Experimental strategies directed at inflammation and coagulation in ARDS and TRALI
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Summary, general discussion and future directives
Introduction

Numerous clinical trials have assessed different types of pharmacologic interventions in patients with the acute respiratory distress syndrome (ARDS) [1]. While most trials showed no effect of the studied intervention, it is suggest that specific subsets of patients could benefit from pharmacologic interventions [2]. The lack of efficacy of pharmacologic interventions for ARDS at least in part could be the result of the relative late timing of the intervention. Indeed, preclinical studies show certain pharmacologic interventions to be very effective for lung injury, but in these studies interventions are done early after or even before the insult. In the human setting, pharmacologic interventions are applied relatively late after development of ARDS.

The pathophysiology of transfusion–related acute lung injury (TRALI) and ARDS are very similar, as discussed in chapter 1. All pathways result in the same downstream event, namely inflammation, activation of platelets and an imbalance between coagulation and fibrinolysis [3,4]. The result is capillary damage and leakage of fluid into the alveolar space [5]. Considering these similarities it is conceivable that strategies aimed at treating ARDS may also benefit patients with TRALI.

In this thesis, the effect of blood transfusion on pulmonary inflammation and coagulation was studied. We investigated the role of different mediators in ARDS or TRALI. Furthermore, we tested several therapeutic strategies in murine models of both ARDS and TRALI. The effect of aspirin on TRALI incidence was tested. Lastly, we performed a systematic review on nebulized anticoagulants in the setting of lung injury in animals and humans.

Inflammation

Blood transfusion is associated with increased morbidity and mortality in cardiac surgery patients, but cause and effect relations remain unknown. In chapter 2, we hypothesized that blood transfusion is associated with changes in pulmonary and systemic inflammation and coagulation. Cardiac surgery patients (n = 45) were grouped as having received no transfusion, limited transfusion (1-2 units) or multiple transfusions (≥5 units). Transfusion during cardiac surgery is associated with activation of inflammation and coagulation in the pulmonary compartment of patients that do not meet TRALI criteria, an effect that was partly dose-dependent. These pulmonary changes were accompanied by systemic derangement of coagulation. These results suggest transfusion to be a mediator of acute lung injury. In chapter 3 we show that the plasma levels of sRAGE are elevated following cardiac surgery and associated with an increase in pulmonary vascular permeability, an established marker of lung injury. In two university hospital intensive care units in The Netherlands, sRAGE and PLI were measured in a cohort of cardiac surgery patients (n = 60). Plasma sRAGE discriminated well between those with an elevated PLI and those with a normal PLI, so that sRAGE may serve as a diagnostic indicator of lung injury before full onset of symptoms. Previous
experimental data suggest that recombinant sRAGE may neutralize the ligand-mediated damage by acting as a decoy, thus protecting sensitive cells from the deleterious effects of ligand-RAGE hyperactivity. Our results contribute to the rationale of exploring sRAGE as a diagnostic and interventional target in cardiac surgery patients at risk of developing ARDS. However, this may not be the case for sRAGE in TRALI. As shown in chapter 3, the level of sRAGE in cardiac surgery patients was not affected by transfusion. In addition, in chapter 4 we found no significant role for the DAMPS, sRAGE, HMGB-1 and S100A12, in TRALI in a cohort of cardiac surgery patients. 14 TRALI cases were matched with transfused and non-transfused controls without lung injury. No difference in sRAGE and HMGB-1 levels were found between groups. Of note, we might have measured levels of sRAGE and HMGB-1 to early in the course of TRALI. There was a trend towards higher S100A12 levels in TRALI patients compared to the controls. In addition, S100A12 was associated with prolonged ventilation and hypoxemia after cardiac surgery and seems to be a DAMP of interest in future research in this field.

Considering the dominant role of inflammation in ARDS, corticosteroid treatment has been studied extensively in ARDS [6]. They were found to have beneficial effects when given at low or moderate doses sooner than two weeks, but appear to be harmful if started later. In contrast, besides anecdotally reports of corticosteroid therapy in TRALI patients, studies are lacking for use of corticosteroid use in TRALI treatment. In chapter 5, methylprednisolone failed to prevent the development of lung injury in a ‘two hit’ murine TRALI model. Given that steroids reduced the systemic inflammatory reaction as well as IL-6 levels in the BALF, the dose seemed adequate. Also, the dose used in this study corresponds to doses which have been beneficial in ARDS patients. Thereby, it seems that steroids are less effective in TRALI then in ARDS. Considering that TRALI is a relatively rare phenomenon, it is not feasible to conduct large clinical trials with clinically relevant end points on efficacy of steroids in TRALI. Instead, studies on the effect of steroids on inflammatory markers in the BALF may serve as an endpoint. Until then however, results of this animal study do not underline the rationale of giving steroids in TRALI.

Platelets, coagulation and fibrinolysis

Platelets play a pivotal role in ARDS pathogenesis. Platelet inhibition, by high dose aspirin, has been shown to attenuate lung injury in multiple preclinical models of ARDS. In observational trials in humans, data indicating a protective effect of aspirin on ARDS are less convincing [7,8]. In line with the clinical data in ARDS, we found in no beneficial effect of pre-admission use of low dose aspirin on TRALI incidence, as shown in chapter 7. We performed a post-hoc analysis of a nested case-control study that had been undertaken in a tertiary referral hospital. Transfusion-related acute lung injury cases (n = 109) were matched with controls (transfused patients’ not developing lung injury, n = 109). Use of aspirin did not alter the risk of transfusion-related acute lung injury after transfusion. Adjustment for confounding
variables using propensity scoring also did not affect the risk of acquiring transfusion-related acute lung injury. Although it is possible that this study was not large enough to demonstrate a positive association, there may also be an alternative explanation for the absence of a beneficial effect of aspirin in TRALI. In chapter 6, we show that high dose aspirin corresponding to doses used in previous preclinical trials is superior to low dose aspirin (as used in the human observational trials) in preventing lung injury in a direct murine model of ARDS. Furthermore, we show high dose aspirin is a better choice than clopidogrel, another frequently used platelet-inhibitor. These results show that clinical trials exploring the effects of antiplatelet agents in ARDS and TRALI patients should take into account the dose and type of antiplatelet drug. High dose aspirin seems to be the therapy of choice at this point. One must take into account that antiplatelet therapy in a bleeding patient in need of transfusion may expose these patients to an additional risk by increasing bleeding tendency.

To further explore the therapeutic potential of platelets in TRALI, we studied the role of CD40ligand, which is a primarily platelet derived pro-inflammatory mediator. CD40-CD40L interaction has been found to play a role in various models of acute lung injury and possibly also in TRALI. To our surprise, as shown in chapter 8 inhibiting or antagonizing CD40-CD40L interaction did not abrogate the TRALI reaction in a mouse model of TRALI. In line with this, we found no difference in plasma levels of sCD40L between TRALI patients and controls. These results suggest that CD40-CD40L is not a platelet inflammatory mediator of importance in TRALI pathogenesis. These results are in contrast with preclinical studies in ARDS.

Data from systemic anticoagulants on beneficial effects on ARDS are conflicting. Severe systemic bleeding due to anticoagulation may have offset the possible positive effects. Nebulization of anticoagulants may allow for improved local biological availability and as such may improve efficacy in the lungs and lower the risk of systemic bleeding complications. In chapter 9, evidence is summarized that local anticoagulant therapy through nebulization of anticoagulants attenuates pulmonary coagulopathy and frequently also inflammation in preclinical studies. Notably, nebulized danaparoid and heparin but not activated protein C and antithrombin, were found to have an effect on systemic coagulation. Recent human trials suggest nebulized heparin for ARDS to be beneficial and safe, but data are very limited. We think further studies on nebulized anticoagulant therapy are justified in ARDS patients. Mechanistic studies addressing issues as the optimal dose and timing should be carried out before RCTs can be undertaken.

Data on the role of coagulation and fibrinolysis in TRALI are limited. In chapter 2, we show that transfusion results in activated coagulation and impaired fibrinolysis in both the pulmonary and systemic system in cardiac surgery patients. These results suggest transfusion to be a mediator of lung injury. In chapter 10, we summarize evidence for a role of activated coagulation and impaired fibrinolysis in TRALI patients. Notably, systemic anticoagulant or fibrinolytic therapy seems an undesirable approach in TRALI patients, since patients in need for a blood transfusion are often bleeding patients. For these patients,
administrating anticoagulant or fibrinolytic therapy locally in the lung offers potentially great benefit by maximizing drug efficacy and minimizing possible adverse events. We suggest future research into this approach.

**Limitations of the preclinical models**

The models for ARDS and TRALI in this thesis have several limitations. Of course, all animals models for ARDS and TRALI have their shortcomings. The LPS used in the direct ARDS model allows for accurate dosing in a reproducible manner. However, it is an oversimplification of the complex clinical scenario of pneumonia induced lung injury. The same applies for the TRALI model used. Furthermore, there are important differences in the ratio of circulating platelets and neutrophils of mice compared to humans. The higher platelet to neutrophil ratio in mice may render antiplatelet therapy more effective in mice compared to humans.

**Limitations of the clinical studies**

Our clinical trials have several limitations. Using a propensity score, may not completely exclude (unknown) confounders. In addition, the numbers of patients in our trials is limited, possibly causing the studies to become ‘underpowered’. The population studied was sometimes heterogeneous, thereby rendering it more susceptible for inconclusive results. One can imagine, that in such a heterogeneous population, some patients will benefit from an intervention while others will not, leading to an overall neutral or negative result. Furthermore, we measured some markers at one or two time points, precluding conclusions about the time course of the role of these mediators.

**Future directives**

First, in distinction from traditional studies, which employs 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 to patients at risk of ARDS or even more applicable (since a transfusion can be anticipated) TRALI, before the full blown syndrome has emerged. With this in mind, our results suggest future studies exploring the therapeutic potential of the early DAMP sRAGE in ARDS and S100A12 in TRALI. Also, an interventional trial studying high dose ASA in preventing ARDS or TRALI is suggested. Of note, our suggestion that blood transfusion is a mediator of lung injury may contribute to the risk-benefit assessment of the decision to initiate blood transfusion in patients at risk of developing lung injury.

Secondly, a major limitation of the majority of pharmacological trials in ARDS patients is the not sufficiently homogenous patient population studied. Differences are for example in primary insult (e.g. sepsis, aspiration or trauma) and patient factors (e.g. gender, age, comorbidities and genetic factors). In such 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 and TRALI lies in careful characterization of patients which are 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.

Thirdly, despite almost exclusively negative results from pharmacological therapies in ARDS, 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 preventive measure, ARDS/TRALI therapy is in part to alleviate symptoms and to ‘buy time’. These therapies should be safe. In line with this, administrating anticoagulant or fibrinolytic therapy locally in the lung offers potentially great benefit by maximizing drug efficacy and minimizing possible adverse events. We suggest future research into this approach for both ARDS and TRALI.


