Pulmonary Activation of Coagulation and Inhibition of Fibrinolysis After Burn Injuries and Inhalation Trauma

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Background: Pulmonary coagulopathy is intrinsic to pneumonia and other forms of acute lung injury. We hypothesized patients with burn injuries and inhalation trauma to have similar alterations in pulmonary coagulation and fibrinolysis.

Methods: We performed a prospective study on changes in pulmonary and systemic thrombin generation and fibrinolytic activity in patients with burn injuries and inhalation trauma requiring mechanical ventilation. Nondirected bronchial lavage was performed on alternate days. Patients requiring mechanical ventilation for nonpulmonary reasons who did not meet the North American European Consensus Conference criteria for acute lung injury functioned as control patients.

Results: We studied 13 patients with burn injuries and inhalation trauma and 15 control patients. On admission, patients with burn injuries and inhalation trauma showed a significant increase in thrombin generation in the airways compared with control patients, as reflected by increased lavage fluid levels of thrombin-antithrombin complexes and fibrin degradation products, and decreased lavage fluid levels of activated protein C and antithrombin. Simultaneously, burn patients showed a significant decrease in fibrinolytic activity, as reflected by decreased lavage fluid levels of plasminogen activator activity. Pulmonary coagulopathy persisted throughout the period of mechanical ventilation and was accompanied by similar changes in systemic coagulation and fibrinolysis. There was no significant correlation between changes in coagulation and fibrinolysis and the extent of burn injury.

Conclusions: Patients with burn injuries and inhalation trauma requiring mechanical ventilation show a distinct and sustained procoagulant and antifibrinolytic shift in the pulmonary compartment. Pulmonary coagulopathy could be an important therapeutic target in these patients.

Key Words: Burn injuries, Inhalation trauma, Coagulation, Fibrinolysis.

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In the United States, each year ~50,000 patients are hospitalized after burn injuries. Concomitant inhalation trauma is reported in 20% to 35% of burn patients requiring hospitalization,1,2 and it is most frequently encountered after house fires, explosions, and other disasters involving fire with smoke formation. Inhalation trauma is an important independent predictor of morbidity and mortality in patients with burn injuries and increases mortality by 20% to 60%. Although advanced care for burn injuries has improved outcome, respiratory complications of inhalation trauma remain a serious threat.3 To date, no specific intervention is available for the treatment of inhalation trauma and therapy is merely supportive (i.e., intubation and mechanical ventilation).

Pulmonary coagulopathy is intrinsic to pneumonia and other forms of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS).4 Alveolar fibrin depositions in pneumonia and ALI/ARDS are the result of local activation of coagulation on the one hand and inhibition of fibrinolysis on the other. Preclinical studies in models of ALI that specifically targeted pulmonary coagulopathy have demonstrated that treatment with anticoagulants has the potential to attenuate pulmonary coagulopathy and to reduce inflammation.5

We hypothesized burn patients with inhalation trauma to show similar alterations in pulmonary coagulation and fibrinolysis as patients with pneumonia or ALI/ARDS. If present, systemic or maybe even local anticoagulant or pro-fibrinolytic therapies could benefit these patients. In this observational clinical study, we investigated pulmonary and systemic coagulation and fibrinolysis in patients with burn injuries and concomitant inhalation trauma during mechanical ventilation.

PATIENTS AND METHODS

Study
An observational study was performed in the intensive care units (ICUs) of an academic hospital and a regional teaching hospital specialized in care for burn patients in the Netherlands. The study protocol was reviewed and approved by the local medical ethics committee. Admissions were screened from January 2006 to December 2008, and written informed consent was obtained from patients or their closest relatives before enrollment.

Subjects
Patients older than 18 years with burn injuries and inhalation trauma, expected to need mechanical ventilation for >72 hours, were eligible for participation. The clinical
diagnosis of inhalation trauma traditionally rests on indirect observation: inhalation trauma was suspected in patients who were trapped in a confined space or who lost consciousness during a fire, the presence of soot in throat or sputum, and presence of facial and upper cervical burns, singed eyebrows, and nasal vibrissae. For the purpose of this study, the clinical diagnosis had to be confirmed with bronchoscopy showing soot or infralaryngeal mucosal damage, indicating exposure of the tracheobronchial tree to the physical products of combustion.

Patients older than 18 years with neither burn injuries nor inhalation trauma who did not meet the North American European Consensus Conference (NAECC) criteria for ALI/ARDS and were expected to require mechanical ventilation for >72 hours functioned as control patients. Participation in interventional trials, pregnancy, increased intracranial pressure, and preexisting severe chronic respiratory disease (defined as a forced expiratory volume in 1 second to forced vital capacity ratio <0.64 and daily medication), and suspected pneumonia were exclusion criteria in addition to the use of corticosteroids or other immunosuppressive agents and intravenously administered heparin. Treatment of patients (i.e., ventilator settings, fluid resuscitation, use of vasopressors, and antibiotic treatment) was based on local guidelines that were in accordance with international guidelines.

All patients were ventilated with Evita 4 ventilators (Dräger, Zoetermeer, The Netherlands) in volume-controlled mode, with tidal volumes of 6 mL/kg predicted body weight. The levels of positive end-expiratory pressure (PEEP) and inspired oxygen (FIO₂) were titrated on PaO₂ according to the local mechanical ventilation guideline. The ventilator was routinely (3 times/d) switched to the pressure support mode. If the pressure support mode was tolerated, this mode was used for further mechanical ventilation.

Data Collection

Demographic data, admission diagnosis, the Acute Physiology and Chronic Health Evaluation (APACHE) II scores and data on the extent of burn injury, expressed as a percentage of the total body surface area (TBSA), were calculated using Lund-Bowder charts. On ICU admission and each alternate day, ventilator settings, blood gas parameters, and chest radiographs data were recorded until the patient was completely weaned from the mechanical ventilator.

The oxygenation index was calculated from the ventilator and blood gas analyses data using the following formula: mean airway pressure × FIO₂ × 100%/PaO₂. Mean airway pressure was measured by the ventilator. The lung injury score (LIS) was calculated.

All patients were monitored for development of ALI/ARDS. ALI/ARDS was diagnosed if a patient met the NAECC criteria. Two independent physicians who were blinded to patient or group identity scored chest radiographs for the presence of bilateral interstitial changes. During the review process, they had access to echocardiography results and data on fluid balance and pulmonary capillary wedge pressures if measured. When interpretation differed, consensus was obtained while reviewing the patients together.

Specimen Collection

Blood sample collection followed by nondirected bronchial lavage was performed on ICU admission and each alternate day throughout the period of mechanical ventilation. Blood samples were drawn into sterile Vacutainer tubes containing citrate, using an already in place arterial catheter. Lavage was performed by instilling 20 mL of sterile 0.9% saline via a standard 50 cm, 14-French tracheal suction catheter as described previously. In short, the distal end of the catheter was introduced via the endotracheal tube and advanced until significant resistance was encountered. Immediately after instillation over 10 seconds, fluid was aspirated before withdrawal of the catheter. Generally, 4 mL to 8 mL of fluid was recovered.

Specimen Processing

The lavage fluids and blood samples were centrifuged at 1,500 × g for 10 minutes at 4°C. The specimens were centrifuged at 1,500 g for 10 minutes at 4°C. The supernatants were stored at −80°C until assays were performed. Blood samples were centrifuged at 1,500 g for 10 minutes at 4°C and supernatants were stored at −80°C until assays were performed.

Assays

Thrombin generation was assessed by measuring thrombin-antithrombin complex (TATc) levels by means of an enzyme-linked immunosorbent assay (Behring, Marburg, Germany). Antithrombin (AT) levels were measured by an automated amidolytic assay. Levels of activated protein C (APC) were measured with an enzyme capture assay, using monoclonal antibody HAPC 1555 and chromogenic substrate Spectrozyme PCa (American Diagnostica, Greenwich, CT). Plasminogen activator activity (PAA) and plasminogen activator inhibitor (PAI)-1 activity were measured by amidolytic assays.

Statistical Analysis

Data are expressed as mean ± 95% confidence interval or median with interquartile ranges, as appropriate. Comparisons between groups on the different time points were made by the Student’s t test or Mann-Whitney U test, as appropriate. Correlation between percentage of TBSA involved in burn injury and markers of coagulation and fibrinolysis were assessed by linear regression analysis of mean, minimum, and maximum values of each patient during the complete study period. The balance between procoagulant and anticoagulant activity was assessed by determination of the TATc to PAA ratio. A two-tailed p value <0.05 was considered as statistically significant. Data were analyzed using SPSS, version 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Patients

Data and sample collection were completed for 28 patients; 13 patients with burn injuries and inhalation trauma and 15 control patients. Baseline characteristics are shown in Table 1. Burn patients were younger than control patients and
had lower APACHE II scores. Two burn patients suffered from ALI/ARDS on ICU admission and three burn patients developed ALI/ARDS during the course of mechanical ventilation (on days 2, 4, and 6, respectively). There was no difference between groups with regard to the recovered volume of lavage fluid.

### Ventilator and Respiratory Data

Tidal volume size, respiratory rate, minute volume, static compliance, PEEP, and peak pressure levels on the day of ICU admission and alternate days are presented in Figure 1. Levels of PEEP were higher in patients with burn injuries and inhalation trauma. Figure 2 shows $P_aO_2$ and $P_aCO_2$, pH, PF ratio, oxygenation index, and LIS. On enrollment, patients with burn injuries and inhalation trauma had a higher pH than control patients. This difference in pH disappeared after the first day of the study period. On day 4, LIS was significantly higher in patients with burn injuries and inhalation trauma compared with control patients and remained higher throughout the study period.

### Pulmonary Coagulopathy

Activation of pulmonary coagulation was observed in patients with burn injuries and inhalation trauma, as reflected by increased lavage levels of TATc compared with control patients (Fig. 3). Lavage levels of natural anticoagulants AT and APC were significantly reduced in patients with burn injuries and inhalation trauma compared with control patients. At baseline, pulmonary fibrinolysis was significantly reduced in patients with burn injuries and inhalation trauma, as reflected by reduced lavage levels of PAA compared with control patients. Attenuation of fibrinolysis seemed to result, at least in part, from upregulation of the main inhibitor of fibrinolysis PAI-1. Lavage levels of PAI-1 were significantly increased in patients with burn injuries and inhalation trauma compared with control patients. In patients with burn injuries and inhalation trauma, there was no relationship between the

### Table 1: Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients With Burn Injuries With Inhalation Trauma (n = 13)</th>
<th>Control Patients (n = 15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>42 (31–45)</td>
<td>61 (46–69)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>77</td>
<td>67</td>
<td>NS</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>9 (6–12)</td>
<td>22 (18–26)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LIS</td>
<td>1.5 (1.2–1.8)</td>
<td>1.0 (0.8–1.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Burn injuries</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TBSA (%)</td>
<td>23 (8–43)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>TBSA third-degree burns (%)</td>
<td>6 (0–24)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Admission diagnose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn injuries with inhalation trauma</td>
<td>13</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td>—</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>—</td>
<td>5</td>
<td></td>
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<tr>
<td>Resuscitation</td>
<td>—</td>
<td>1</td>
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</tbody>
</table>

Data represent median (interquartile range). Comparison between groups was done using Mann-Whitney U test.
Figure 2. Respiratory values and lung injury score of patients with burn injuries and inhalation trauma (filled circles) compared with control patients (open circles). Data represent mean (95% confidence interval). *p < 0.05.

Figure 3. Levels of TATc, AT, APC, PAA, and PAI-1 in lavage fluid of patients with burn injuries and inhalation trauma (filled circles) compared with control patients (open circles). Data represent median with interquartile ranges. **p < 0.001 for all time points.
extent of pulmonary coagulopathy and the presence or development ALI/ARDS.

Correlations between pulmonary coagulopathy and the extent of burn injury, expressed as percentage TBSA burn, were poor to medium (Fig. 4). Only the minimum level of APC in each patient was significantly associated with burn size, similar to the correlations between pulmonary coagulopathy and the severity of the burn injury as measured by percentage third-degree TBSA burn (data not shown).

**Systemic Coagulopathy**

All patients with burn injuries and inhalation trauma had systemic hemostatic disturbances as reflected by increased plasma levels of TATc and reduced plasma levels of PAA (Fig. 5). Correlations between systemic coagulopathy and the percentage TBSA burn (Fig. 6), and third-degree TBSA burn (data not shown) were poor. The procoagulant-anticoagulant balance, expressed as TATc to PAA ratio, was significantly higher in the burn and inhalation trauma patients compared with the control patients (0.04 ± 0.02 vs. 0.009 ± 0.003, p < 0.0001).

**DISCUSSION**

In this study, marked procoagulant and antifibrinolytic changes were found in the airways of patients with burn injuries and inhalation trauma compared with patients without burn injuries and inhalation trauma and no ALI/ARDS. Pulmonary coagulopathy seems to be intrinsic to inhalation trauma. All patients with burn injuries and inhalation trauma demonstrated pulmonary coagulopathy to a similar degree, irrespective of the presence or development of ALI/ARDS or the extent of burn injury, expressed as TBSA burn. This is the first study on the presence and extent of pulmonary coagulopathy in patients with burn injuries and inhalation trauma.

Previous studies have reported increased procoagulant activity in the alveolar space in patients with pneumonia or ALI/ARDS. Günther et al. found disturbances in the alveolar hemostatic balance in patients with severe pneumonia, both spontaneously breathing and those requiring mechanical ventilation. Schultz et al. demonstrated disturbances in the local hemostatic balance in mechanically ventilated patients who developed ventilator-associated pneumonia. In this study, there was a marked increase in procoagulant activity with an increase in PAI-1 levels even before the diagnosis of pneumonia was made clinically, suggesting that a shift in the hemostatic balance in the lungs toward a procoagulant state is an early feature of pulmonary inflammation. This study confirms the hypothesis that similar procoagulant changes are present in the lungs of patients with burn injuries and inhalation trauma.

Interestingly, similar to the early procoagulant changes in pneumonia, the procoagulant changes after burn injuries and inhalation trauma occur shortly after the insults. Although pulmonary coagulopathy was a consistent finding in burn patients, only a minority of the 13 patients developed ALI/ARDS. At first glance, this contradicts with the above-mentioned hypothesis; however, ALI/ARDS was diagnosed only when patients fulfilled the NAEC criteria. These criteria may be difficult to interpret, especially when higher levels of PEEP are being applied. Indeed, higher levels of PEEP may erroneously give the impression that ALI/ARDS is not present or resolving. Hence, pulmonary coagulopathy may be present in patients not (yet) fulfilling the NAEC criteria. The correlation data of coagulopathy and LIS underline this hypothesis. The LIS corrects for level of PEEP and consequently an increase in coagulopathy was correlated with a higher LIS. Notably, levels of PEEP were already higher on postburn day 1 in the burn patients with inhalation compared with the control patients. Since mechanical ventilation protocols were similar for burn patients and patients without burns, in particular with respect to the titration of levels of PEEP and FIO2, this suggests that burn patients with inhalation already developed lung injury early after the insult.

Higher systemic levels of TAT and lower levels of PAA were found in patients with burn and inhalation trauma. With this design of the study, we were unable to analyze whether this was caused by the burn or by the inhalation trauma.

Ware et al. demonstrated the degree of pulmonary coagulopathy to be an independent risk factor for mortality and adverse clinical outcomes of ALI/ARDS patients. Although being a precondition for secondary reparative processes, there is a substantial body of in vitro and in vivo data, suggesting that pulmonary coagulopathy and persistent deposition of fibrin in the alveolar compartment contribute to lung injury. Thrombin exerts proinflammatory effects through its regulation of cytokine transcription and release and is responsible for the increased production of interleukin-6, interleukin-1 beta, and monocyte chemotactic protein-1. Binding of fibrin to monocytes activates the transcription of NF-kB and AP-1, which in turn regulates the production of various cytokines. Furthermore, the binding of fibrin to monocytes and other cells enhances the inflammatory response by facilitating and enhancing cell migration. Fibrin may also directly compromise lung function by inactivating surfactant leading to loss of lung compliance and atelectasis. Fibrin has been shown to be an important composite of obstructive cast material in an ovine model of combined smoke inhalation and burn. Pathology studies in this model have shown that obstructing materials are mainly composed of infiltrated neutrophils, shed bronchial epithelial cells, mucus, and fibrin. Indeed, in this model, treatment with aerosolized tissue-plasminogen activator significantly improved pulmonary function. Interestingly, nebulized tissue-plasminogen activator has also been shown to be of benefit in the removal of mucous clots in a pediatric patient with plastic bronchitis.

Activation of coagulation during inflammation is a physiologic response, which helps containing inflammatory activity (or infection) at the site of injury. However, it may also aggravate inflammation and thus contribute to disease. Blocking coagulation or stimulating fibrinolysis could theoretically dampen the inflammatory response thereby reducing lung injury. Moreover, many anticoagulant and profibrinolytic compounds have also been shown to have anti-inflammatory effects that seem to be independent of their anticoagulant and pro-fibrinolytic properties. Of interest, in our study, the extent of burn injury, expressed as TBSA burn was associated with a...
Figure 4. Linear regression analysis of the correlation between burn percentage and levels of TATc, AT, APC, PAA, and PAI-1 in lavage fluid of patients with burn injuries and inhalation trauma. Coagulation data as (A) the mean (SD), (B) the maximum value, and (C) the minimum value for each individual patient. The p values and $r^2$ are given in each panel.
decrease in pulmonary APC levels, suggesting that burn injury results in a loss of anticoagulant function.

Several preclinical models of inhalation trauma investigated the effects of anticoagulants on the formation of airway obstructive casts. Infusion of unfractionated heparins reduced pulmonary fibrin depositions and improved oxygenation in a combined ovine model of smoke inhalation and barotraumas.\textsuperscript{18} However, inflammatory responses in lungs were similar to control patients. However, local administration of heparin to the lungs through nebulization did show a reduction in cellular infiltration of the lungs in an ovine model of smoke inhalation injury combined with intratracheal instillation of bacteria.\textsuperscript{19} In an ovine model of smoke inhalation injury combined with intratracheal administration of bacteria, infusion of APC improved oxygenation but did not affect pulmonary edema.\textsuperscript{20} Intravenously administered and aerosolized recombinant human AT in combination with aerosolized heparin in an ovine model of ALI/ARDS induced by skin burn and cotton smoke inhalation attenuated all observed pulmonary pathophysiology.\textsuperscript{21,22} It remains unclear to what extent the beneficial effects of these aerosolized agents rest on the breakdown of central bronchial obstructions or on the effects on alveolar fibrin, the end product of coagulation. To date, only one rather small clinical trial investigated the effect of anticoagulant therapy for inhalation trauma. In pediatric patients suffering from inhalation injury,
treatment with a combination of nebulized heparin reduced mortality. However, since treatment with heparin in this study was combined with the mucolytic agent, N-acetylcysteine, it is impossible to determine the exact role of alveolar anticoagulation on mortality in these patients.

A limitation of our study is that we only compared patients with combined burn injuries and inhalation trauma with a control group of ventilated patients without lung injury. Ideally, we should have compared patients with solely smoke inhalation with patients with solely burns. However, burn patients without inhalation trauma frequently do not need intubation and mechanical ventilation for several days unless the burn is very large, and patients with solely inhalation trauma have self-limited injury with fairly rapid extubation as well. As a consequence, we were only able to add a control group of ventilated patients without burns and inhalation trauma. This makes it difficult to analyze to what extent burn injuries and inhalation trauma separately contributed to the disturbances in pulmonary coagulation. The effects of burn injuries on systemic coagulation and fibrinolysis as described previously may have contributed to pulmonary coagulopathy. In other words, the changes in coagulation and fibrinolysis found in the pulmonary compartment (as measured in the lavage fluid) could be the result of leakage from the system. Indeed, we also observed increased activation of coagulation in the systemic compartment. However, this seems unlikely for the following reasons: (1) with the used lavage technique, there is a >100-fold dilution of alveolar concentrations, so the levels of coagulation and fibrinolysis markers were much higher in the pulmonary compartment than systemic levels; and (2) the correlation between pulmonary coagulopathy and the extent and severity of the burn injuries was poor.

Another shortcoming is that because of the absence (in the literature) of a satisfactory means to assess the severity of inhalation injury, we were not able to quantify inhalation injury in our patients. It can be speculated that more coagulopathy exists with more severe inhalation trauma.

Another limitation of our study is that at baseline, the patients in the control group differed from the patients with burn injuries and inhalation trauma with regard to age and APACHE II scores. However, it is unlikely that age affected pulmonary coagulation and fibrinolysis in our study. The difference in baseline APACHE II score seems to indicate that the control patients were more severely ill than the patients with burn injuries and inhalation trauma. However, it should be noted that the APACHE II score is of limited use in burn patients with inhalation trauma since neurologic, metabolic, hemodynamic, and respiratory problems usually do not occur until hours after admission. Also, a considerable part of difference in APACHE II scores was due to the fact that the control patients were mostly neurologic admissions with a lower Glasgow Coma Score.

Finally, the samples obtained from these patients may not adequately reflect changes in coagulation and fibrinolysis in the alveoli because of the sampling strategy. We used a nondirected lavage technique to obtain samples from the pulmonary compartment. This technique only yields samples from the larger airways. Procoagulant changes in larger airways may not reflect changes in the smaller airways and alveoli.

In conclusion, pulmonary coagulopathy seems intrinsic to burn injury and inhalation trauma. It could be an important therapeutic target in these patients. Further clinical research should investigate whether anticoagulant interventions are beneficial in patients with burn injuries and inhalation trauma.

REFERENCES
