Summary, general discussion and conclusions
Chapter 1 introduces the concept of systemic inflammation after major surgery. The understanding of (systemic) inflammation in the context of surgery throughout previous centuries is described. Chapter 1 also describes the two patient groups studied: patients who had undergone orthotopic liver transplantation (OLT) on the one hand, and patients with ruptured abdominal aortic aneurysms (RAAA) on the other. The concept of the Systemic Inflammatory Response Syndrome (SIRS) is reviewed, and the role of monocyte function after OLT and RAAA is explored.

In chapter 2 the pathogenesis of sepsis is described with a focus on the role of monocytes, immunomonitoring and treatment. In this review article, the two phase theory of sepsis is presented. The first phase represents the inflammatory response leading to the activation of monocytes with a systemic release of pro-inflammatory cytokines like TNF-α, IL-1 and IL-6. The first phase is generally described as the Systemic Inflammatory Response Syndrome (SIRS). The second phase represents the anti-inflammatory phase with the release of anti-inflammatory cytokines as IL-10, and is referred to as the Compensatory Anti-inflammatory Response Syndrome (CARS). Despite enormous effort, most randomised controlled trials investigating a single drug-intervention in sepsis did not show an improvement in survival. The intervention drugs used in these trials were almost always designed to counteract the inflammatory response, which is the first phase. The anti-inflammatory response may already have been in effect at the time the drugs were administered, which may account for the lack of successful intervention seen in these studies. In this chapter we elaborated on the role of HLA-DR expression on monocytes as a parameter for the inflammatory response. A normal or high HLA-DR expression on monocytes corresponds with SIRS while a low HLA-DR indicates CARS. The expression of HLA-DR may be used as an indicator for immunomodulation. In patients exhibiting a predominantly systemic inflammation, anti-inflammatory interventions may improve prognosis, while patients with sepsis and low HLA-DR expression may benefit from immunostimulation.

Sepsis following orthotopic liver transplantation (OLT) is a complication that causes significant morbidity and mortality. Chapter 3 describes a prospective
Summary, discussion and conclusions

observational study of 20 adult OLT patients. HLA-DR expression on monocytes was monitored during the first 4 weeks after transplantation. The seven patients who developed sepsis had a significantly lower HLA-DR expression on monocytes, both before and after they developed sepsis. In the two patients with sepsis who died, HLA-DR expression remained low, while in the five septic patients who recovered, HLA-DR expression returned to normal values. The patients who developed sepsis had received significantly more prednisolone than the patients who did not. Incubation with prednisolone in vitro lowered the expression of HLA-DR in a dose-dependent manner. Low HLA-DR expression on monocytes appears to be a reliable risk marker for impending sepsis in OLT patients. The level of risk may be, at least in part, related to the dose of prednisolone. If HLA-DR is treated as a risk indicator for sepsis, treatment aimed at improving monocyte function or a reduction in steroids should be promptly initiated in those patients who show a low expression of HLA-DR to prevent the development of sepsis.

Laboratory values are routinely determined after the surgical repair of a ruptured abdominal aortic aneurysm (RAAA). In chapter 4 we present the results of a retrospective analysis of the course of all routinely determined laboratory values in 290 RAAA patients. The purpose of the study was to describe the ‘normal or benign’ course of RAAA patients who survive until hospital discharge and to identify factors that distinguished them from the non-survivors. Both the survivors and the non-survivors showed a wide range of laboratory abnormalities after surgery. The laboratory results were grouped into six categories (haematology and coagulation, systemic inflammation, metabolic, renal, liver, and electrolytes). The categories we chose are strongly related to the causes of death. For example, the patients who die of massive haemorrhage usually do so within the first 24 hours following surgery, whereas systemic inflammation and MOF develop more than 24 hours after surgery. We believe that recognition of the associated laboratory abnormalities is important as it may help in the early prediction and identification of post-operative complications.

Chapter 5 describes systemic inflammation and HLA-DR expression on monocytes in RAAA patients in a prospective observational study. Thirty patients were
included and analysed consecutively. The primary outcome parameter was hospital mortality. Mortality was higher in older patients, patients with a higher APACHE-II (Acute Physiology and Chronic Health Inquiry) score, and the vital need for suprarenal clamping. The Sequential Organ Failure Assessment score (SOFA) was significantly higher in non-survivors from day 1 through day 14 after surgery. IL-6 and IL-10 were transiently higher on days 1 and 3 in non-survivors. HLA-DR expression on monocytes dropped after surgery in both survivors and non-survivors. However, in non-survivors HLA-DR expression was significantly lower from day 3 through day 14 after surgery compared with the survivors. Low HLA-DR expression was associated with multiple organ failure and death, although death from secondary infections was not seen in our patients. We conclude that shock and surgery lead to an overwhelming inflammatory response which sets the stage for fatal multiple organ failure.

Rapid surgical intervention is one of the most important principles in the clinical management of patients with RAAA. Chapter 6 describes the importance of a multidisciplinary approach with clear protocols and dedication to the treatment of these patients. Our treatment protocol consisted of rapid transport by ambulance to the emergency department while accepting a low systolic blood pressure. During transport, the hospital was notified of imminent arrival of a patient with a possible RAAA. Only three of the 126 patients with ruptured abdominal aortic aneurysms were denied surgery. The average intrahospital delay was 25 minutes. Hospital mortality was 33%. Despite our liberal policy of offering surgery to almost every patient, hospital mortality was comparable to the best reported in the literature.

The multicentre aneurysm screening study group has investigated the potential benefit of screening men aged 65-74 years for an abdominal aneurysm. In chapter 7, we emphasize that guidelines for the prompt treatment of ruptured aortic aneurysms should also be included when screening programs are implemented. Patients who develop a ruptured AAA despite the presence of a screening program should not be denied treatment due to an overly fatalistic view on their outcome. In our opinion, immediate surgery for RAAA patients is as cost effective as a screening program.
GENERAL DISCUSSION AND CONCLUSIONS

Our results show that both OLT and RAAA patients develop an intense inflammatory response and depressed HLA-DR expression after surgery which is associated with high morbidity and mortality. Therefore, understanding the inflammatory response after major surgery is relevant and may provide opportunities to improve outcome in these patients.

The implications for understanding the pathophysiology of SIRS

The previously postulated SIRS/CARS sequence of events is depicted in the left panel of figure 1. A catastrophic event causes an inflammatory reaction that is followed by a counter-inflammatory response that theoretically should restore the balance of health. We also see these two phases in the OLT patients’ inflammatory response, but here they occur against the background of immunosuppression present as a result of pre-existing illness and immunosuppressive drugs.

**Figure 1.** left panel the ‘normal’ pro- and anti-inflammatory response, illustrated by a mildly enhanced pro- and subsequently a counter-inflammatory response which re-establishes equilibrium. The right panel shows the response in a patient with an overwhelming counter-inflammatory response which can be counteracted by early intervention (i.e. decreasing immunosuppressants).
In OLT patients, the intensity of the pro-inflammatory phase may be less intense as a result, and the counter-inflammatory phase becomes more pronounced than in other patients. This might explain the high incidence of sepsis related complications in our patient cohort. A timely reduction in immunosuppression (indicated by the arrow in the right panel of figure 1) might be effective in preventing these infections, as discussed below.

To complicate matters, our observations in RAAA patients seriously challenge the SIRS/CARS concept of events. We did not see the development of any opportunistic infections which are the classical clinical manifestation of immunodeficiency. Instead, patients died from the complications of multiple organ failure which developed almost immediately after surgery. Additionally, our data (and those of others) show that the pro and counter inflammatory responses co-exist from the start (136,171,172). This is represented in figure 2.

Whether these two types of inflammatory response contribute equally to the development of multiple organ failure is not clear. This warrants further research as it has important consequences for intervention.

![Figure 2](image)

**Figure 2:** The co-existing pro- and anti-inflammatory response.

*Diagnostic and prognostic aspects of monocyte HLA-DR expression*

Previously, HLA-DR expression on monocytes was measured to predict septic complications after major surgery, trauma, severe burns, and for patients on a
ventricular assist device awaiting cardiac transplantation (23-27,62). Most studies showed that a persisting low HLA-DR expression was associated with poor outcome (79,136,142,173-176), however a few others could not confirm these results (177,178). These latter 2 studies measured HLA-DR expression on monocytes only during the first 2 post-operative days. Our studies on OLT and RAAA patients confirmed that HLA-DR expression on monocytes is an accurate predictor of outcome. Low HLA-DR expression on monocytes was present 1-8 days before the onset of clinical symptoms in all 7 patients who developed sepsis, and remained low in the 2 patients who died from sepsis. RAAA patients with a low HLA-DR expression on monocytes had a very poor prognosis. Non-survivors could be identified by a significantly lower intensity of HLA-DR expression on monocytes as early as day 3, and preceding death by a median of 8 days.

The usefulness of monocyte HLA-DR expression, at least in RAAA patients, is somewhat limited by our finding that 3 days were required before a distinction between survivors and non-survivors could be made with any degree of statistical significance. This observation was confirmed by Monneret et al, who showed that HLA-DR expression on monocytes is significantly lower in the non-survivors after 3-4 days (175).

Laboratory markers such as lactate levels, platelet counts, CRP, pro-calcitonin, or cytokine levels also show marked differences between survivors and non-survivors. It remains to be determined which of these will serve as the most useful prognostic markers, and whether different markers will be required for different disease processes.

Mechanism(s) of decreased HLA-DR expression
It is not known which pathophysiological mechanism is responsible for the (low) HLA-DR expression on monocytes; for example signal transduction or mRNA secretion might be important. A study by Fumeaux et al showed that IL-10 decreases HLA-DR expression. In patients with septic shock, this is probably due to re-endocytosis of HLA-DR and intracellular sequestration (179).
Our studies did not clarify whether low HLA-DR expression is “just” a clinically useful epiphenomenon or whether it constitutes a relevant element in the pathophysiologic chain that underlies SIRS. In the first theory, monocytes might be useful as surrogate markers in (immuno-)intervention studies (which by itself is important given the large numbers of patients required in randomised studies having survival as outcome parameter). In the second theory, monocytes (or their HLA-DR expression) might constitute a target for intervention.

Therapeutic implications of monocyte HLA-DR expression

Our observations in OLT patients strongly suggest that it is the combination of chronic illness, major surgery and immunosuppression that predisposes the patients to septic complications. While the first two of these factors cannot be changed, the intensity of immunosuppression can be easily adapted. The potential impact of such changes is illustrated by our observation that in vitro, prednisolone decreases HLA-DR expression on monocytes in a dose-dependent way.

**Figure 3.** The abrogated pro- and anti-inflammatory response by early intervention.

Consequently, monitoring HLA-DR expression provides us with the opportunity to decrease immunosuppression pre-emptively in OLT patients at a high risk of developing sepsis in an effort to prevent this complication and improve outcome. Our findings justify undertaking a prospective, randomised study to determine whether the lowering of immunosuppression guided by low monocyte HLA-DR expression decreases the incidence of septic complications. Indeed, our
observations on the effect of steroids on HLA-DR expression provides an argument for low-dose prednisolone or even steroid-free immunosuppression regimens that may be associated with a lower incidence of sepsis (180,181).

The therapeutic consequences of low HLA-DR expression in other categories of patients such as those with RAAA are more difficult to envisage. As pro- and counterinflammatory responses co-exist, immuno-intervention should probably be aimed at lowering both these two responses. Intuitively it might be disadvantageous to “block” inflammation, thereby leaving counter-inflammation unopposed. Measures directed at both components of the response might be more beneficial (Figure 3).

After Ronco et al. showed that a high ultrafiltration rate leads to a better prognosis in ICU patients with renal failure (182), the early initiation of renal replacement therapy in septic ICU patients became more accepted (183-186). Other investigators showed that high cutoff haemofiltration leads to lower levels of IL-6 and IL-1ra, which had a beneficial effect on leukocyte proliferation, polymorphonuclear function, and the amount of norepinephrine administered (187-189). Furthermore, selective extracorporeal immunoabsorption lowers LPS, IL-6, and C5a levels, and leads to an increase in HLA-DR expression on monocytes. However, the effect on outcome in patients with sepsis has not yet been established (190).

Hydrocortisone suppletion might also be beneficial, as a significant number of RAAA patients have relative adrenal insufficiency (153). Hydrocortisone restores haemodynamic stability and modulates the immune response and probably improves prognosis in patients with septic shock (151,152,191-193). One can speculate that this effect also holds for RAAA patients. In a subgroup of RAAA patients even high dose steroids might be beneficial. However, after the negative results of high dose steroids in patients with sepsis and the significant increase in secondary infections in the group of patients receiving methylprednisolone no more trials with high dose steroids were conducted (194). Nevertheless, hypothetically there may be a two group phenomenon. One group would have a relatively low pro- and anti-inflammatory response, and the second group would display a high inflammatory response. The first group would have
a good prognosis anyway, and would not benefit from the administration of methylprednisolone as they are less susceptible to multiple organ failure to begin with. The second group, which has the massive immune response may benefit from the administration of high dose steroids to mitigate that response. To our knowledge, these two groups have not been separately explored in the literature. It would be worthwhile to investigate a two group model, although more evidence supporting this theory would first be necessary before a study of high dose steroid treatment for patients with sepsis can be re-initiated.

Prevention of RAAA

In order to improve prognosis for RAAA patients, the benefits of screening for AAA need to be evaluated. The MASS study showed that screening elderly men for AAA reduced AAA-related mortality (54). However, it is unclear if screening remains beneficial when all the family members of an AAA patient are screened. Furthermore, the prevention of AAA related deaths may just change the cause of death in elderly patients without delaying it, i.e. a lower AAA related mortality may lead to more death from myocardial infarction and cancer (195). Meticulous surgical technique and increasing experience with endovascular repair for RAAA patients will likely also improve outcome. Hopefully this will be confirmed in a randomised controlled trial (196). In the meantime liberal and immediate surgery for RAAA patients is probably the best treatment for these patients (35).

HLA-DR expression on monocytes is an important and relevant parameter in patients following liver transplantation and ruptured abdominal aortic aneurysm repair. Persistent low HLA-DR expression is associated with a high mortality in several patient groups. In this situation, OLT patients may benefit from a reduction in immunosuppressive medications, especially corticosteroids. In patients who have undergone RAAA repair, the low HLA-DR expression on monocytes is the result of an overwhelming pro- and anti-inflammatory response. These patients might benefit from hydrocortisone administration in the case of relative adrenal cortical insufficiency or
early high-volume venovenous haemofiltration. More knowledge on the intracellular mechanism responsible for modulating HLA-DR expression on monocytes is necessary to make individualised immunointervention treatment a success.