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The acute pulmonary inflammatory response to the graded severity of smoke inhalation injury

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Abstract

Objective—To determine if the graded severity of smoke inhalation is reflected by the acute pulmonary inflammatory response to injury.

Design—In a prospective observational study we assessed the bronchoalveolar lavage fluid (BALF) for both leukocyte differential and concentration of 28 cytokines, chemokines, and growth factors. Results were then compared to the graded severity of inhalation injury as determined by Abbreviated Injury Score criteria (0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Massive).

Setting—All patients were enrolled at a single tertiary burn center.

Patients—The BALF was obtained from 60 patients within 14 hours of burn injury who underwent bronchoscopy for suspected smoke inhalation.

Interventions—None.

Measurements and Main Results—Those who presented with worse grades of inhalation injury had higher plasma levels of carboxyhemoglobin and enhanced airway neutrophilia. Patients with the most severe inhalation injuries also had a greater requirement for tracheostomy, longer time on the ventilator, and a prolonged stay in the intensive care unit. Of the 28 inflammatory mediators assessed in the BALF, 21 were at their highest in those with the worst inhalation injury scores (Grades 3 and 4), the greatest of which was interleukin (IL)-8 (92,940 pg/ml, Grade 4). When compared in terms of low inhalation injury (Grades 1–2) versus high inhalation injury (Grades 3–4), we found significant differences between groups for IL-4, IL-6, IL-9, IL-15, interferon- γ , granulocyte-macrophage colony-stimulating factor, and monocyte chemotactic protein-1 ($p < 0.05$ for all).

Conclusions—These data reveal that the degree of inhalation injury: 1) has basic and profound effects on burn patient morbidity; 2) evokes complex changes of multiple alveolar inflammatory proteins; and 3) is a determinant of the pulmonary inflammatory response to smoke inhalation. Accordingly, future investigations should consider inhalation injury to be a graded phenomenon.

Keywords

Burn; Inhalation Injury; Inflammation; Cytokine; Chemokine; Growth Factor

Introduction

In the United States, 40,000 hospital admissions for burn injury occur each year (1). Approximately 6–20% of fire victims have a concurrent inhalation injury, which is a chemical insult rather than a thermal tracheobronchiolitis (2, 3). Acutely, those with an inhalation injury have more pronounced arterial hypotension and require more extensive fluid resuscitation, and the diffuse epithelial sloughing, bronchorrhea, and plugging of sub-segmental bronchi characteristic of inhalation injury can result in subsequent pneumonia rates of 20–50% (4–8). Moreover, the combination of pulmonary and dermal injury may double a patient's mortality (8). Prior investigations in patients with smoke inhalation have revealed a temporal increase in pro-inflammatory cytokine levels in the airway (9), coupled with alveolar leukocytes that are “primed” for an enhanced response to stimulation with lipopolysaccharide (10). However, these early studies did not examine inflammatory mediators in the context of severity of inhalation injury. As such, we hypothesized that pro-inflammatory mediators at the alveolar level would increase as the degree of inhalation injury increased, highlighting the importance of classifying inhalation injury as a graded phenomenon.

Materials and Methods

From January 2007 to April 2010, bronchoalveolar lavage fluid (BALF) was collected from 60 patients admitted to the Burn Intensive Care Unit in whom inhalation injury was suspected by history and/or physical findings, such as injury within a confined space or soot at the nares or tracheal secretions. Patients were excluded from the study for the following: age less than 18 years, malignancy, immunosuppressive medications or known autoimmune or chronic inflammatory diseases. Clinical variables and outcomes collected were: age, gender, race/ethnicity, % total body surface area (% TBSA) burn, inhalation injury grade, partial pressure of oxygen in arterial blood to fraction of inspired oxygen (P:F) ratio at the time of bronchoscopy, initial 24 and 72 hour fluid requirements, incidence of pneumonia, incidence of sepsis, ICU and hospital length of stay, and mortality. Pneumonia was defined as any BAL culture of >100,000 colony forming units (CFUs), and sepsis defined as hemodynamic instability in combination with a documented source of infection (blood, urine, BAL, or wound). All samples were collected before any aerosolized pulmonary medications had been administered. Six non-smoking, non-intubated, non-hospitalized healthy adult volunteers (free of pulmonary, cardiac, infectious, and allergic disease) were also included as a control group for comparison. This study was approved by the Loyola University Medical Center Institutional Review Board (LU#107372).

Inhalation injury scoring system

The degree of inhalation injury was determined using a standardized bronchoscopic scoring system based on Abbreviated Injury Score (AIS) criteria as we have previously published (11). The severity of inhalation injury was graded into five categories (0, 1, 2, 3, and 4) with 0 being the absence of visible inhalation injury (Table 1).

Bronchoalveolar lavage

Bronchoscopy and BAL were performed by a standardized protocol within 14 hours in all burn-injured patients (12). That which was not required for routine clinical analysis was

pooled for research use. In control subjects, the bronchoscope was directed into a subsegment of the right middle lobe and wedged; the first 50 mL aliquot of saline was instilled, and the aspirate discarded. Subsequent 50 mL aliquots were instilled into the same subsegment, and immediately aspirated with gentle hand aspiration into sterile syringes. These were immediately transferred into sterile 50 mL conical tubes, placed on ice, and transported to the laboratory for additional processing. There were no immediate complications to bronchoscopy in either the patient or control groups.

Sample processing

BALF samples were strained through sterile 100 micron nylon cell strainers (BD Biosciences, Bedford, MA). The raw sample was centrifuged at 1200 RPM for 5 minutes to separate the supernatant from the cellular component. The supernatant was then aliquoted and frozen at -80° . Cytokine concentrations in BALF were measured by Bio-Rad Multiplex Assays (Hercules, CA) according to manufacturer protocol. All samples were assayed in duplicate and the results analyzed using the Bio-Plex manager software, version 5.0.

Statistical analysis

Patient demographics, outcomes, and inflammatory mediator concentrations were assessed for normality and parametric or non-parametric tests applied where appropriate. Correlations were performed with Spearman's rank correlation coefficient, and the r value reported. Continuous variables of parametric tests are reported as mean with standard deviation, and non-parametric tests reported as median with 25th and 75th percentiles. Otherwise dichotomous variables are reported as a number and percent. Statistical analyses were calculated with SAS Version 9.1 (SAS Institute Inc., Cary, NC) and corresponding graphs created with GraphPad Prism 5 for Windows (GraphPad Software, La Jolla, CA). A difference between observed variables was considered statistically significant when $p < 0.05$.

Results

Patient characteristics by inhalation injury grade

As shown in Table 2, patients with each grade of inhalation injury were similar in terms of age, gender, race/ethnicity, and % TBSA burn across grades of inhalation injury. The percent of plasma COHb measured nearest the time of injury was predictably higher among patients with higher grades of inhalation injury. Likewise, the BALF white blood cell (WBC) distribution changed across grades of inhalation injury with a significant shift from a macrophage predominant population to one dominated by neutrophils. Though the initial P:F ratio was lower in those with a Grade 4 injury no significant difference was reached.

Patient outcomes by inhalation injury grade

As shown in Table 3, the degree of inhalation injury had neither a significant effect on subsequent pneumonia, sepsis, hospital length of stay, or mortality, nor did it appear to be a risk factor for mortality when adjusting for the effects of age and % TBSA. However, those with a more severe inhalation injury required greater ventilator days and a trend toward a greater rate of tracheostomy and ICU length of stay. When comparing those without inhalation injury (Grade 0) to those with any inhalation injury as a single group (Grades 1–4) initial 24-hour fluid requirements were greater (median 4.2 Liters (L) vs 8.8 L, respectively; $p=0.036$), as were the number of ventilator days (median 3 vs 15; $p<0.001$) and tracheostomies (0 vs 51%; $p=0.021$).

Inflammatory mediators by inhalation injury grade

Inflammatory mediators with statistical significance and/or the potential for biological relevance are shown in Figure 1. Across grades of inhalation injury (and excluding controls for statistical comparison), the overall median value differed significantly ($p < 0.05$) for complement component 5a (C5a), IL-6, IL-15, interferon (IFN)- γ , monocyte chemotactic protein-1 (MCP-1), regulated on activation, normal T expressed and secreted (RANTES), tumor necrosis factor (TNF)- α , and vascular endothelial growth factor (VEGF). IL-7 and platelet derived growth factor (PDGF) were not graphically represented though had similar differences between medians ($p < 0.05$). Of all the mediators tested, only VEGF demonstrated a pattern of decreasing concentrations across the grades of inhalation injury. No protein demonstrated a linear relationship to the grade of inhalation injury.

Patient characteristics when grouped by inhalation injury severity

In order to minimize clinician bias during inhalation injury grading, and to better assess the effects of inhalation injury severity, patients were grouped and compared according to low inhalation injury severity (Grades 1 and 2) and high inhalation injury severity (Grades 3 and 4). Patient characteristics by group are reported in Table 4. Most importantly there were no differences in age, gender, race/ethnicity, and % TBSA; moreover, the groups did not differ by initial P:F ratio, BALF WBC differential, or initial BALF culture for microorganisms. On the contrary, those with a high compared to low inhalation injury severity appeared to have a greater % COHb (median 10.4% vs 5.1%), though this difference only approached statistical significance ($p=0.059$).

Table 5 demonstrates the comparison of BAL fluid immune mediator concentrations not only of those with low and high inhalation injury severities, but also of subjects without visible inhalation injury (Grade 0), and controls. Compared to controls, IL-1RA, IL-7, IL-8, IL-10, and MIP-1 β were elevated in Grade 0 patients, while the concentration of GM-CSF was lower. With the addition of inhalation injuries, IL-1 β , IL-1RA, IL-6, IL-8, IL-9, G-CSF, IFN- γ , MCP-1, MIP-1 β , PDGF, and RANTES were increased over controls and/or Grade 0 patients, while IL-7 and VEGF tended to be lower.

Patient outcomes when grouped by inhalation injury severity

As with worsening inhalation injury grades, the group with the highest inhalation injury severity required significantly more ventilator days ($p=0.036$) and ICU days ($p=0.040$) compared to those with a more mild inhalation injury severity (Table 6). Though the severity of inhalation injury had no effect on either the resuscitation fluid requirements or mortality, patients with the worst inhalation injury severity also tended to have a greater percentage who required a tracheostomy (33% vs. 52%), percentage who developed pneumonia or sepsis (43% vs. 67% and 13% vs. 29%), and a longer hospital length of stay (11 vs. 25 days).

Inflammatory mediators when grouped by inhalation injury severity

In contrast to the comparison of patient characteristics, numerous differences were found between the inflammatory mediator profile of those with low vs. high inhalation injury severities (Figure 2), and the respective differences are as follows: IL-4 (median 0.2 vs. 1.8 pg/ml; $p=0.048$), IL-6 (median 141.7 vs. 351.3 pg/ml; $p=0.001$), IL-9 (median 1.2 vs. 10.0 pg/ml; $p=0.015$), IL-15 (median 0.7 vs. 4.7 pg/ml; $p=0.001$), granulocyte-macrophage colony-stimulating factor (GM-CSF) (median 4.3 vs. 9.5; $p=0.040$), IFN- γ (median 39.8 vs. 142.8 pg/ml; $p=0.048$), and MCP-1 (median 49.9 vs. 185.1; $p=0.001$).

Correlations with Presenting Characteristics and Outcomes

Given that carbon monoxide (CO) has known immune modulating effects (namely anti-inflammatory), we assessed for a correlation between the initial % COHb and BALF immune mediator concentrations. We found differences that were only suggestive of significance ($p < 0.10$) between % COHb and the BALF concentrations of C5a, IL-1 β , IL-8, FGF-basic, IFN- γ , MCP-1, MIP-1 β , and PDGF. Furthermore, the % COHb did not correlate with initial P:F ratio (r : -0.184; p =0.187).

Correlations with outcomes were also assessed for inhalation injury grade, % TBSA, and the concentration of 13 immune mediators measured in the BALF selected for either concentrations found at physiologically relevant levels or those that have been previously studied in burn care (Table 7). Inhalation injury grade correlated with frequency of tracheostomy, number of ventilator days, ICU LOS, hospital LOS ($p < 0.05$ for all) and nearly the frequency of pneumonia ($p = 0.051$). Likewise, % TBSA correlated with fluid resuscitation requirements, pneumonia, ventilator days, ICU LOS, hospital LOS, and mortality ($p < 0.05$ for all). No BALF mediator concentration correlated with resuscitation requirements. However, we did find the following differences: IL-1 β correlated with sepsis and ICU LOS, and inversely with mortality; IL-1RA correlated with sepsis and inversely with mortality; IL-8 correlated inversely with mortality; IL-10 correlated inversely with mortality; G-CSF correlated inversely with mortality; MCP-1 correlated with the frequency of tracheostomy; RANTES correlated with the frequency of tracheostomy; and TNF- α correlated with ICU LOS ($p < 0.05$ for all).

Discussion

It is well-established that patients suffering smoke inhalation injury in addition to dermal injury have poorer outcomes than those with skin burns alone (4–8, 13, 14). Animal experiments suggest that these effects are modulated by an augmented inflammatory response (15–18), and in humans, smoke inhalation injury seems to evoke enhanced pulmonary leukocyte activity and pronounced alveolar neutrophilia (9, 10). Though these initial reports focused on the early time-course of the pulmonary inflammatory response to inhalation injury, none addressed the magnitude of inflammatory response as it relates to the degree of inhalation injury. The present study of human burn victims not only reaffirms the effects of smoke inhalation on many burn patient outcomes, but is the first to demonstrate that the severity of inhalation injury may play a role in the overall pulmonary inflammatory response.

One such feature of the burn patient with smoke inhalation that we have re-demonstrated is the association of elevated COHb levels in patients with higher grades of inhalation injury. In addition to the potential for cerebrototoxicity, CO has been shown in a large animal model of combined burn and smoke inhalation injury to be associated with an increased pulmonary shunt fraction (18, 19). Although this feature could have profound effects on the burned patient through hypoxemic pulmonary vasoconstriction and ventilation/perfusion mismatching, we failed to note any substantial differences in the initial P:F ratio over the range of inhalation injury severities. Moreover, the anti-inflammatory effects of CO have been previously noted in the setting of burn and smoke inhalation injury, sepsis, and trauma (20–24). One might theorize that CO, if present at higher levels in patients with greater inhalation injury severities, would mute the inflammatory response to worsening injury. That we still see a greater immune response with worse grades of inhalation injury suggests a limited effect of CO on reducing the pulmonary inflammatory response in these patients.

Another finding of our study was a shift in the BALF WBC differential to one of neutrophil predominance, confirming earlier findings observed in smoke inhalation by Wright and

Murphy, and Riyami et al. (10, 25). In fact, in patients with a Grade 4 inhalation injury, 94% of the leukocyte population was neutrophils. This contrasts sharply with the normal pulmonary leukocyte distribution, which is upwards of 95% macrophages (26). In light of previous studies on burn and inhalation injury, we speculate that a robust production of chemotactic factors (such as IL-8), in addition to the extensive and narrow pulmonary vascular bed, are contributory to this phenomenon (9, 27, 28). Regardless of the mechanism, the preponderance of neutrophils might contribute to later immune dysfunction, bacterial overgrowth, and pneumonia (14, 29).

Though differences in many initial patient characteristics fell short of statistical significance when contrasting low and high severities of inhalation injury, certain outcomes across inhalation injury grades persisted with this method of comparison. For instance, the requirement for tracheostomy, the number of ventilator days, and the ICU length of stay tended to be increased with worse grades of inhalation injury. Likewise, when placing emphasis on the difference between inhalation injury severities, those with the worst injuries had more tracheostomies, ventilator days, pneumonia, sepsis, days in the ICU, and overall hospital length of stay. Perhaps of greatest importance is that patients in our study with an inhalation injury of any kind had a mortality of 27% as compared to only 11% for those without, which is similar to findings by Shirani et al. in 1987, who also showed that inhalation injury in addition to burns increased mortality by approximately 20% (30). Finally, the inverse correlation of numerous pulmonary immune mediator concentrations with mortality deserves mention. On close inspection of these patients, Davis et al found that not only were many BALF immune mediator concentrations lower in those who would succumb to their injuries, but that pulmonary leukocyte production of these mediators in culture, particularly after stimulation with lipopolysaccharide, was blunted (31).

Although many of our results substantiate already held tenets of burn and inhalation injury, our having demonstrated an enhanced pulmonary inflammatory response to worse degrees of inhalation injury is a new contribution. Previous studies directed at delineating the alveolar response to inhalation injury have shown that leukocytes recruited to the alveolar space have been primed for further stimulus, and that alterations of the inflammatory mediator profile of the lung may correlate with pulmonary physiologic dysfunction and later pneumonia (9, 27). Our results indicate that many inflammatory mediators present in the BALF of those with an inhalation injury are significantly altered, even as early as within 14 hours of injury. Of these, we have identified IL-1 β , IL-1 α , IL-6, IL-8, and IFN- γ as best representing physiologically relevant levels and among the most important in regards to lung injury. Indeed, each of these immune mediators has been extensively studied in both humans and animal models of burn injury, lending further credibility for their importance and potential for therapeutic intervention (10, 32–47).

We concede that our study has certain limitations. First, there is an inherent selection bias in that only those suspected of an inhalation injury were candidates for enrollment. Therefore, much of the burn patient population outside of this category were not included for comparison, which may or may not have had significant effects on specific findings, those in particular being resuscitative fluid requirements and mortality. Second, the precision of BALF for measuring pulmonary inflammatory mediators is subject not only to various degrees of dilution but iatrogenic trauma to the airway which may result in contamination from blood and other cellular debris. Furthermore, at present, there is no standard denominator to correct for these possible confounders. Nonetheless, adjustment for total protein content in the BAL fluid had little impact on the results of our study and the overall discussion would have remained unchanged. Third, we were unable to account for the possibility that aspiration of oral, pharyngeal, or gastroduodenal contents may have had any effect on the inflammatory response that we observed. Finally, bronchoscopic grading of

inhalation injury grade is inherently subject to inter-rater differences, even though our system is based on Abbreviated Injury Score criteria. To minimize such effects, we also demonstrated our findings according to low and high severities of inhalation injury in a manner that we feel allowed for better clarity into the primary inflammatory response to inhalation injury.

Conclusions

Our study, emphasizing smoke inhalation injury in the burn-injured patient, demonstrates several correlates with worse grades of inhalation injury. Specifically, we have found that worse inhalation injury is associated with: 1) a greater degree of alveolar neutrophilia; 2) elevated plasma COHb levels; 3) a greater likelihood of tracheostomy, prolonged ventilator requirements, and longer length of stay in both the ICU and the hospital; and 4) enhanced pulmonary inflammatory mediator production. These data, we hope, will serve the purpose of better understanding the biological mechanisms behind smoke inhalation injury, and we believe that future investigations should consider inhalation injury to be a graded phenomenon.

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Figure 1.

Inflammatory mediator concentrations in the BAL fluid of controls (C) and burn patients with inhalation injury graded 0–4 by bronchoscopic criteria (0 = no injury; 1 = mild injury; 2 = moderate injury; 3 = severe injury; 4 = massive injury). Statistical comparison by Kruskal-Wallis and post-hoc analysis do not include controls, which are included for visual comparison. * $p < 0.05$ between groups.

Figure 2. Inflammatory mediator concentrations in the BAL fluid of burn patients with low inhalation injury severity (Grades 1–2) vs high inhalation injury severity (Grades 3–4). Differences considered statistically significant when $p < 0.05$.

Table 1

Coding and grading of inhalation injury by bronchoscopy

AIS code	Grade	Class	Description
919201.2	0	No injury	Absence of carbonaceous deposits, erythema, edema, bronchorrhea or obstruction
919202.3	1	Mild injury	Minor or patchy areas of erythema, carbonaceous deposits in proximal or distal bronchi
919204.4	2	Moderate injury	Moderate degree of erythema, carbonaceous deposits, bronchorrhea, or bronchial obstruction
919206.5	3	Severe injury	Severe inflammation with friability, copious carbonaceous deposits, bronchorrhea or obstruction
919208.6	4	Massive injury	Evidence of mucosal sloughing, necrosis, endoluminal obliteration

Table 2

Patient characteristics by inhalation injury grade

	Grade 0 n=9	Grade 1 n=15	Grade 2 n=15	Grade 3 n=18	Grade 4 n=3	p Value
Age	47 (27–67)	57 (44–61)	48 (37–57)	50 (31–68)	75 (55–80)	0.162
Gender						0.944
Male	5 (56)	10 (67)	9 (60)	12 (67)	1 (33)	
Female	4 (44)	5 (33)	6 (40)	6 (33)	2 (67)	
Race/Ethnicity						0.524
Caucasian	7 (78)	10 (67)	9 (60)	9 (50)	1 (33)	
African Am.	2 (22)	3 (20)	3 (20)	5 (28)	1 (33)	
Hispanic	-	-	1 (7)	3 (17)	1 (33)	
Asian	-	1 (7)	-	1 (6)	-	
Other/Unknown	-	1 (7)	2 (13)	-	-	
Initial P:F ratio	356.4 (±45.9)	354.3 (±26.6)	311.7 (±29.5)	358.1 (±31.44)	282.3 (±48.8)	0.685
Initial COHb (%)	2.8 (0.8–4.2)	3.8 (1.1–5.5)	9.9 (2.6–13.7)	10.4 (4.7–27.9)	20.5 (0.9–40.0)	0.014
BAL Cell Diff (%)						
Neutrophils	41 (26–69)	79 (45–93)	84 (64–95)	87 (72–91)	94	0.042
Lymphocytes	6 (3–17)	4 (1–10)	2 (1–15)	2 (1–11)	0	0.242
Monocytes	1 (0–4)	0 (0–1)	0 (0–2)	1 (0–3)	0	0.619
Macrophages	54 (10–68)	14 (4–46)	11 (4–32)	7 (3–12)	6	0.133
Eosinophils	1 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0	0.679
Basophils	0	0	0	0 (0–2)	0	0.289
Initial BAL > 100,000 CFUs	3 (60)	1 (7)	1 (7)	0	0	0.005
TBSA (%)	15 (7–24)	20 (5–65)	2 (0–39)	18 (0.8–35)	7 (7–15)	0.354

Data presented as n (%), median (interquartile range), or mean (±SEM), where appropriate BAL, Bronchoalveolar Lavage; CFUs, Colony Forming Units; COHb, Carboxyhemoglobin; TBSA, Total Body Surface Area skin burn

Table 3

Patient outcomes by inhalation injury grade

	Grade 0 n=9	Grade 1 n=15	Grade 2 n=15	Grade 3 n=18	Grade 4 n=3	p Value
Initial 24 hour fluid requirements (L)	4.2 (2.6–9.9)	12 (3–21)	7.7 (3.4–23)	10 (6.1–16)	7.0 (5.0–13)	0.424
Initial 72 hour fluid requirements (L)	15 (8.8–22)	22 (10–28)	16 (12–37)	20 (12–27)	14 (11–24)	0.715
Ventilator days	3 (2–4)	6 (3–30)	10 (3–33)	23 (12–38)	23 (15–33)	0.003
Tracheostomy	0	4 (27)	6 (40)	10 (56)	1 (33)	0.070
Pneumonia	3 (33)	6 (40)	7 (47)	12 (67)	2 (67)	0.424
Sepsis	1 (11)	2 (13)	2 (13)	5 (28)	1 (33)	0.681
ICU LOS	10 (5–21)	7 (6–41)	13 (7–37)	22 (15–38)	24 (23–53)	0.076
Hospital LOS	20 (7–30)	8 (6–46)	14 (6–40)	25 (17–38)	24 (23–53)	0.352
Mortality	1 (11)	7 (47)	2 (13)	4 (22)	1 (33)	0.210

Data presented as n (%) or median (interquartile range), where appropriate ICU, intensive care unit; LOS, length of stay

Table 4

Patient characteristics by inhalation injury severity

	Low n=30	High n=21	p Value
Age	54 (39–60)	55 (36–72)	0.509
Male Gender			0.917
Male	19 (63)	13 (62)	
Female	11 (37)	8 (38)	
Race/Ethnicity			0.387
Caucasian	19 (63)	10 (48)	
African Am.	6 (20)	6 (29)	
Hispanic	1 (3)	4 (19)	
Asian	1 (3)	1 (5)	
Other/Unknown	3 (10)	-	
Initial P:F ratio	333.0 ($\pm$ 19.9)	347.3 ($\pm$ 28.1)	0.671
Initial COHb (%)	5.1 (2.2–10.2)	10.4 (4.5–30.7)	0.059
BAL Cell Diff (%)			
Neutrophils	84 (53–94)	87 (74–93)	0.553
Lymphocytes	2 (1–11)	2 (1–10)	0.855
Monocytes	0 (0–2)	0 (0–3)	0.549
Macrophages	11 (4–38)	6 (4–12)	0.355
Eosinophils	0 (0–1)	0 (0–1)	0.691
Basophils	0	0 (0–1)	0.117
Initial BAL > 100,000 CFUs	2 (7)	0	0.503
TBSA (%)	15 (1–58)	15 (1–28)	0.437

Data presented as n (%), median (interquartile range), or mean ($\pm$ SEM), where appropriate. BAL, Bronchoalveolar Lavage; CFUs, Colony Forming Units; COHb, Carboxyhemoglobin; TBSA, Total Body Surface Area skin burn

Table 5

Comparison of BAL fluid immune mediator concentrations from health controls, burn patients with no visible inhalation injury, and burn patients with low and high inhalation injury severities

Mediator (pg/ml)	Control	No INI (Grade 0)	Low INI (Grade 1/2)	High INI (Grade 3/4)	p-value
C5a	638.2 (269.0–1034)	307.3 (98.0–475.7)	249.0 (127.0–567.7)	559.9 (237.2–1152)	0.167
IL-1 β	0.1 (0.0–5.0)	10.4 (2.9–49.4)	15.5 (2.6–76.3)*	42.4 (13.9–111.4)*	0.001
IL-1RA	31.1 (20.1–69.9)	176.4 (125.2–683.9)*	291.5 (90.9–728.4)*	301.8 (159.1–1362)*	0.004
IL-2	0.0 (0.0–0.7)	0.0 (0.0–7.3)	0.5 (0.0–2.6)	2.5 (0.0–4.6)	0.245
IL-4	0.0 (0.0–0.2)	1.4 (0.0–3.3)	0.0 (0.2–2.7)	1.8 (0.3–5.1)	0.042
IL-5	0.0 (0.0–0.5)	0.3 (0.1–0.5)	0.2 (0.0–0.4)	0.0 (0.1–0.5)	0.482
IL-6	2.0 (0.8–5.4)	51.4 (20.1–138.2)	141.7 (47.2–245.0)*	351.3 (160.8–1047)*†‡	<0.001
IL-7	1.7 (0.8–2.7)	11.9 (6.7–14.8)*	3.9 (0.7–7.2)†	3.7 (0.6–6.0)†	0.002
IL-8	0.0 (0.0–0.0)	2.9 (1.4–9.4)*	4.0 (1.1–13.4)*	4.5 (1.5–13.1)*	<0.001
IL-9	0.0 (0.0–5.8)	3.1 (0.0–19.9)	1.2 (0.0–12.6)	10.0 (6.0–16.6)*	0.021
IL-10	0.7 (0.5–5.3)	10.8 (4.2–12.8)*	3.4 (1.9–13.7)	7.3 (4.4–10.1)	0.036
IL-12	17.8 (11.0–21.1)	9.0 (0.0–19.4)	0.0 (0.0–5.8)*	0.0 (3.4–15.0)	0.006
IL-13	1.7 (1.4–1.8)	4.1 (3.0–7.9)	3.0 (1.4–17.3)	3.2 (1.1–7.2)	0.081
IL-15	1.1 (0.3–2.2)	7.6 (0.3–17.9)	0.7 (0.0–5.8)	4.7 (2.9–17.0)†	0.003
IL-17	0.0 (0.0–3.5)	0.0 (0.0–27.3)	0.0 (0.0–0.0)	0.0 (0.0–9.6)	0.477
Eotaxin	0.0 (0.0–45.9)	46.9 (5.3–130.5)	30.3 (0.9–92.6)	48.6 (14.2–93.3)	0.210
FGF-basic	0.0 (0.0–0.6)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.512
G-CSF	15.7 (6.4–34.1)	67.8 (22.6–151.9)	103.9 (56.5–261.5)*	75.9 (41.0–168.0)*	0.010
GM-CSF	12.8 (11.7–84.1)	2.1 (0.0–6.8)*	0.0 (0.0–4.3)*	3.7 (0.0–9.5)	0.001
IFN- γ	4.2 (1.8–9.0)	38.9 (6.3–87.0)	39.8 (14.2–94.2)	142.8 (33.1–212.9)*	0.005
IP-10 ^c	0.4 (0.2–1.9)	3.9 (1.4–12.1)	0.9 (0.2–2.7)	0.9 (0.3–1.6)	0.115
MCP-1	22.6 (16.1–29.6)	74.2 (29.5–459.5)	49.9 (31.0–120.4)	185.1 (105.9–551.2)*†	<0.001
MIP-1 α	0.0 (0.0–0.5)	0.0 (0.0–15.9)	0.0 (0.0–14.2)	0.0 (0.0–18.9)	0.911
MIP-1 β	6.9 (5.3–8.9)	69.0 (39.7–96.9)*	36.8 (16.5–85.9)*	21.1 (78.0–179.2)*	0.011
PDGF	0.0 (0.0–10.1)	22.7 (13.5–102.3)	10.0 (0.0–34.6)	28.7 (9.8–40.9)*	0.031

Mediator (pg/ml)	Control	No INI (Grade 0)	Low INI (Grade 1/2)	High INI (Grade 3/4)	p-value
RANTES	0.3 (0.0–9.0)	22.0 (18.8–47.4)	35.0 (19.7–98.0) *	56.1 (21.9–119.9) *	0.004
TNF- α	0.0 (0.0–5.3)	56.9 (0.0–232.8)	4.8 (0.0–58.6)	34.8 (5.5–63.4)	0.050
VEGF	402.7 (207.2–775.6)	355.7 (235.5–614.6)	131.1 (43.2–270.9)	58.2 (27.6–127.6) * $\dagger$	< 0.001

Data represented as median (interquartile range); INI, inhalation injury;

* p<0.05, vs control;

$\dagger$ p<0.05, vs No INI;

$\dagger$ p<0.05, vs Low INI;

ϵ represented as ng/ml; Kruskal-Wallis with post-hoc analysis.

Table 6

Patient outcomes by inhalation injury severity

	Low n=30	High n=21	<i>p</i> Value
Initial 24 hour fluid requirements (L)	10 (3.3–22)	8.7 (5.8–15)	0.579
Initial 72 hour fluid requirements (L)	19 (12–27)	17 (11–28)	0.716
Ventilator days	7 (3–31)	23 (13–35)	0.036
Tracheostomy	10 (33)	11 (52)	0.174
Pneumonia	13 (43)	14 (67)	0.162
Sepsis	4 (13)	6 (29)	0.177
ICU LOS	13 (6–37)	24 (16–39)	0.040
Hospital LOS	11 (6–41)	25 (19–39)	0.081
Mortality	9 (30)	4 (19)	0.626

Data presented as n (%) or median (interquartile range), where appropriate ICU, intensive care unit; LOS, length of stay

Table 7

Spearman correlation of inhalation injury grade, % TBSA, and select BAL fluid immune mediators with patient outcomes

	Initial 24 hr Fluid	Initial 72 hr Fluid	Ventilator Days	Tracheostomy	Pneumonia	Sepsis	ICU LOS	Hospital LOS	Mortality
INI Grade	0.189	0.127	0.491	0.341	0.254	0.177	0.375	0.261	-0.038
p	0.149	0.348	<0.001	0.008	0.051	0.176	0.003	0.044	0.774
% TBSA	0.744	0.800	0.373	0.045	0.343	0.088	0.331	0.397	0.436
p	<0.001	<0.001	0.004	0.730	0.007	0.502	0.010	0.002	<0.001
IL-1 β	0.037	-0.023	0.117	0.189	0.058	0.358	0.280	0.220	-0.270
p	0.779	0.865	0.384	0.151	0.664	0.007	0.031	0.094	0.039
IL-1RA	-0.184	-0.231	0.080	0.212	0.016	0.284	0.186	0.119	-0.313
p	0.163	0.087	0.549	0.107	0.905	0.030	0.160	0.369	0.016
IL-6	0.079	0.002	0.141	0.179	0.128	0.092	0.155	0.064	-0.087
p	0.553	0.989	0.291	0.176	0.336	0.488	0.240	0.632	0.513
IL-8	-0.110	-0.229	-0.052	0.055	-0.064	0.164	0.074	0.072	-0.387
p	0.433	0.110	0.713	0.693	0.648	0.241	0.598	0.608	0.004
IL-10	-0.003	-0.026	-0.101	-0.056	0.002	0.180	0.122	0.067	-0.360
p	0.981	0.846	0.450	0.673	0.988	0.172	0.359	0.612	0.005
G-CSF	-0.171	-0.151	0.146	0.164	0.045	-0.014	0.197	0.239	-0.338
p	0.200	0.272	0.277	0.219	0.735	0.914	0.139	0.071	0.010
IFN- γ	0.095	0.005	0.112	0.185	0.084	0.207	0.212	0.136	-0.208
p	0.475	0.974	0.402	0.160	0.528	0.115	0.107	0.304	0.114
MCP-1	-0.183	-0.006	0.250	0.345	0.196	-0.082	0.230	0.243	-0.152
p	0.170	0.967	0.061	0.008	0.141	0.542	0.082	0.066	0.256
MIP-1 β	-0.179	-0.118	0.166	0.137	0.064	0.011	0.067	0.092	-0.126
p	0.182	0.397	0.221	0.309	0.635	0.936	0.620	0.500	0.351
PDGF	-0.302	-0.187	0.055	0.197	0.161	-0.027	0.046	0.089	-0.218

	Initial 24 hr Fluid	Initial 72 hr Fluid	Ventilator Days	Tracheostomy	Pneumonia	Sepsis	ICU LOS	Hospital LOS	Mortality
p	0.052	0.254	0.728	0.210	0.309	0.868	0.771	0.574	0.165
RANTES	0.021	0.107	0.253	0.263	0.051	0.084	0.234	0.245	-0.247
p	0.875	0.433	0.055	0.044	0.702	0.525	0.075	0.062	0.059
TNF- α	0.076	-0.014	0.019	0.093	0.094	0.241	0.280	0.232	-0.183
p	0.569	0.917	0.889	0.482	0.476	0.066	0.032	0.077	0.165
VEGF	-0.140	-0.054	-0.115	0.027	0.170	-0.075	-0.168	-0.077	-0.189
p	0.296	0.699	0.394	0.842	0.201	0.576	0.207	0.568	0.155

INI, inhalation injury; TBSA, total body surface area burn. Each cell represents Spearman's rank correlation coefficient (top) and p-value (bottom).