Part IV

General Discussion
Summary, general discussion and future perspectives
SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In this thesis we have focused on the pharmacological treatment of acute respiratory distress syndrome (ARDS) and have tried to gain more insight into one of the important contributors to the development of ARDS: transfusion of red blood cells, fresh frozen plasma and platelets. Numerous clinical trials have evaluated different types of pharmacological interventions [1,2]. In the first part of this thesis, we have focused on the potential use of specific anticoagulants in frequently occurring clinical syndromes (sepsis and ARDS) in the intensive care unit (ICU), where the final common pathway consists of coagulation activation and impaired fibrinolysis. The second part encompasses preclinical trials. In different animal models of pneumonia, the efficacy of several locally applied anticoagulants and fibrinolytic agents was evaluated. The third part consists of clinical studies. We evaluate the effects of systemically administered recombinant human activated protein C (rh-APC) in patients with ARDS as a single-organ failure. Furthermore, we retrospectively investigated the pulmonary effects of blood transfusion in critically ill patients, and prospectively in patients having undergone cardiac surgery.

CHAPTER 2

In Chapter 2, we reviewed the effects of heparin and heparin-like compounds on coagulation and beyond. We addressed the properties of heparin as a chemical compound, an anticoagulant and an anti-inflammatory drug. Heparin may have important immune-modulating properties and anti-inflammatory properties that could relate to the inhibition of thrombin formation. Additionally, heparin may play a modulating role in vascular adherence, migration and activation of leukocytes by downregulation of several cellular adhesion molecules and reducing complement activation. Although conflicting, pro-inflammatory actions of heparin have been described as well. However, heparin treatment in animal sepsis models appeared to be beneficial. We performed a meta-analysis of animal sepsis survival studies available at the time and found an overall survival benefit in the animals treated with heparin. In post-hoc analyses of the placebo arms of large multicenter studies, where some patients received (low molecular weight) heparin as thromboprophylaxis and others did not, there seemed to be a trend towards improved survival in patients treated with (low molecular weight) heparin. At that time, a randomized clinical trial (HETRASE trial) to evaluate this concept, was underway [3].

CHAPTER 3

We reviewed the potential of activated protein C (APC) in modulating the pro-inflammatory and procoagulant state for enhancing pulmonary endothelial barrier function in animal models and human ARDS. We underlined the extensive crosstalk between inflammation and coagulation, occurring not only in sepsis, but in ARDS as well. At that time, APC was the only biological agent that reduced mortality in patients with severe sepsis, many of them having pneumonia...
or ARDS. Therefore, APC might be a rational choice to address coagulopathy underlying ARDS. The anticoagulant, anti-inflammatory, anti-apoptotic and pro-fibrinolytic properties of APC were discussed as well as the direct effect of APC on endothelial barrier enhancement. Numerous animal models of ARDS were discussed, where treatment consisted of intravenous APC. Most studies found an attenuation of transcapillary albumin leakage, lung wet dry ratios or extravascular lung water (all markers for endothelial permeability) and reduction of inflammatory markers in animals treated with intravenous APC. In human ARDS, post-hoc analyses of the PROWESS trial suggested that patients with severe pneumonia had a faster resolution of pulmonary dysfunction and increased survival if treated with APC [4]. At that time, there was one randomized clinical trial evaluating the effect of intravenous APC on the course of human ARDS [5]. In this trial, APC did not seem to improve outcome. Nevertheless, for a number of reasons this trial was inconclusive. Considering the pathophysiology of ARDS - damage to alveolar epithelium and vascular endothelium, defective barrier function, activation of coagulation and inflammation – we concluded that APC appeared to be the best candidate to be effective in the pharmacological treatment of ARDS.

Chapter 4

In Chapter 4 we hypothesized that local treatment (i.e. nebulization) with the anticoagulants antithrombin (AT), APC, heparin and danaparoid would be equally effective as systemic treatment in attenuating pulmonary procoagulant changes. Furthermore, we hypothesized there could be anti-inflammatory and antibacterial effects. Pneumonia was induced by intratracheal instillation of Streptococcus pneumoniae in Sprague-Dawley rats. The rats were randomized to treatment with AT, APC, heparin, danaparoid or saline 0.9% (n=7 per group). Healthy animals without pneumonia served as controls (n=4). We found a reduction of coagulation activation in the pulmonary compartment in the animals treated with AT, APC, heparin and danaparoid. Additionally, the effects of nebulized APC, AT and heparin were restricted to the pulmonary compartment, whereas nebulized danaparoid affected systemic coagulation as well. AT also reduced bacterial outgrowth, thereby possibly exerting an (indirect) lung-protective effect. These data support the hypothesis that locally applied anticoagulants can alter procoagulant changes in the pulmonary compartment following pneumonia.

Chapter 5

In this chapter, our hypothesis was similar as the one described in chapter 4, namely that local treatment with the anticoagulants antithrombin (AT), APC, heparin and danaparoid would be effective in attenuating pulmonary procoagulant changes. In this series of experiments, pneumonia was induced by intratracheal instillation of Pseudomonas aeruginosa in Sprague-Dawley rats. The rats were randomized to treatment with AT, APC, heparin, danaparoid or saline 0.9% (n=7 per group). Healthy animals without pneumonia served as controls (n=4). We found that nebulized
AT and APC potently inhibit coagulation activation. However, both AT and APC affected systemic coagulation, suggesting leakage from the alveolar compartment into the circulation. Heparin and danaparoid did not modify pulmonary fibrin turnover in these experiments. There were no changes in pulmonary inflammation or bacterial clearance from the respiratory tract, in spite of marked anticoagulant effects of AT and APC in the lung.

**Chapter 6**

In the two previous chapters, we used anticoagulant agents aiming to ameliorate pulmonary coagulopathy. In Chapter 6 we investigated the effect of nebulized fibrinolytic agents on pulmonary coagulopathy, for which we used recombinant tissue-type plasminogen activator (rt-PA) and anti-plasminogen activator inhibitor 1 (PAI-1) antibodies. Two models of lung injury were used in Sprague-Dawley rats: a direct model where *Pseudomonas aeruginosa* was intratracheally administered and an indirect model where the animals were challenged with intravenous lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4. We found that nebulization of both rt-PA and anti-PAI-1 increased fibrinolysis in the pulmonary compartment in both models of lung injury. Nonetheless, neither rt-PA nor anti-PAI-1 affected inflammation or bacterial clearance from the respiratory tract. Systemic fibrinolysis, however, was increased as well. Again, suggesting leakage from the alveolar compartment into the circulation.

**Chapter 7**

In Chapter 7 we described a sub study of the INFALI (INFectious of INFlammatory Acute Lung Injury/acute respiratory distress) study: a multicenter open-label randomized controlled trial of patients with ALI/ARDS. When we initiated this study, no data existed on the effects of an infusion of APC on the pulmonary coagulopathy present in patients with ARDS. We hypothesized that administration of intravenous APC would attenuate this coagulopathy which could be beneficial to these patients. Patients were randomized to receive intravenous APC (24 mcg/kg/hr during 96 hours) or placebo. Patients with (severe) sepsis were excluded. In 27 patients serial non-directed bronchoalveolar lavage fluid (NBLF) samples were obtained; 16 patients were treated with APC and 11 patients were treated with placebo. Infusion of APC resulted in higher plasma levels of APC during the infusion period, and higher levels of APC in NBLF, when compared to placebo. Furthermore, systemic administration resulted in reduced activation of coagulation and enhanced fibrinolysis in the alveolocapillary compartment. In this sub study administration of APC also decreased the lung injury score and the simplified acute physiology score on day 5 when compared with baseline. We concluded that administration of intravenous APC in patients with ARDS resulted in attenuation of pulmonary coagulopathy and injury.
The results of the INFALI study, a multicenter open-label randomized controlled trial of APC versus placebo in patients with infectious or inflammatory ALI/ARDS, were reported here. A key factor in the pathogenesis of ARDS is alveolocapillary inflammation, leading to endothelial barrier dysfunction and increased permeability. The latter can be assessed at the bedside with help of the non-invasively measured pulmonary leak index (PLI) of $^{67}$Gallium($^{67}$Ga)-transferrin [6]. We hypothesized that administration of intravenous APC would attenuate the increase in pulmonary vascular permeability and would therefore be beneficial to patients with ARDS. Patients were randomized to receive intravenous APC (24 mcg/kg/hr during 96 hours) or placebo. 71 patients were enrolled, 33 patients were assigned to APC and 38 to placebo. Unfortunately, the study was prematurely ended because APC was withdrawn from the market and no longer commercially available. The most frequent reason for inclusion was pneumonia. With regard to disease severity, groups did not differ. Nearly all patients needed invasive mechanical ventilation. At baseline, the PLI was increased in 87% of patients. There were no differences between groups in the course of ventilator pressures, tidal volumes, gas exchange and oxygen requirements. After the treatment period there was no difference in PLI between groups. Nor did we find a difference in the number of ventilator-free days or mortality. There were no bleeding complications, yet two pneumothoraces occurred, one in each group. We concluded that a 4-day course of intravenous APC did not ameliorate the increased permeability and clinical course in critically ill patients with ARDS, mostly caused by pneumonia. However, this study was likely to be underpowered. Firstly, because of the stringent exclusion criteria we applied in order to prevent bleeding complications. Secondly, because APC was withdrawn from the market.

Another clinical syndrome in which increased pulmonary vascular permeability is of pivotal importance in its pathogenesis is transfusion-related acute lung injury (TRALI). Epidemiologic studies suggest that even non-massive red blood cell (RBC) transfusions may be an independent risk factor for TRALI and resultant mortality [7]. In this chapter, we performed a retrospective analysis on critically ill patients without overt bleeding in the ICU of a university hospital. Our hypothesis was that RBC transfusions may induce subtle pulmonary changes, reflecting a subclinical form of TRALI, which may go unseen and therefore unreported. We analyzed 83 patients from a 5-month period, who had received at least 1 RBC unit and stayed at least 24 hours in the ICU. The $\text{PaO}_2/\text{FiO}_2$ (PF) ratio dose-dependently decreased, whereas the lung injury score dose-dependently increased. Both returned to baseline 48 hours after transfusion. The total number of RBCs given in the ICU did not directly contribute to ICU- and 1-year mortality. We concluded that the effects of RBC transfusion in non-bleeding critically ill patients are subtle and may represent subclinical TRALI, but do not adversely affect outcome.
Chapter 10
In Chapter 10, we performed a prospective cohort study in two university hospital intensive care units on patients who had undergone cardiac surgery. There is an association between blood transfusion and pulmonary complications following cardiac surgery [8]. We hypothesized that factors contributing to these complications may include antibodies or bioactive lipids, which have been implicated in TRALI. We measured the PLI, within 3 hours postoperatively, in cardiac surgery patients who had received no, restrictive (one or two transfusions) or multiple (five or more transfusions) transfusions (n=20 per group). Transfused blood products were screened for bioactive lipid accumulation and the presence of antibodies. The PLI was elevated in all groups after cardiac surgery, but transfused patients had a higher PLI than non-transfused patients. The amount of RBCs, but not fresh-frozen plasma or platelets was associated with an increase in PLI. Surgery risk and time on cardiopulmonary bypass did not influence the risk of enhanced pulmonary leakage. We concluded that blood transfusion during cardiac surgery is associated with an increase in pulmonary vascular leakage, an effect that is dose dependent for RBC products. There was no association between the level of bioactive lipids or the presence of specific antibodies and increased pulmonary vascular leakage.

General Discussion
As stated before, several clinical trials have evaluated different types of pharmacological interventions in ARDS, however none of these interventions resulted in clinically beneficial effects [1,2]. In this thesis we have evaluated the potential benefits of locally administered anticoagulants and fibrinolytic agents in animal models of ARDS, as well as the systemic administration of the naturally occurring anticoagulant APC, in patients with ARDS. The INFALI Study did not demonstrate unequivocal beneficial effects in patients treated with a 4-day course of intravenous APC: we did not find a survival benefit, nor a reduced number of days on the ventilator for the patient group as a whole. There was, however, a trend towards improved survival in patients treated with APC when ARDS originated from pneumonia, which became statistically significant when alveolocapillary permeability was increased at the start of APC infusion. However, these are post hoc analyses, which should be interpreted with caution. In our sub study of the INFALI Study we demonstrated that infusion of APC resulted in higher plasma levels of APC during the infusion period, and higher levels of APC in NBLF, when compared to placebo. Additionally, we demonstrated that systemic administration resulted in reduced activation of coagulation and enhanced fibrinolysis in the alveolocapillary compartment. These findings are proof of the concept that systemic administration actually affects coagulopathy locally, i.e. in the pulmonary compartment.
An important downside of systemically administered anticoagulants is the risk of (potentially life-threatening) bleeding. To avoid this risk, there might be benefit in nebulizing anticoagulants
into the compartment where they are supposedly needed the most, which are the lungs. The animal studies in this thesis support the concept that the intrapulmonary procoagulant state can be affected by nebulization of anticoagulants, but this does not seem to alter pulmonary inflammation, with the exception of antithrombin in a model of *S. pneumoniae* pneumonia. However, reduction of inflammation should perhaps not be the objective of anticoagulant therapy. Perhaps fibrin should be, as fibrin depositions can activate neutrophils and fibroblast, reduce alveolar fluid clearance and surfactant production [9]. Notably, in spite of local administration systemic coagulation was affected as well.

The two final studies in this thesis underline the potential downsides of blood transfusion. Both retrospectively and prospectively, there appeared to be a dose-dependent relationship between RBC transfusion and increased pulmonary vascular permeability, as expressed by surrogate markers such as PF-ratios and lung injury score or by the PLI.

These studies have several limitations. The animal models we used obviously are a simplification of a complex and heterogeneous patient population. We used a pretreatment model, the anticoagulants of fibrinolytic agents were nebulized prior to the bacterial or LPS challenge. Post treatment models obviously more closely resemble the clinical situation. Additionally, no antibiotics were administered and all animals were breathing unassisted, whereas most patients with ARDS are receiving mechanical ventilation. With regard to the clinical studies, the pitfalls of a retrospective analysis are clear. The number of patients in Chapter 7 and 8 is limited, as the INFALI Study was prematurely stopped since APC was withdrawn from the market and patient enrollment was challenging because of the large number of exclusions due to prior use of therapeutic anticoagulation. This resulted in underpowering of the study. Furthermore, the populations studied were heterogeneous, reflecting clinical practice. It is conceivable that in a heterogeneous population, some patients might benefit from an intervention while others might not, leading to an overall neutral or even negative result.

**Future directives**

From a pathophysiologic point of view, it seems rational to pharmacologically intervene in the pulmonary coagulopathy arising during ARDS [10]. However, some points need to be addressed. Firstly, an important limitation of previous trials in patients with ARDS is heterogeneity. There was a wide range in disease severity, primary insult (i.e. pneumonia, aspiration, near-drowning, trauma) and patient factors (i.e. age, sex, comorbidities). As pointed out earlier, while some patients in a heterogeneous population might benefit from an intervention, others might not, leading to neutral or even negative result. More specifically, post-hoc analyses of the PROWESS Study [4] and our own INFALI study (Chapter 8) indicate that patients developing ARDS following pneumonia might benefit most from an intervention with APC. A future trial should be designed to include a more selected, and more homogeneous patient population. Ideally, to ensure an adequate amount of patients enrolled, this should be a multicenter trial.
Secondly, what would be the preferred way to administer the anticoagulant. Nebulization has the theoretical advantage of local administration of the anticoagulant, without systemic side effects (i.e. risk of bleeding). However, in our animal experiments, we observed systemic effects of the nebulized anticoagulants resulting from the increased vascular permeability, with the anticoagulants leaking from the alveolar compartment into the circulation. Furthermore, the animals were breathing unassisted, whereas most patients are mechanically ventilated. There is ongoing debate as to how much of a nebulized compound actually reaches the alveolar compartment of a mechanically ventilated patient, as it is sensitive to numerous types of bias [11]. Furthermore, atelectasis is commonly occurring in mechanically ventilated patients with diseased lungs [12]. During atelectasis a lung compartment is not aerated, while blood flow is still present. Nebulized anticoagulants would by definition never reach the lung tissue distal to atelectasis. In a future trial, the preferred way of administering the anticoagulant would be intravenously, ensuring distribution throughout the lung, even the collapsed parts.
REFERENCES

9. Ware LB. Pathophysiology of acute lung injury and acute respiratory distress syndrome. Semin Respir Crit Care Med 2006;27:337-349