our findings indicate that SAA and hsCRP are measurable in this patient group, are higher in patients with NIs than in healthy newborns and have a temporal association to the onset of NIs. In adults suffering bacterial infections, the interindividual variability of the levels of body temperature, SAA, CRP and IL-6 was considerable. During the first 24 h of admission, SAA and CRP levels increased in patients with illness duration less than 24 h in contrast to the temperature level that decreased. The temporal course of SAA and CRP was similar. SAA and IL-6, in comparison with CRP, did not add any important information on disease activity or therapeutic effect.
Future remarks

During the study period, new biomarkers have emerged. Of these new biomarkers, the possibility to detect CRP down to 0.16 mg/L has gained the most attention. SAA is known to be produced also in other tissues than liver and is possibly suited for detecting local inflammatory events (i.e. in cerebrospinal fluid, urine, sinuses fluids, middle ear secretion, pleural fluid and sputum). The “new” high sensitivity CRP will also be just as good in detecting systemic inflammation as SAA in low-virulent infections and infections in newborns. Further studies are proposed in the following areas: the newborn patient group in which new possibilities emerge in predicting, diagnosing and monitoring NIs, and presumably also early onset infections after delivery as well as distinguishing respiratory distress syndromes from infections; patients (especially aging individuals) with asymptomatic bacteriuria without clear cut urinary tract symptoms in aiding the decision on antibiotic treatment or not; patients with low-virulent infections such as in prostheses and patients with low-virulent intracellular pathogens (i.e. Chlamydia pneumoniae); and patients with viral infections aiding the decision on duration of antiviral treatment. For the determination of SAA in local fluids (i.e. urine and cerebrospinal fluids), new studies are suggested.
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