CHAPTER 10

SUMMARY, DISCUSSION AND LINES OF FURTHER INVESTIGATION
SUMMARY
Physiological parameters and circulating markers in humans who display systemic inflammation were the main focus of this thesis. The patients investigated in this thesis varied from trauma patients with or without fat embolism (FES), patients with burns, patients receiving cytokine treatment to critically ill patients.

Fat embolism syndrome
In Chapter 2 we hypothesized that the nature of a femoral fracture, timing of the operation and early inflammatory responses would affect the risk for subsequent development of FES. We studied the incidence of FES in patients with an isolated fracture of the femoral shaft admitted to our hospital in the period 1968-1985. In a detailed analysis of the factors associated with the occurrence of FES an elevated temperature was the only factor that discriminated FES patients from other patients on day 0, suggesting that early acute phase responses are intimately connected with the onset of FES.

Chapter 3 addressed the hypothesis that an open foramen ovale is necessary for large fat globules gain entrance the systemic arterial circulation. We demonstrated that the presence of a patent foramen ovale with a right-to-left shunt is not relevant in FES, thus indicating that the fat globules are able to reach the systemic circulation through the lungs.

Interleukin-6 and acute phase responses
In chapter 4, it was hypothesized that IL-6 is an endogenous pyrogen as well as an inducer of acute phase responses. Thanks to the availability at that time of the sensitive and specific B9.9 assay for IL-6, we were the first to show that circulating IL-6 is increased after inflammation in humans. IL-6 was already sharply increased when burns patients presented at the hospital. IL-6 was correlated with fever, and the IL-6 peak preceded increases in acute phase proteins. In chapter 5, all major subsequent parts of the acute phase response were looked for and actually observed in patients with severe burns: fever, tachycardia, leukocytosis, left shift in the leukocyte differentiation, increased CRP and increased α1-proteinase inhibitor. As late parts of the inflammatory response thrombocytosis, a distinct and short-lasting IgM-peak and finally increased IgG levels were observed. There was no relation between the extent of the burns and IL-6 levels, but patients with more severe burns had elevated IL-6 for longer periods. The early parts of the acute phase response were positively correlated with increased IL-6 levels, compatible with a causal role of IL-6 in the induction of these responses.
Procalcitonin

Chapter 6 addressed that hypothesis that endotoxin is not a sine qua non for the induction of PCT. We found that in vitro, PCT is produced by human liver slices in significant amounts and that PCT-production could be stimulated with IL-6 or TNFα. In vivo, it was also found that IL-6 and TNFα induce PCT. Thus was concluded that cytokines without the presence of endotoxin can induce PCT. In the patients treated with TNFα, the very pronounced acute phase response that was induced also allowed the comparison of response times of PCT and CRP. PCT reached half-maximal levels within 8 hours, compared to 20 hours for CRP.

Primary changes in platelet count - thrombocytopenia

Early decreases in platelet count occur in nearly all trauma patients and in other critically ill patients. It is also known that the magnitude of the early drop in platelet count is related with outcome.

Chapter 7 investigated early platelet sequestration after major trauma, and the effect of high-steroids on platelet counts. Changes of the platelet count and hemoglobin in the first two days after moderate injury were measured retrospectively. In two groups of matched patients, one group received a very high dose of methylprednisolone starting 4 hours after the injury. Over 48 hours, the platelet count dropped by 39% whereas hemoglobin only dropped by 14%. By taking transfusions and changes in hemoglobin into account, it was concluded that the post-traumatic thrombocytopenia is mainly caused by sequestration of platelets, not blood loss. Methylprednisolone had no effect on platelet-sequestration. Methylprednisolone is a very powerful and pleiotropic inhibitor of inflammation that can prevent platelet sequestration if administered before an inflammatory stimulus. However, inflammation-induced platelet sequestration apparently becomes refractory to steroid treatment within hours.

Secondary changes in platelet count - (blunted) thrombocytosis

Based on extensive evidence that links systemic inflammation with the sequestration of platelets we hypothesized that secondary changes in platelet count would be also be related with outcome.

In chapter 8 this conjecture was verified in patients in our own ICU. Surgical ICU patients were analyzed for temporal changes in the platelet count as well as the leukocyte count. Both in the large (N=1415) overall group and in all subgroups (trauma, abdominal surgery, vascular surgery and liver transplantation) the same phenomena were observed: Platelet counts decrease to nadir values at 2 to 3 days after ICU admission and failure of the platelet count to recover to normal or supranormal levels is associated with mortality. Leukocyte counts were not associated with mortality. The parameter \( \Delta PC/\Delta t \) between day 2 and day 10 was a better predictor of mortality then the APACHE-II score at ICU-admission.

In chapter 9 we tested the assumption that the observations for surgical ICU patients in chapter 8 are also true for ICU patients in general. In addition we verified if secondary changes in PC (i.e. after ICU day 2) have a stronger correlation with outcome than initial
changes in PC. Thus the relation between temporal changes in PC and mortality were verified in a larger mixed patient set from the second European ICU Study (EURICUS-II; N=5206). In this population as well, low $\Delta PC/\Delta t$ values were associated with mortality. Changes after day 2 indeed had a stronger relation with outcome than initial changes in platelet counts.

**DISCUSSION AND FUTURE DEVELOPMENTS**

Although we formally only studied patients and not healthy persons, trauma patients can be effectively considered as previously healthy persons who subsequently sustained an injury. This is the justification for the title of this thesis "Acute systemic inflammation in health and disease". Except for chapters 8 and 9 where a substantial number of patients with underlying chronic disease were also included, the other chapters concern patients who were basically previously healthy.

| Table 10.1. (See cover) Sequence of inflammatory parameter changes studied in this thesis |
|---------------------------------------------|---------------------------------------------|
| **Time after inflammatory stimulus**       | **Parameter**                               |
| Hour                                       | TNF$\alpha \uparrow$, IL-6 $\uparrow$       |
|                                            | fever, tachycardia                          |
|                                            | platelet count $\downarrow$                 |
| Hours                                      | leukocyte count $\uparrow$                  |
|                                            | PCT $\uparrow$                              |
| Day                                        | CRP $\uparrow$, SAA $\uparrow$              |
| Days                                       | $\alpha_1$-antiproteinase $\uparrow$        |
|                                            | albumin $\downarrow$                        |
| Week                                       | platelet count $\uparrow$/=                 |
|                                            | IgM $\uparrow$                              |
| Weeks                                      | IgG $\uparrow$                              |

The main purpose of this thesis was to define the time course of systemic inflammatory parameters and thus help address hypotheses concerning the role of these parameters in the inflammatory sequence. The implications of the findings are discussed in more detail below. Table 10.1 summarizes the time-scale and the associated order in which parameter changes were observed. The final paragraph of this chapter discusses what should be the properties of an ‘ideal’ inflammatory marker and why this is not necessarily the same as a marker of disease severity. In this context, procalcitonin and the platelet count are considered as markers of inflammation and severity respectively.

*Inflammation in health and disease - Physiological and pathophysiological response*
Classifying responses as either physiological or pathophysiological depends to a certain degree on an arbitrary cut-off. Nobody would probably call wound healing an unhealthy response. On the other hand, septic shock due to an exaggerated release of cytokines after endotoxin stimulation is definitely not a beneficial response. Thus calling a response physiological depends on the extent of the primary stimulus and the induced responses and whether the host is overwhelmed or not. We consider the trauma patients previously healthy and assume that their primary responses are physiological. The severity of illness varied in the different studies: the patients studied in chapters 2, 3, 6 and 7 were mainly admitted to the ward, patients in chapters 4 and 5 to the burn unit, while patients studied in chapters 8 and 9 were admitted to the ICU. The systemic inflammatory responses in the moderately injured patients who stayed at the ward could still reasonably be called physiological, since these responses still are beneficial and do not appear to worsen the patient's condition. In intensive care patients on the other hand, frequently disproportional host responses are observed that can be interpreted as pathophysiological.

The fat embolism syndrome: a peculiar case of inflammation

The first two chapters concerned the fat embolism syndrome (FES). A femoral shaft fracture is the typical trauma associated with FES. FES is the secondary systemic result (generalized petechiae, cerebral and respiratory disturbances) of a primary local trauma. Since FES develops in far fewer patients than the number of patients in which release of bone marrow fat is observed, additional pathogenic mechanisms must be present. In chapter 2 we found that local factors, in particular a closed femoral fracture coupled with late operative stabilization of this fracture, was associated FES. Thus we interpreted that femoral fractures where the surrounding soft tissues (skin and muscle) were not decompressed, led to FES. A conceivable mechanical explanation is that increased pressure around the fracture enhances the entrance of fat into the systemic circulation. The other major observation in chapter 2 is the association of FES with an early onset of fever. Nowadays with modern early fracture stabilization and supportive volume therapy, FES is rarely seen as an isolated syndrome [1]. But regardless of the decreasing incidence, FES remains a specific entity with an unknown pathogenesis. Causal treatment of FES is still not possible, while positive end-expiratory pressure ventilation is the mainstay of supportive therapy in cases of pulmonary insufficiency. Many prophylactic schemes have been tested, but none have shown real benefit. As we and others have observed, early operative stabilization of fractures lowers the incidence of FES. The higher incidence of FES [2] when multiple femoral fractures are present [3] implies that in multiple injured patients FES is often present, but can sometimes not be recognized due to the concomitant injuries to the brain or lungs. Several years after the our publication that dismissed patent foramen ovale as relevant in FES (chapter 3), a report based on one patient with presumed FES and a patent foramen ovale appeared in the New England Journal of Medicine [4] The authors claimed that patent foramen ovale is important in the pathogenesis of FES. As pointed out in chapter 3 we
disagree since the single patient described in this report did not conform to important diagnostic criteria of classical FES. Later, Forteza [5] and colleagues showed the presence of cerebral fat emboli with transcranial doppler in 5 patients with long bone fractures. 4 of these 5 patients did not have a patent foramen ovale. It should be noted that, in contrast to FES, the presence of persisting foramen ovale is an important risk factor in ‘macro-embolic’ entities such as cerebral thrombo-embolism or air-embolism.[6,7].

The association of fat embolism with early fever, and thus with early systemic inflammation, that we found has also been observed by others [8]. Elevated levels of CRP have been implicated as a cause, since CRP has fat agglutinating potential under certain circumstances [9].

Some remaining questions regarding FES:

- Fat and marrow embolization can be imaged by TEE nearly always during orthopedic surgery [10]. Why does it not harm the vast majority of patients?
- Neutral fat which is relatively non-toxic can be converted to much more reactive fatty acids by lipase or lipoprotein lipase. These fatty acids can be toxic to the pulmonary vasculature [11]. But it is still not clear if this biochemical process plays a significant role in FES.
- How do large (up to 100 µm) fat globules manage to pass the glomerulus [16], a filter that otherwise prevents the leakage of much smaller macro-molecules or lipoproteins?

After decades of experiments, a suitable animal model still needs to be developed. The so-called models of FES typically involved rather extreme stimuli, rapidly resultant in the animals death. Typical models executed were: severe experimental shock [13], acute right heart failure [14] after massive fat infusion or acute oleic acid pulmonary injury models [15]. Rapidly developing shock is part of these models, but shock is not part of the classic FES. Also, none of these models reproduces the intriguing interval free of symptoms before the FES becomes manifest.

Full-blown FES with petechiae and respiratory distress and cerebral disturbances has become less frequent, but subclinical manifestations with permanent damage may frequently occur. When a successful animal model of fat embolism is developed the mechanisms by which fat emboli are pathogenic may be understood more clearly. Such models could lead to directed therapy that may limit subclinical damage by fat emboli that may be more pervasive and irreversible [17] than the acute clinical condition suggests.
Chapter 10

**IL-6**

Since our first studies on IL-6, an extraordinary amount of research has been performed on IL-6. Together with many other cytokines, such as TNFα and IL-1, IL-6 is produced shortly after the local tissue damage [18] or systemic endotoxin stimulation occurs. Amongst others, monocytes and endothelial cells can produce large amounts of IL-6 [19,20]. After the discovery of many other cytokines that have many functions, IL-6 still stands out for its very broad range of actions. Platelet production is stimulated by IL-6 [21,22] and thrombopoietin [23]. The sharp IgM-peak during the second week and later the more sustained elevation in IgG, reflect immunoglobulin stimulation by the cytokines, especially by IL-6 [24]. IL-6 appears to be involved in the pathogenesis of multiple myeloma [25] and Castleman's disease [26]. In fact, sustained inflammation is in general associated with hypergammaglobulinemia and under these conditions high IgG levels contribute to the elevated ESR [27] as is shown by determining the ‘defibrinated’ ESR. Although obsolete now, measuring the ESR after fibrin removal was an elegant way of quantifying chronic inflammation [28].

Unlike in patients with mechanical injury, in sepsis cytokines often do more harm than good. It has now been extensively shown that shock in sepsis is mediated by sometimes extremely high levels of cytokines such as TNFα, IL-6 and IL-1 [29], due to massive activation by endotoxin. Thus after limited trauma the inflammatory often is in a compensated state, but in sepsis it is overwhelmed, resulting in a decompensated state. At that point cytokines have lost their beneficial function. It is only logical to assume that the inflammatory system was 'designed' by evolution for local purposes, i.e. containment of infection and restoration after trauma. The exquisite sensitivity of some myeloid cell types to endotoxin is beneficial under local circumstances. But when local defenses are overwhelmed, the massive induction of cytokines serves no purpose, and will be lethal unless rapid medical intervention is performed. Depending on the sort of disease, on the stage of the disease and on the investigator, some cytokines have been classified as inflammatory or anti-inflammatory. Although IL-6 levels are especially increased in sepsis, and higher levels associated with poor outcome, some have nevertheless called IL-6 anti-inflammatory [30]. The observation that elevated cytokine levels are correlated with increased mortality has generated an array of sepsis intervention trials. In addition to high-dose corticosteroids, agents aimed at blocking or inhibiting the effects of endotoxin, TNFα, IL-1-receptor, platelet-activating factor receptor, bradykinin, prostacyclin and thromboxane-A2 were tested in randomized controlled trials [31]. Although it may appear conceptually helpful to divide cytokines into pro-inflammatory and anti-inflammatory, such a division is artificial, because many cytokines have multiple “functions” and because many functions are performed by multiple cytokines. Thus it appears more appropriate to view cytokines not as sharply defined hormones, but as connections in a signalling network in which considerable overlap exists. Overlap and parallel pathways may partly explain the failure of nearly all sepsis intervention trials to affect mortality. These trials have been aimed at blocking a single pathway [31]. At the effector level it possibly easier to divide proteins into pro-inflammatory and anti-inflammatory. For example, the enzyme elastase that is released by neutrophils could be considered pro-inflammatory. Many acute
phase reactants appear to be anti-inflammatory, like for example $\alpha_1$-proteinase inhibitor, a serine protease inhibitor that inactivates elastase by binding it. The high molar circulating concentration of $\alpha_1$-proteinase inhibitor compared to elastase keeps the proteolytic activity of elastase restricted to a limited space in the direct neighborhood of the activated neutrophils [32].

**Procalcitonin**

Two recent studies [32,33] found that in septic patients (35 and 24 patients respectively) PCT was superior to IL-6, CRP, soluble CD14 and TNF$\alpha$ in early detection of non-survivors (13 and 8 patients respectively).

PCT levels may be an attractive tool for clinical monitoring, but the origin of increased PCT-synthesis may be even more important pathophysiologically. Endocrine cells in the lung have been initially proposed as a source of increased PCT synthesis after pulmonary injury like inhalation burns [35] or pneumonitis [36] although others found no relation of PCT elevation with the presence or absence burn injury with or without inhalation [37]. Despite the intensive search for a source of PCT, at the time of this writing no significant *in vitro* production equaling or surpassing the release of PCT that we found in liver slices [38], has been reported, regardless of cell type or tissue used. In support of our finding that the liver produces PCT, hepatic vein levels of PCT were higher than central venous levels in extracorporeal bypass patients [39].

The *in vitro* PCT-concentrations that were found in the supernatant after stimulation ($\approx 1$ $\mu$g/L) were considerable, both when compared to *in vivo* PCT concentrations, and when compared to *in vitro* CRP and SAA concentrations (<1 mg/L). The measured liver synthesis rate of PCT could account for observed levels to *in vivo*. We could not find reports of PCT-measurements of supernatants of hepatocytes cultured with various cytokines, and cytokine-stimulated HepG2 cells produced no PCT (unpublished data). Such measurements may

| Table 10.2. Comparison of PCT with cytokines and acute phase proteins |
|---|---|---|
| **Function** | **Cytokines** | **Procalcitonin** | **Acute Phase Proteins** |
| | Signalling | ? | Effector. (Innate bacteriostatic, coagulation, protease inhibition) |
| Source | Leukocytes, endothelial cells | Liver ? | Hepatocytes |
| Molecular weight | <50 000 | 13 000 | > 50 000 |
| Mass concentration | 0.1-1 ng/l | 1-100 $\mu$g/l | 10 mg/L – 10 g/l |
| Induction time to half maximal levels | minutes - hours | 8 hrs | >20 hrs |
| Half life | minutes - hours | day | day - days |
clarify the role of the liver and which cell type is involved in PCT synthesis, which is particularly important as this organ is the sole producer of most acute phase proteins.

**PCT - cytokine or acute phase protein**

When Kushner’s broad definition of an acute phase protein is applied [40], PCT fits the definition since it is increased by more than 25% in inflammation. But the more important question is whether PCT behaves as other ‘established’ acute phase proteins like CRP, fibrinogen or α1-proteinase inhibitor. Whereas CRP has been established as important in aspecific antibacterial defenses, the potential function of PCT is still unknown. Although, as the name implies, PCT is a precursor of calcitonin, circulating PCT levels appear to be largely unrelated to calcitonin levels. This is impressively illustrated by the fact that PCT levels can increase orders of a magnitude, with unchanged calcitonin levels. The relatively large quantities of PCT that were produced *in vitro* by human liver slices, when extrapolated to the *in vivo* situation, could account for most PCT-levels observed in septic patients. Since the time of publication, no other convincing source of PCT has been reported. But the question remains which cell type within the liver (e.g. hepatocyte, endothelial cell or Kupffer cell) is the source of PCT. The characteristics of PCT as indicated in the table are intermediate between cytokines and acute phase proteins.

In experiments in Syrian hamsters, Nylen [41] found that PCT exacerbates mortality in experimental sepsis. The methodology of this study has been criticized [42] on several points. The investigators used non-recombinant human PCT in hamsters, and it was inhibited by goat anti-calcitonin antibodies. Thus the effects observed may not necessarily be due to PCT. Analogous to the debate if some cytokines are pro-inflammatory or anti-inflammatory, the fact that very high levels of PCT are associated with poor outcome does of course not automatically imply that PCT is 'bad' or pro-inflammatory. Related to calcitonin's actions, it has been suggested that PCT could play a role in osteoclast activity [43]. Maybe PCT is neither a cytokine, nor an acute phase protein. PCT could also simply have no function, or it may be a useless by-product.

**Clinical value of determining cytokines, acute phase proteins and PCT**

In clinical practice CRP has generally substituted the ESR as a marker of inflammation, although for some acute diagnostic problems and especially chronic diseases clinicians still use the ESR [44]. The direct measurement of cytokines, such as IL-6 has limited practical applicability. Due to the short half-life of cytokines (minutes to hours) relatively infrequent point measurements are only of limited value in assessing the area under the curve. Nevertheless it has been shown that high IL-6 levels are related to mortality in sepsis patients, and IL-6-levels >1000 pg/ml have been used as a criterion to enroll patients in the anti-TNF intervention trials.
The published evidence indicates that the overall response of parameters of the acute phase response appears to be one-dimensional in the sense that the extent of the overall inflammatory stimulus determines the height of both cytokine levels and APP’s. A local infection will hardly result in practically measurable cytokine levels and may give a discrete elevation of CRP. A major burn will result in elevated cytokine levels and a large increase in CRP and other acute phase proteins. Sepsis can result in extreme increases of cytokines and maximal levels of acute phase proteins. According to this view daily CRP-determinations will inform the clinician on the extent of ongoing inflammation. As reported in chapter 6, PCT achieves half-maximal levels 12 hours earlier than CRP. This can be a very important clinical advantage of PCT since in acute situations the clinician is informed 12 hours earlier on the extent of the systemic inflammation present.

Whatever may be the ultimate role of PCT in addition or even instead of CRP, it appears of little interest to pursue a role for SAA as an inflammatory marker [45]. SAA dynamics may be somewhat more pronounced than CRP-dynamics, but both SAA and CRP display a time to half-maximal levels of 20 hours, as opposed to only 8 hours for PCT [38]. Thus measuring SAA levels appears to present little additional information when PCT and CRP levels are known.

Open questions on the role of PCT

- PCT shows an extraordinary rise after severe inflammation. This rise may be more pronounced than that of any known acute phase protein. Baseline levels of PCT appear to be below 0.01 µg/L [46,47]. To fully define its dynamic range, reliable baseline levels of PCT in healthy persons should be determined.
- Does most PCT originate in the liver? Is the endothelial cell the source of PCT, because inducing PCT in hepatocytes and myeloid cells has not succeeded?
- Can endotoxin reproducibly induce PCT in liver slices? If so, this might represent an unique in vitro inflammatory model. If cytokine-induction is a prerequisite for PCT-synthesis, this would be a two stage system (stage 1: endotoxin induces cytokines; stage 2: cytokines induce PCT).

Platelet counts

Causes of secondary (relative) thrombocytopenia

We found that patients who do not survive after admission to a surgical ICU have a blunted or absent secondary increase in platelet counts [48]. Data on the development of platelet counts in medical ICU patients in our hospital indicated that these patients also show a relation between platelet count and outcome [49]. Bleeding had only a minimal overall contribution to this phenomenon in both groups. Bone marrow synthesis in these patients appears to be more than adequate [50]. Probably very few of the surgical ICU patients (chapter 8) and few of the
EURICUS-patients (*chapter 9*) had a compromised bone marrow before ICU-admission. Besides, leukocyte counts in the non-survivors were at least as high as in survivors [48], also not indicative of marrow failure. Thus drops in platelet count, or inappropriately low platelet counts, very probably result from sequestration. We interpret this sequestration of platelets as a reflection of ongoing disease activity, i.e. SIRS or infection. The prognostic importance of platelet counts led to its inclusion (with the exclusion of the leukocyte count in some cases) in ICU severity scores such as the SOFA-score [51].

Two main causes of platelet sequestration can be distinguished: aggregation and adhesion. Aggregation occurs in the process of (disseminated) coagulation. The prime target of platelet adhesion is activated endothelium or subendothelium, a process that can occur independent of coagulation. Although we believe strong arguments exist to assume that platelets adhere to activated endothelium that is associated with systemic inflammation, as also expanded on elsewhere in this thesis, some alternative hypotheses exist.

*Alternative explanations for the disappearance of platelets*

Gando [52,53] contends in an number of studies that DIC with platelet consumption is nearly always present in several critically ill patient groups. But others found only in 40% of the thrombocytopenic surgical ICU patients proof of coexisting DIC. In a liver transplant model extensive adherence of solitary platelets to apoptotic sinusoidal endothelial cells in the absence of (occlusive) clots was detected and even visualized [54]. Tissue factor (TF), the generally accepted starting point of the (extrinsic) coagulatory cascade has been detected in elevated levels in trauma and sepsis [55] as further evidence of disseminated activation of coagulation. But TF-levels as well as changes in other coagulation parameters are relatively modest in many patients compared to the extensive disappearance of platelets from the circulation. For example platelet consumption often occurs in the absence of fibrinogen depletion [56]. Others have observed so-called hemophagocytosis in bone marrow in very small, strongly selected patients sets [57], while another study has implicated that autoimmune phenomena such as platelet associated immunoglobulins have been detected in critically ill patients [58]. The importance of the qualitative observation of hemophagocytosis is not established, and quantifying platelet immunoglobulins is methodologically difficult. Patients who underwent a liver transplantation present a special case, since in these patients thrombocytopenia is not only strongly coupled to graft function [59] but also to an increased splenic volume [60].

*Early changes in PC and methylprednisolone*

Steroids, including methylprednisolone, have been shown in many experimental models to strongly inhibit endotoxin- or trauma-induced inflammation and limit or prevent the thrombocytopenia that is seen in these models. In healthy persons high-dose steroids do not affect the platelet count [61]. But evidently it fails to affect the early deposition of platelets in trauma patients when given after within hours the trauma. Steroids have failed to affect outcome in sepsis intervention trials. Unfortunately other sepsis-intervention studies have also
not resulted in decisive benefits for other immunomodulatory agents. The fact that these studies also showed no impact on platelet counts or subscores based on the platelet count underscores the apparently important relation of platelet counts and survival.

**ΔPC/Δt as a marker**

Recently a study was published that related the incidence of thrombocytopenia in two medical ICUs with ICU mortality [62]. After admission, nadir platelet counts below $150 \times 10^9$/L or a decrease of more than 50% was associated with higher death rates with an odds ratio of 6.0 (CI 3.0-12). The occurrence of thrombocytopenia had more predictive power than either APACHE-II, SAPS II and MODS scores. The study did not address the relevance of a blunted rise in platelet counts. In chapter 9 we comprehensively studied time-dependent changes in PC. In was found that $\Delta PC/\Delta t$ after day 2 had a stronger relation with outcome than early changes in PC or nadir PC, thus pointing to the importance of day 2 as a ‘turning point’ with regard to platelet counts.

**Open questions on time-dependent changes in platelet counts.**

- Based on periodic recordings of the ESR (or CRP) and platelet count [63], one could try to deduce the ‘true individual normal’ platelet count in patients with chronic inflammatory diseases. The platelet count that is measured at times when ESR and CRP are low could be assumed to be normal for that particular individual. The individual $\Delta$ platelet count could subsequently be analyzed in relation with elevated ESR and CRP levels.
- Where do the platelets go to, when platelet consumption is observed during SIRS or sepsis? In one study that imaged labeled platelets in critically ill patients [68], the intestinal organs showed the highest platelet activity. It would be interesting to know if the homing behavior of the platelets is associated with clinical organ damage, for example acute renal failure or ARDS.
- What are the clinically relevant receptors and ligands in platelet - endothelial cell interaction in critically ill patients (in the absence of DIC)? In cardiovascular medicine it has recently been proven that systemic inflammation is an important independent risk factor for ischemic events [69,70], underscoring the clinical relevance of the relation between inflammation and coagulation. The great success of glycoprotein IIb/IIIa receptor (GP-IIb/IIIa) inhibitors in the CCU is [71] might in turn also be related with the anti-inflammatory effects of inhibiting GPIIb/IIIa. The P-selectin receptor, that is expressed by activated endothelial cells, has an important role both in leukocyte adhesion and in fibrinogen-independent platelet-adhesion [69]. Sindram has suggested that P-selectin is important in mediating reperfusion injury in transplanted livers [54].
- Is the platelet endothelial interaction (adhesion) always irreversible or is it under some conditions still reversible? Maybe marginated platelets, analogous to leukocytes, can reenter the circulation. Both in *in vitro* and *in vivo* [73] experiments this phenomenon has been observed. The spleen is an example of a microenvironment where platelets are reversibly segregated from the circulation. If platelet re-entry is relevant, part of the
effects of potential agents that selectively block platelet-endothelial interaction might be reflected by increases in platelet counts.

The ideal inflammatory marker

After discussion of the merits of the several parameters that were studied as markers of inflammation and disease severity, it is useful to define the “ideal” inflammatory marker as a thought experiment. What should be its properties?

It must have very low values in healthy persons. It increases rapidly once an inflammatory state develops. Its increase is proportional to the extent of the underlying inflammatory process - for example proportional to the total amount of damaged tissue or the total quantity of cytokines produced. After the marker has rapidly increased to this proportional level, it predictably decreases. The half-time should be around 1 day - a convenient unit of time in clinical practice. The marker can be determined for little cost in serum or plasma and is not sensitive to in vitro changes during procurement or storage.

Figure 10.1. Comparison of levels of ideal inflammatory marker (solid line) with levels of cytokine (dashed line) after an acute inflammatory event. An ideal marker rises rapidly after inflammation in a manner proportional to the inflammatory stimulus. When the inflammation disappears, the marker decreases with a half-life of one day. The cytokine rises rapidly but also decreases rapidly.
Indicators of inflammation and severity - Procalcitonin and the platelet count.
On the basis of the formulated profile of the ideal inflammatory marker, procalcitonin probably comes closest to these requirements. Disadvantages of PCT as an inflammatory marker may be blunted responses in neutropenia [74] and interindividual differences that are larger than for CRP [75]. The combination of CRP and PCT may turn out to be a particularly effective indicator of the inflammatory state and the possibility of bacterial infection.

It is important to note that a marker of inflammation is not the same as a marker of severity. A good marker of severity should be associated with outcome (i.e. mortality, hospital stay), whereas a marker of inflammation reflects the amplitude of the inflammatory response as such, regardless of the impact this inflammatory response has on ultimate outcome. Well-known examples of markers of severity are the severity scores: Acute physiological and chronic health evaluation (APACHE-II) [76], injury severity score (ISS) [77] or multiple organ dysfunction score (MODS) [78].

Changes in platelet counts (nadir platelet count and ∆PC/∆t) are also strongly related to outcome. The fact that ∆PC/∆t after ICU-day 2 is as good a predictor of outcome as the APACHE-II score in surgical ICU patients [48] emphasizes that platelets are deeply related to outcome. Correlating platelet count with PCT and mortality may show to what extent the platelet count reflects inflammation and organ dysfunction respectively.

Interactions of acute systemic inflammation with other physiological systems.
Many interactions between the inflammatory system and other (patho)physiological systems have been uncovered. Except where platelets and coagulation are concerned, these systems were not subject of this thesis. To illustrate that inflammation has connections with many

![Figure 10.2. Comparison of CRP (dashed line) with ideal inflammatory marker (solid line).](image)
other important physiological processes some examples:

- Inflammation ↔ specific immune response:
  Example: induction of antigen specific immunoglobulin and T-cell responses

- Inflammation ↔ stress system
  Examples: Astronauts produce increased levels of IL-6 during launch and landing [79]; exercise induces cytokines [80] and acute phase proteins [81]

- Inflammation ↔ central nervous system
  Example: inflammation and IL-6 induce sleep [82]; sleep induces IL-6 [83]

- Inflammation ↔ metabolism
  Example: down-regulation of albumin and induction of cachexia by inflammation [84,85,86]

**Systemic inflammation in health and disease**

To summarize, in this thesis several aspects of acute systemic inflammation were studied. FES is a special form of systemic inflammation in which fat globuli are involved. Why this response occurs only in a small subset of patients with long bone fractures and why it does not occur in the vast majority of apparently similar patients has only partly been clarified. SIRS with it’s associated increases in cytokines, PCT and acute phase proteins, is a universal response in patients who are critically ill, whether due to trauma, surgery or infection.
Sequestration of platelets accompanies SIRS initially in virtually all patients, and secondarily in many patients with complicated courses. For a number of reasons, PCT and the platelet count stand out for their diagnostic and prognostic value compared to many other parameters. Although we do only partly understand why this is the case, further investigation into these parameters must lead to phenomena central to the outcome of critically ill patients.

REFERENCES


51. Vincent et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multi-center, prospective study. Working group on "sepsis-


