CHAPTER 1

INTRODUCTION AND SCOPE OF THIS THESIS
INTRODUCTION

Wounds, healing and inflammation

When the skin is damaged, for example by a small cut, a blood clot will start forming within seconds, and within minutes bleeding will stop. We should realize that the process of wound repair has then already started. Under normal circumstances, healing will be the end result of overlapping processes: inflammation, tissue formation and tissue remodelling [1]. Inflammation is a response of the immune system, since the immune system guards the individual against infection. Inflammation is defined in Webster's Medical Dictionary as "A local response to cellular injury that is marked by capillary dilatation, leukocyte infiltration, redness, heat, pain, swelling, and often loss of function and that serves as a mechanism initiating the elimination of noxious agents and damaged tissue[2]."

Since minor wounds are sustained very regularly, the local inflammatory responses initiated by the immune system are as crucial part of body homeostasis as is feeding or sleeping. Inflammation in an extremely complex homeostatic response that involves neutrophils, platelets, macrophages, endothelial cells and the coagulation and complement systems. The immune system can be divided into the innate immune system and the adaptive immune system. Furthermore, the system has been divided into a humoral and a cellular system. Combined, these two divisions result in the four major parts (Table 1.1) of the immune system.

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<tr>
<th>Table 1.1. Major divisions in the immune system</th>
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<tr>
<td><strong>Humoral</strong></td>
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<td><strong>Cellular</strong></td>
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This thesis focuses on a number of aspects of the innate humoral immune response. The early inflammatory response is part of the innate immune system. In contrast to the adaptive immune system, which provides improved responses upon repeated infection, the innate response is not based on immune memory, but on evolutionary highly preserved mechanisms of pattern recognition of potential pathogens. For example, bacterial endotoxin (lipopolysaccharide; LPS) is recognized by the innate immune system in even very low concentrations, and thus serves as a very strong stimulus of subsequent responses. Acting together, CD-14 and so-called Toll-like receptors (e.g. TLR-4) present on myeloid cells, are believed to form a single pathway common to all mammals to transduct the LPS-signal [3]. The importance of these Toll-like receptors is underscored by the discovery that the receptors are coupled to a pathway that activates genes mediating innate immune defenses in mammals, insects, and even plants [4,5].
Whereas local inflammation in the setting of tiny wounds may not have significant systemic effects, at a certain size of the wounds, systemic effects will become evident.

Clinical signs of systemic inflammation
The systemic manifestations of the innate immune response that result after trauma, burns or ICU-admission are the subject of this thesis. When an inflammatory stimulus is sufficiently strong to cause systemic effects (Table 1.2), the so-called systemic inflammatory response syndrome (SIRS) will develop. SIRS has been defined by a consensus conference [6] as present if two of the clinical manifestations listed in Table 1.3 are observed. At the ward many patients may have SIRS, at the intensive care unit nearly all patients have SIRS.

Grades and definitions of systemic inflammation

**Figure 1.1.** To preserve a stable healthy situation (homeostasis), like other physiologic systems, the immune system reacts to disturbances such as a trauma with phased responses. The purpose of these responses is to eliminate the disturbance and restore homeostasis. The induction phase the presence of tissue damage or infection (the stimulus) is recognized and transduced into cytokine or chemokine signals that direct the response that eliminates the injury. (Adapted from T.H.The et al. 1995)
The American consensus statement contained, apart from the definition of SIRS three additional definitions. The main purpose of these definitions has been to illuminate the distinction between inflammation and infection:

**SIRS** = fever + leukocytosis;
**Sepsis** = SIRS + infection;
**Severe Sepsis** = Sepsis + multiorgan dysfunction;
**Septic shock** = Severe Sepsis + refractory hypotension [6].

<table>
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<th>Table 1.3. SIRS (Systemic Inflammatory Response Syndrome) criteria [6]</th>
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<tr>
<td>Temperature &gt;38° or &lt;36° C</td>
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<tr>
<td>Heart rate &gt; 90 /min</td>
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<tr>
<td>Respiratory rate &gt; 20</td>
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<tr>
<td>Hyperventilation (PaCO₂ &lt; 4.3 kPa)</td>
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<tr>
<td>Leukocytes &gt; 12 10⁹/l or &lt; 4 10⁹/l</td>
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<tr>
<td>Immature neutrophils &gt; 10%</td>
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**Organ failure**
Multiple organ failure has been defined in 1985 by Goris as 'generalized, autodestructive inflammation' [7]. As more organs are affected by the systemic inflammatory process, the chances of survival decrease.
Two examples of how organ failure has been graded are shown in table 1.4, a minimal total score has a high probability of survival, a maximal score has a very low probability of survival.

**Biochemical signs of systemic inflammation - acute phase proteins**
Although not strictly defined, the acute phase response can be considered to consist of the initial systemic clinical and biochemical responses that follow for trauma or infection. Fever is the clinical hallmark of the acute phase response, and it is usually accompanied by tachycardia [8]. These clinical signs are paralleled by many biochemical changes. Many of the proteins induced by the innate immune response are called acute phase proteins.
An elevated erythrocyte sedimentation rate (ESR) [9] was the first widely used laboratory

<table>
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<th>Table 1.4. Examples of two multiple organ failure scores</th>
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<tr>
<td>Organ/system</td>
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<tr>
<td>Cardiovascular</td>
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<tr>
<td>Pulmonary</td>
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<tr>
<td>Renal</td>
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<tr>
<td>Neurologic</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Hematologic/ coagulation</td>
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<tr>
<td>Gastrointestinal</td>
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<td>Total score</td>
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parameter of inflammation. Increased levels of the acute phase protein fibrinogen are a main determinant of an elevated ESR [10]. A large number of proteins have turned out to be acute phase proteins (APP). What constitutes an acute phase protein has not clearly been defined. An increased levels after inflammation and some sort of effector function (as opposed to signalling cytokines) are the most important characteristics of acute phase proteins. Kushner [11] somewhat sweepingly defined APP as those proteins whose plasma concentration rises 25% or more following an inflammatory stimulus. Albumin, the most abundant protein in the circulation, is sometimes called a negative acute phase protein since it is down-regulated during inflammation. The metabolic effects and the reasons why albumin levels are decreased, are only partly understood [12]. Although not subject of this thesis, decreased albumin levels are an important illustration of the relation between inflammation and metabolism.

Nowadays, C-reactive protein (CRP) is the most widely directly measured acute phase protein. In health it is hardly detectable in plasma, but with inflammation it can rise rapidly to 10- or 100-fold levels. Although CRP’s function is not clearly identified, CRP is probably involved in early non-specific antibacterial defences [13]. Thanks to reliable assays and CRP’s half-life which is in the order of one day, CRP has gained wide popularity for monitoring inflammation in many disease states.

On the basis of the rapidity and magnitude of increase in concentration during the acute phase response, acute phase proteins can be divided into 3 groups (Table 1.5).

**Source of acute phase proteins**

The liver is quantitatively and qualitatively the major source of acute phase proteins. In several hepatocyte models for different species, the induction of many acute phase protein-production has been demonstrated. For example in 1966 Hurlimann et al. already performed \textit{in vitro} studies that showed that hepatocytes produce CRP [15].

### Table 1.5. Classification of some acute phase proteins

<table>
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<tr>
<th>Slow response</th>
<th>Intermediate response</th>
<th>Rapid response</th>
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<tr>
<td>Antitrombin III</td>
<td>α1-proteinase inhibitor</td>
<td>C-reactive protein (CRP)</td>
</tr>
<tr>
<td>Complement C3, C4</td>
<td>α1 acid glycoprotein</td>
<td>Serum Amyloid A (SAA)</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>α1 anti-chymotrypsin</td>
<td></td>
</tr>
<tr>
<td>C1-inhibitor</td>
<td>Haptoglobin</td>
<td></td>
</tr>
<tr>
<td>α2-antiplasmin</td>
<td>Fibrinogen</td>
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Adapted from Heinrich et al. [14]
Function of acute phase proteins
A close look at the functions - as far as understood - of the acute phase proteins illustrates the purpose of the acute phase response: provide the organism with those proteins that are necessary to keep up both an adequate and a restrained inflammatory response. Table 1.5 shows that proteins for the complement system, the coagulation system and the fibrinolytic system are provided by the acute phase response. Most acute phase proteins have larger molecular weights (>50,000 Dalton) and higher concentrations (mg/L to g/L range) when compared to cytokines which have a lower molecular weight and much lower concentrations. This is due to fact that acute phase proteins are often effector molecules (e.g. thrombus formation, protease inhibitor, bactericidal factor) as opposed to cytokines that are hormone-like proteins. Understandably, effector functions require much higher concentrations than necessary for signalling functions.

Function unknown or no function
Currently it is possible to detect many biological substances and study their interactions with many cell types. This results in a huge number of possible relations that can be studied. It is helpful to try to understand these mechanisms in a teleological manner. Teleology is the use of design or purpose as an explanation of natural phenomena [2]. In understanding inflammation this implies that evolution should have provided the organism with defenses that can plausibly be expected to help the organism survive. A mammal will certainly have a survival benefit from adequate protection against minor trauma, since every animal will continuously sustain such traumas during its life. But an animal cannot logically be expected to have an elaborate defense against the results of septic shock. An animal with septic shock or severe trauma will in all probability not survive anyway, so evolution has no interest in providing for defenses in ‘lost cases’. Of course the animal (or man) with septic shock may survive with medical intervention, but at this stage we cannot rely on inflammation responses behaving in a ‘logical’ way. Thus, we may expect every protein found near a small uncomplicated wound to have a relevant function, and search aggressively for this function if we do not know it yet. We also may understand why TNFα levels are very high in septic patients, but we should not expect that these high levels have a specific function. In fact in these patients many molecules with elevated or depressed levels will not have a function, may have lost their function or may even function inappropriately.

That a distinct degree of systemic inflammation might be beneficial to the host, was already believed centuries ago [16,17], when patients who did not properly recover from wounds or infection were treated by inducing additional inflammation. The widely practiced cauterization of wounds with hot irons or hot oil was also performed on healthy skin to enhance recovery of wounds elsewhere (see illustration). This practice of counter-irritation was also performed in the 20th century, albeit in the slightly more humane form of turpentine abscesses. In fact turpentine in still used in animal models to induce a sterile acute phase response [18].
History of endogenous pyrogen and IL-6

The genesis of fever has long been a subject of investigation. Decades ago it became apparent that an 'endogenous pyrogen' had to exist. This substance is made by the body in response to tissue damage or infection and it induces fever by changing the temperature setpoint in the hypothalamus - a process that can be inhibited by prostaglandin inhibitors like aspirin. With modern molecular biological technology it became clear that several cytokines not only induce fever but also induce acute phase proteins. They can thus be considered not only endogenous pyrogens but turned out to be true hormones of inflammation.

In the 1980’s interleukin-1 (IL-1) became the first contender for the role of endogenous pyrogen. Experiments by Aarden et al. showed that hybridoma growth factor (HGF) was active in the IL-1
assay [19]. A very sensitive bio-assay for HGF was developed, and this assay did not respond to IL-1, indicating that HGF had to be another cytokine. After cloning of cDNA, HGF was discovered to be identical with a new cytokine named interleukin-6 [20]. In many in vitro and in vivo studies that followed, the B9.9 bioassay for HGF/IL-6 has been very important in defining the pleiotropic role of IL-6, to be later replaced by ELISAs [21].

The cytokines TNFα, IL-6 and IL-1 are elevated within hours after a major inflammatory stimulus [22]. TNFα peaks earlier than IL-6 and also induces IL-6. For example van Gameren and colleagues [23] showed that administration of IL-6 to patients induced most of the responses listed in Tables 1.2 and 1.3. Several studies have assessed the predictive value of cytokine levels for clinical outcome. In general, cytokine levels in patients with sepsis are much higher than in trauma patients [24]. In addition it was found by Hack et al. that higher IL-6 levels were associated with mortality in sepsis patients [25]. Partly due to the fact that cytokine levels can rapidly change, and due to difficulty in routinely assaying cytokines, these measurements are not used in clinical practice. IL-6 levels measured at the bedside (positive at > 1000 pg/ml) have been used to triage patients for anti-TNFα sepsis trials [26].

Methodological advantages of studying patients with mechanical trauma or burns

The pathophysiology of inflammation has received intensive attention in patients that are critically ill, but it is useful as well to study the physiology of inflammation in patients that are not critically ill, e.g. in healthy persons that sustain a nonlethal trauma. Trauma patients usually are healthy before the injury and show a responses roughly proportional to the severity of the injury [27]. Since the time of the injury is a well-defined moment as opposed to sepsis, the timing and order of the elicited responses can be studied in more detail.

Concerning inflammatory responses in trauma patients, patients with burns represent a special group. Thermal injury is a one-dimensional trauma, with the percentage of burned total body surface (TBSA) being the key parameter. With only the age of the patient and TBSA it is possible to make a good prediction of mortality and hospital stay [28]. Severely burned patients can have extensive tissue damage without accompanying organ damage. Early after sustaining burn injury, patients show a pronounced inflammatory response. This occasionally extreme response has made burns patients a subject of many studies on virtually all known aspects of host responses.

Differentiating infectious from non-infectious causes of inflammation.

Both trauma and infection can induce an acute phase reaction, clinically characterized by SIRS. When SIRS persists for a number of days after admission to the hospital, this can be due to the trauma itself or secondary infection. In the first case a wait-and see policy is often justified. In the second case it may be crucial to search for a focus, or to start antibiotics and even perform an operation. Since a majority of trauma patients at the ICU develop fever, with only proven infection in half the these patients [29] - it is very useful to possess additional tools to recognize bacterial infection. Especially in immune suppressed patients (e.g. after transplantation) it would be very useful to possess a rapid assay to detect bacterial infection, since in these patients the margins of error are small. Now that many inflammatory markers can be measured in the circulation, researchers have looked for those markers that can discriminate bacterial infection
from other causes of systemic inflammation. But discriminating causes of inflammation on patterns of cytokine or acute phase protein responses have proven an elusive goal. Measuring the bacterial product endotoxin itself would seem to be an obvious solution to identify bacteremia and endotoxemia early [30]. Unfortunately circulating endotoxin assays are difficult to use and reproduce [31].

In accordance with the concept of the spectrum of increasing systemic inflammatory responses with increasing infection: SIRS → sepsis → severe sepsis → septic shock, systemic inflammation with infection is associated with higher cytokine levels and higher levels of CRP. Thus, serial quantitative measurements of the extent of the acute phase response, usually in the form of CRP have been a major clinical tool in detecting infection. The acute phase response appears to modulate mainly in intensity and duration, not in the relative enhancement or inhibition of components of the response. This is of course in agreement with the non-specific nature of the acute phase reaction.

**Procalcitonin to differentiate infection and non-infection?**

The recent introduction of a convenient assay for procalcitonin (PCT) and the first experiences with measurements of circulating PCT have been promising. Some authors have proposed that a practical parameter that differentiates bacterial infection from other causes of inflammation is now at hand. Although PCT is biochemically a precursor of calcitonin (a hormone involved in calcium homeostasis) it is functionally not related to calcitonin. Both the cellular origin and function of PCT are unknown. Nevertheless, circulating PCT measurements in many patients groups [32] have shown that:

- Circulating PCT is elevated proportional to the inflammatory stimulus.
- PCT can be induced by infusing endotoxin in volunteers. Elevated PCT-levels are detectable at 6 hours and disappear with a half-life of about a day [33].
- The dynamic range of PCT is very large: PCT rises at least a factor 400 within 6 hours in volunteers receiving endotoxin. Where one report [34] claims that PCT levels are <0.01 ug/l in normal persons, PCT can reach 1000 ug/l in sepsis [35]. Thus PCT's dynamic range may be even greater than that of CRP or SAA.

The most interesting aspect of PCT is the evidence that PCT compared to CRP is superior in discriminating bacterial from non-bacterial inflammation. De Werra [36] compared patients with septic shock, cardiogenic shock, and bacterial pneumonia. The best predictive value for septic shock was found for PCT in septic shock, with PCT varying from 72 to 135 ng/mL, compared with approximately 1 ng/mL in the other groups. The monograph on PCT by Meisner [32] contains a number of early studies that indicate that PCT is better than CRP in discriminating serious infection from other causes of inflammation. More recently Gendrel et al. reported that PCT was more specific and sensitive than CRP for the differentiation of bacterial and viral infections in children [37]. In 236 children with viral infections they found a median PCT of 0.2 ug/l and a median CRP of 10 mg/l. In 46 children with bacterial sepsis or meningitis PCT was 18 ug/l and CRP 144 mg/l. Thus PCT is about a 100-fold higher in sepsis compared to viral infection, whereas CRP is 'only' 15 times higher.
Still the literature is not conclusive on this very important issue. Those disease categories where determining PCT may not be reliable need to be better defined. For example de Bont [38] found that in neutropenic patients PCT responses are much lower than would be expected on the basis of other signs and elevated CRP-levels. The various results that suggest that preferential induction of PCT by bacterial infection exists, have led some authors to hypothesize that endotoxin can induce PCT directly. A logical question is whether cytokines like TNFα or IL-6 are necessary and sufficient for the induction of PCT. And although increased levels of IL-6 and TNFα were also observed after endotoxin infusion, this does not prove that TNFα and IL-6 are necessary for the induction of PCT synthesis, as they are for the acute phase protein induction. Does PCT originate in the liver? Or more general, should PCT be viewed as an acute phase protein?

Platelets and endothelium in inflammation
Platelets can be considered inflammatory agents as much as coagulatory agents. In systemic inflammation platelets first disappear from the circulation (thrombocytopenia), and as the patient recovers uneventfully platelets will reappear in increased amounts [39] (thrombocytosis). Several cytokines can induce this sequence. After administration to humans, IL-6 will induce a complete acute phase response, including the characteristic sequence of a nadir platelet count at day 2 or 3 and a maximal platelet count at day 12 [23]. Concerning the causes of premature disappearance of platelets from the circulation, two important possibilities exist. The platelet end up as part of a clot or the platelet can (temporarily) adhere to the endothelium. In both cases platelets can amplify inflammation through the release of powerful mediators.

Consumption of platelets is an integral part of the syndrome of diffuse intravascular coagulation (DIC). The reverse, that DIC always accompanies platelet consumption, is not true. DIC is characterized by extensive intravascular formation of fibrin, in severe cases resulting in vascular occlusion and organ failure. DIC occurs secondary to diverse range of serious diseases, like sepsis or trauma [40]. It is important to note that significant decreases in platelet count are observed in virtually all patients with trauma or sepsis. Although a significant proportion of these patients may have indicators of 'low-grade' DIC, many do not have DIC, indicating that platelet sequestration and DIC are not the same [41].

Endothelium, the single cell layer that separates the blood from the organs and tissues, is a very active cell system. Endothelium regulates hemodynamics and the transport of molecules and cells between blood and the tissues. Activation of endothelium occurs early and universally [42] in the process of inflammation. Endothelial cell activation [43] is characterized by the loss of vascular integrity, expression of adhesion molecules, transition from an antithrombotic to a prothrombotic state and cytokine production. Exposure of the subendothelium with tissue factor and von Willebrand Factor and rapid expression of P-selectin can initiate the binding of platelets as well as the adhesion molecules such as GPIIb/IIIa, P-selectin, CD-31, LFA-1, CD-36 [44] and CD-87 (urokinase plasminogen activator receptor; uPAR) [45]. Cardiovascular research has produced extensive evidence of the importance of platelet-endothelium interaction. This is underscored by the clinical effectiveness of aspirin and the recently introduced GPIIb/IIIa-inhibitors in limiting or preventing coronary thrombosis [46]. Despite the evidence of the importance of the platelet-endothelial interaction in inflammation, direct studies on this interaction are methodologically
very difficult. *Ex vivo*, endothelial cells and platelets are not the same as *in vivo*. Whereas the erythrocyte has a circulating precursor that can be clearly identified and quantified (the reticulocyte), no such equivalent exists for the circulating platelet. Therefore, in thrombocytopenia it is difficult to decide if decreased synthesis or increased aggregation or adhesion is present, even when one resorts to a bone marrow biopsy or radioactively labeled platelet studies [47]. Re-injected radioactively labeled autologous platelets are not identical to native circulating platelets.

**Serial platelet counts as an indicator of endothelial activation**

Instead of performing highly specialized platelet studies in a limited set of patients, in this thesis it was preferred to study serial platelet counts and study them in larger patient groups. The assumption was that changes in the platelet count are to a large extent correlated to the magnitude of endothelial activation, which in turn is related to systemic inflammation. A low admission platelet count is known to be a relatively strong predictor of adverse outcome in a variety of disease states. In meningococcal sepsis [48], after ruptured aortic aneurysm [49], at admission to the intensive care [50] the early platelet count is one of the strongest predictors of outcome. As a result the platelet count has been introduced in several intensive care scoring systems, while the leukocyte count has been eliminated from some scoring systems. The multi-organ dysfunction score (MODS) [51], the sequential organ failure assessment score (SOFA) [52] and the pediatric risk of mortality score (PRISM-III) [53] use the platelet count and not the leukocyte count as one of its component parameters. In contrast to the well-described importance of initial platelet counts, the relevance of subsequent changes has received little attention. Within the healthy individual the platelet count is quite stable with an intra-individual variation that is only 20% to 30% (or 60 · 10^9/L) of the inter-individual variation [54,55]. Thus serial platelet counts might in principle provide additional information. As long as the platelet count is within the normal range (i.e. 150 to 350 · 10^9/L) it has been implicitly assumed that such a count is normal. Yet, in patients with an uneventful clinical recovery after a moderate trauma, thrombocytosis normally develops during the second week. Thrombopoietin has been identified as a major regulator of thrombopoiesis [56]. After trauma IL-6 is released, which is also capable of inducing thrombocytosis [23]. In critically ill patients thrombocytosis is often not present, although such patients typically have cytokine levels (including IL-6) much higher than those observed after uncomplicated trauma. Thus despite the fact that many ICU patients are in a more advanced state of inflammation (ranging from SIRS to sepsis, severe sepsis or even septic shock) compared to uncomplicated trauma patients (usually SIRS), they have lower platelet counts. Since in many instances these decreased platelet counts are still in the 'normal range' these changes have received little attention. That platelet counts decrease as a result of increased endothelial activation may be the explanation of the inverse relation between inflammation and platelet count in critically ill patients.
Fat embolism syndrome

This thesis originated in studies on the incidence and causes of the fat embolism syndrome (FES). FES is typically seen in young patients who sustain isolated long bone fractures [57]. After an interval of 8 hours to 2 days the three cardinal symptoms of the syndrome appear:

• petechiae with a typical upper-body distribution, unlike that seen in severe thrombocytopenia
• respiratory distress
• cerebral disturbances

As the name of the syndrome denotes, fat is involved in these three cardinal symptoms, since fat emboli of bone marrow origin can be recovered from skin lesions, the lung and the brain during pathological examination. In the process of embolization, the venous fat apparently easily (by)passes the lungs. Aggressive, early operative stabilization of fractures, and the improved level of supportive care are assumed to have contributed to the decrease in FES-incidence. Although FES is seen less frequently than for example 25 years ago, both its causes and the reasons for its decreased incidence are unclear. Fever and tachycardia are among the 'minor symptoms' described for FES [57], indicating a link of FES with inflammatory responses. Instead of being a result of FES, systemic inflammation might be a factor in inducing FES. CRP can agglutinate fat globules into emboli [58] - a property of CRP that has even led to the development of a bed-side CRP-test based on fat-agglutination [59]. Here we looked at the relation of early inflammatory signs with the subsequent development of FES.

Prolonged increases in pressures in (closed) wounds around the fractured bone may assist the process of fat intravasation and subsequent embolization. Early operation may decompress the fractured bone and prevent further embolization of fat. The potential detrimental effect of delayed treatment on the incidence of FES, and the relevance of a persisting open foramen ovale [60] as a shunt for fat globules are explored in this thesis.
OUTLINE AND AIMS OF THIS THESIS

In this thesis the systemic counterparts of acute local inflammation were studied. As a part of the innate immune response, systemic manifestations of inflammation were primarily studied in trauma patients.

Hypothesis

The various stages of the systemic inflammatory response are expressed by distinct markers (pro-inflammatory cytokines as well as acute phase proteins and platelet counts) in a typical time sequence. They reflect the underlying pathophysiological mechanism and may offer possibilities for monitoring (new) intervention strategies by providing better clinical prognostic and diagnostic tests.

The clinical model used to study these marker kinetics were trauma patients since this patient category allows to note the starting point of the (pathophysiologic) chain of events.

Trauma patients are young and in general have no comorbidity. By a better understanding of the time sequence of the marker responses in trauma patients with single injuries physician may subsequently better interpret responses in critically ill patients, most of whom have preexistent co-morbidity.

Aims of this thesis

To study the various stages of the systemic inflammatory response, we selected a number of (circulating) markers that we assumed would adequately reflect and be correlated with (patho)physiological signs and symptoms. The protein responses of IL-6, PCT, CRP, SAA and α1-antiproteinase as well as the platelet count and leukocyte count and physiological parameters as temperature or heart rate were studied.

The first studies concern:
- Fat embolism syndrome (chapters 2 and 3)

The subsequent chapters are ordered according to the sequence of inflammatory events:
- Interleukin-6 (chapters 4 and 5)
- Procalcitonin (chapter 6)
- Platelet counts (chapters 7, 8 and 9)

Two studies (chapters 7 and 9) were an intervention studies; all studies were retrospective in design, although in chapters 4, 5, 6 and 9 some samples and data were prospectively collected. Not studied in this thesis were: animals, local responses, adaptive immune responses.

Fat embolism syndrome

Chapter 2 is a retrospective study of the incidence of FES in 172 patients with an isolated fracture of the femoral shaft. The goal was to find associations of the incidence of FES with the type of fracture, the timing of operation and an early inflammatory response.

Chapter 3 verifies if a right-to-left shunt in the heart has a causal role in FES. Such a shunt could explain the transit of large fat globules from the venous to the arterial circulation.
**Interleukin-6 and acute phase responses**

**Chapter 4** describes IL-6 levels in patients with burns at a time that IL-6 had never been measured in patients with acute systemic inflammation. The aim was to correlate IL-6 levels with basic acute phase responses: i.e. fever, CRP-levels and $\alpha$1-antiproteinase levels, and address the question if IL-6 was the long sought endogenous pyrogen.

**Chapter 5** examines IL-6 and acute phase responses in burns patients in detail. The goal was to study time dependent changes of phenomena in which IL-6 could play a causal role. Also correlations of these parameters with IL-6 and the potential causal relations on the basis of published evidence (as it was published at that time) are formulated.

**Procalcitonin**

**Chapter 6** it is assumed that endotoxin is not necessary for the induction of PCT. Thus, the direct effects of TNF$\alpha$ and IL-6 on the expression of PCT, SAA and CRP were measured. *In vitro*, human liver slices were used. *In vivo*, two groups of cancer patients, treated with TNF$\alpha$ and IL-6 respectively, were studied.

**Primary and secondary thrombocytopenia**

Early and late decreased platelet counts in trauma and ICU patients may reflect increased sequestration due to systemic endothelial activation.

The aim in **chapter 7** was to examine early changes in platelet count as they occur during the first 2 days after trauma. We also investigated if high-dose methylprednisolone administered shortly after the injury affected platelet consumption. This may indicate if the pleiotropic effects steroids are able to affect inflammation related platelet sequestration.

In **chapter 8** the aim was relate late changes (i.e. > 2 days) in platelet count with mortality, since persisting systemic inflammation is known to be associated both with platelet sequestration and mortality. Patients from one surgical ICU were studied. The value of platelet counts was also compared to leukocyte counts.

The goal in **Chapter 9** was to generalize the observations of the previous study and address changes in platelet counts and outcome in a very large heterogeneous European multi-center population of ICU patients. The predictive power for mortality of early and late changes in platelet count respectively, was investigated. For this purpose a simple mathematical model to describe time-dependent changes in platelet counts was used. We also investigated the behavior of platelet counts according to admission groups (medical, surgery scheduled or unscheduled.)

Finally **Chapter 10** attempts to integrate the results and give suggestions for further research. In particular the timing of all responses observed in the various studies is combined into one scheme. The question what would constitute an ideal inflammatory marker is discussed, as well if such a marker exists.
REFERENCES


