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Receptor for Advanced Glycation Endproducts in Sepsis-Induced Lung Injury

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Receptor for Advanced Glycation Endproducts in Sepsis-Induced Lung Injury

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An abstract of

A thesis submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Master of Science in Clinical Research

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Abstract

Receptor for Advanced Glycation Endproducts in Sepsis-Induced Lung Injury

By Annette Esper, MD

Objective: Acute lung injury (ALI) is a common, lethal disease that is associated with a high mortality. Sepsis is the most common cause of ALI and is common in the critically ill patient. However, not all patients with sepsis develop ALI; therefore, it is imperative to understand what may alter the development of ALI in patients with sepsis. The receptor for advanced glycation endproducts (RAGE) is known to be a marker of cell injury in ALI. Therefore, we sought to determine the association between RAGE and ALI development and to further determine if RAGE levels predicted mortality in sepsis patients.

Study design: Prospective cohort study of patients with severe sepsis or septic shock.

Methods: Patients in the intensive care unit were enrolled within 72 hours of the development of severe sepsis or septic shock. Bronchoscopy with bronchoalveolar lavage (BAL) and blood draws were performed at enrollment. Primary outcomes were development of ALI and death. BAL fluid (BALF) and blood were assayed for sRAGE using an Elisa based kit. TH1 and TH2 cytokines were measured in BALF using a multiplex kit.

Results: Forty-six patients with severe sepsis or septic shock were enrolled, 24 of who developed ALI. BALF sRAGE levels in ALI patients were significantly higher than in non-ALI patients (median, 4256 pg/ml vs. 1433 pg/ml, respectively, p=0.048. Plasma sRAGE levels in ALI patients (median, 1555 pg/ml) were not significantly higher than in non-ALI patients (median, 1103 pg/ml), p=0.15. There was poor correlation between BALF sRAGE levels and the ratio of TH1/TH2 cytokines (correlation coefficient of 0.21, p=0.34). Median BALF sRAGE level in survivors was 2428 pg/ml compared to 1354 pg/ml in non-survivors, p=0.38; median plasma sRAGE level in survivors was 945 pg/ml vs. 1518 in non-survivors, p=0.14.

Conclusion: BAL fluid sRAGE is elevated in patients with ALI compared to non-ALI patients with sepsis. There was no difference detected in plasma sRAGE between the two groups. BAL fluid and plasma sRAGE levels were not predictive of mortality. Further studies are needed to understand the role of RAGE in ALI pathogenesis.

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Table of Contents

Introduction:

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS), the severest form of ALI, are acute and life-threatening forms of injury to the lung, that affect 200,000 people each year in the US and as many as 25% of mechanically ventilated patients.(20) ALI results in significant morbidity and, because there is no defined medical therapy, mortality rates remain between 25% and 40% (21, 26).

Whether from a direct injury to the lung such as pneumonia, or from an indirect injury such as abdominal sepsis, ALI falls on a final common pathway of oxidative stress, alveolar inflammation and increased pulmonary capillary permeability. The inflammatory response in ALI is driven by activation of key signaling pathways that involve alveolar macrophage (AM) responses, activation of transcription factors, oxidative stress, and cytokine release(28). Not all critically ill patients develop ALI, and of the disorders that cause ALI, sepsis carries the highest risk at approximately 40% (10, 18). If we can determine the factors and/or mechanisms that render specific populations at risk of developing ALI, we can better understand the pathophysiology of the disease.

The receptor for advanced glycation endproducts (RAGE) is a member of the immunoglobulin superfamily and has been suggested as a biomarker for ALI (25). RAGE is involved in propagating the inflammatory response which involves activation of transcription factors and the production of proinflammatory cytokines(5). Animal models of ALI have shown increased RAGE levels in the bronchoalveolar lavage fluid (BALF) (25). Human studies have shown increased RAGE in pulmonary edema fluid of patients with ALI compared to patients with cardiogenic pulmonary edema (25). However, there is conflicting data about whether RAGE is elevated in sepsis. Many of the studies on RAGE in ALI have been retrospective in nature, and

therefore it is difficult to determine the role of RAGE in the pathogenesis of sepsis-induced ALI and how it impact different parts of the inflammatory response.

The primary objective of this study was to determine if RAGE levels in patients with sepsis were associated with the development of ALI and with an increase in proinflammatory cytokines. The second objective was to determine if RAGE levels predicted mortality in patients with sepsis. We hypothesized that higher BALF sRAGE levels in septic patients would be associated with the development of ALI when compared to those septic patients that do not develop ALI, and that elevated sRAGE levels would be associated with an increased risk of death.

Background:

Acute lung injury (ALI) is a common lethal disease and has a significant impact on public health. Diverse biological insults including sepsis, trauma, and aspiration of gastric contents cause ALI injury even in previously healthy individuals. Though defined by the development of hypoxemia and diffuse bilateral infiltrates on chest radiograph in the absence of left atrial hypertension, ALI is characterized by lung inflammation, increased pulmonary alveolar-capillary permeability and the subsequent accumulation of pulmonary edema(27). ALI represents an important source of morbidity, mortality and health-care cost. The economic burden of ALI is tremendous, as these patients account for over 2 million intensive care unit (ICU) days and 3.6 million hospital days in the United States each year(22). Despite multiple clinical studies, there are presently no effective medical therapies for ALI, and its mortality rate remains unacceptably high at 40% (22, 27).

Only 7-34% of critically ill patients develop ARDS (11, 19), with certain diagnoses, such as sepsis, associated with a higher incidence of ARDS. We studied 351 critically ill patients with various at-risk diagnoses and found that the incidence of ARDS was 44% with aspiration, 31% with pancreatitis, and 36% with sepsis, indicating that not all critically ill patients develop ARDS(16). Therefore, factors other than an at-risk diagnosis must be important determinants of which patients eventually develop ARDS. Predisposing factors for sepsis (age, chronic liver disease, HIV, cancer) (1, 9, 15, 17, 23) do not equally contribute to the risk of ALI. Accurate identification of populations at risk is crucial to improve our understanding the mechanism of ARDS and to develop effective therapies for these patients.

ALI is a complex disease, and much uncertainty exists regarding biological mechanisms that place specific populations at risk. Experimental models of ALI have been used to better define this role. Central to the inflammatory response in ALI are activation of key signaling pathways, including alveolar macrophage (AM) responses, activation of transcription factors, oxidative

stress, and cytokine release.(29) Understanding how these signal pathways may be altered and how they contribute to the development of ALI, may lead to potential therapeutic options.

The receptor for advanced glycation endproducts (RAGE) is a multi-ligand binding receptor that is highly expressed in the lungs, specifically in alveolar epithelial cells and alveolar macrophages(5). The receptor consists of an extracellular domain, a transmembrane domain, and a cytosolic domain. RAGE binds to various proteins, such as AGEs (advanced glycation end products) which are end products of glycation and oxidation and can be increased in disease states such as diabetes, age, renal failure and inflammation(12). RAGE-ligand interaction results in activation of proinflammatory transcription factors and cellular activation, thus promoting tissue injury(24). Extensive studies have indicated a role for RAGE signaling in macrophage activation, defined by upregulation of proinflammatory factors such as cytokines.

RAGE is implicated in ALI as an important pathway to alveolar inflammation, and as a marker of alveolar injury when assayed in the plasma(8). Based on experimental ALI studies in rats, RAGE can be detected in bronchoalveolar lavage fluid (BALF) and plasma (25). Furthermore, the level of RAGE has been shown to correlate with the severity of ALI (13, 25). Human studies evaluating pulmonary edema fluid have shown increased RAGE levels in edema fluid when compared to plasma. RAGE levels in plasma and BALF are higher in patients with ALI compared to patients with hydrostatic pulmonary edema and healthy volunteers (13, 25). Plasma rage levels decrease over time(13) and may be associated with poorer outcomes(6). The presence of RAGE-related disease, such as diabetes and the cause of lung injury have not been shown to influence RAGE levels (13).

RAGE may also play a role in the acute systemic inflammatory response that is characteristic of sepsis; however, there is conflicting data regarding the role of RAGE in sepsis. RAGE knockout mice have been shown to be resistant to septic shock (7, 14). Elevated sRAGE levels have been

described in surgical ICU patients with severe sepsis when compared to healthy volunteers(4). Plasma rage levels are increased in patients with ALI (with or without severe sepsis) when compared to patients with severe sepsis alone (13). Based on these preliminary studies, it remains unclear the specific role that RAGE plays in the pathogenesis of ALI in sepsis patients and therefore further investigation is warranted.

Methods:

a. Hypotheses

1. ALI is associated with increased BALF sRAGE levels in patients with severe sepsis or septic shock and increased sRAGE levels are associated with increased production of proinflammatory cytokines.

2. Increased BALF sRAGE levels are associated with increased risk of death in patients with severe sepsis of septic shock.

b. Study Design

To test the first hypothesis, we performed a cross-sectional study of patients with severe sepsis with and without ALI. To determine the impact of RAGE on mortality, we performed a prospective, observational cohort study of patients with severe sepsis or septic shock.

c. Characteristics of study population

Patients were enrolled from the adult Intensive Care Units (ICU) at Grady Memorial Hospital, Atlanta, GA, USA. Members of the study team screened the ICU daily for patients that met the inclusion criteria. Informed consent was obtained from patients' next of kin or durable power of attorney.

1. Inclusion criteria: Patients were eligible for enrollment within the first 72 hours of severe sepsis development. Patients were required to meet all the inclusion criteria within a 24-hour period. The definition of severe sepsis and septic shock is based on the American College of Chest Physicians/Society of Critical Care Medicine criteria for sepsis(3):
    a. Patients must meet at least two of the following criteria for SIRS: a)temp > 38C; b)white blood cell count $>12X10^9$/L or $<4X10^9$, or presence of >10% bands/immature neutrophils; c)respiratory rate >20 breaths/min; d)heart rate >90 beats/min
    b. Patients must have a clearly defined source of infection

c. Patients must meet the criteria for severe sepsis: sepsis associated with organ dysfunction, hypoperfusion, or hypotension: hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status

d. Septic shock is defined as meeting at least one of the following: a) systolic blood pressure <90 mm Hg for >1 hr; b) the requirement for vasopressor therapy, excluding dopamine at a dose <5 mug/kg/min.

2. Exclusion Criteria: No informed consent, greater than 72 hours since sepsis criteria met and age <18.

d. *Protocol:* A blood draw and bronchoscopy with bronchoalveolar lavage (BAL) were performed within 72 hours of sepsis development and within 24 hours of enrollment into the study. All enrolled patients were mechanically ventilated. A 5.2 mm bronchoscope was used for an 8F endotracheal tube (ETT, or smaller for a smaller ETT). The airway was entered via the ETT with minimal suctioning and the bronchoscope wedged in the right middle lobe at baseline. The lavage procedure was performed by installation of 60mL aliquots of 0.9% non-bacteriostatic normal saline solution, followed by immediate withdrawal with low-pressure hand suction. The lavage was continued, as tolerated, until 50mL of collected fluid or a total of 200mL had been administered.

Data was collected using established case report forms (CRFs) and then later entered into a web-based database. Study personnel (research nurses/coordinators and assistants) collected the following data on severe sepsis/septic shock patients: demographics, comorbid conditions, medication history, glucose control during ICU stay, insulin administration, ventilator settings (e.g. tidal volume, compliance), severity of illness measures (APACHE, SOFA, lung injury score), duration of mechanical ventilation, development of ALI, survival status. Data was collected until death or patient discharge.

e. *Outcome variables:* The primary outcomes for the aims were the development of ALI and 28-day mortality. Patients were considered to have ALI if they met the American-European Consensus Conference definition(2) within 5 days of meeting criteria for septic shock: PaO_2/FIO_2 <300 mm Hg, bilateral infiltrates on frontal chest radiograph, and pulmonary artery occlusion pressure <18mm Hg or no clinical evidence of left atrial hypertension.

f. *Predictor variables:* The primary variables of interest included sRAGE levels and TH1/TH2 cytokine levels. Potential confounders included APACHE, SOFA score and age because they have been shown to be associated with the outcome and potentially the exposure in prior studies.

g. *Assays*

BAL processing: Cell count and viability were determined on the Countess Automated Cell Counter (Invitrogen; Carlsbad, CA). The BAL fluid was then centrifuged to separate the cells from the fluid. Once the cells were separated from the BAL, the fluid was retained and stored for future analysis. Plasma was processed in a similar fashion. sRAGE concentrations were determined using an ELISA-based Human RAGE immunoassay, which measures the extracellular domain of RAGE. Optical density (OD) was measured at 450nm using a microplate reader. The mean minimum detectable dose of RAGE utilizing this assay is 4.12 pg/ml. The assay has not demonstrated any cross-reactivity with other proteins tested. TH1 and TH2 cytokines were measured using a multiplex assay kit (Human Cytokine Lincoplex kit).

h. *Sample size*

In our cohort of sepsis patients, we expected approximately 50% to develop ALI. In a previous study in sepsis patients, the mean difference in plasma sRAGE between ALI and non-ALI sepsis patients was found to be 2463 pg/ml with an SD of 1585 pg/ml. Other studies examining edema fluid between ALI and non-ALI patients found smaller differences; therefore, we adopted a more conservative expectation for detectable differences for BALF sRAGE between sepsis patients with and without ALI. We expected that the true difference in BALF sRAGE between sepsis and ALI patients will be 1585 pg/ml, and therefore we will need to study 22 sepsis subjects and 22

ALI subjects to be able to reject the null hypothesis that the population means of the two groups are equal with probability 0.9. The Type I error probability associated with this test of this null hypothesis is 0.05.

i. *Statistical Analysis*

Univariate comparisons between patients who did and did not develop ALI and between survivors and nonsurvivors were calculated and evaluated for statistical significance at an alpha of 0.05 using a chi-squared test for categorical variables and a two-sample t-test for continuous variables. Wilcoxon Rank Sum test was used for variables not normally distributed. To examine RAGE levels among those with and without ALI, a bivariate logistic model was created with ALI as the dependent variable and the variables of interest and potential known confounders as independent variables. A similar bivariate logistic model was constructed for the outcome of 28 day mortality. The Hosmer-Lemeshow test was used to test the goodness of fit of the logistic model. The relationship between RAGE and TH1/Th2 ratio was determined using the Pearson correlation coefficient. ROC curve analysis was used to determine a cut-off for RAGE that differentiates ALI and non-ALI patients. RAGE level was dichotomized at the median level and a chi-squared test was used to test the association between RAGE and the primary outcomes. Finally, the Kaplan-Meier method was used to estimate the survival function for mortality.

Results:

Baseline Patient Characteristics

A total of 46 mechanically ventilated patients with severe sepsis were enrolled, 70% of whom had septic shock. Twenty-four patients in the cohort (52%) developed ALI. The 28-day mortality for the entire cohort was 48%. Table 1 summarizes the basic demographics and clinical characteristics of these patients. Patients without ALI had higher baseline SOFA scores and were more likely to be in shock when compared to ALI patients. Pneumonia was the most common source of sepsis, followed by genitourinary, blood, skin/soft tissue and other. Patients with ALI were more likely to have a pulmonary source of sepsis. There were no differences between the two groups with respect to other sources of sepsis. Mortality rates were higher in patients without ALI. There were no differences in other baseline demographics, clinical characteristics or comorbidities known to possibly influence RAGE levels.

BAL fluid and plasma RAGE in ALI

BALF sRAGE levels were significantly higher in patients with ALI compared to non-ALI patients (4255 pg/ml vs. 1433 pg/ml, respectively; p= 0.04) (Figure 1a). There was no difference in plasma sRAGE levels between the 2 groups (1555 pg/ml in ALI vs. 1102 pg/ml in non-ALI, p=0.15) (Figure 1b). There was no association between plasma and BALF sRAGE levels (Figure 2). The median BALF sRAGE level for the entire cohort of 1848 pg/ml was used as a cut-off to categorize high vs. low RAGE levels. Those with high RAGE were more likely to have ALI with a $\chi 2$ 4.46, P=0.03 and OR 3.75. In patients with ALI, median RAGE levels in BALF vs. plasma were 4256 pg/ml vs. 1555 pg/ml respectively; p=0.02 (Figure 3). In non-ALI patients, median RAGE levels in BALF vs. plasma were 1433 pg/ml and 1103 pg/ml, respectively, p=0.34. After logistic regression analysis was performed with APACHE, SOFA, respiratory source and BAL RAGE entered as potential confounders into the model, every 100-unit increase in BALF sRAGE was associated with an OR of 1.05 for the development of ALI

(Table 2). There was no interaction detected between predictor variables. The area under the receiver-operating characteristic curve when BALF sRAGE was used to differentiate the presence or absence ALI in patients with severe sepsis or septic shock was 0.73 (Figure 4). A cut-off value of 2945 pg/ml had a sensitivity of 68% and a specificity of 67%. A logistic regression model was run using RAGE dichotomized based on the median levels for the cohort and the cut-off value from ROC curve analysis. At the median cut-off value for the cohort, high rage was associated with an OR of 11.57 for having ALI (Tables 3 and 4).

Relationship between sRAGE levels and severity of illness

The association between sRAGE levels and indicators of illness severity was determined in patients with severe sepsis or septic shock. Higher levels of BALF sRAGE were poorly associated with organ dysfunction, as measured by the SOFA score (r=0.14, p=0.36), and severity of lung injury, as measured by the lung injury score (LIS) (r=.15, p=0.51, Figure 5a and 5b). Higher plasma sRAGE levels were not associated with organ dysfunction or severity of lung injury(r= 0.17, *p*=0.28 and r=-0.03, p=0.89, respectively).

Association between sRAGE levels and TH1/TH2 cytokines.

TH1 and TH2 cytokine production in patients with severe sepsis was quantified in the BALF. The cytokines IFN-gamma, IL-2 and TNF-alpha are characteristic of a TH1 phenotype, while IL-4, IL-10, and IL-13 are characteristic of a TH2 phenotype. To determine if a patient had more of a TH1 vs. a TH2 profile, the results were reported as a TH1/TH2 ratio. There was poor correlation between BALF sRAGE and TH1 profile, TH2 profile and TH1/TH2 ratio (r=.21, p=0.34, Figure 6).

sRAGE levels and mortality

The primary outcome measure was 28-day mortality. Table 5 represents the baseline characteristics between survivors and nonsurvivors. Non-survivors had a higher APACHE and SOFA score and were more likely to be in shock. Survivors were more likely to have ALI. There were no other observed differences between the 2 groups. There was no difference in plasma and BALF sRAGE between survivors and non-survivors (Figure 7). Median BAL RAGE in survivors was 2428 pg/ml vs. 1354 pg/ml in nonsurvivors, p=0.33. Median plasma RAGE was 945 pg/ml in survivors vs. 1517 pg/ml in nonsurvivors, p=0.14. After logistic regression analysis, with APACHE, SOFA and ALI entered into the model; BALF sRAGE was not associated with mortality at 28 days. Mortality was then stratified by dichotomizing BALF sRAGE using the median cut-off. Survival analysis was performed using the Kaplan Meier method (Figure 8). There was no statistically significant difference in survival time between patients with high vs. low levels of sRAGE in the BALF (Log Rank $\chi 2=0.94$, $p=0.33$).

Discussion:

The results of this prospective observational study show that sRAGE levels in the BALF fluid of patients with sepsis-induced ALI are higher than levels in non-ALI patients. This confirms what has been shown in other studies of ALI. Although prior studies have shown an increase in plasma sRAGE in patients with ALI (25), we did not find a difference in plasma sRAGE between ALI and non-ALI patients with sepsis. If in fact RAGE is localized to the pulmonary inflammatory process specific to ALI and not related to the systemic inflammatory response in sepsis, then our findings may be expected. Interestingly, patients with ALI were more likely to have a respiratory source of infection than non-ALI patients. This alone however does not explain the differences in sRAGE levels since there were no significant differences in BALF sRAGE levels based on source of infection. Furthermore, in contrast to prior studies, high BAL sRAGE levels poorly correlated with severity of organ dysfunction and severity of lung injury. There may be characteristics of our study population that may have confounded this association.

It is well known that RAGE is highly expressed in the lungs. Consistent with other studies, we found that BALS sRAGE levels were higher than plasma sRAGE levels in patients with ALI, suggesting that the inflammatory process involving RAGE is localized to the lung. We did not observe a difference in RAGE levels between BALF and plasma in sepsis patients without ALI, which confirms that RAGE may be specific to the inflammatory process in ALI. However, we did not compare are cohort to non-septic controls in order to determine if sepsis itself is associated with elevated RAGE levels.

RAGE propagates the inflammatory response which includes the production of proinflammatory cytokines(5). Therefore, we would have expected that increased sRAGE levels would lead to an increase in the TH1 (proinflammatory) cytokines. Animal studies have shown that rage knockout mice have a decrease in proinflammatory cytokine production in response to an insult(4). The

current study found no association between BAL sRAGE levels and the levels of TH1 and TH2 cytokines. The fact that we did not find a correlation between RAGE and cytokines speaks to the complexity of ALI pathogenesis. There may be potential confounders that impact the balance of pro- and anti-inflammatory cytokine production in our cohort of patients. Future studies need to be performed to understand the impact of RAGE on intermediators of the inflammatory process in ALI.

In our cohort of patients, sRAGE levels in BALF and plasma were not different between survivors and nonsurvivors. After logistic regression modeling, BALF sRAGE was not found to be an independent predictor of mortality. This is consistent with other studies that also found no association between sRAGE levels and mortality (13). However, some studies have found that plasma sRAGE levels are independently associated with outcome(6). The reasons for these discrepancies may be due to the differences in the patient population in each study and in other management practices that may influence outcome. Surprisingly in our study population we found an increased mortality in the patients without ALI which is likely explained by the increased severity of illness in the non-ALI group.

It is generally accepted that sRAGE is a marker of ALI; however, a cut-off level of RAGE has not been well defined. The cut-off value of sRAGE to diagnose ALI in our cohort of severe sepsis patients was not associated with a high sensitivity and specificity. However, there are no other studies to our knowledge that have attempted to determine a cut-off value for sRAGE in BAL fluid. Our cut-off value is higher when compared to a study in mechanically ventilated patients where a plasma sRAGE cut-off value of 980 pg/ml differentiated the presence from absence of ALI in patients with severe sepsis or septic shock, with a sensitivity of 94% and specificity of 100% (13). Based on our findings, we would expect the cut-off in BALF to be higher.

The strengths of this study include the prospective nature of the data collected which decreases the risk of information bias. While RAGE levels don't appear to differ based on the cause of ALI, focusing this study on a cohort of patients with the same risk factor for ALI is an additional strength and allows us to study a more homogenous population. Another strength of this study is our ability to collect BALF which allows us to sample the epithelial lining fluid of the lung and isolate cells for potential analysis.

Our study had some limitations. First, we initially sought to determine if sRAGE levels in patients with severe sepsis or septic shock predicted the development of ALI. Due to challenges associated with obtaining informed consent in a timely manner, the majority of patients had already developed ALI at the time of bronchoscopy. As a result, we were only able to compare differences in RAGE between ALI and non-ALI patients at one point in time and could not establish the temporal causal direction. Furthermore, since previous studies have shown that plasma and edema fluid sRAGE levels decrease with time, it would be useful to obtain BALF sRAGE levels at different time points and determine if there is an association between change in sRAGE levels and important clinical outcomes. Future studies should attempt to investigate the role of RAGE in sepsis patients free of ALI.

Another potential limitation of this study is the selection of sepsis patients. There are cases where patients were not enrolled because they were too unstable to undergo bronchoscopy. As a result, there may be an extent of selection bias introduced and this may have impacted our results to an extent. If these patients were enrolled, we may have observed an association between sRAGE levels and mortality.

Conclusions

Our study confirms that sRAGE levels are elevated in the BALF of patients with sepsis-induced lung injury. sRAGE was not associated with severity of disease or an increased risk of death. BALF may be a useful biomarker for ALI; however, additional studies are necessary to better understand the role of RAGE in the pathogenesis of ALI. Futures studies need to address downstream factors that are regulated by RAGE and the impact RAGE has on cellular activation and function. Additional evidence is also needed to support a role for RAGE in the pathogenesis of acute systemic inflammation. An understanding of these basic mechanisms may provide more than diagnostic utility, and more importantly a potential therapeutic role for sepsis and ALI.

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Table 1. Baseline characteristics of sepsis patients.

Patient Characteristics	**ALI N=24**	**Non-ALI N=22**	**P value**
Age (mean, SD)	53 (15.8)	58 (16.2)	0.28
Gender (% male)	15 (63%)	14 (64%)	0.51
Race White Black Other	 1 (4%) 22 (92%) 1 (4%)	 2 (9%) 20 (91%) 0	 0.51
APACHE (mean, SD)	23.5 (6.5)	27.2 (6.2)	0.05
SOFA (mean, SD)	9.2 (3.6)	11.7 (3.0)	0.02
BMI, kg, mean, SD	23.3 (4.1)	23.8 (6.7)	0.78
Tobacco use	13 (65%)	15 (75%)	0.49
Alcohol abuse	7 (35%)	6 (35%)	0.99
Diabetes	4 (17%)	7 (32%)	0.23
Source of Sepsis Respiratory Blood Genitourinary Gastrointestinal Other	 21 (91%) 1 (4%) 1 (4%) 0 0	 9 (41%) 3 (14%) 5 (23%) 1 (4%) 4 (18%)	 0.02
Shock	11(50%)	19 (91%)	0.004
PaO_2/FIO_2 ratio (median, IQR)	134 [97-241]	N/A	
Lung Injury Score (mean, SD)	2.27 (0.56)	N/A	
Ventilator days, median, IQR	12 [7-16]	12.5 [7-24]	0.92
Length of Stay, median days, IQR Hospital ICU	 24.5 [15-38] 14 [7-25]	 19.5 [10-37] 13 [10-18]	 0.40 0.95
Mortality	8 (33%)	14 (64%)	0.04

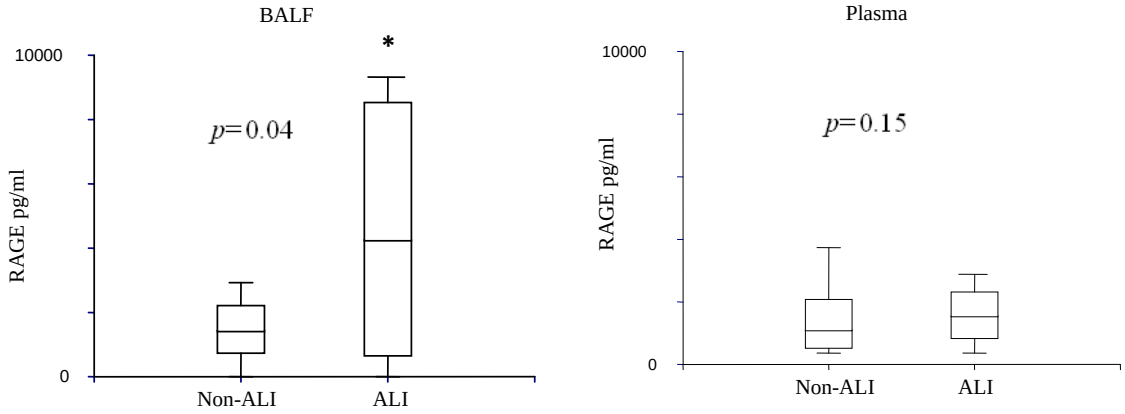

Figure 1. Box plots of sRAGE levels measured in (a) BAL fluid and (b) plasma of patients with and without ALI.

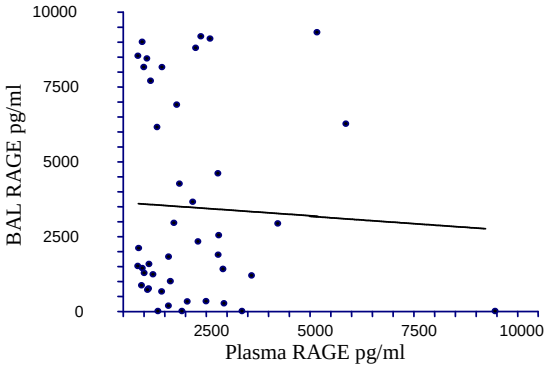

Figure 2. Association between BALF and plasma sRAGE levels.

Correlation coefficient = -0.04, p=0.76

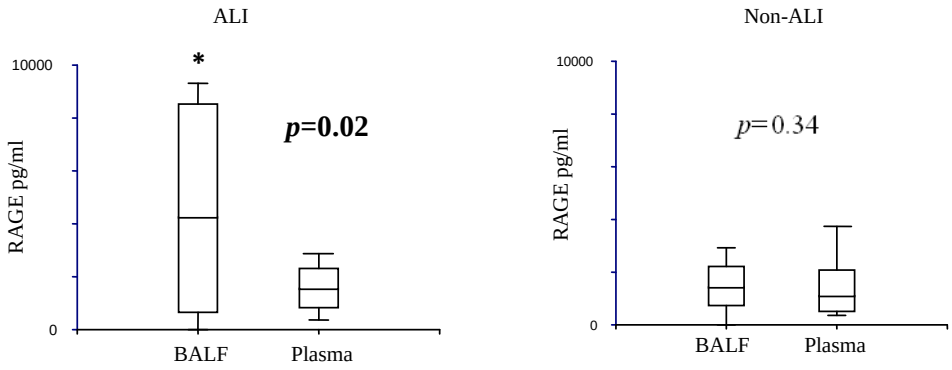

Figure 3. Differences in sRAGE levels in BALF versus plasma in (a) ALI vs. (b) non-ALI patients.

Table 2. Logistic Regression Model for ALI

Parameter	**Beta**	**Wald *P* value**	**OR [CIs]**
APACHE	-.063	0.34	0.93[.78,1.13]
SOFA	-0.219	0.26	0.80 [0.55,1.17]
Respiratory source	2.39	0.045	10.96 [1.06,114]
BAL RAGE pg/ml	0.049	0.008	1.05 [1.01,1.09]

Logistic regression model for ALI. The following potential confounders were included in the model: APACHE, SOFA score and respiratory source of infection. Log Likelihood =-14.91462, Model R-squared 0.488, H-L goodness of fit: $\chi 2$ 5.83, p=0.67.

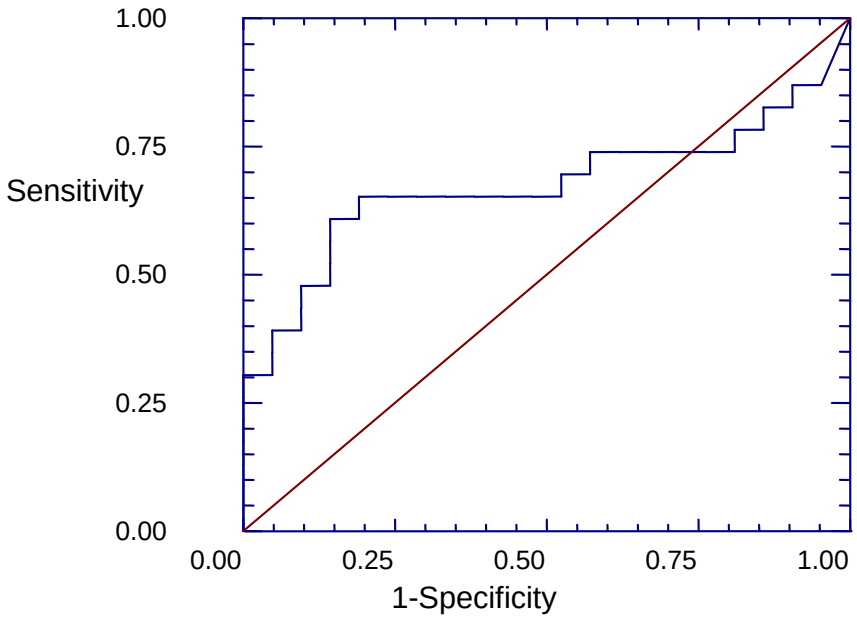

Figure 4. Receiver-operating characteristic (ROC) curve of BALF sRAGE levels in differentiating between the presence and absence of ALI. The area under the ROC curve was 0.73 for a cut-off of value of 2945 pg/ml, with a sensitivity of 68% and specificity of 67%.

Table 3. Logistic regression model for ALI with RAGE dichotomized.

Parameter	**Beta**	**Wald *P* value**	**OR [CIs]**
APACHE	-0.12	0.17	0.88[.74,1.05]
SOFA	-0.36	0.08	0.69[0.46,1.05]
Respiratory source	3.13	0.07	22.8 [0.78,667]
BAL RAGE	3.55	0.004	34.8[3.18,380]

Logistic regression model for ALI with RAGE dichotomized as high or low. The ROC curve cut-off sRAGE level was used to dichotomize the variable. Model using ROC RAGE cutoff: Log Likelihood -14.114; Model R-squared 0.515; H-L goodness of fit: $\chi 2$ 4.68, p=0.79

Table 4. Logistic regression model for ALI with RAGE dichotomized.

Parameter	**Beta**	**Wald *P* value**	**OR [CIs]**
APACHE	-0.10	0.19	0.90[.77,1.05]
SOFA	-0.35	0.048	0.70 [0.49,0.998]
Respiratory source	2.58	0.01	13.2 [0.99,175]
BAL RAGE	2.45	0.006	11.57 [1.61,83]

Logistic regression model for ALI with RAGE dichotomized as high or low. The median sRAGE level for the cohort was used to dichotomize the variable. Model using median RAGE cutoff: Log Likelihood -17.156; Model R-squared 0.411; H-L goodness of fit: $\chi 2$ 5.47, p=0.71

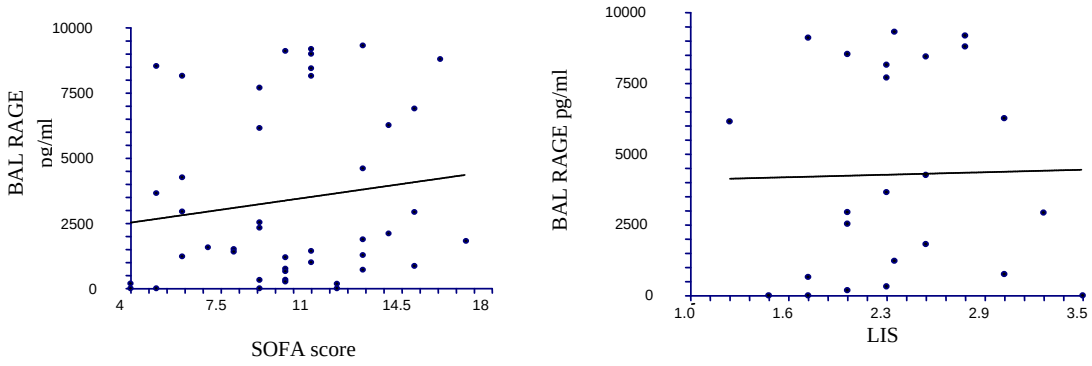

Figure 5. Correlation of BALF sRAGE with (a) organ dysfunction as defined by SOFA score and (b) lung injury score (LIS). Correlation coefficient for organ dysfunction was 0.54, p=0.008 and correlation coefficient of 0.15 for LIS, p=0.51.

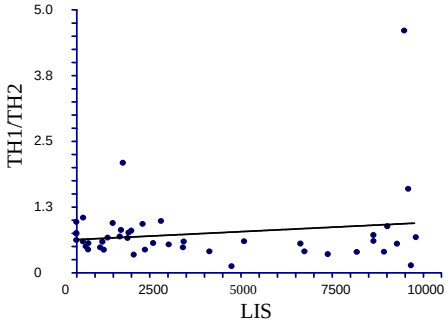

Figure 6. Association of BALF sRAGE levels and TH1/TH2 cytokine ratio with a correlation coefficient of 0.21, p=034.

Table 5. Baseline characteristics of survivors versus nonsurvivors in the septic cohort.

Patient Characteristics	**Survivors N=24**	**Non-Survivors N=22**	**P value**
Age (mean, SD)	53 (16.5)	58 (15.4)	0.24
Gender (% male)	15 (63%)	14 (64%)	0.94
Race White Black Other	 1 (4%) 22 (92%) 1 (4%)	 2 (9%) 20 (91%) 0	 0.52
APACHE (mean, SD)	22.7(5.4)	28 (6.7)	0.004
SOFA (mean, SD)	9.1 (3.35)	11.7 (3.26)	0.01
BMI, kg, mean, SD	24.7 (5.37)	22.2 (5.31)	0.13
Tobacco use	12 (63%)	16 (76%)	0.37
Alcohol abuse	8 (44%)	5 (26%)	0.25
Diabetes	5 (21%)	6 (27%)	0.23
Source of Sepsis Respiratory Blood GU GI Other	 18 (75%) 3 (13%) 2 (8%) 0 1 (4%)	 12 (57%) 1 (5%) 4 (19%) 1 (5%) 3 (14%)	 0.34
Shock	12 (55%)	18 (86%)	0.03
ALI	16 (67%)	8 (36%)	0.04
LIS (mean, SD)	2.33 (0.55)	2.37 (0.64)	0.85
Ventilator days, median, IQR	13 [6-21]	12 [7-19]	0.95
Length of Stay, median days, IQR Hospital ICU	 24.5 [18-41] 15 [8-27]	 17 [19-31] 13 [8-24]	 0.09 0.62

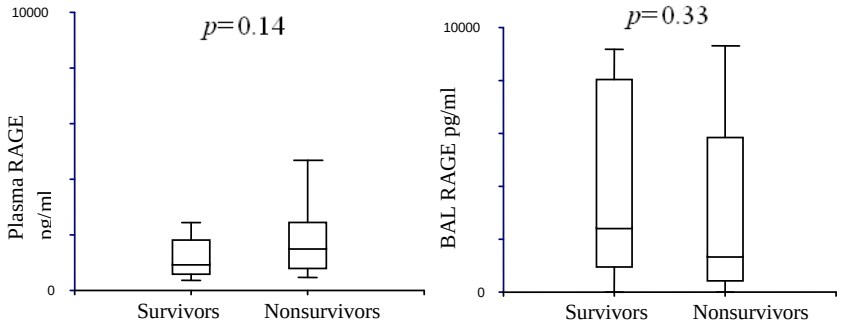

Figure 7. sRAGE levels in (a) plasma and (b) BALF of survivors vs. nonsurvivors.

Table 7. Logistic regression model for mortality.

Parameter	**Beta**	**Wald *P* value**	**OR**
APACHE	0.11	0.10	1.12 [0.98,1.28]
SOFA	0.11	0.42	1.11 [0.86,1.44]
ALI	-0.55	0.52	0.58 [0.11,3.10]
BAL RAGE pg/ml	-0.00009	0.50	1.0 [0.9997,1.0002]

The following predictors were included in the logistic model: APACHE, SOFA score, ALI and BALF sRAGE. Log Likelihood for the model was -25.25812 with a model R-squared of 0.167. H-L goodness of fit test: $\chi 2$ 10.38, *p=0.32.*

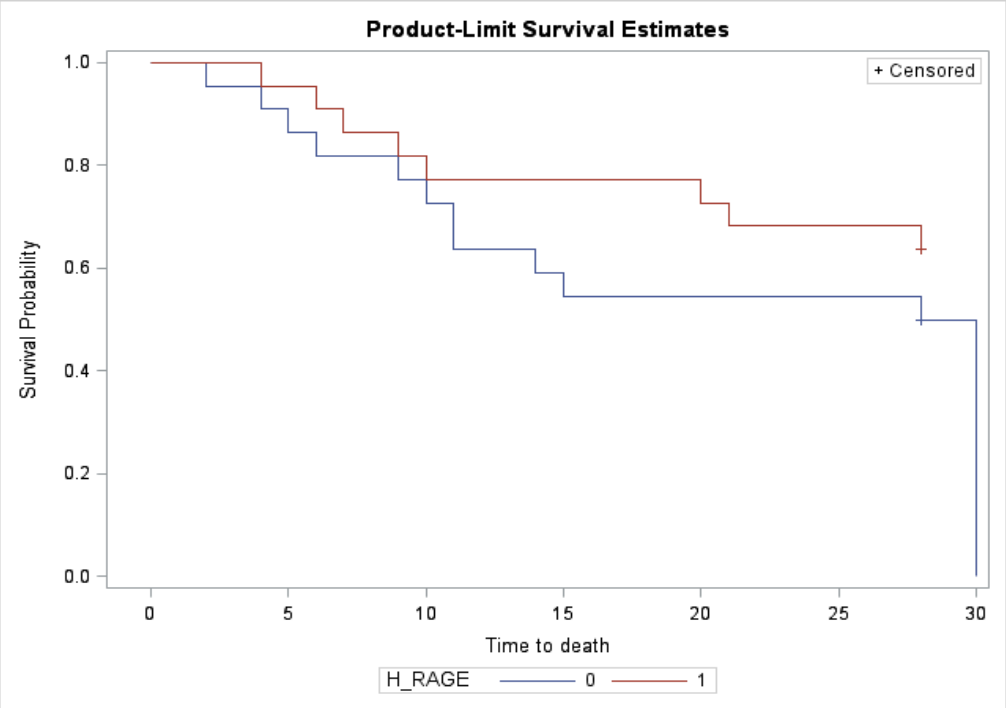

Figure 8. Kaplan Meier Survival Curve in patients with high versus low RAGE levels. H_RAGE=0 defines refers to low BALF sRAGE, and H_RAGE=1 refers to high sBALF sRAGE. The median BALF sRAGE for the cohort was used to dichotomize RAGE into high and low.