Experimental strategies directed at inflammation and coagulation in ARDS and TRALI
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Coagulopathy as a therapeutic target for TRALI: rationale and possible sites of action

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Abstract

Transfusion-related acute lung injury (TRALI) is a subcategory of acute lung injury (ALI). As such, there are many similarities between the syndromes, both clinically and pathophysiologically. Pulmonary changes in fibrin turnover have emerged as a hallmark of ALI, thereby initiating studies investigating the potential of therapeutic interventions aimed at ameliorating this so-called pulmonary coagulopathy. Enhanced coagulation and impaired fibrinolysis are probably also important features of TRALI. In particular, platelets play an important role in mediating injury during a TRALI reaction. In this narrative review, the evidence of the role of coagulopathy and platelet activation in TRALI is discussed. Given that host risk factors for acquiring TRALI have been identified and that there is a time frame in which a preventive strategy in patients at risk for TRALI can be executed, preventive strategies are suggested. In this review, we discuss potential preventive anticoagulant interventions.
Introduction

Transfusion-related acute lung injury (TRALI) is a subcategory of acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS). There is a clear distinction in etiology, and also in associated mortality,[1,2] suggesting different pathologic mechanisms and sequelae. The main difference in pathologic mechanism is that TRALI can be induced by donor antibodies, which are likely to be cytotoxic and cause endothelial damage, capillary leak and ALI.[3] However, there are also many similarities. Both syndromes have a two hit mechanism. The first is the underlying clinical condition of the patient, that causes activation of the pulmonary endothelium and leads to priming of neutrophils.[3] In ALI/ARDS, different exposures are implicated, including pneumonia or sepsis, which can induce lung injury in the susceptible host.[1,4] An eliciting event is even part of the definition of ALI. Alike in ALI, a two hit mechanism is postulated in TRALI, in which the second event is the infusion of donor antibodies or bioactive lipids present in the transfused blood product.[3] In line with this, the incidence of ALI/ARDS is particularly high in critically ill patients, and there is recent evidence that the same holds true for TRALI.[2,5] In addition to the two hit mechanism, the same clinical diagnostic criteria for ALI also apply to TRALI (acute onset of hypoxemia, bilateral pulmonary infiltrates and absence of left ventricular overload). The only addition for TRALI is that symptoms should occur within proximity of a blood transfusion.[6] Thereby, ALI and TRALI are clinically and radiologically indistinguishable. In addition, there are histopathologically similarities.[6,7]

Changes in pulmonary fibrin turnover are a hallmark of ALI/ARDS.[8] There is evidence from experimental and preclinical studies that anti-coagulant therapies have the potential to reduce pulmonary coagulation and inflammation, and to improve outcome in ALI.[9] There is histopathological[10] and functional evidence that platelets play an important role in ALI.[11-13] Anti-platelet therapy may protect against ALI in at risk patients[14], suggesting platelets as an important factor in mediating lung injury. Although less well studied, also in TRALI there is evidence for pulmonary coagulopathy.[15,16] In addition, platelets have been identified as key players in mediating the inflammatory response in TRALI.[13,17]

TRALI is not an uncommon finding in critically ill patients.[2] Presumably, a high incidence is due, at least in part, to the frequent presence of an inflammatory condition. In line with this, sepsis and cardiac surgery are identified as risk factors for TRALI.[18,19] Also, these patients are frequently transfused. Thereby, TRALI contributes substantially to morbidity, increased length of stay and mortality in the intensive care unit.[2,18,19] Treatment for TRALI to date is supportive.[2] Development of a specific treatment, especially in patients at high risk for developing lung injury after transfusion, may be a rational goal of further TRALI research.

This narrative review summarizes the evidence for a pathophysiologic role of enhanced pulmonary coagulation and impaired pulmonary fibrinolysis in TRALI, also focusing on the role of platelets. Potential rational therapeutic anticoagulant interventions are discussed.
Materials and Methods

Data sources
An electronic search in the databases Medline and Embase is performed. Reference lists of retrieved papers are screened for potentially important papers.

Keywords
The Medline database is used to identify medical subject’s headings (MeSH) to select search terms. In addition to MeSH terms, also free-text words are used. Search terms refer to aspects of the condition (“transfusion-related lung injury”) as well as related conditions (“acute lung injury” and “acute respiratory distress syndrome”). In addition, we search on disease mediators (“coagulation” and “fibrinolysis”) and interventions (“activated protein C” (APC) OR “antithrombin” (AT) OR “heparin” OR “thrombomodulin”(TM) OR “tissue factor pathway inhibitor” (TFPI) OR “inactivated factor VIIa”(IFVIIa) OR “tissue plasminogen activators” (TPA)). The types of anticoagulants are chosen based on preclinical and clinical studies with anticoagulants in sepsis and ALI/ARDS.[8,9,20]

Fibrin turnover in ALI/ARDS

Systemic coagulopathy in inflammation
Ample evidence demonstrates that coagulopathy is an important event in systemic inflammatory conditions, such as sepsis.[21] Nowadays, coagulopathy and impaired fibrinolysis are accepted as intrinsic to sepsis and therapeutic interventions for patients with sepsis target this pathway.[22] Pro-inflammatory cytokines, e.g. tumor necrosis factor (TNF)–α, interleukin (IL)–1 and IL–6, are released during the initial phase of the inflammatory response and activate TF through activation of the tissue factor (TF) pathway.[8,21] TF binds and activates the downstream coagulation cascades through factor (F) VII (Figure 1).[23] Only small amounts of TF are exposed to the circulating blood under normal conditions, but in sepsis, TF-expression is increased by endotoxins and pro-inflammatory cytokines.[9] The natural inhibitors of coagulation (APC, AT and TFPI) regulate the coagulation cascade (Figure 1). The pro-coagulant state of sepsis is not sufficiently counterbalanced by natural inhibitors, because levels of the natural anticoagulants are low due to an enhanced breakdown and impaired production.[24] At the same time, fibrinolysis is attenuated by an increased synthesis of inhibitors of plasminogen activators, including the main inhibitor of fibrinolysis plasminogen activator inhibitor type-1 (PAI-1).[21] In short, while fibrin production is increased, fibrin breakdown is impaired.
In short, activation of tissue factor (TF)-factor VII (FVIIa) starts the coagulation cascade. A key step is the activation of prothrombin to form thrombin, which converts fibrinogen to fibrin. Several coagulation factors accelerate the conversion of prothrombin to thrombin. Activated protein C (APC) can inactivate several coagulation factors after forming a complex with thrombomodulin. Antithrombin (AT) serves to block the action of multiple coagulation factors (e.g. Xa and IIa). Tissue factor pathway inhibitor (TFPI) inhibits stepwise the activation of coagulation factors. The fibrinolytic system is designed to degrade clots. Specific proteases cleave the fibrin networks that are formed during coagulation leading to fibrin degradation products (FDP). The main inhibitor of the plasminogen activators is plasminogen activator inhibitor type-1 (PAI-1). Adapted and modified from Hofstra et al. [9] Thick arrows: stimulating effect; Thin arrows: inhibiting effect.

Pulmonary coagulopathy in ALI/ARDS

While in systemic inflammation a systemic coagulation imbalance is present, it is not surprising that pulmonary inflammation causes a similar but local pro-coagulant state. [9,25-27] Indeed, pulmonary inflammation is associated with activation of coagulation and attenuation of fibrinolysis, resulting in alveolar fibrin depositions. In the lung, fibrin inhibits surfactant function and enhances neutrophil recruitment.[28]

Furthermore, fibrin degradation products are capable of stimulating fibroblast proliferation. [28] In addition, intravascular coagulation results in increased pulmonary vascular resistance and pulmonary hypertension.[29] The local changes in coagulation and fibrinolysis in the pulmonary compartment during ALI/ARDS very much resemble those found in the blood compartment during sepsis.[8] Increased concentrations of TF in the bronchoalveolar lavage fluid have been observed in various etiologies of ALI/ARDS, suggesting a common pathologic mechanism.[26,30] Inadequate levels of natural anticoagulants APC[31] and TFPI[32] are found in patients with pneumonia, as well as prominent changes in the alveolar fibrin production and breakdown, which correlate with disease severity, [26] whereas in patients
with ALI/ARDS, lower levels of APC in the bronchoalveolar lavage fluid correlate with worse outcome.[24,33,34]

Of note, the procoagulant activity in ALI/ARDS[35] occurs independent of its cause (direct, e.g. pneumonia or aspiration or indirect, e.g. sepsis). With different types of ALI/ARDS, the same three processes are recognized: activation of the TF pathway resulting in coagulation, enhanced breakdown and decreased production of natural anticoagulants and impairment of fibrinolysis. [8,21,24] This procoagulant state is present already in the early phase of ARDS and tends to decrease after 7–14 days.[36]

**Role of platelets in ALI/ARDS**

Platelets play a role in the haemostatic process, leading to the formation of a haemostatic plug. In addition, activated platelets provide a surface for catalysis of the extrinsic clotting cascade, enhancing coagulation and the consolidation of the thrombus.[37] Furthermore, platelets play a role in inflammation and wound healing. In response to activation, platelets change their shape, up-regulate the expression of adhesion molecules such as glycoprotein (GP) IIb/IIIa, P-selectin, PECAM-1 and thrombospondin and secrete the content of granules.[38] These granules contain factors relevant for coagulation and fibrinolysis.[39]

In ALI/ARDS, the pulmonary microvascular endothelium upregulates adhesion molecules upon activation.[40,41] Activated platelets have an increased expression of P-selectin, which mediates secondary capturing (the initial binding) of neutrophils to the vessel wall.[11,42] Secondary capture is the interaction of a freely flowing neutrophil with an adherent platelet or leukocyte, leading to subsequent attachment to the endothelium and vascular damage.[11] A key pro-inflammatory mediator involved in this interaction in ALI/ARDS is thromboxane A2 (TXA2)[11], which is released by platelet-neutrophil aggregates.[43]

**Fibrin turnover in TRALI**

Given the fact that coagulopathy is an important feature of ALI/ARDS, the same may hold true for TRALI. In animals, massive transfusion results in extensive numbers of microthrombi in the pulmonary vasculature.[44] In line with this, in a two-hit murine transfusion model, we ourselves show that transfusion with outdated blood products causes lung injury characterized by activation of pulmonary coagulation and impaired pulmonary fibrinolysis. [15,16] Indeed, in this model pulmonary levels of thrombin-antithrombin complex (TATc), PAI-1 and fibrin degradation products increase, whereas the level of plasminogen activator activity (PAA) reduces. Aged red blood cells have a pro-coagulant activity,[45] which may contribute to lung injury by increasing thrombin generation.

In accordance, blood transfusion in cardiac surgery patients is associated with enhanced pulmonary coagulation and inhibition of fibrinolysis.[46] In the pulmonary compartment of patients who receive blood products, increased levels of TATc, PAI-1 and fibrin degradation
products are found compared to patients who do not receive blood products. Furthermore, pulmonary levels of PAA are decreased. These pulmonary changes are accompanied by systemic derangement of coagulation and fibrinolysis. Of note, transfusion is dose-dependently associated with an increase in the levels of PAI-1. An increase in PAI-1 levels is of prognostic significance in patients with ALI/ARDS,[47] sepsis[48] and pneumonia.[49] The foregoing indicates that in the presence of a first hit, coagulopathy and impaired fibrinolysis may be important pathways in mediating lung injury in TRALI and that coagulopathy induced by transfusion is comparable to the pathogenesis of ALI/ARDS.[1]

Platelets in TRALI

Thrombocytopenia is a prominent feature in an animal model of TRALI, suggesting that platelets might be involved in TRALI pathogenesis.[17] Similar to ALI/ARDS, in which the platelet-neutrophil interaction seems important,[12] in TRALI both neutrophils and platelets sequestrate in the lungs of mice challenged with TRALI-inducing antibodies.[13] Alike with ALI/ARDS, with TRALI platelets roll along and firmly adhere to lung endothelial cells in TRALI,[17] as shown by intravital microscopy.[50] However, there are also differences between ALI/ARDS and TRALI. Whereas blocking of P-selectin seems protective in acid-induced ALI/ARDS by reducing platelet-neutrophil aggregates,[43] a role for P-selectin is not established so far in TRALI.

Suggested therapeutic interventions targeting coagulation in ALI/ARDS

Activated protein C (APC) is a natural anticoagulant with cytoprotective, immunomodulatory and pulmonary neutrophil chemotaxis inhibitory properties.[22,51-55] Treatment with APC benefits patients with severe sepsis, in particular patients with the highest disease severity scores.[22] Of interest, treatment with APC results in a more rapid resolution of respiratory failure.[22] Furthermore, patients with sepsis due to severe community-acquired pneumonia seem to benefit most from treatment with APC.[56] Treatment with APC restores depleted APC levels in the lungs of patients with ALI/ARDS.[55,57] APC significantly reduces leukocyte accumulation and neutrophil chemotaxis in the alveolar space in a human model of endotoxin-induced lung injury.[53] APC attenuates pulmonary inflammation and coagulation, reduces lung injury and improves outcome in both direct and indirect models of ALI/ARDS.[9] These results were not supported by a randomized controlled trial in critically ill patients diagnosed with ALI, in which intravenous APC did not improve outcome compared to placebo.[57] Suggested reasons for this contrasting results are the possible difference in timing between administration of APC and the onset of ALI, a non-significant difference in baseline lung function and mechanical ventilation,[58] and above all the study showing no benefit may have been underpowered to show a difference in mortality.[59]

Antithrombin (AT), another natural anticoagulant, has anti-inflammatory properties and the potential to correct endothelial microvascular dysfunction.[60,61] AT attenuates pulmonary
inflammation and coagulation, reduces lung injury and improves outcome in different models of ALI.[9] Many of these effects are likely to result from an AT-induced production of prostacyclin.[63,64] Treatment with AT reduces the prevalence of new respiratory dysfunction in patients with severe sepsis.[65] High-dose AT therapy failed to show an effect on 28-day all-cause mortality in adult patients with severe sepsis and septic shock.[66]

Heparin, a frequently used anticoagulant, also has anti-inflammatory properties.[67] Heparin limits fibrin deposition in the microcirculation and alveolar spaces.[68] In addition, heparin has inflammation-reducing capacities.[69-72] In line with this, pre-operative heparin limits the development of pulmonary microvascular fibrin deposition following cardiac surgery.[73] Furthermore, in patients on mechanical ventilation, treatment with nebulized heparin is associated with fewer days of mechanical ventilation and tends to improve oxygenation.[74] In animal models of acute lung injury, heparin attenuates pulmonary inflammation and coagulation, reduces lung injury and improves outcome.[9]

Other anticoagulants and fibrinolytics have been less extensively studied. Pre-clinical data obtained from studies in ALI models however, suggest that treatment with TFPI, TM, FVIIa and TPA reduces pulmonary coagulation and inflammation.[9,75]

Targeting platelets is a topic of interest in ALI/ARDS. Blocking or eliminating P-selectin reduce neutrophil recruitment into the lung and rescue mice from acid-induced ALI.[43] In addition, in both in vivo and in vitro models of LAI, blocking TXA2 by aspirin or a thromboxane receptor antagonist protects against ALI.[43,76-81] Anti-platelet therapy is associated with a reduced incidence of ALI/ARDS in a recent cohort study of critically ill patients, even when adjusting for confounding variables.[14]

**Side–effects of anticoagulant therapies**

The occurrence of serious bleeding during anticoagulant therapy may offset potential beneficial effects of anticoagulants.[22,82-84] Alternatively, successful treatment requires adequate concentrations of medication for sufficient duration at the site of action. To date, the achieved concentration of study medications in the lung is still undetermined. Of interest is local administration of anticoagulants in the pulmonary compartment, since it is feasible, has the potential to administer higher doses at the site of action and may reduce unwanted systemic effects.[85-88]

**Rationale for therapeutic interventions targeting coagulation in TRALI**

To date, treatment of TRALI is supportive.[1,2,18,89] Considering the two-hit model of lung injury in these diseases, a preventive strategy in patients with a first hit (e.g. surgery, active infection or aspiration) should be applied, including lung-protective ventilator settings and a
restrictive transfusion practice. However, whereas transfusion cannot be avoided altogether, a preventive strategy can be considered, particularly in patients at risk. In some of the preclinical studies with anticoagulants, the animals are pre-treated before ALI is induced. For transfused patients at risk, prophylactic treatment against lung injury would be a major breakthrough. Targeting coagulation or platelets seems to be a promising and rational approach.

Currently, there are no studies in animal models or trials of patients with TRALI targeting the coagulation cascade. Blood transfusion is associated with pulmonary and systemic coagulopathy before clinical lung injury is apparent.[46] We suggest that this activation of coagulation and impaired fibrinolysis may be a first manifestation of lung injury and a possible pre-stadium of TRALI. In line with this, in preclinical TRALI models, lung injury after transfusion with outdated blood products is characterized by activation of coagulation and impaired fibrinolysis.[15,16] Furthermore, in trials in patients with severe sepsis, anticoagulant treatment seems to especially affect pulmonary outcome.[22] Lastly, there is evidence from pre-clinical studies in both direct and indirect models of ALI that anticoagulants attenuate lung injury and improve outcome.[9] Following this line of thought, it is reasonable to explore anticoagulant strategies, which have shown to be involved in ALI/ARDS, including treatment with APC, AT or heparin. Furthermore, the membrane of erythrocytes is suggested to activate factor IX, which in turn is capable of activating X leading to thrombin generation,[90] which may play a role in activating the pulmonary coagulation cascade following a red blood cell transfusion. APC, heparin and AT are known to inhibit thrombin formation by inhibiting factor IX or X and therefore constitute potential therapeutic targets in TRALI. Also, there is evidence that fibrinolysis is impaired in TRALI. Nebulizing anticoagulants has the potential benefit to deliver high concentrations in the lung without affecting systemic coagulation and we hypothesize that this may be an elegant treatment option for TRALI prevention, which needs to be explored. However, direct evidence that these strategies will work is still missing.

Platelet activation plays a major role in ALI/ARDS and TRALI pathophysiology[11,13,91] and a high dose of aspirin improves outcome in an animal model of TRALI.[13] The precise pathway through which aspirin attenuates lung injury is not known, but may depend on the dose. A low dose of 30 mg aspirin per day is capable of complete suppression of platelet TXA2 production via inhibition of isoform COX-1,[92,93] inducing an adequate antithrombotic effect. In higher doses, aspirin is also capable of inhibiting COX-2, resulting in anti-inflammatory effects [94]. Prostanoids, such as prostaglandin E2, generated by the COX-2 enzyme, are present in high concentrations in the lung of animals and humans with sepsis and ALI/ARDS.[95-97] Thereby, the anti-inflammatory properties of aspirin may have contributed to outcome in the animal studies. Although the appropriate dose of aspirin for preventing lung injury needs to be assessed in clinical trials in which the benefit will need to be determined in light of the additional risk of bleeding, the present results suggest aspirin as a promising preventive approach in TRALI.
Conclusion

There is evidence for pulmonary coagulopathy in patients with TRALI, which appears early in the course of the disease. There is a rationale for studying anticoagulants in the setting of TRALI. Furthermore, from preclinical studies, treatment with aspirin seems a promising approach. In ICU patients, blood transfusion is common and associated with adverse outcome. Because TRALI is caused by a precipitating factor (the transfusion of a blood product), pre-treatment is possible and lung injury might be preventable. We suggest future research to study anticoagulant treatment as a prophylactic strategy for TRALI in the critically ill.
Reference List


COAGULOPATHY AS A THERAPEUTIC TARGET FOR TRALI


