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TITLE: Acute Lung Injury Following Smoke Inhalation: Predictive Value of Sputum Biomarkers and Time Course of Lung Inflammation

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13. ABSTRACT (Maximum 200 Words)

Background: The role of lung inflammatory mediators in the development of lung injury following smoke inhalation is unknown.
Objectives: To evaluate the predictive value and role of inflammatory mediators in acute lung injury following smoke inhalation.
Specific aims: 1) Determine the predictive value of initial inflammatory markers in bronchial secretions of smoke inhalation victims for subsequent lung injury.
2) Measure longitudinal changes in inflammatory mediators in smoke inhalation victims.
Study design: Bronchial secretions from 200-250 intubated patients with smoke inhalation injury will be evaluated for initial and longitudinal changes concentrations of substance P, TNF-α, IL-1, IL-8, and IL-10, as well as cell count and differential every two hours to a maximum of 72 hours. Initial lung inflammation and changes in inflammatory markers will be compared in patients without and without subsequent significant lung injury.
Progress to date: We have enrolled 25 subjects to date in the study, almost all of whom have developed acute lung injury. We have collected detailed clinical outcome data on these subjects and have started to analyze substance P, TNF-α, IL-1, IL-8, and IL-10 concentrations. Determination of the concentrations predictive of the severity of subsequent lung injury await the recruitment and analysis of additional subjects.

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Smoke inhalation, acute lung injury, inflammation, cytokines

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INTRODUCTION

The goal of this research is to identify inflammatory mediators playing key roles in acute lung injury (ALI) following smoke exposure. Our objectives are to determine the value of initial concentrations of these mediators in predicting later development of ALI, and to determine how the mediator concentrations change over time, which may also have predictive value and improve our understanding of the mechanism of smoke injury. We hypothesize that smoke inhalation results in rapid changes (within two hours) in lung inflammatory mediators, initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury, and changes over time in lung inflammatory mediators will precede clinical findings of acute lung injury. Over the remaining years of this grant, we will be evaluating initial concentrations and changes over time of inflammatory mediators in pulmonary secretions of approximately 100 ventilated patients with smoke inhalation. The clinical course of these patients will be tracked, including % body surface area burn, days on a ventilator, days in ICU, pulmonary infiltrates, white blood cell count, fever, sputum volume, oxygen requirements, blood oxygenation, and development of ALI.

BODY

The two main specific aims of the study are: 1) Determine the predictive value of initial inflammatory markers in bronchial secretions of smoke inhalation victims for subsequent extent of lung injury; and 2) Measure longitudinal changes in bronchial inflammatory mediators in smoke inhalation victims. The specific aims have been divided into five tasks as shown in the approved Statement of Work timetable (with the task description modified to clarify the meaning of each step).

	Year 1	Year 2	Year 3	Year 4
Recruitment/Enrollment	→→→→→→→→→→→→→→→→			
Tracheobroncheal fluid sample collection	→→→→→→→→→→→→→→→→			
Medical outcome data collection	→→→→→→→→→→→→→→→→			
Sample Analysis		→→→→→→→→→→→→→→→→		
Data analysis/Manuscript preparation		→→→→→→→→→→→→→→→→		

The major activity of the first year of this research was to obtain Institutional Review Board (IRB) approval from the Army, the University of Arizona, and the Maricopa Integrated Health System (MIHS), which is the parent institution of the Arizona Burn Center where the subjects are enrolled in the study and the bronchial suction material and clinical outcome data collected. This process took much longer than anticipated and therefore required shifting the start of all of the timetable tasks into year 2. We therefore plan to continue subject recruitment and sample collection through year 4.

Due to problems obtaining timely consent, we revised our initial goal of enrolling 80-100 subjects per year with the new goal of 30-50 subjects per year, which we have achieved. During this third program year, we have consented 44 additional subjects, bringing the total number of subjects to 69.

We have collected clinical outcome data on 69 subjects and have entered this data into our database (Table 1). Among these patients, there have been 8 deaths (13.1%). The mean age of patients who died was significantly higher than that of survivors (61.8 vs. 37.1 yrs), and had significantly higher scores on the bronchial severity scale.

Arterial blood gas data, including PaO_2, FIO_2, and positive end respiratory pressures (PEEP), were collected on 64 subjects, generally starting within 6 hrs of acute smoke exposure (0.33-20 hrs, median 3.25 hrs) and continuing up to 72 hrs post-intubation. Of the patients with acceptable air blood gas data, 23 out of 43 (53.5%) had a PaO_2/FIO_2 ratio less than 200 within 72 hrs, almost all within 24 hours of intubation. A PaO_2/FIO_2 ratio less than 200 in concert with pulmonary infiltrates on chest x-rays is considered diagnostic of acute respiratory distress syndrome (ARDS).

Laboratory assays on the bronchial lavage samples have required considerable experimentation with different preservation techniques and different types of assays. In the past year, we have collected and processed BAL samples on 28 smoke inhalation subjects. We completed assays of Interleukin-1 beta, Interleukin-8, Tumor Necrosis Factor-alpha (IL-1β, IL-8 and TNF-α, respectively) using the R&D Systems Quanti-Glo Elisa Kits High, medium, and low controls were added to the testing protocol for all assays after 11/15/04 to insure accuracy of the testing protocol. Measurement of protein concentration using the Sigma BCA-1 Protein Determination Reagent Kit was performed on 32 subjects, after kit verification for use on sputum and bronchial lavage samples. Urea nitrogen was performed using the Pointe Scientific, Inc., Reagent Set # B7550-400 for 32 subjects, after the methodology was verified using the protocol for verification of new tests. The levels of urea were consistently low. Both soluble Fas Ligand (sFASL) and Transforming Growth Factor-beta1 (TGF-β1) were measured on 23 subjects using the R&D Systems Quanti-Kine Elisa Kits, after verification of the testing procedure.

Cell counts and differentials were completed on 52 subjects. The initial procedure called for preservation of the sample in methanol. Because of the unavoidable time delay between collection and processing, methanol did not preserve the cells sufficiently for accuracy for either the cells counts or the differentials. 40% Glycerol was used with better cell counting results, but cells were too degenerated upon storage to accurately perform the differential. Cytolyte was then used yielding cells that displayed less cellular disintegration; however, the cells contracted, which made cell counting difficult and the staining characteristics for the differential were unreliable. Histochoice was then used which showed much better results for cell counting and differentiation and is the method of preservation used at this time.

Substance-P was assayed on 16 subjects using the R&D Elisa Assay Kit. Results were consistently under the detection limit. After conferring with company representatives and laboratorians experienced in testing Substance-P levels, an additional preliminary concentration/purification protocol using a C-18 reverse phase cartridge (Sep-Pak) was performed on several subjects. Repeated Substance-P analyses remained under the detection limits in our BAL samples, so these assays were discontinued.

Over 1800 BAL samples have been collected on 69 consented subjects. Time of sample collection and laboratory results for 28 patients have been incorporated into our database to date. An initial BAL sample was collected within 3 hours of exposure in 68% of subjects and within 4 hours of exposure in 89% of subjects. Following the initial sample collection, subsequent samples were collected at approximately two hour intervals.

Data analysis is ongoing. We have found that PaO_2/FIO_2 decreases over time in these patients ($p<0.0001$), generally reaching its nadir at about 20-30 hours post-intubation (Figure 1). Production of (log-transformed) IL-1β, IL-8, and TNF-α is highly correlated, and all three cytokines increased significantly over time (all $p<0.001$), but most steeply in the first four hours post-intubation (Figure 2). Early cytokines tended to be higher in patients with sepsis, trauma, fracture and increased percent full thickness burn, but only IL-8 production in the first 6 hours was significantly higher in patients with sepsis ($p<0.023$). PaO_2/FIO_2 ratio had a significant inverse relation to IL-1β and TNF-α ($p<0.025$ and $p<0.035$, respectively). We are beginning to construct random coefficients models to predict the rate of decline in PaO_2/FIO_2 by levels of IL-1β in BAL, adjusted for patient's age, positive end respiratory pressures (PEEP) over time, percent full body surface burn, and fractures.

Table 1. Population characteristics of all subjects and subjects with BAL samples in first six hours.

	All subjects N=61	Subjects with early BAL samples N=31
N (%) Males	51 (83.6)	29 (93.5)
Females	10 (16.4)	2 (6.5)
Mean (±s.d.) age in years	39.2 (±18.7)	42.5 (±16.9)
N (%) total thickness burn		
<15%	23 (37.7)	13 (41.9)
15-35%	17 (27.9)	6 (19.4)
>35%	21 (34.4)	12 (38.7)
N (%) with fracture	6 (10.0)	4 (12.9)
N (%) with trauma	9 (15.0)	6 (19.4)
N (%) with organ failure	31 (51.7)	14 (45.2)
N (%) with chest infiltrates	32 (59.3)	21 (70.0)
N (%) with sepsis	27 (45.0)	11 (35.5)
N (%) died	8 (13.1)	3 (9.7)

Figure 1. PaO2/FIO2 Ratio over Time Since Intubation

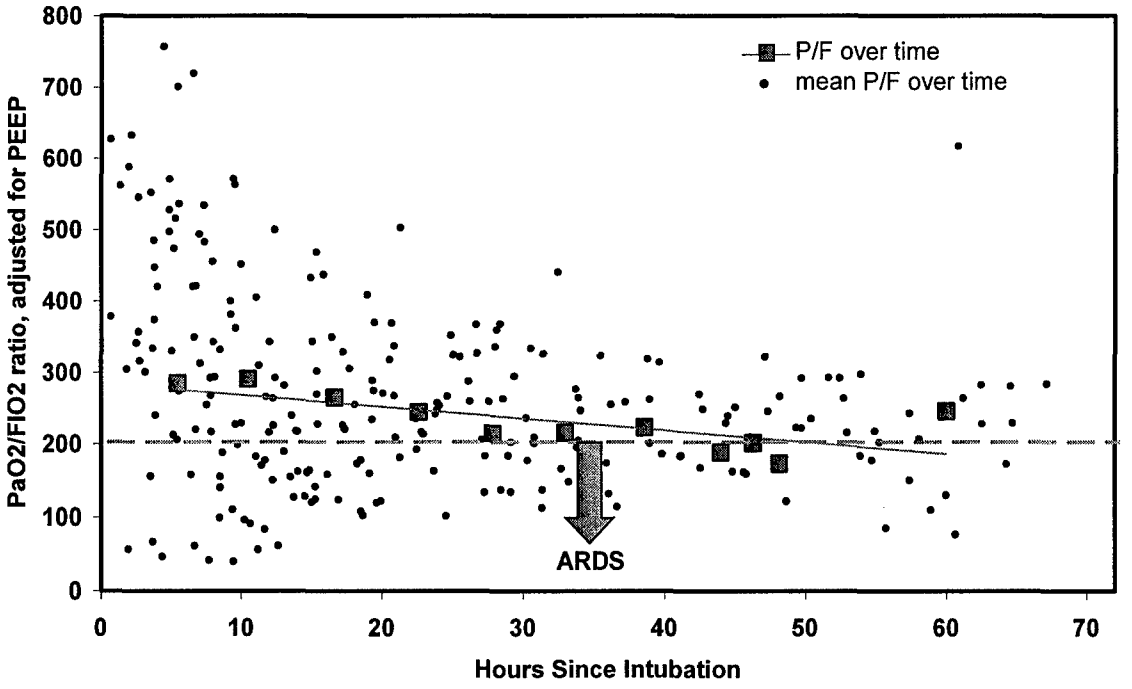
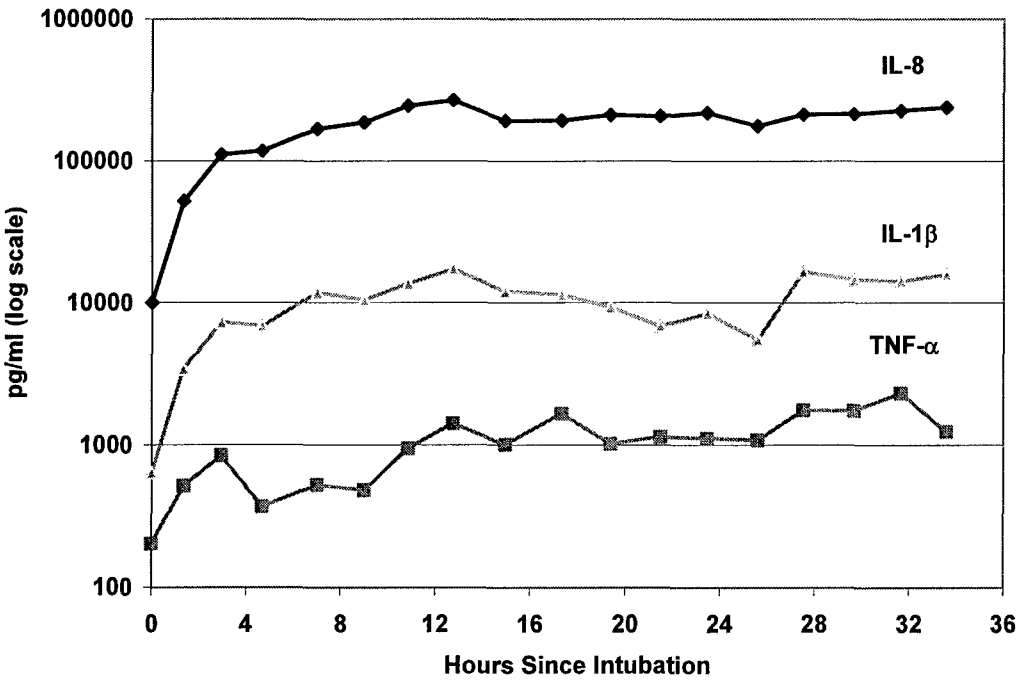


Figure 2. Mean BAL Cytokine Values over Time Since Intubation



KEY RESEARCH ACCOMPLISHMENTS

- We have demonstrated that in our patient population smoke inhalation victims almost uniformly manifest a decline in their PaO_2/FIO_2 ratio to below 300, which is consistent with development of acute lung injury.
- We have demonstrated that bronchial suction material can be used for longitudinal analysis of cytokines using commercially available ELISA kits. We have also found a significant negative correlation between IL-1β and TNF-α and PaO_2/FIO_2 ratio.
- We have begun to look at other biomarkers, including transforming growth factor beta (TGF-β) and soluble FAS ligand (sFASL), and currently have 315 samples analyzed from 23 subjects.
- We are in the process of modeling the rate of decline of PaO_2/FIO_2 over time by levels of IL-1β in BAL, accounting for possible confounders.

REPORTABLE OUTCOMES

We presented a poster at a scientific meeting describing our preliminary findings and will present a second this month. The details are as follows:

1) American Thoracic Society 100th International Conference, Orlando, FL May 25, 2004

"Longitudinal changes in tracheobronchal suction fluid inflammatory mediators following smoke inhalation"

2) American Thoracic Society 101st International Conference, San Diego, CA May 23, 2005

"Use of tracheobronchial suctionate inflammatory markers to predict subsequent lung injuring in smoke inhalation victims"

CONCLUSIONS

Smoke inhalation injury continues to cause significant morbidity and even mortality, as demonstrated by the clinical outcomes of our subjects to date. No diagnostic test or specific pharmaceutical therapy is available for acute lung injury following smoke exposure. We have shown that longitudinal evaluation of tracheobronchial suctionate from smoke inhalation victims can be analyzed for measurement of inflammatory mediators. If we can show that specific inflammatory mediators secreted in the lungs in the first 2-6 hours following smoke exposure are predictive of later decline in PaO_2/FIO_2 ratio, then it will be reasonable to consider evaluation in animal models and, if successful, in human clinical trials, of pharmacological agents working through antagonism or promotion of the effects of these mediators.

Future directions include the analysis of additional inflammatory mediators and measurement of our present and additional inflammatory mediators in small animal models of smoke exposure. We plan to analyze for concentrations of C5a in our subjects given the availability of C5a receptor antagonist which has shown promise in animal models of neutrophil-mediated injury. The selection of additional inflammatory mediators will also be based in part on the availability of potential therapeutic interventions associated with the selected mediators.

REFERENCES

None

APPENDICES (see following pages)

1) Abstract, American Thoracic Society 100th International Conference May 25, 2004
Orlando, FL
"Longitudinal changes in tracheobronchal suction fluid inflammatory mediators following smoke inhalation"

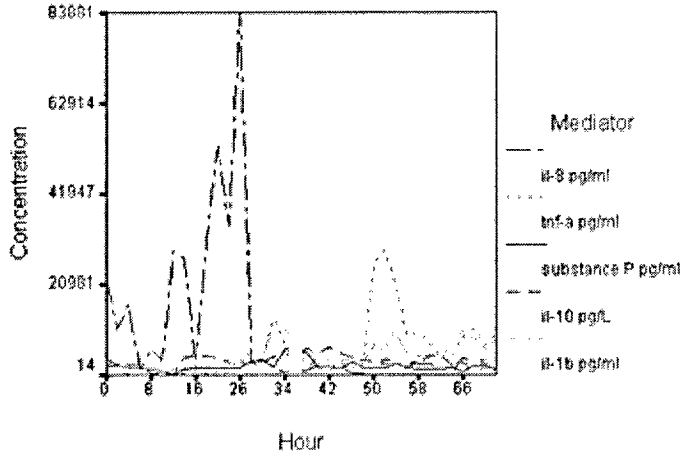
2) Abstract, American Thoracic Society 101st International Conference May 23, 2005
San Diego, CA
"Use of tracheobronchial suctionate inflammatory markers to predict subsequent lung injuring in smoke inhalation victims"

[C52] [Poster: E14] Longitudinal Changes in Tracheobronchal Suction Fluid Inflammatory Mediators Following Smoke Inhalation

J.L. Burgess, A. Josyula, K.N. Foster, T.A. Hysong, N.S. Francis University of Arizona, Tucson, AZ; Maricopa Integrated Health System, Phoenix, AZ

Rationale: The role of inflammatory mediators in the development of human smoke inhalation injury is not well understood. We hypothesize that initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury. *Methods*: As a first step in investigating this process, mediator concentrations in tracheobronchal secretions of ventilated patients were collected every two hours over the first 72 hours following smoke inhalation. Sample supernatants were analyzed by ELISA. *Results*: For the first four subjects for which samples have been analyzed, comparing the initial mediator concentration with the peak level gave the following fold increases: interleukin (IL)-1β 70-106; IL-8 6-115; IL-10 1-7; TNF-α 225-2560; and substance P 1-4. The longitudinal changes in one of the subjects enrolled in the study are illustrated in Figure 1. *Conclusions*: Longitudinal collection of tracheobronchal suction material provides a means of measuring changes in inflammatory mediators which will be evaluated for association with development of acute lung injury.

Figure 1. Longitudinal changes in inflammatory mediator concentrations in a single subject



Tuesday, May 25, 2004 8:15 AM

[**] Thematic Poster Session (Abstract Page: A641) Session: 8:15 am-4:15 pm, ENVIRONMENTAL AND OCCUPATIONAL PULMONARY TOXICOLOGY

American Thoracic Society : Abstract # 956596

Title: **Use of tracheobronchial suctionate inflammatory markers to predict subsequent lung injury in smoke inhalation victims.**

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[2] Arizona Burn Center, Maricopa Integrated Health System, Phoenix, AZ.

Rationale: Smoke inhalation victims are at high risk of developing acute respiratory distress syndrome (ARDS). Given the delay of 12 or more hours from exposure to development of ARDS, a prognostic test applied early in the clinical course could potentially identify patients for whom specific interventions may be of value. *Methods*: Patients with inhalation injury admitted to a regional burn center and requiring intubation were eligible for the study. Tracheobronchial suction fluid was collected every two hours. Sample supernatants were analyzed for interleukin-1, -8, (IL-1, IL-8) and tumor necrosis factor alpha (TNF-) by ELISA. Medical history and clinical course including arterial oxygenation (PaO2) and fraction of inspired oxygen (FIO2) were collected. *Results*: Mean PaO2/FiO2 decreased over time, generally reaching its nadir at 18-28 hours post-intubation. Regression models were run to assess the relationship between early IL-1, IL-8 and TNF- concentrations and subsequent PaO2/FiO2 measurements, adjusting for potential confounders including age, asthma, COPD, percent of full thickness body surface burned, and fractures suffered. In an analysis of eight patients with complete information, the log of IL-1 from a bronchial sample at 4 hours post-intubation (p=.008), age (p=.023), and % body surface burned (p=.022) were all significant predictors of PaO2/FiO2 at 18 hours. At similar time points, tracheobronchial TNF-, but not IL-8, was predictive of later PaO2/FiO2. *Conclusion*: In patients admitted to a burn center with smoke inhalation requiring intubation, IL-1 concentrations in tracheobronchial suction material at four hours were predictive of PaO2/FIO2 at 18 hours after exposure.

This research was supported by the U.S. Army Peer Review Medical Research Program, grant DAMD17-02-1-0673.