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Original Article

Bronchoalveolar lavage and plasma Antithrombin and cytokines in inhalation and burn injury: a pilot study

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Abstract: Systemic inflammatory response syndrome (SIRS) is initiated during the acute phase of thermal injury. The objective was to determine the SIRS impact on cytokine and Antithrombin (AT) levels in smoke inhalation and burn injury. This observational pilot study compared plasma and bronchoalveolar lavage fluid (BAL) cytokine and AT levels in the first six days post smoke inhalation and burn injury. Twenty-five patients, 14 with inhalation + burn injury > 10% total body surface area (TBSA) and 11 with inhalation injury and ≤ 10% TBSA participated. Human Th1/Th2 cytometric bead array kit from BD Biosciences Pharmingen determined cytokine levels; AT levels with Sigma Diagnostics and spectrophotometry. Results indicated no significant age difference between the two groups (42.1 ± 7.2) versus 49.6 ± 6.4 years. On admission, the inhalation group had 5.4 ± 3.9% TBSA compared to 35.0 ± 22.2% TBSA in the inhalation + burn group, $P < 0.001$. Comparing groups, AT plasma levels were significantly decreased ($P = 0.025$) and IL-2 levels significantly increased ($P = 0.025$) in the inhalation + burn group compared to the inhalation group; there was no significant difference in BAL AT or cytokine levels. Combined group plasma AT levels (65.41 ± 4.44%) were significantly increased compared to BAL AT levels (1.06 ± 0.71%), $P < 0.001$. In contrast, BAL TNF-α levels (35.61 ± 16.01 pg/ml) were significantly increased in relation to the plasma levels (4.68 ± 1.27 pg/ml), $P = 0.02$. On days 1-2, AT plasma levels were significantly decreased in the inhalation + burn group (41.01 ± 5.24%) compared to the inhalation group (81.02 ± 10.99%), $P = 0.002$. IL-6 plasma levels were higher in the inhalation + burn group compared to the inhalation group on admission, but both levels decreased by days 3-6. IL-6 BAL levels were elevated in both groups on days 1-2 and decreased by days 3-6. In the first six days of resuscitation, all plasma cytokines were increased in the two groups compared to controls. AT plasma and BAL levels were significantly reduced in both groups, contributing to the coagulopathy. Increased BAL TNF-α and IL-6 levels may have contributed to the pulmonary perturbations during the initial SIRS response in both groups.

Keywords: Inhalation, burns, cytokines, antithrombin, IL-2, TNF-α, IL-6

Introduction

Pulmonary complications and lung injury-mediated death in burn patients have been recognized in the literature for more than 50 years [1-3]. Despite major advances in burn treatments, the 10%-20% frequency of inhalation injury still has a deleterious effect on burn morbidity and mortality [4]. In 2018, experts from the American Burn Association, federal government, firefighters, and commercial enterprises met in Washington, D.C. to update the status of thermal and inhalation injury dynamics, diagnoses and evaluations [5]. A review of published inhalation injury studies noted the limitations of current treatments and discussed future research initiatives utilizing cytokines and biomarkers [4]. Inhalation and burn injuries initiate a Systemic Inflammatory Response Syndrome (SIRS), which produces a "cytokine storm", in some cases progressing to Acute Respiratory Distress Syndrome (ARDS); systemic coagulation and fibrinolysis are also activated [6]. The purpose of this study was to determine the impact of SIRS by comparing concurrent bronchoalveolar lavage fluid (BAL) with plasma cytokine and AT levels in patients who had mainly inhalation injury to those with inhalation + burn injury, in the acute phase of resuscitation. The hypothesis was that compared to plasma levels, BAL levels would reflect a greater perturbation of the cytokines due to more severe lung involvement in the acute phase of injury.

Materials and methods

Sample population

This was a prospective pilot study in which all patients admitted to the burn intensive care unit and their families were approached for study participation. Enrollment criteria were: 1) inhalation injury requiring mechanical ventilation; 2) inhalation injury with ≤ 10% total body surface area (TBSA) and inhalation + burn > 10% TBSA; 3) age > 10-years old; 4) no coagulopathic blood disorder or other medical contraindications; 5) no anticoagulant therapy; 6) consent obtained from family and/or patient within the first 48 hours of injury. Associated trauma, such as fractures, were acceptable for study inclusion. This study was supported by the Rush University-Cook County Hospital Collaboration Grant Program and approved by the Institutional Review Board.

Burn care

In the first 24 hours after admission, burn patients were resuscitated according to the modified Parkland formula. On admission, arterial blood gas measurement, carboxyhemoglobin (COHb) levels, chest x-ray and fiberoptic bronchoscopy were performed in intubated patients suspected of having an inhalation injury. Although severity of inhalation injury was not graded, patients were diagnosed with inhalation injury based on the clinical history, burn etiology, COHb levels, and bronchoscopic findings. Positive bronchoscopic findings included intramucosal lesions such as edema, erythema, blebs, or mucosal slough and extra mucosal lesions such as soot, charring or bronchorrhea. All patients required ventilatory support on admission. Bennett 7200 ventilators provided high-flow humidified oxygen maintaining oxygen saturation (SaO_2) ≥ 95%. All patients received mechanical ventilation, nebulized albuterol, and N-acetylcysteine. Wound management was performed in the operating room or the burn center hydrotherapy room, depending on the necessity for initial escharotomy and/or fasciotomy. If hemodynamically stable, patients with full thickness burns were taken to surgery within the first 72 hours of admission for excision and temporary wound coverage.

Biological sample collection and handling

Anti-inflammatory IL-4 and Antithrombin (AT), pro-inflammatory TNF-α and IL-6, and immune modulators IFN-γ, IL-10 and IL-2 were studied. Blood samples were drawn within 48 hours of injury and at 3-6 days later (in conjunction with bronchoscopy at the time of BAL specimen collections) via a two-syringe butterfly technique using a 21-gauge needle and polypropylene syringes or through the central venous line into 3.2% sodium citrate tubes. Three normal control plasma specimens, obtained from the hematology laboratory, were tested with the patient samples in all assays. For cytokine and AT assays, the tubes were immediately centrifuged (1200 × g, 20 minutes, 4°C) to obtain platelet-poor plasma. Plasma was aliquoted and stored frozen at -70°C until analysis. All laboratory assays on the patients were performed within three months of entry into the study.

Procedures for the collection and processing of BAL fluid

Intubated patients suspected of having smoke inhalation based on history or findings of soot in the mouth or oropharynx underwent flexible fiberoptic bronchoscopy with BAL; 30 cc of normal saline was lavaged into the tracheobronchial tree and withdrawn into a Leukin sputum trap. Approximately 40-60% of the initial BAL total volume was returned with each procedure. The fluid was centrifuged at 3000 g for 10 minutes, separated from the cells, and stored at -70°C prior to analysis. All laboratory assays on the patients were performed within three months of entry into the study. Further mention of bronchoalveolar fluid will be abbreviated as BAL.

Antithrombin

Both plasma and BAL were tested for AT levels by Sigma Diagnostics (Dorset, UK). The spectrophotometer was a Beckman Coulter Du 640, (Fullerton, CA). Four normal plasma samples were titrated individually; because of minimal variation, they were pooled and used as the normal plasma standard. The samples were assayed according to the manufacturer's directions. Diluted samples or standards (200 ul) were warmed to 37°C and a bovine thrombin and heparin mixture was added. Substrate was added and allowed to react for 2 minutes with uninhibited thrombin. The reaction was stopped with citric acid and read at 405 nm.

Table 1. Demographic characteristics of inhalation and burn + inhalation groups

Parameters	Inhalation (11)	Inhalation + Burn (14)	*P value
Patient Characteristics (mean ± sd)			
Age (years)	42.1 ± 23.7	49.6 ± 23.9	0.44
Male/Female	8/3	8/6	0.42
% TBSA	5.4 ± 3.9	35.0 ± 22.2	0.015
LOS (days)	12.3 ± 11.2	35.5 ± 35.1	0.06
Ventilator (days)	6.8 ± 7.5	19.5 ± 26.2	0.15
SIRS Score	1.9 ± 1.3	2.1 ± 1.3	0.73
Baux Score	62.1 ± 23.6	101.6 ± 26.2	0.001
Baux M%	4.5 ± 7.8	35.6 ± 34.6	0.027
Laboratory (mean ± sd)			
WBC (4.5-11.0 × 10^9/L)	14.2 ± 10.3	13.8 ± 6.8	0.93
Platelets (150-400 × 10^9/L)	224.0 ± 65.0	283.7 ± 93.5	0.10
Glucose (72-99 mg/dl)	181.9 ± 78.4	196.2 ± 92.2	0.70
COHB (%)	16.3 ± 9.2	12.1 ± 12.5	0.48
Complications n (%)			
Pneumonia	9 (81.82)	6 (42.86)	0.04
Mortality	2 (18.18)	6 (42.86)	0.18

Mean ± sd = mean ± standard deviation; LOS, length of stay; Vent, mechanical ventilator days; WBC, white blood count; COHB, carboxyhemoglobin; Inhalation, Inhalation and ≤ 10% TBSA burn; Inhalation + Burn. Inhalation with > 10% TBSA burn. *one way-ANOVA and Mann Whitney U tests.

Cytokines

Flow cytometry (BD Biosciences, San Diego, CA) utilized the Cytometric Bead Array kit for Human Th1/Th2 cytokines (BD Biosciences Pharmingen, San Diego, CA) to measure Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), Interleukin-10 (IL-10), Interleukin-6 (IL-6), Interleukin-4 (IL-4), and Interleukin-2 (IL-2) plasma levels, using manufacturer reagents and standards, according to manufacturer's instructions. Normal control samples were run simultaneously. This multiplexed bead assay was a series of spectrally discrete particles that were used to capture and quantitate soluble analytes. Normal levels for the cytokines studied were IFN-γ (3 ± 3 pg/ml); TNF-α (1 ± 1 pg/ml; IL-10 (2 ± 2 pg/ml); IL-6 (4 ± 1 pg/ml); IL-4 (1 ± 2 pg/ml); IL-2 (< 1 pg/ml).

Statistical analysis

Statistical analyses were performed utilizing Statistica® (STATSOFT, Tulsa, OK). The study compared plasma and BAL levels in two groups: patients with inhalation with ≤ 10% TBSA burn (inhalation), inhalation with ≥ 10% TBSA burn (inhalation + burn) groups; plasma and BAL specimens on days 1-2 and days 3-6 post injury; survival. Descriptive statistics (mean ± sd) for demographic parameters, (mean ± se) for cytokine and antithrombin values were used. One way-ANOVA with the Tukey test for unequal numbers, and nonparametric Mann-Whitney U tests were applied for age, length of stay (LOS), ventilator days, carboxyhemoglobin (COHb), % TBSA, white blood count, SIRS Score, Baux score, plasma and BAL cytokine/AT levels. Chi-square (Pearson and Maximum Likelihood) were utilized for gender, pneumonia, mortality, and subset comparisons. Two patients with a history of chronic obstructive lung disease and IL-6 levels of > 25,000 pg/ml were removed as outliers from the IL-6 analysis. Because there were results for only one patient for the Inhalation BAL levels on days 3-6, they were not included in the graphs. Cytokine and AT graphs were constructed utilizing GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA USA). A p value of < 0.05 was considered significant.

Results

Table 1 shows days 1-6 cumulative plasma and BAL specimen levels comparing the inhalation and inhalation + burn groups. The inhalation group had a mean ± sd: 5.4 ± 3.9% TBSA compared to 35.0 ± 22.2% TBSA in the inhalation + burn group, $P = 0.015$; LOS (12.3 ± 11.2 versus 35.5 ± 9.4 days), $P = 0.06$. There was no signi-

Table 2. Plasma cytokines and antithrombin in inhalation and burn injury groups (Days 1-6)

Parameters	Control	Inhalation	Inhalation + Burn	*P value
Pro-Inflammatory Cytokines				
TNF-α	1 ± 1 pg/ml	5.25 ± 2.67	4.38 ± 1.37	0.46
IL-6	4 ± 1 pg/ml	439.61 ± 187.89	1011.84 ± 423.47	0.17
Anti-Inflammatory Modulators				
AT	100 ± 20%	79.98 ± 6.25	58.13 ± 5.17	0.025
IL-4	1 ± 2 pg/ml	3.58 ± 1.89	3.41 ± 0.63	0.25
Immunity Modulators				
IFN-γ	3 ± 3 pg/ml	16.05 ± 4.81	38.44 ± 18.77	0.59
IL-2	< 1 pg/ml	1.63 ± 0.75	4.68 ± 1.10	0.025
IL-10	2 ± 2 pg/ml	19.00 ± 4.25	40.21 ± 12.27	0.89

Mean ± se = mean ± standard error; AT = Antithrombin; TNF-α = Tumor Necrosis Factor-a; IL-6 = Interleukin-6; IL-4+ Interleukin -4; IFN-γ = Interferon-γ; IL-2 = Interleukin-2; IL-10 = Interleukin-10; Inhalation = Inhalation and ≤ 10% TBSA burn; Inhalation + burn = Inhalation with > 10% TBSA burn; *Mann Whitney U test.

Table 3. BAL cytokines & antithrombin in inhalation and burn injury groups (Days 1-6)

Parameters	Inhalation	Inhalation + Burn	*P value
Pro-Inflammatory Cytokines			
TNF-α	42.82 ± 31.76	30.21 ± 16.83	0.52
IL-6	878.37 ± 424.92	918.55 ± 187.97	0.69
Anti-Inflammatory Modulators			
AT	0	1.77 ± 1.13	0.29
IL-4	4.60 + 1.97	3.71 ± 1.29	0.74
Immunity Modulators			
IFN-γ	18.55 + 6.87	15.26 + 5.33	0.75
IL-2	3.07 + 1.21	2.94 + 0.97	0.95
IL-10	10.35 + 6.06	35.20 + 16.93	0.37

Mean ± se = mean ± standard error; AT = Antithrombin; BAL = bronchoalveolar lavage fluid; TNF-α = Tumor Necrosis Factor-α; IL-6 = Interleukin-6. Inhalation = Inhalation and ≤ 10% TBSA burn; Inhalation + Burn = Inhalation with > 10% TBSA burn; There are no normal values noted for BAL. For comparison, these are normal plasma levels: IFN-γ (3 ± 3 pg/ml); TNF-α (1 ± 1 pg/ml; IL-10 (2 ± 2 pg/ml); IL-6 (4 ± 1 pg/ml); IL-4 (1 ± 2 pg/ml); IL-2 (< 1 pg/ml). *Mann Whitney U test analysis.

ficant difference between the inhalation and inhalation + burn groups in age (42.1 ± 23.7 versus 49.6 ± 23.9 years), $P = 0.44$. The Baux score and Baux M% were significantly increased in the inhalation + burn groups compared to the inhalation group. The inhalation group had a higher frequency of pneumonia in this study, $P = 0.04$. During the early resuscitation phase (days 1-6), comparing all plasma versus all BAL AT and cytokine levels, only plasma AT levels (65.41 ± 4.44%) were significantly increased compared to the BAL AT levels (1.06 ± 0.71%), $P < 0.0001$. In contrast, BAL TNF-α levels (35.61 ± 16.01 pg/ml) were significantly increased in relation to the plasma TNF-α levels (4.68 ± 1.27 pg/ml), $P = 0.02$.

There were two (18.18%) non-survivors in the inhalation group compared to the inhalation + burn group with six (42.86%). The combined mean ± sd age was non-survivor to survivor (49.4 ± 30.9 versus 44.9 ± 20.3 years). On admission, compared to survivors, non-survivors had a higher mean ± sd% TBSA (42.9 ± 24.5 versus 17.1 ± 17.2), $P = 0.03$; COHb (22.7 ± 11.6 versus 12.2 ± 9.8%), white blood count (21.0 ± 1 2.0 versus 11.33 ± 4.71 × 10^9/L), $P = 0.02$; SIRS scores (2.9 ± 1.1 versus 1.6 ± 1.1), $P = 0.03$; higher Baux scores (103.9 ± 44.4 versus 75.0 ± 19.1), ventilator days/LOS ratios (0.9 ± 0.2 versus 0.5 ± 0.2), $P < 0.001$, and shorter hospitalizations (21.6 ± 39.2 versus 27.0 ± 24.6 days), $P = 0.04$ (by the Mann Whitney U test). There was no difference between the inhalation and inhalation + burn groups, except for plasma AT, which was significantly higher in the survivors compared to the non-survivors, (70.22 ± 5.95% versus 42.97 ± 10.02%), $P < 0.001$. There were no significant differences in plasma or BAL cytokine levels in survivors compared to non-survivors in the early phase of resuscitation.

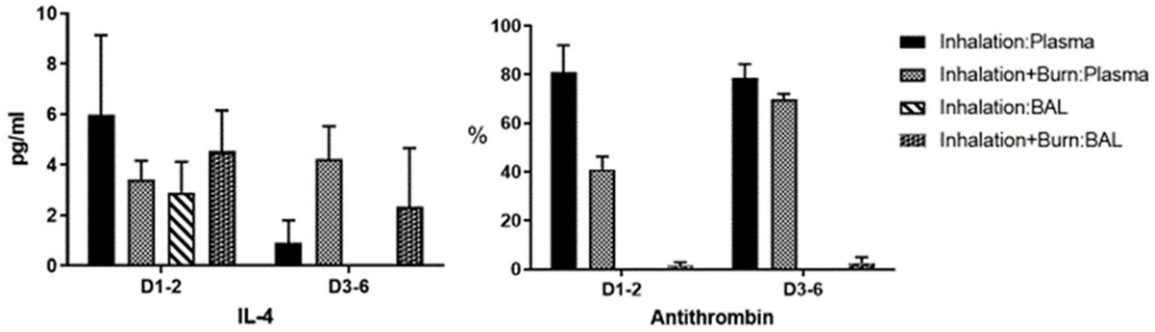

Figure 1. Days 1-2 and days 3-6 for Antithrombin, and IL-4 plasma and BAL fluid levels in the first six days of resuscitation after smoke inhalation and burn injury.

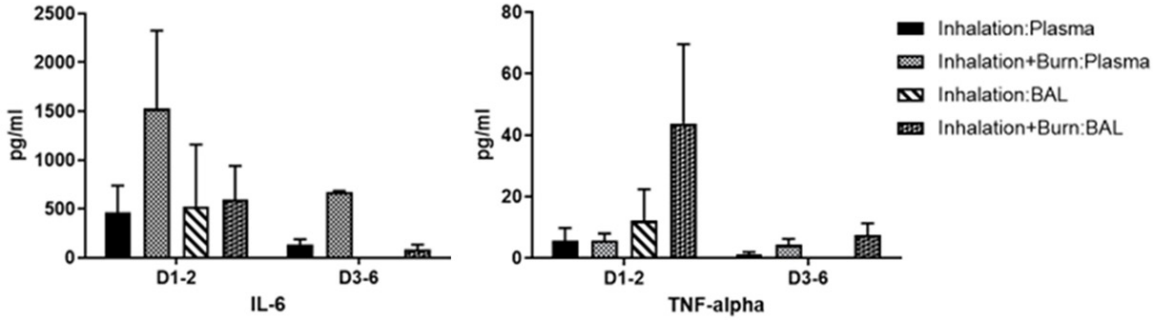

Figure 2. Days 1-2 and days 3-6 for TNF-α, and IL-6 plasma and BAL fluid levels in the first six days of resuscitation after smoke inhalation and burn injury.

Plasma and BAL AT and cytokines in inhalation and inhalation + burn groups

Tables 2 and **3** depict the plasma and BAL AT and cytokine values during the combined days 1-6 of early resuscitation. If one compared the groups by the Mann Whitney U test, only AT plasma levels are significantly decreased (P = 0.025) and IL-2 levels significantly increased (P = 0.025) in the inhalation + burn group compared to the inhalation group. There was no significance difference in BAL AT or any cytokine levels between the two groups. If one compared AT and cytokines on days 1-2 to days 3-6 in the two groups, there was no difference in plasma or BAL AT or cytokines in the inhalation group. However, the AT plasma and BAL levels in the inhalation + burn group were significantly different on days 1-2 compared to days 3-6.

In the 6-day early phase of resuscitation, compared to the inhalation group, IL-10, IL-6, IFN-γ, IL-2 levels were more elevated in the inhalation + burn group, similar in the TNF-α and IL-4 levels, and significantly decreased in AT levels. Compared to the inhalation group, BAL levels were more elevated in IL-10, similar in TNF-α, IL-6, IL-4, IFN-γ, IL-2, and minimally increased in AT levels in the inhalation + burn group. **Figures 1-3** illustrate the differences in plasma and BAL levels of the AT and cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α) in this study. On days 1-2, BAL IL-10, Il-6, Il-4, IL-2, IFN-γ, and TNF-α levels were higher in both groups compared to days 3-6, except for AT levels, which were minimally elevated or decreased in the first 6 days. Compared to days 3-6, plasma TNF-α and IL-6 levels were more elevated on days 1-2, **Figure 1**. AT levels were decreased to a greater extent on days 1-2, especially in the inhalation + burn group but improved by days 3-6, **Figure 2**. Compared to days 1-2, plasma IL-2 level remained elevated on days 3-6 in the inhalation + burn group and decreased in the Inhalation group; plasma IFN-γ level increased on days 3-6 in the inhalation + burn group, **Figure 3**.

Discussion

Within the SIRS-initiated cytokine increase, there was no significant difference between

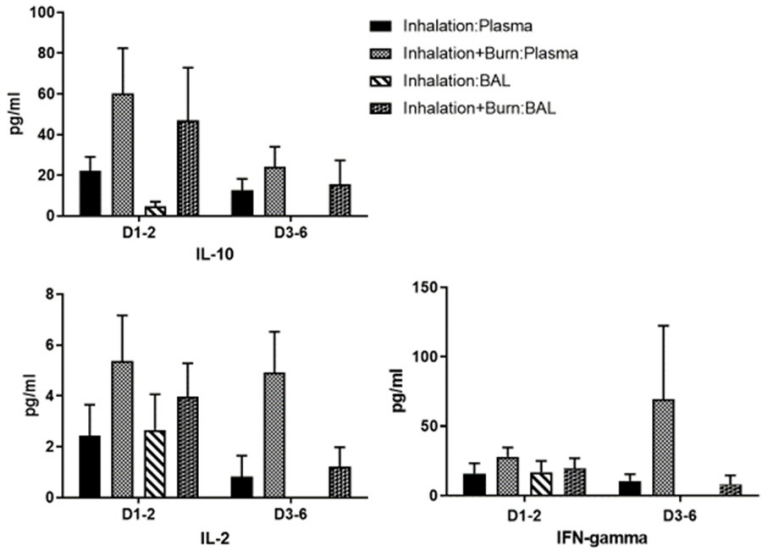

Figure 3. Days 1-2 and days 3-6 for IFN-γ, IL-10, IL-2 plasma and BAL fluid levels in the first six days of resuscitation after smoke inhalation and burn injury.

BAL and plasma cytokines except for TNF-α ($P = 0.02$) and Antithrombin ($P < 0.001$) in the inhalation + burn group and only antithrombin ($P = 0.01$) in the inhalation group. Cytokines play a major role in thermal and inhalation injury. Compared to normal levels, days 1-6 AT plasma levels were significantly decreased in inhalation and inhalation + burn patients commensurate with injury severity. Although all cytokine levels were significantly increased in plasma compared to controls, there was no significant difference between the two study groups. This was partly the result of small numbers and a large coefficient of variation in the statistical analyses of these cytokines.

There are few current studies correlating plasma and BAL cytokine and AT levels in patients in the acute phase of inhalation and thermal injury. One study noted that on day 1 of ARDS, non-survivors had significantly persistent elevations of BAL TNF-alpha, IL-6, IL-2, and IL-4 compared to survivors; initial BAL IL-2 and Il-4 levels were significantly higher in patients with sepsis [7]. Another study determined that in patients with ARDS, BAL TNF-α was negatively correlated to PaO_2/FiO_2 and serum levels were related to outcome [8]. A study of lung alveolar leukocyte function after thermal injury and smoke inhalation noted that by day 4, inhalation + burn injury alveolar macrophages produced more TNF-α and IL-6 than burn or control patient alveolar macrophages [9]. These laboratory findings support our BAL results in both the inhalation and inhalation + burn patients. In more recent reports, BAL fluid cytokine and immune modulator studies evaluated the pulmonary aspect but did not compare concurrent BAL and plasma levels of AT and cytokines at the time of injury [10-12]. These inhalation + burn injury studies assessed survival in patients with inhalation injury and > 10% TBSA in their analyses [10-12]. In a pediatric study of cytokines in severely burned patients in the first seven days after admission, serum levels of IL-4, IL-6 and IL-10 were significantly increased in non-survivors compared to survivors [10]. Sixty burn patients were studied for their BAL cytokine concentrations; non-survivors had decreased levels of IL-10 compared to survivors [11], In a subset of patients with the highest Baux scores, non-survivors also had lower levels of IL-2, IL-4, IFN-γ, and TNF-alpha [11]. Another study reported that IL-6 levels were significantly elevated, while IL-4 levels were decreased in non-survivors compared to survivors [12].

After the initial pulmonary insult, patient studies have indicated that the lung is a repository of cytokines even during recovery post hospitalization. A six-month follow-up of patients who had smoke inhalation demonstrated that compared to controls, their main symptoms were a productive cough and lymphocytic infiltrate on a bronchial biopsy [13]. These patients had serum TNF-α and IFN-γ levels that were significantly increased, $P < 0.05$ [13].

Unlike the literature studies, this pilot study did not find significant cytokine difference between the survivors and non-survivors or between the plasma and BAL levels except for AT and TNF-α. AT was not investigated by the previous authors when other cytokines and biomarkers in inhalation and burn injury were studied. AT is a serine protease, which functions as a natural anticoagulant and an anti-inflammatory agent;

it is decreased in thermal injury [14-16]. High levels of AT human concentrate have improved lung function and reduced IL-6 levels in burn patients [15]. It removes thrombin from the circulation by forming thrombin-antithrombin (TAT) complexes. There are few studies in the literature comparing plasma and BAL AT and cytokine levels in burn patients with inhalation injury. In this study, we have shown that patients with inhalation and inhalation + burn injury had decreased AT plasma and BAL levels during the initial resuscitation period.

Lung pathophysiology research in burn trauma and smoke inhalation injury executed at the University of Texas Medical Branch in Houston, Texas has shown therapeutic benefits of recombinant Antithrombin (rhAT) for inhalation/burn injury and pulmonary function in their ovine burn and smoke inhalation-induced model of acute lung injury/ARDS [17-20]. A sepsis investigation after smoke inhalation in sheep showed that rhAT attenuated the septic shock and acute lung injury [17]. Airway fibrin clots formation (causing airway obstruction and ARDS) was prevented with aerosolized anticoagulants (rhAT and heparin) and reduced the pulmonary pathology [18]. The current study has demonstrated that AT was minimally present in the lung during the early phases of inhalation and burn injury. Ovine studies have shown that rhAT (ATryn) is a viable treatment for patients with inhalation and burn injury [17-20]. It has been used in hereditary AT deficiency; as a recombinant medication, it is less expensive than the human concentrate. Leitner et al performed a randomized, double blind, placebo-controlled study with rhAT infusions to 200%-500% of normal levels (100 ± 20%) in 30 healthy male volunteers As a dose-dependent medication, rhAT inhibited tissue-factor-triggered coagulation, reduced IL-6 levels by 40%, and transiently decreased neutrophil and monocytes counts [21]. The infusion or aerosolization of AT, a natural anticoagulant, into injured human pulmonary parenchyma may alleviate or reduce the formation of the airway fibrin clots and decrease mortality and morbidity.

More recent literature and research on Antithrombin has been from Japan. In 2017, a national study for antithrombin use in patients with burn and inhalation injury was undertaken [22]. Results of 103 propensity score matched (PSM) pairs indicated that patients receiving antithrombin had a lower 28-day mortality rate compared to controls [33.0% vs 47.6%; 95% confidence interval [CI] 1.2-28.0) [22]. In addition, the PSM pairs receiving antithrombin had more ventilator-free days compared to controls (16.4 days vs 12.6 days, $P = 0.04$ [22]. Since this was an observational study, further investigations in Antithrombin for burn and inhalation injury was recommended. Currently, Antithrombin research has been advocated for sepsis associated disseminated intravascular coagulation (DIC) patients. The Japanese Society on Thrombosis and Haemostasis has adopted an Antithrombin level of < 70% as one of the diagnostic criteria for DIC in their scoring systems for infection [23].

Inhalation and burn patients are also at risk for DIC, sepsis, pneumonia, wound and other infections. In future studies addressing the impact of smoke inhalation and burn injury on the lung, AT, TNF-α and IL-6 levels may be predictive for injury severity in these patients. Increased BAL and plasma TNF-α and IL-6 levels in the current study reflected literature findings in patients with other lung injuries such as ARDS and infection. rhAT may be a worthwhile treatment option for qualified patients with inhalation and burn injury. This pilot study may serve as an impetus for human smoke inhalation and burn studies using rhAntithrombin, especially since ATryn has been used successfully in human hereditary AT deficiency and ovine inhalation and burn studies.

Limitations

The limitations of this pilot study were mainly the small patient numbers, and patient variability and heterogeneity (time from injury to presentation and injury severity). Inhalation injury was not graded but was treated based on the bronchoscopy visualization, history, with the clinical and physical presentations.

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Disclosure of conflict of interest

None.

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