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Tracheobronchial Protease Inhibitors, Body Surface Area Burns, and Mortality in Smoke Inhalation

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Abstract

The objective of this study was to assess tracheobronchial protease inhibitor concentrations longitudinally and determine whether initial concentrations predict subsequent lung injury and mortality in intubated burn victims. Tracheobronchial suction fluid was collected every 2 hours for 36 hours. Alpha-1-antitrypsin (AAT), secretory leukocyte peptidase inhibitor (SLPI), alpha-2-macroglobulin (A2M), and cell and differential counts were assayed. Partial pressure of oxygen in arterial blood/fraction of inspired oxygen (PaO₂/FIO₂) and peak airway pressure (PAP) were recorded for 72 hours. Standard statistics were used to evaluate cross-sectional relationships; random coefficient (mixed) models were used to evaluate temporal trends in marker concentrations and relation to clinical outcomes. Among 29 patients, 24 (83%) developed hypoxemia (PaO₂/FIO₂ <200); six died within 2 weeks. When adjusted for gender, age, %TBSA burn, and positive end-expiratory pressure setting, A2M ($P = .007$) and neutrophils ($P = .032$) increased linearly during 36 hours, and SLPI decreased ($P = .038$). Initial SLPI concentration was a negative predictor of maximum PAP ($P = .009$). None of the markers predicted longitudinal change in PaO₂/FIO₂. Mean levels of AAT and A2M in initial samples were significantly lower in patients with >35% TBSA burn ($P = .010$ and $.033$, respectively), when compared with patients with less severe burns. However, patients with increased A2M in combination with >35% TBSA burn had a 6-fold (95% CI: 1.8–20) increased relative risk of death. Tracheobronchial AAT and A2M levels were significantly lower in patients with more severe burns and increased over time. Initial SLPI levels predicted subsequent PAP. Increased early A2M in combination with extensive burn predicted early mortality.

Smoke is a complex mixture including aldehydes, acid gases, particulates, free radicals, carbon monoxide, and cyanide. The effects of smoke inhalation depend on the extent of exposure, with high concentrations and/or prolonged duration causing more severe injury.¹ Rapidly following the exposure, airway epithelial cells slough into the lumen, necessitating routine suctioning to prevent obstruction.² Additional tracheobronchial injury typically occurs within 12 hours, while parenchymal injury may be delayed up to 24 hours.³ Activated neutrophils entering the lung produce a number of inflammatory mediators, including neutrophil elastase, which play a role in the development of lung injury.⁴

Two common lung protease inhibitors, alpha-1-antitrypsin (AAT) and secretory leukocyte peptidase inhibitor (SLPI), bind to and inactivate neutrophil elastase^{5,6} as well as other serine proteases.^{7,8} AAT is produced predominantly in the liver and passes into the lung with increased lung–blood permeability, although some is also produced within the lung.^{9–12} Tracheobronchial AAT is therefore predominantly a marker of injury in the airway. SLPI is produced in the lung by submucosal bronchial glands, nonciliated epithelial cells, macrophages, and other mucosal surfaces.^{13–15} Alpha-2-macroglobulin (A2M) is a nonspecific, extracellular protease inhibitor produced in the liver that irreversibly binds to a broad array of proteases, which are then engulfed by phagocytic cells.¹⁶ Elevated A2M has been associated with increased alveolar–capillary permeability due to oxidant-induced damage.¹⁷

The combination of smoke inhalation and skin burns is associated with a higher risk of lung injury and mortality than is seen in patients with either exposure alone.^{18–21} This has been ascribed to augmentation of pulmonary microvascular permeability,²² increased bronchial, bronchiolar, and terminal bronchiolar obstruction caused by mucosal hyperemia, increased numbers of neutrophils in the epithelial lining of the small bronchi and bronchioles, and increased influx of acute inflammatory cells²³ in combined inhalation and burn injury. Increased release of proteases is considered a mechanism for several of these findings. Total proteolytic activity is increased in the circulation of burn patients and correlates with both %TBSA burn and mortality.²⁴

In this study, we measured levels of AAT and SLPI in tracheobronchial fluid for 36 hours and A2M for 12 hours following smoke exposure and intubation. We hypothesized that, because of increased protease release, smoke exposure would consume protease inhibitors, leading to a decline in their levels, and that increased BSA burns would exacerbate this reduction. We also hypothesized that, given their role in neutralizing excess protease activity, protease inhibitor levels would be predictive of subsequent lung injury as measured by partial pressure of oxygen in arterial blood/fraction of inspired oxygen (PaO₂/FIO₂) <200 and maximum peak airway pressure (PAP) at any point within 72 hours of exposure. Our objectives were to evaluate early temporal changes in tracheobronchial fluid AAT, SLPI, and A2M following smoke exposure, to determine whether initial (earliest measured) protease inhibitor concentrations can predict the extent of subsequent measures of lung injury, and to assess the effect of skin burns on protease inhibitor levels.

METHODS

This study was approved by the Institutional Review Boards of the University of Arizona, Tucson, Arizona, and Maricopa Integrated Health System, Phoenix, Arizona. The study methods are described in additional detail elsewhere.²⁵ Briefly, all patients admitted to the Arizona Burn Center during a 23-month period starting June 2003, who were considered by the admitting team to have suffered smoke inhalation based on a history of smoke exposure in a confined space, who had a physical examination finding of carbonaceous sputum, and who required intubation, were eligible for study participation. For the current study, 29 patients who had an initial tracheobronchial sample collected within 8 hours of intubation were selected. Medical history, concurrent injuries, and %BSA burn were assessed on admission. PaO₂, FIO₂, and positive end-expiratory pressure (PEEP) settings were recorded on average every 6 hours, and PAP was recorded approximately every 2 hours for up to 72 hours.

Patients who presented intubated or who required intubation during resuscitation underwent bronchoscopy generally within 2 to 6 hours of admission. Based on initial bronchoscopy, inhalation injury was scored on a scale of 1 to 5 (none, mild, moderate, severe, and copious and severe), for carbon staining/soot, edema/swelling, secretions/purulence, and erythema/bleeding. The highest score on any of these signs was the score given to the patient. For example, a score of "1" indicated no carbon staining or soot; "2," staining affecting the pharynx and trachea; "3," staining of the mainstem/1-degree bronchi; "4," more than the 1-degree bronchi; and "5," copious staining and soot throughout. Evaluation of the other signs used similar measurement scales.

Tracheobronchial suction fluid was collected at approximately 2-hour intervals for up to 36 hours as part of routine pulmonary care after instillation of normal saline (15 ml in adult and 5 ml in pediatric patients) through the endotracheal tube. A small volume was mixed 1:1 with fixative (Histochoice MB; Amresco, Solon, OH) and kept at 2 to 8°C before measurement of cell count and differential count. The remainder of the sample was frozen at -80°C before thawing and vortex mixing with an equal volume of 0.1% dithiothreitol solution (Sputolysin; Calbiochem, San Diego, CA). After centrifugation, the supernatant was then refrozen at -80°C for later analysis. AAT and A2M were assayed in duplicate using ALPCO enzyme-linked immunosorbent assay (ELISA) kits (Alpco Diagnostics, Salem, NH) and SLPI using R&D Systems' Quantikine ELISA kits (Minneapolis, MN). By following kit protocols, dilutions were established for AAT and SLPI (1:1000) and for A2M (1:8). Samples with levels falling outside the standard curve were rediluted and reassayed to obtain valid results. Taking into account the lowest dilution used, the limits of detection for AAT, A2M, and SLPI were 344 ng/ml, 285 ng/ml, and 12.5 ng/ml, respectively. None of the protease inhibitors had more than 5% of their values below the detection limit. Protein concentration was measured using the Sigma BCA-1 Protein Determination Reagent Kit (Lincoln Park, MI).

Statistical Methods

Absolute neutrophil count was calculated by multiplying the percent of neutrophils in the differential count by the number of white blood cells per milliliter. The frequency

distributions of the protease inhibitors, protein, and neutrophils were right skewed, and $\log_{(e)}$ -transformations (ln) were used to normalize these distributions. Protease inhibitor-protein ratios were calculated as ratios of the $\log_{(e)}$ of the inhibitors divided by the $\log_{(e)}$ of the total protein. For certain analyses, marker concentrations were dichotomized at the median, and hypoxemia was categorized as $\text{PaO}_2/\text{FIO}_2 < 200$ vs > 200 within 72 hours. Percent TBSA burn was coded as a 3-category ordinal variable (< 15 , $15-35$, and $> 35\%$) and as a binary variable (< 35 vs $> 35\%$). Age at injury ($13-20$, $20-40$, and > 40 years) was also categorized for inclusion as covariates in regression analyses. Analysis of variance (ANOVA) and t -tests were used to compare the mean levels of normally distributed outcome variables by categorical explanatory variables. Relative risk (RR) and Fisher's exact test were computed from contingency tables assessing the relation between dichotomous variables, such as marker concentrations above or below the median and death or $\text{PaO}_2/\text{FIO}_2$ ratio < 200 within 3 days of exposure. Gender, age, PEEP setting, and %TBSA burn were included as potential confounders in the multivariate models. The effects of initial (earliest measured) marker concentrations on binary injury outcomes were assessed using logistic regression models, with and without adjustment for confounders. Random coefficient mixed models were used to: 1) analyze temporal patterns in protease inhibitor and neutrophil levels during the first 36 hours postintubation; 2) determine whether the ratio of $\text{PaO}_2/\text{FIO}_2$ was dependent on tracheobronchial marker concentrations; and 3) estimate correlations between markers, taking into account within-subject correlation. In these models, subjects and sample times were fitted as random effects and other covariates as fixed effects. Mixed models are an extension of regression models but can model repeated measures on subjects over time, account for the autocorrelation among these measures, and handle cases with incomplete data. SPSS 15.0²⁶ and Stata 10.0²⁷ were used for data management and statistical analyses. Tests of significance were all two sided; a critical value of $[\alpha] = 0.05$ was used in all analyses.

RESULTS

Demographic, medical record, and exposure data collected on the 29 patients are presented in Table 1. The study population was predominantly men and ranged in age from 13 to 73 years. Percent TBSA burn ranged from 0 to 95%. A total of eight patients died; six within the first 2 weeks and two after 2 months of hospitalization. Of those who died, causes of death were multisystem organ failure (4 patients), 95% TBSA burn injury (2 patients), inhalation injury (1 patient), and brain death (1 patient). Patients were intubated between 20 minutes and 4 hours postinjury (mean 1.1 ± 0.78 hours). Bronchoscopy results were missing for three patients: two because they had sustained unsurvivable burn injuries and were provided with comfort care only, and for one, the results were missing from the records. On an average, $13.9 (\pm 2.9)$ tracheobronchial samples were collected per subject within 36 hours of intubation, and $14.9 (\pm 3.2)$ blood gas measures were recorded per subject during the 72 hours of observation. More than 50% of patients required a PAP of > 30 mm Hg to maintain adequate oxygenation, and the majority (83%) developed hypoxemia ($\text{PaO}_2/\text{FIO}_2 < 200$) at a minimum of one time point within 72 hours of exposure. Mean PAP during the 72 hours was significantly higher in subjects who died within 2 weeks (38 vs 29 mm Hg) or at any time point (36 vs 29 mm Hg) compared with that of surviving patients ($P = .005$ and $.020$,

respectively). Number of days intubated was not related to %TBSA burn and did not differ among those who died (at any time point) and those who survived.

Correlation between tracheobronchial markers over time were evaluated using bivariate mixed models, treating repeated measures within subjects as random effects. Neutrophils were found to be positively correlated with AAT ($P < .001$) and negatively correlated with SLPI ($P = .001$), and concentrations of total protein were positively correlated with AAT and A2M (both $P = .001$) but not with SLPI.

Multivariate mixed models of tracheobronchial AAT, SLPI, A2M, and neutrophils over time, adjusted for age category, %TBSA burn, and PEEP setting, are given in Table 2. Levels of AAT measured during 36 hours showed a nonsignificant increase. TBSA burn >35% was associated with a 149% decrease in AAT, when compared with subjects with 35% burn ($P = .036$), and a 1-unit increase in PEEP was associated with an increase in AAT of 15% ($P = .006$). SLPI concentrations showed a slight decrease over time after adjustment for potential confounders. Subjects older than 40 years had significantly lower levels of SLPI compared with those younger than 20 years ($P = .010$). A2M showed an increase of 17% per hour ($P < .007$). TBSA burn >35% was associated with a 510% decrement in A2M compared with that of TBSA burn 35% ($P = .010$), and a 1-unit increase in PEEP setting was associated with a 26% increase in A2M. Although time from injury was a positive predictor of neutrophil count ($P = .032$), each unit increase in PEEP was associated with a 17.5% decrease in neutrophils ($P = .005$). Neutrophil count increased by 2.8% per hour. When the neutrophil model was restricted to patients who did not receive inhaled steroids, heparin, or beta-agonists (13 patients with a total of 104 tracheobronchial samples), there was no increase or any other trend in neutrophil count over time.

The effects of %TBSA burn on mean AAT levels in initial (mean 2 hour), 12, 24, and 36 hours tracheobronchial samples are shown cross-sectionally in Figure 1A. Greater than 35% TBSA burn was associated with significantly lower AAT at 2 and 24 hours ($P = .010$ and $P = .024$, respectively). Similar to AAT, A2M was significantly lower among patients with >35% TBSA burns at both 2 and 10 hours ($P = .033$ and $.019$, respectively; Figure 1B). Neither SLPI nor neutrophil counts varied by burn severity (Figure 1C, D). The geometric mean marker concentrations from the initial samples, stratified by %TBSA burn and by survival vs death within 2 weeks of injury, are given in Table 3. Initial protein ($P = .013$), as well as AAT and A2M (as mentioned above), showed negative trends with percent burn. In contrast, initial A2M concentration was significantly increased in those patients who died within 2 weeks of injury ($P = .046$). In unadjusted models of marker levels over time stratified by burn severity, $\log_{(e)}$ AAT concentration increased 4.9% per hour in patients with >35% TBSA burn ($P = .002$), but it showed no trend among patients with less severe burns. A2M showed an increase of 11.6% per hour in patients with 35% TBSA burn ($P = .039$) and more than double the rate of increase (27.6% per hour) in patients with >35% TBSA burn ($P = .040$). Neither SLPI nor neutrophil counts were influenced by stratification by burn severity.

In linear regression models, the $\log_{(e)}$ of the initial tracheobronchial SLPI concentration was a negative predictor of maximum PAP recorded within 72 hours of exposure ($P = .011$,

adjusted $r^2 = .188$). Although additional covariates (%TBSA burn, AAT, A2M, and neutrophil count) were not significant predictors, the adjusted r^2 increased to .223 with their inclusion in the model. Initial tracheobronchial antiprotease concentrations were not predictive of maximum PEEP.

In logistic regression models adjusted for age category and %TBSA burn, initial tracheobronchial marker concentrations, total protein, and neutrophil count showed no influence on the development of hypoxemia ($\text{PaO}_2/\text{FIO}_2 < 200$) within 72 hours of injury. Neither initial nor longitudinal measures of AAT, SLPI, A2M, and neutrophils were predictive of $\text{PaO}_2/\text{FIO}_2$ over time in mixed models, with or without adjustment for age, gender, %TBSA burn, tracheobronchial protein, and PEEP.

The RR of mortality within 2 weeks of injury in relation to %TBSA burn and initial marker levels is given in Table 4. Percent TBSA burn was not by itself a predictor of mortality, nor were high vs low levels of any of the markers measured. However, patients who suffered TBSA burn $>35\%$ and had an initial A2M concentration above the median had a risk of death 6.0 (95% CI: 1.8–20) times greater than subjects without the combination of high A2M and high TBSA burn (Fisher's exact test, $P = .022$). Patients with high TBSA burn and either a high initial AAT (RR, 4.2; 95% CI: 1.3–13.9) or a high initial neutrophil count (RR, 3.7; 95% CI: 0.98–13.7) also had an increased risk of death, although these associations did not reach significance when applying Fisher's exact test ($P = .11$ and .091, respectively).

DISCUSSION

Tracheobronchial concentrations of A2M increased and SLPI decreased during 36 hours in intubated, smoke-inhalation victims. AAT increased during this time, but only in patients with burns covering more than 35% TBSA, whereas the rate of increase in A2M was more than double in patients with more extensive burns. Initial SLPI concentration was a negative predictor of maximum PAP within the initial 72-hour postintubation period. Stratification by extent of skin burn indicated that the temporal increases in AAT and A2M were due to markedly reduced initial concentrations in those patients with burns covering more than 35% TBSA, when compared with patients with less extensive burns. This depletion of tracheobronchial AAT among patients with more extensive burn injury resolved within 36 hours. Although the clinical significance of the reduction in AAT and A2M with more severe burn injury is not clear, we found a several-fold increase in risk of mortality within 2 weeks of injury in those patients with $>35\%$ TBSA burn and an early high A2M levels, and a similar pattern emerged with AAT. Neither $>35\%$ TBSA burn alone nor the markers alone predicted mortality.

SLPI concentrations did not follow the same pattern of the other antiproteases studied. We believe that the major site of production of SLPI, the lung, helped to determine the observed findings. AAT and A2M, with their primary site of production being the liver, increased in the lungs over time, likely because of leakage into the lung from the systemic circulation, whereas SLPI concentrations did not demonstrate a temporal increase. The mechanisms explaining the negative association between initial tracheobronchial SLPI and maximum

PAP are not clear, although reduced SLPI concentrations may have allowed continuing protease-derived damage in the lung, leading to the need to increase PAP to maintain oxygenation. The significant association of PAP with mortality in this study emphasizes the importance of this clinical end point. The negative relationship between SLPI and tracheobronchial neutrophils suggests that neutrophils may directly cause decreased SLPI concentrations through increased protease release.

Much of what we found is contrary to our original hypotheses. We expected increased concentrations of protease inhibitors to be protective against lung injury, but instead we found that extensive skin burns were associated with lower concentrations of AAT and A2M, and that extensive skin burns in conjunction with high concentrations of these markers were associated with an increased risk of death. One possible explanation for these results is that protease inhibitors are being rapidly depleted by an increase in protease activity, as reported in sheep with smoke inhalation injury.¹⁶ The increase in AAT and A2M over time seen in our study could subsequently lead to a decreased level of protease activity, which would be consistent with a rabbit model of smoke inhalation in which elastase activity in bronchoalveolar lavage fluid was high for 12 hours postinhalation, then decreased after 24 hours.²⁸ An increase in lung permeability caused by concomitant smoke inhalation and more severe skin burns would be expected to result in increased tracheobronchial AAT and A2M because of their relatively high concentration in the systemic circulation, and not in a decrease as we observed. However, the increased mortality associated with severe burns in combination with high levels of A2M and, to a lesser extent, AAT could be explained by increased lung permeability caused by more severe smoke exposure.

Concomitant dermal burns with smoke inhalation have been found to markedly increase mortality due to lung injury.^{29,30} Combined burn and inhalation injury in mice induced greater lung damage and edema and more severe impairment of pulmonary gas exchange.³¹ Patients in a burn unit who suffered inhalation injury alone had 12% mortality, whereas those with combined thermal and inhalation injury had a 20% mortality rate.³⁰ Mortality in mice with third-degree burns covering 40% TBSA had a 30% mortality rate, but when combined with smoke injury, suffered a 70% mortality rate.³¹ Given the findings of the current study, increased initial concentrations of A2M in combination with more severe skin burns may have clinical relevance and may underscore the contribution of increased lung permeability to mortality in smoke inhalation victims.

There are a number of limitations to this study. Given the relatively small number of patients included in this study, our findings should be considered preliminary, and replication with a larger population of smoke inhalation and burn victims is required to assess clinical significance. We were not able to directly measure the duration and magnitude of smoke exposure, which can greatly affect the extent and time course of lung injury, and the wide range of bronchoscopy findings suggests that the extent of smoke inhalation varied greatly among our subjects. We were only able to measure A2M up to 12 hours postexposure due to cost factor. Our study measured tracheobronchial mediator concentrations, whereas there may be differential effects on protein biomarkers in large and small airways because of markedly increased bronchial circulation with smoke inhalation.³² Furthermore, measurement of protease inhibitor or neutrophil elastase concentration and activity at the

alveolar level may not correlate well with tracheobronchial measurements. Although bronchoalveolar lavage would be an ideal assay to perform to evaluate alveolar protease inhibitor concentrations, it is more invasive than the tracheobronchial suctioning used in our study. Ideally, protease inhibitor activity should be directly measured in addition to the measurement of proteins by ELISA, because smoke exposure may alter the binding sites recognized by the antibodies used in the ELISA assay.³³ All patients in the Arizona Burn Center are managed with a specific fluid resuscitation protocol based initially on the Parkland formula and then adjusted based on urine output. Hence, neither underfluid nor overfluid resuscitation is expected to have occurred to such an extent that they would fully explain our findings related to the more severely burned patients. However, future studies should track the extent of fluid resuscitation and fluid balance.

In conclusion, tracheobronchial AAT and A2M increased linearly with time in the 36 hours after smoke exposure, especially in patients with the most extensive burn injuries. Initial SLPI, but not AAT or A2M, was predictive of maximum PAP within 72 hours, but none of the markers were associated with the extent of hypoxemia. High initial tracheobronchial A2M concentrations in combination with extensive burn were predictive of subsequent mortality within a 2-week period.

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Figure 1.

Log_(e)-transformed marker concentrations (means and SE) in tracheobronchial secretions over cumulative time from exposure, stratified by %TBSA burn: A, Alpha-1-antitrypsin; B, Alpha-2-macroglobulin; C, Secretory leukocyte peptidase inhibitor; and D, Neutrophil count. *P* values are based on ANOVA of mean concentrations by percent burn.

Table 1

Patient characteristics and clinical symptoms (n = 29)

Characteristic	Total, N (%)	Survivors, N (%)	Nonsurvivors, N (%)
N (%)	—	23 (79)	6 (21)
Sex			
Male	23 (79)	18 (78)	5 (83)
Female	6 (21)	5 (22)	1 (17)
Age (yr)			
13–20	5 (7)	4 (17)	1 (17)
20–40	11 (77)	8 (35)	3 (50)
>40	13 (17)	11 (48)	2 (33)
TBSA burn			
<15%	5 (18)	4 (18)	1 (17)
15–35%	10 (36)	9 (41)	1 (17)
>35%	13 (46)	9 (41)	4 (67)
Missing	1	—	—
Inhalation injury score			
1	4 (15)	4 (18)	0
2	13 (50)	11 (50)	2 (50)
3	4 (15)	3 (14)	1 (25)
4	5 (19)	4 (18)	1 (25)
5	—	—	—
Missing results	1	1	—
PaO ₂ /FIO ₂ within			
72 h			
200	24 (83)	20 (87)	4 (67)
>200	5 (17)	3 (13)	2 (33)
Maximum PEEP setting within 72 h			
5–9	16 (55)	13 (57)	3 (50)
10–14	10 (34)	8 (35)	2 (33)
15	3 (10)	2 (9)	1 (17)
Maximum PAP within 72 h*			
<30	13 (45)	13 (57)	0 (0)
30–35	11 (38)	8 (35)	3 (50)
>35	5 (17)	2 (9)	3 (50)

Likelihood ratio χ^2 or Fisher's exact tests were used to compare proportions between those who died within 2 weeks of exposure (nonsurvivors) and survivors.

* $P = .007$.

PEEP, positive end-expiratory pressure; PAP, peak airway pressure.

Table 2

Multivariate mixed models of markers over time postinjury

Model	Ln AAT (ng/ml)		Ln SLPI (pg/ml)		Ln A2M (ng/ml)		Ln Neutrophils (Cells/ml)	
	β	P	β	P	β	P	β	P
Fixed effects parameters								
Intercept	8.69	.001	16.0	.001	7.16	.001	6.34	.001
Time from intubation (h)	0.020	.102	-0.027	.038	0.158	.007	0.027	.032
Gender								
Male (ref)	—	—	—	—	—	—	—	—
Female	0.531	.338	0.644	.361	0.250	.780	-0.405	.560
Age (yr)								
13–20 (ref)	—	—	—	—	—	—	—	—
>20–40	0.167	.770	-1.01	.164	0.478	.615	0.322	.653
>40	0.181	.763	-1.98	.010	0.919	.366	1.17	.126
TBSA burn								
35% (ref)	—	—	—	—	—	—	—	—
>35%	-0.913	.036	0.088	.875	-1.81	.010	-0.740	.173
PEEP setting	0.138	.006	-0.078	.148	0.232	.019	-0.161	.005
Random-effects parameter estimates $\pm$ SE								
SD (intercept)	1.43 $\pm$ 0.26		1.70 $\pm$ 0.30		2.31 $\pm$ 0.42		1.26 $\pm$ 0.25	
SD (slope)	0.05 $\pm$ 0.01		0.53 $\pm$ 0.12		0.23 $\pm$ 0.05		0.05 $\pm$ 0.01	
Correlation (intercept, slope)	-0.80 $\pm$ 0.12		-0.73 $\pm$ 0.16		-0.76 $\pm$ 0.13		-0.36 $\pm$ 0.25	
SD (residual)	0.94 $\pm$ 0.04		0.81 $\pm$ 0.04		0.86 $\pm$ 0.82		0.91 $\pm$ 0.04	
Log likelihood	-413.1		-386.2		-168.2		-382.2	

Regression coefficients and *P* values (*z* test).

AAT, alpha-1-antitrypsin; SLPI, secretory leukocyte peptidase inhibitor; A2M, alpha-2-macroglobulin.

Table 3

Initial geometric mean concentrations (SD) of tracheobronchial markers by % TBSA burn and by survival vs death within 2 wk of injury

Initial Marker	<15% TBSA (n = 5)	15–35% TBSA (n = 10)	>35% TBSA (n = 13)	Survived (n = 23)	Died (n = 6)
AAT (µg/ml)	118.5 (114.0)	38.6 (46.1)	9.7 (13.4) *	26.8 (42.9)	81.6 (112.0)
A2M (µg/ml)	137.9 (159.6)	88.7 (215.2)	15.5 (34.7) *	29.4 (75.2)	183.7 (269.6) *
SLPI (µg/ml)	2.9 (3.1)	14.4 (20.9)	4.6 (4.3)	8.3 (14.7)	4.4 (4.7)
Neutrophils (cells/ml)	1136 (2418)	590 (757)	172 (171)	547 (1204)	208 (202)
Total protein (µg/ml)	10320 (7817)	6269 (3640)	3732 (1547) *	5065 (2882)	8575 (8099)

ANOVA based on log(e)-transformed values.

* $P < .05$.

AAT, alpha-1-antitrypsin; SLPI, secretory leukocyte peptidase inhibitor; A2M, alpha-2-macroglobulin.

Table 4

Relative risk and 95% CI for death within 2 wk of injury for specific risk factors alone and in combination

Risk Factors	Relative Risk	95% CI for Relative Risk	P*
TBSA burn >35%	2.31	0.50–10.6	.372
A2M >median	5.4	0.71–40.4	.080
AAT >median	2.14	0.46–9.9	.390
SLPI >median	0.93	0.22–3.9	.999
Neutrophil count >median	1.07	0.26–4.5	.999
Protein >median	1.07	0.26–4.46	.999
TBSA burn >35% and A2M >median	6.00	1.81–19.9	.022
TBSA burn >35% and AAT >median	4.17	1.25–13.9	.107
TBSA burn >35% and SLPI >median	3.67	0.98–13.7	.091
TBSA burn >35% and neutrophil count >median	3.67	1.98–13.7	.091

TBSA burn >35%, initial tracheobronchial marker levels > median, and the combination of TBSA burn >35% and initial marker levels >median.

* Relative risk and Fisher's exact test based on 2×2 tables and χ^2 analyses.

AAT, alpha-1-antitrypsin; SLPI, secretory leukocyte peptidase inhibitor; A2M, alpha-2-macroglobulin.