

REVIEW

Anticoagulants for ARDS: facts and future

A.D. Cornet¹, H.M. Oudemans-van Straaten¹, M.J. Schultz², N.P. Juffermans², P.R. Tuinman¹

¹Department of Intensive Care Medicine, REVIVE (Research VUmc Intensive Care), VU University Medical Center, Amsterdam, the Netherlands

²Department of Intensive Care Medicine, Academic Medical Center, Amsterdam, the Netherlands

Correspondence

P.R. Tuinman – email: p.tuinman@vumc.nl

Keywords – Lung injury, inflammation, coagulation, ARDS, anticoagulants, therapy

Abstract

Acute respiratory distress syndrome (ARDS) is a clinical syndrome affecting both medical and surgical patients which frequently necessitates mechanical ventilation in an intensive care unit and carries considerable morbidity and mortality. At present, there is no treatment specifically directed at the underlying pathophysiology. Central in its pathophysiology is the inflammation-induced activation of coagulation, and the resulting depletion of naturally occurring anticoagulants.

This narrative review is based on a PubMed search of relevant clinical studies published in English and discusses the role of systemic administration of activated protein C, antithrombin, tissue factor pathway inhibitor, inactivated factor VIIa and heparin in the treatment of ARDS. Clinical trials of systemic anticoagulant strategies in ARDS patients have proven unsuccessful in improving patient outcomes. We suggest some future directives.

Introduction

Acute respiratory distress syndrome (ARDS), with its mildest form previously known as acute lung injury (ALI), is an important contributor to the total number of intensive care admissions. ARDS is a clinical syndrome, affecting both medical and surgical patients, which may be triggered by direct insults to the lung (e.g., aspiration or pneumonia), but can also occur secondary to a systemic insult (e.g., sepsis or multiple transfusions) (*table 1*). Since the first description of ARDS in 1967, considerable progress has been made in understanding the pathophysiology and pathogenesis of this form of lung injury.^{1,2} Despite better insight into its pathophysiology, ARDS remains a major cause of morbidity and mortality in the critically ill, which may be as high as 30-60%.³ Survivors of ARDS experience exercise limitation, decreased physical quality of life and psychological sequelae.⁴ In this narrative review, we will discuss the pathophysiology of ARDS

focussing on the extensive crosstalk between inflammation and coagulation. Subsequently, we will discuss potential therapeutic interventions directed at pulmonary coagulation in ARDS, after which we will conclude with possible directions for further research.

Pathogenesis: extensive crosstalk between inflammation and coagulation

Inflammation and coagulation are two important host defence mechanisms against injury and infection. Inflammation and coagulation, obviously, require strict regulation. After an insult the host response must be counterbalanced by inhibition or deactivation in order to restore homeostasis. Actually, this should not come as a surprise. A perfect example is the mutual activation and augmentation of coagulation and inflammation in a fresh wound. To prevent exsanguination, clotting is instantaneously activated. However, with an injured natural barrier against micro-organisms, the role of the inflammatory response is also clear: to prevent invasion of pathogens. On the other hand, when micro-organisms induce inflammation, activation of coagulation rapidly ensues. Teleologically, this is viewed as an adjunct to contain the micro-organism and prevent haematogenic spread. As such, coagulation and inflammation can be regarded as ‘brothers-in-arms’.⁵ In ARDS, however, these regulatory mechanisms fail, resulting in an exaggerated and sustained activation of inflammation and coagulation. Pulmonary inflammation in ARDS patients causes remarkably similar haemostatic disturbances to those observed in systemic inflammation, as seen in septic patients.

Inflammation

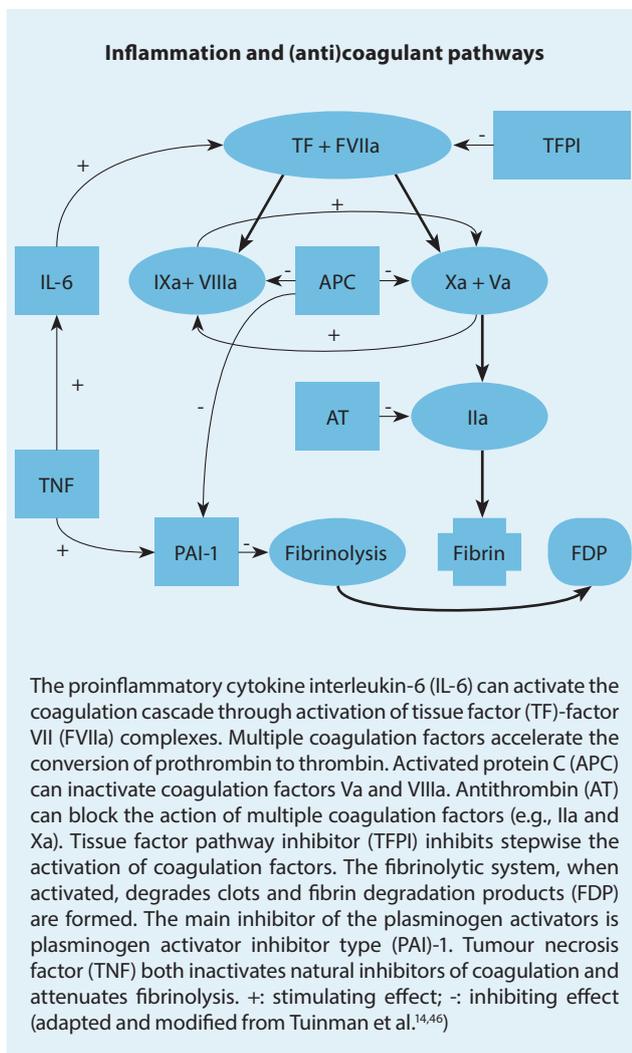
Upon insult, immune cells are immediately directed to the site of injury or infection to initiate a pro-inflammatory response. The pulmonary endothelium is responsible for a number of physiological functions, including the control of vasomotor

tone and the trafficking of leukocytes as a cornerstone of the innate immune system.⁶ The quiescent state of the endothelium expresses an anti-adhesive, anticoagulant and vasodilatory phenotype, whereas endothelial activation results in a pro-adhesive, procoagulant and vasoconstrictive phenotype.⁷

Table 1. Clinical conditions associated with the development of ARDS

Direct lung injury	Indirect lung injury
Pneumonia	Sepsis
Aspiration of gastric contents	Severe trauma with shock
Mechanical ventilation	Cardiopulmonary bypass
Lung contusion	Acute pancreatitis
Fat emboli	Drug overdose
Near drowning	Transfusion of blood products
Inhalational injury	

Figure 1. Schematic and simplified presentation of interaction between inflammation, coagulation, fibrinolysis and anticoagulant pathways.



The interaction between endothelium and neutrophils is crucial for maintaining homeostasis and eradicating bacterial and fungal pathogens.⁶ The pulmonary circulation contains about 28% of the blood neutrophil pool that is available upon demand for host defence.⁸ Activated neutrophils are larger than the diameter of pulmonary capillaries and have a decreased ability to deform within the capillary. As a result, their transit time in the lung is prolonged, allowing for more interaction time with the vessel wall.⁹ In ARDS, neutrophils adhere to the activated capillary endothelium, also referred to as capture, and migrate through the interstitium into the air space. In this air space, alveolar macrophages secrete cytokines, e.g., interleukin (IL)-1, -6, -8 and -10 and tumour necrosis factor (TNF) α , which act locally to stimulate chemotaxis and activate neutrophils, further enhancing inflammation.² These cytokines represent the pivotal link between inflammation and changes in coagulation and fibrinolysis.

It is within this inflammatory response that the lung barrier breaks down and allows transit of protein-rich oedema fluid into air spaces.² Furthermore, damage to epithelial cells involves the basal membrane and reduces the amount and function of surfactant. This increases alveolar surface tension, decreases lung compliance and causes atelectasis.^{2,10} Interaction between inflammatory cells and haemostatic effector cells, such as platelet-neutrophil interaction, is important in ARDS.¹¹ Evidence is emerging that platelets are important mediators of lung injury as well.^{12,13}

Coagulation and fibrinolysis

Normally, the coagulation system is locally activated in order to limit blood loss or to prevent invading pathogens from spreading beyond initial infection. Pulmonary coagulopathy is intrinsic to ARDS. Indeed, both microvascular thrombi and alveolar fibrin deposits are hallmarks of ARDS, irrespective of its cause,^{2,14-17} and are much more outspoken than the fibrin deposition associated with severe inflammation in other organs. In addition, the extent of pulmonary coagulopathy depends on the severity of lung injury¹⁸ and is clearly linked to clinical outcome.¹⁹

Changes in pulmonary coagulation and fibrinolysis in ARDS resemble those found in the systemic circulation during sepsis.¹⁷ Pulmonary coagulopathy with lung injury is characterised by activated coagulation via activation of the tissue factor (TF) pathway, where different cytokines (TNF- α and IL-1 α) enhance expression of TF on endothelial cells.²⁰ TF complexes with factor (F) VII, to activate FX, which in turn activates FV. Activated FV interacts with prothrombin (FII) to form thrombin, which turns fibrinogen into fibrin, the end product of the coagulation cascade. Additional characteristics are attenuation of fibrinolysis, and enhanced breakdown and/or decreased production of the natural anticoagulant activated protein C (APC), antithrombin and tissue-factor pathway inhibitor (TFPI) (figure 1).^{14,16,21,22}

All three processes may lead to excessive alveolar fibrin depositions. Furthermore, soluble levels of thrombomodulin are increased in the alveolar fluid of patients with ARDS. The thrombin-thrombomodulin complex is responsible for the conversion of protein C into APC.²³ The extensive crosstalk between coagulation and inflammation may result in a downward spiral and further inflame the lungs.¹⁷ Activated coagulation factors may initiate or exaggerate injury,²⁴⁻²⁶ impair alveolar aeration and perfusion²⁷ and promote fibrosis.²⁸ Attenuated fibrinolysis is evidenced by increased levels of plasminogen activator inhibitor-1 (PAI-1) (the main natural inhibitor of fibrinolysis) in the bronchoalveolar lavage fluid of ARDS patients.¹⁹

The delicate balance between protective and injurious innate and adaptive immune responses and haemostatic pathways may determine whether alveolar injury continues or is repaired and resolved.¹¹

Treatment directed at coagulation in ARDS

Activation of coagulation is both a consequence and a contributor to ongoing lung injury. Hence, pulmonary coagulopathy could serve as a target for therapeutic interventions in patients with lung injury. The majority of preclinical studies have demonstrated that anticoagulant strategies reduce lung injury and improve oxygenation in various lung injury models.²⁹ In contrast, clinical trials do not suggest beneficial effects of systemic anticoagulants in patients with ARDS (table 2).

Activated protein C

The anticoagulant activity of APC involves the proteolytic inactivation of FVa and FVIIIa. Additionally, APC inactivates PAI-1 which results in enhanced fibrinolysis.²³ Anti-inflammatory effects consist of downregulation of several cytokines, chemokines and adhesion molecules as well as direct inhibition of chemotaxis and leukocyte degranulation.³⁰ Infusion of APC appeared to improve outcome of patients with sepsis.³¹ Indeed, in the pivotal PROWESS trial of patients

with sepsis, infusion of APC was associated with improved survival.³¹ These results have been offset by the results from the recent PROWESS-SHOCK trial,³² which showed no benefit of APC infusion compared with placebo in patients with septic shock, causing the producing company to withdraw APC from the market and to discontinue all other ongoing clinical trials. The PROWESS trial showed that infusion of APC resulted in a more rapid resolution of respiratory failure, indicated by reduced number of days of mechanical ventilation in patients treated with APC.³³ Of note, the beneficial effect of infusion of APC was most prominent in patients with severe community-acquired pneumonia,³⁴ suggesting that patients with a pulmonary cause of their sepsis may benefit more from treatment with APC than patients with sepsis from another source. A clinical trial of patients with ARDS showed decreased pulmonary dead space fraction with infusion of APC, although this was not associated with an improved clinical outcome.³⁵ Seventy-five critically ill patients who met the American/European consensus criteria for ALI were included. Patients with an APACHE II score of 25 or more and severe sepsis were excluded. Participants were randomised to receive APC (24 µg/kg/h for 96 h) or placebo within 72 hours of onset of ARDS. Plasma levels of APC were increased with treatment. However, no effects on the number of ventilator-free days or 60-day mortality were found, despite a decrease of dead space fraction. There was no difference in the number of bleeding events between groups. Of note, the study was prematurely stopped after the interim analysis of 60 patients because of futility. The study population of this study was significantly different from that of most clinical trials of ARDS because of exclusion of more severely ill patients, which may have influenced the results. Remember, in those days, the more severely ill patients with sepsis received APC as part of standard sepsis treatment according to sepsis guidelines and for that reason could not participate in this trial. A multicentre randomised controlled trial of our group showed that infusion of APC actually attenuated pulmonary coagulopathy in ARDS patients and

Table 2. Clinical trials with anticoagulants in patients with ARDS

Intervention	N (patients)	Primary outcome	Effect	Secondary outcomes	Effect	Reference
APC 24 µgr/kg/h for 96 hrs	75	Ventilator-free days	No difference	Dead space fraction 60-day mortality	Reduced No difference	Liu KD et al, 2008 ³⁵
APC 24 µgr/kg/h for 96 hrs	71	Pulmonary Leak Index	No difference	Ventilator-free days LIS 28-day mortality	No difference No difference No difference	Cornet AD et al, 2014 ³⁷
TFPI 25 µgr/kg/h for 96 hrs	1897	28-day mortality	No difference	Ventilator-free days ICU-LOS Hospital-LOS	No difference No difference No difference	Wunderink RG et al, 2011 ⁴¹
Inactivated FVIIa 400 µgr/kg (dose escalation)	214	28-day mortality	Increased in treatment group	Serious bleeding events	Increased in treatment group	Vincent JL et al, 2009 ⁴²

See manuscript for details. APC = activated protein C; TFPI = tissue factor pathway inhibitor; ICU = intensive care unit; LIS = lung injury score; LOS = length of stay

was not associated with bleeding complications.³⁶ However, no effect on alveolocapillary permeability nor the clinical course of ARDS was observed, although underpowering cannot be excluded.³⁷

Antithrombin

Antithrombin predominantly inhibits thrombin and FXa, but also exerts its anticoagulant effect by inhibition of the TF-FVIIa complex and FIXa. Anti-inflammatory effects have been demonstrated in human models of endotoxaemia: amelioration of IL-6 production and reduced leukocyte rolling on the endothelium.²⁰

Infusion of antithrombin had no beneficial effects on outcome in patients with sepsis. In the KYBERSEPT trial (a large multicentre trial in septic patients), mortality was not affected by infusion of antithrombin.³⁸ However, there was a trend toward less (new onset) severe pulmonary dysfunction in patients treated with antithrombin.³⁸ No clinical trials with antithrombin in ARDS patients have been conducted to date.

Tissue factor pathway inhibitor

TFPI is attached to the endothelium by glycosaminoglycans, so it can optimally exert its anticoagulant effects by inhibiting TF-FVIIa and FXa.²⁰

In the OPTIMIST trial in patients with sepsis, no benefit was found with infusion of TFPI.³⁹ In this trial it was hypothesised that infusion of TFPI would improve survival of patients with a community-acquired pneumonia.⁴⁰ These results, however, have been offset by the negative results from the recent CAPTIVATE trial. In this trial, 2100 patients with severe community-acquired pneumonia were randomised to intravenous TFPI or placebo. High-dose TFPI (75 µg/kg/h) was discontinued after the first interim analysis according to prescribed futility criteria. No benefit of TFPI infusion (25 µg/kg/h) was shown compared with placebo despite evidence of biological activity.⁴¹ Possible explanations for these disappointing results are, among others, irreversible activation of coagulation and inflammation before onset of treatment, or local inactivation of TFPI by proteases in the alveoli.

Inactivated recombinant factor VIIa

A multicentre randomised placebo-controlled trial in patients with ARDS, in which 214 patients were randomised to inactivated recombinant factor VIIa or placebo, no beneficial effect of the intervention was seen on morbidity or overall outcome. In fact, the cohort of patients receiving a higher dose (4 x 400 µg/kg) had increased mortality rates and there was a trend to an increased risk of bleeding.⁴² Of interest, no treatment effect on IL-6 (activation of coagulation) or D-dimer (activation of coagulation) were seen in the intervention group. Suggested limitations of this study are timing of intervention, heterogeneity of the patient population and the limited number of patients.

Heparin

Heparin exerts its anticoagulant effects by binding to antithrombin and heparin cofactor II. This bond between heparin and antithrombin results in a conformational change of the latter, potentiating its ability to inactivate factors IX, X, XI and XII.⁴³ Heparin has anti-inflammatory effects besides anticoagulant properties. Post-hoc analysis of four large clinical trials of patients with sepsis suggested that low-dose heparin improves survival.⁴⁴ Pre-operative infusion of heparin reduced pulmonary microvascular fibrin deposits following cardiac surgery.⁴⁵ To our knowledge, no clinical trials have studied the effects of systemic heparin in ARDS.

Systemic versus local treatment

High pulmonary concentrations of an anticoagulant may be necessary to have any effect on pulmonary coagulation, which may not be achievable with intravenous administration. Similar to systemic antimicrobial therapy for pneumonia, where antimicrobial agents penetrate in dissimilar amounts into the lung, it could be argued that anticoagulants may not penetrate into lung tissue adequately to have a local effect. Local administration of anticoagulants, through nebulisation, may allow higher pulmonary concentrations.

The association between systemic anticoagulation and serious bleeding complications is high in patients with sepsis. Using higher systemic dosages, which may be necessary to have sufficiently high concentrations in the lung, will without a doubt increase the incidence of bleeding events. Local administration of anticoagulants may have a potential benefit over intravenous administration since it could reduce the risk of bleeding, especially at higher dosages. A systematic review of nebulised anticoagulants for ARDS concluded that local anticoagulant therapy through nebulisation of anticoagulants attenuated pulmonary coagulopathy and frequently also inflammation in preclinical studies of lung injury. In addition, the data from human trials suggested nebulised heparin for ARDS to be beneficial and safe, but data are very limited.¹⁴

Future directives

First, a major limitation of the majority of pharmacological trials on anticoagulants in ARDS is that the patient population studied is not homogeneous. There are differences in primary insult (e.g., sepsis, aspiration or trauma) and patient factors (e.g., gender, age, comorbidities and genetic factors). In such a heterogeneous population, it is not surprising that while some patients will benefit from the intervention studied, others will not, leading to an overall negative or at best neutral result. We think the key to the development of new effective pharmacological therapies in ARDS lies in careful characterisation of patients, which is likely to benefit from the pharmacological intervention studied. A shift in trial design from large 'all-inclusive' studies towards trials in smaller more selected populations is warranted.

Secondly, in distinction from traditional studies, which employ interventional strategies after the diagnosis of ARDS (e.g. < 48 hours of the diagnosis), we believe a potential important shift in treatment paradigm is to employ pharmacological interventions in patients at risk for ARDS before the full-blown syndrome has emerged. Thirdly, pharmacological interventions in ARDS have not led to an improvement of patient outcome, but our understanding of the mechanisms of this disease has increased. When we continue to increase our understanding of the mechanisms of this disease, a 'side effect' can be a better outcome for these patients in the future.

Lastly, we think that one should remember that, when not applied as a preventive measure, ARDS therapy is in part to alleviate symptoms and to 'buy time', so the underlying condition of the patient can be treated. These therapies should be safe. In line with this, administering anticoagulant therapy locally in the lung offers a potentially great benefit by maximising drug efficacy and minimising possible adverse events. We suggest future research by this approach for ARDS.

Conclusions

Activation of coagulation is an important hallmark of ARDS. Despite the promising results from experimental studies, clinical trials of anticoagulant strategies in ARDS patients to date have proven unsuccessful in improving patient outcomes. Considering the complex effects of modulation of coagulation on both haemostasis and inflammatory pathways, further studies are needed to improve our understanding of this disease. We suggest emphasis in future studies on specific subgroups of ARDS patients, and timing and mode of administration of anticoagulants.

References

- Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet*. 1967;2:319-23.
- Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000;342:1334-49.
- Rubinfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med*. 2005;353:1685-93.
- Herridge MS, Tansey CM, Matte A, et al. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med*. 2011;364:1293-304.
- Schultz MJ, Dixon B. A breathtaking and bloodcurdling story of coagulation and inflammation in acute lung injury. *J Thromb Haemost*. 2009;7:2050-2.
- Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood*. 2003;101:3765-77.
- Aird WC. Endothelial cell heterogeneity. *Crit Care Med*. 2003;31:S221-30.
- Peters AM. Just how big is the pulmonary granulocyte pool? *Clin Sci (Lond)*. 1998;94:7-19.
- Worthen GS, Schwab B 3rd, Elson EL, Downey GP. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science*. 1989;245:183-6.
- Adhikari N, Burns KE, Meade MO. Pharmacologic therapies for adults with acute lung injury and acute respiratory distress syndrome. *Cochrane Database Syst Rev*. 2004;CD004477.
- Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. *J Clin Invest*. 2012;122:2731-40.
- Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). *Front Biosci*. 2009;14:150-8.
- Bozza FA, Shah AM, Weyrich AS, Zimmerman GA. Amicus or adversary: platelets in lung biology, acute injury, and inflammation. *Am J Respir Cell Mol Biol*. 2009;40:123-34.
- Tuinman PR, Dixon B, Levi M, Juffermans NP, Schultz MJ. Nebulized anticoagulants for acute lung injury – a systematic review of preclinical and clinical investigations. *Crit Care*. 2012;16:R70.
- Gunther A, Mosavi P, Heinemann S, et al. Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia. Comparison with the acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2000;161:454-62.
- Schultz MJ, Haitsma JJ, Zhang H, Slutsky AS. Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia--a review. *Crit Care Med*. 2006;34: 871-7.
- Bastarache JA, Ware LB, Bernard GR. The role of the coagulation cascade in the continuum of sepsis and acute lung injury and acute respiratory distress syndrome. *Semin Respir Crit Care Med*. 2006;27:365-76.
- Glas GJ, van der Sluijs KF, Schultz MJ, Hofstra JJ, van der Poll T, Levi M. Bronchoalveolar hemostasis in lung injury and acute respiratory distress syndrome. *J Thromb Haemost*. 2013;11:17-25.
- Ware LB, Matthay MA, Parsons PE, Thompson BT, Januzzi JL, Eisner MD. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. *Crit Care Med*. 2007;35:1821-8.
- Schouten M, Wiersinga WJ, Levi M, van der Poll T. Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol*. 2008;83:536-45.
- Levi M, ten Cate H. Disseminated intravascular coagulation. *N Engl J Med*. 1999;341:586-92.
- Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. *Chest*. 1992;101:816-23.
- Christiaans SC, Wagener BM, Esmon CT, Pittet JF. Protein C and acute inflammation: a clinical and biological perspective. *Am J Physiol Lung Cell Mol Physiol*. 2013;305:L455-66.
- Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. *Semin Respir Crit Care Med*. 2006;27:337-49.
- Laterre PF, Wittebole X, Dhainaut JF. Anticoagulant therapy in acute lung injury. *Crit Care Med*. 2003;31:S329-36.
- Gropper MA, Wiener-Kronish J. The epithelium in acute lung injury/acute respiratory distress syndrome. *Curr Opin Crit Care*. 2008;14:11-5.
- Dixon B, Schultz MJ, Smith R, Fink JB, Santamaria JD, Campbell DJ. Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial. *Crit Care*. 2010;14:R180.
- Scotton CJ, Krupiczkoj MA, Konigshoff M, et al. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest*. 2009;119:2550-63.
- Hofstra JJ, Juffermans NP, Schultz MJ, Zweers MM. Pulmonary coagulopathy as a new target in lung injury--a review of available pre-clinical models. *Curr Med Chem*. 2008;15:588-95.
- Cornet AD, van Nieuw Amerongen GP, Beishuizen A, Schultz MJ, Girbes AR, Groeneveld AJ. Activated protein C in the treatment of acute lung injury and acute respiratory distress syndrome. *Expert Opin Drug Discov*. 2009;4:219-27.
- Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med*. 2001;344:699-709.
- Ranieri VM, Thompson BT, Barie PS, et al. Drotrecogin alfa (activated) in adults with septic shock. *N Engl J Med*. 2012;366:2055-64.
- Dhainaut JF, Laterre PF, Janes JM, et al. Drotrecogin alfa (activated) in the treatment of severe sepsis patients with multiple-organ dysfunction: data from the PROWESS trial. *Intensive Care Med*. 2003;29:894-903.
- Laterre PF, Garber G, Levy H, et al. Severe community-acquired pneumonia as a cause of severe sepsis: data from the PROWESS study. *Crit Care Med*. 2005;33:952-61.
- Liu KD, Levitt J, Zhuo H, et al. Randomized clinical trial of activated protein C for the treatment of acute lung injury. *Am J Respir Crit Care Med*. 2008;178:618-23.
- Cornet AD, Hofstra JJ, Vlaar AP, et al. Activated protein C attenuates pulmonary coagulopathy in patients with acute respiratory distress syndrome. *J Thromb Haemost*. 2013;11:894-901.
- Cornet AD, Groeneveld AB, Hofstra JJ, et al. Recombinant human activated protein C in the treatment of acute respiratory distress syndrome: a randomized clinical trial. *PLoS One*. 2014;9:e90983.
- Eisele B, Lamy M, Thijs LG, et al. Antithrombin III in patients with severe sepsis. A randomized, placebo-controlled, double-blind multicenter trial plus a meta-analysis on all randomized, placebo-controlled, double-blind trials with antithrombin III in severe sepsis. *Intensive Care Med*. 1998;24:663-72.

39. Abraham E, Reinhart K, Svoboda P, et al. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit Care Med.* 2001;29:2081-9.
40. Laterre PF, Opal SM, Abraham E, et al. A clinical evaluation committee assessment of recombinant human tissue factor pathway inhibitor (tifacogin) in patients with severe community-acquired pneumonia. *Crit Care.* 2009;13:R36.
41. Wunderink RG, Laterre PF, Francois B, et al.: Recombinant Tissue Factor Pathway Inhibitor in Severe Community-Acquired Pneumonia: A Randomized Trial. *Am J Respir Crit Care Med.* 2011;183:1561-8.
42. Vincent JL, Artigas A, Petersen LC, Meyer C. A multicenter, randomized, double-blind, placebo-controlled, dose-escalation trial assessing safety and efficacy of active site inactivated recombinant factor VIIa in subjects with acute lung injury or acute respiratory distress syndrome. *Crit Care Med.* 2009;37:1874-80.
43. Lindahl U, Backstrom G, Hook M, Thunberg L, Fransson LA, Linker A. Structure of the antithrombin-binding site in heparin. *Proc Natl Acad Sci USA.* 1979;76:3198-202.
44. Cornet AD, Smit EG, Beishuizen A, Groeneveld AB. The role of heparin and allied compounds in the treatment of sepsis. *Thromb Haemost.* 2007;98:579-86.
45. Dixon B, Opeskin K, Stamaratis G, et al. Pre-operative heparin reduces pulmonary microvascular fibrin deposition following cardiac surgery. *Thromb Res.* 2011;127:e27-30.
46. Tuinman PR, Schultz MJ, Juffermans NP: Coagulopathy as a therapeutic target for TRALI: rationale and possible sites of action. *Curr Pharm Des.* 2012;18:3267-72.