Plasma Receptor for Advanced Glycation End-Products
And Clinical Outcomes in Acute Lung Injury

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ABSTRACT

OBJECTIVES: To determine whether baseline plasma levels of the receptor for advanced glycation endproducts (RAGE), a novel marker of alveolar type I cell injury, are associated with the severity and outcomes of acute lung injury, and whether plasma RAGE levels are affected by lower tidal volume ventilation.

DESIGN, SETTING, AND PARTICIPANTS: Measurement of plasma RAGE levels from 676 subjects enrolled in a large randomized controlled trial of lower tidal volume ventilation in acute lung injury.

MEASUREMENTS AND MAIN RESULTS: Higher baseline plasma RAGE was associated with increased severity of lung injury. In addition, higher baseline RAGE was associated with increased mortality (odds ratio for death 1.38 [95% CI 1.13 to 1.68] per one-log increment in RAGE; p=0.002) and fewer ventilator-free and organ failure-free days in patients randomized to higher tidal volumes. These associations persisted in multivariable models that adjusted for age, gender, severity of illness, and the presence of sepsis or trauma. Plasma RAGE was not associated with outcomes in the lower tidal volume group (p=0.09 for interaction in unadjusted analysis). In both tidal volume groups, plasma RAGE levels declined over the first 3 days; however, the decline was 15% greater in the lower tidal volume group (p=0.02; 95% CI 2.4% to 25.0%).

CONCLUSIONS: Baseline plasma RAGE levels are strongly associated with clinical outcomes in acute lung injury patients ventilated with higher tidal volumes. Lower tidal volume ventilation may be beneficial in part by decreasing injury to the alveolar epithelium.

Abstract word count: 240
INTRODUCTION

Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), remain a major cause of acute respiratory failure and death in critically ill patients. Despite therapeutic advances in ventilation strategy and fluid management, mortality and morbidity remain high, and no effective pharmacologic therapies for the syndrome have yet been identified. Increased understanding of the pathophysiology and pathogenesis of this syndrome, identification of predictors of poor outcomes, and insight into the mechanisms of benefit of effective therapies such as low tidal volume ventilation may lead to further therapeutic advances.

The integrity of the alveolar-capillary barrier is critical to normal pulmonary function. Furthermore, impaired alveolar fluid clearance is known to be a central feature of the pathogenesis of ALI/ARDS. Type I epithelial cells cover 95% of the surface area of the alveolus and play a key role in epithelial integrity and alveolar fluid clearance. Given these important functions, the magnitude of damage to the alveolar type I cell could be a major determinant of the severity of ALI/ARDS and of its clinical outcomes. However, the search for a reliable, easily measured marker of alveolar type I cell injury in humans has proved difficult, and thus the importance of alveolar type I cell damage in humans with ALI remains unclear.

The receptor for advanced glycation end products (RAGE) was recently identified as a promising new marker of alveolar type I cell injury. RAGE is a member of the immunoglobulin superfamily that acts as a multiligand receptor and is involved in propagating inflammatory responses. Immuno-electron microscopy has demonstrated that RAGE expression in the lung is primarily located on the basal surface of alveolar type I cells. Although RAGE is produced by other cell types in other organs, such as vascular endothelium and nervous tissues, and is therefore not specific for alveolar type I cell injury, it is most abundant in the lung. In a murine model of ALI, elevated RAGE levels in bronchoalveolar lavage fluid and serum were directly related to the severity of experimentally induced lung injury. Similarly, in human subjects, plasma RAGE levels were significantly higher in patients with ALI than in patients with hydrostatic pulmonary edema or normal controls. Elevated plasma RAGE levels have also been associated with an increased duration of mechanical ventilation and ICU length of stay in patients following lung transplantation. However, the potential pathogenic and prognostic value of RAGE has not been studied in patients with ALI.

The objective of this study was to determine whether plasma levels of RAGE are associated with the severity and clinical outcomes of ALI/ARDS in subjects enrolled in the ARDS Network’s trial of a lower tidal volume, plateau pressure-limited ventilation strategy. We hypothesized that increased baseline levels of RAGE would be associated with increased severity of ALI/ARDS and with poor clinical outcomes, including increased mortality and fewer ventilator-free and organ failure-free days. Further, from a mechanistic perspective, we hypothesized that plasma RAGE levels measured at 3 days after enrollment would be preferentially decreased by a low tidal volume ventilation strategy.

METHODS

Clinical data and biological samples for this study were obtained from patients enrolled in a randomized controlled trial of lower tidal volume, plateau-pressure limited ventilation conducted by the National Heart, Lung, and Blood Institute’s ARDS Network. This trial enrolled 861 patients to test the hypothesis that ventilation with a lower tidal volume, plateau pressure-limited strategy would reduce mortality in patients with ALI. The details and results of the clinical trial have been previously published in full. Briefly, patients were randomized to ventilation with a tidal volume of either 6 or 12 ml/kg predicted body weight; once the benefit of the lower tidal volume strategy had been demonstrated, an additional 41 patients were assigned to the lower tidal volume to complete a factorialized trial of lisofylline versus placebo,
these patients are also included in this analysis. All patients were followed until death, 180 days or until discharge home with unassisted breathing. The trial demonstrated a significant reduction in 180-day mortality in the lower tidal volume treatment group (31% vs 40%, p=0.007). In a factorial design, ketoconazole(18) and lisofylline(17) were also administered to subsets of patients as investigational therapies for ALI, neither of which affected mortality. Inclusion and exclusion criteria are described in the original publication.(2) The institutional review boards of all involved hospitals approved the trial.(2)

Assay Procedures

We measured baseline (Day 0, pre-randomization) and Day 3 plasma levels of RAGE using a commercially available enzyme-linked immunoassay (Human RAGE Quantikine ELISA; R&D Systems, Minneapolis, MN) in 676 patients from the original clinical trial. RAGE levels were not measured in all 902 patients due to limited plasma availability.

Statistical Methods

Statistical analysis was performed with STATA/SE 9.2 (College Station, TX). To compare baseline levels of RAGE in two-group comparisons, we used Mann-Whitney rank-sum tests; for multiple group comparisons, we used the Kruskal-Wallis test. Because RAGE levels were not normally distributed, we applied natural log-transformation to the plasma RAGE levels in order to apply linear models. We first tested the impact of RAGE on mortality in an unadjusted logistic regression, and then created a multivariable logistic model to control for baseline clinical features that may have affected outcomes, including age, gender, sepsis or trauma as etiology of lung injury, and severity of illness, as reflected by APACHE III score(19). These covariates were selected on the basis of the bivariate analysis results (all were associated with mortality with p<0.05 with the exception of gender, which was associated with baseline RAGE) and prior clinical research about predictors of mortality in ALI.(20) We performed similar analyses using linear regression models for the outcomes of ventilator-free days and organ failure-free days. In addition, in a pre-specified analysis, plasma RAGE levels were divided into quartiles to test for a non-linear relationship with outcomes in similar regressions. All analyses were stratified by ventilation strategy due to an observed interaction between baseline plasma RAGE level and ventilation strategy; this interaction was the only interaction tested and was initially sought after a difference in the odds ratios for death stratified by ventilation strategy was observed. Unstratified analyses are presented in the online supplement. A p-value of <0.20 was considered potentially important for interactions;(21) for all other analyses, a p-value of <0.05 was considered significant. Logistic models were checked using the Hosmer-Lemeshow test and the linktest;(22) linear models were evaluated using residual-based diagnostics. Analysis of covariance was used to test the impact of lower tidal volume ventilation on the change in RAGE levels from Day 0 to Day 3.

RESULTS

Baseline Clinical Characteristics of Subjects

Baseline plasma samples were available for RAGE measurements in 676 of the 902 original study subjects. The clinical characteristics of subjects who had plasma samples available were similar to those who did not have plasma samples available (Table 1), with the exception of a lower incidence of trauma as the etiology of ALI and a slightly lower mean APACHE III score. Baseline plasma RAGE levels did not differ by clinical risk factor for ALI (p=0.54) or by race-ethnicity but did differ by gender (median in men 5536 pg/ml vs. 3360 pg/ml in women; p<0.0001). Plasma RAGE levels did not differ based on the presence of end-stage renal disease, diabetes, cirrhosis of the liver, a history of malignancy or immunosuppression, or a history of the acquired immunodeficiency syndrome.
Table 1: Baseline Characteristics of the ARMA Study Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RAGE measured (n=676)</th>
<th>Not measured (n=226)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>51 (17)</td>
<td>52 (18)</td>
<td>0.43</td>
</tr>
<tr>
<td>Female gender</td>
<td>394 (58)</td>
<td>143 (63)</td>
<td>0.19</td>
</tr>
<tr>
<td>Race-ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>497 (69)</td>
<td>164 (73)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>118 (17)</td>
<td>37 (16)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>39 (6)</td>
<td>12 (5)</td>
<td>0.61</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>17 (3)</td>
<td>9 (4)</td>
<td></td>
</tr>
<tr>
<td>Native American</td>
<td>3 (0.4)</td>
<td>3 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (0.3)</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>APACHE III score, mean (SD)</td>
<td>82.0 (27.5)</td>
<td>86.5 (31.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sepsis as primary ALI risk factor</td>
<td>173 (26)</td>
<td>63 (28)</td>
<td>0.50</td>
</tr>
<tr>
<td>Trauma as primary ALI risk factor</td>
<td>63 (9)</td>
<td>33 (15)</td>
<td>0.03</td>
</tr>
<tr>
<td>PaO2:FiO2 ratio, mean (SD)</td>
<td>128 (58)</td>
<td>135 (58)</td>
<td>0.11</td>
</tr>
<tr>
<td>Diabetes</td>
<td>97 (14)</td>
<td>29 (13)</td>
<td>0.55</td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>18 (3)</td>
<td>2 (1)</td>
<td>0.12</td>
</tr>
<tr>
<td>Lower tidal volume arm</td>
<td>352 (52)</td>
<td>121 (54)</td>
<td>0.70</td>
</tr>
<tr>
<td>WBC, thousands, mean (SD)</td>
<td>14.9 (1.0)</td>
<td>14.5 (1.1)</td>
<td>0.55</td>
</tr>
<tr>
<td>Lowest MAP, mean (SD)</td>
<td>62.1 (14)</td>
<td>61.5 (14)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data reported as n (%) unless otherwise specified.
Abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; ALI = acute lung injury; WBC = white blood cell count; MAP = mean arterial pressure.
Severity of ALI/ARDS

Higher baseline levels of plasma RAGE were significantly associated with more severe ALI/ARDS, as reflected by measures of pulmonary physiology including lower PaO₂:FiO₂ ratio, lower compliance, and higher clinical and radiographic lung injury scores (Figures 1 and 2). Likewise, higher baseline plasma RAGE levels were associated with higher pre-randomization tidal volume \((r=0.12; \ p=0.002)\), and plateau pressure \((r=0.22; \ p<0.0001)\). Higher baseline levels of plasma RAGE were also associated with higher APACHE scores \((r=0.17; \ p<0.0001)\).

Clinical Outcomes

In unadjusted analyses, baseline plasma RAGE was strongly associated with poor clinical outcomes. Baseline RAGE was significantly higher in subjects who died compared to those who survived (median [IQR] in non-survivors 5327 [2250 to 10112] pg/ml vs. in survivors 3618 [1916 to 7402] pg/ml; \(p=0.001\)). However, upon further analysis of the association between baseline plasma RAGE and death, an interaction between baseline plasma RAGE and ventilation strategy was detected \((p=0.09)\). When the analysis was stratified by ventilation strategy, baseline RAGE was predictive of death only in the group randomized to the 12 ml/kg strategy (Figure 3 and Table 2; OR per one-log increment in RAGE 1.38; 95% CI 1.13 to 1.68; \(p=0.002\)). Baseline RAGE was not predictive of death in the lower tidal volume group (OR 1.07; 95% CI 0.86 to 1.32; \(p=0.53\)).

Baseline plasma RAGE was also strongly associated with increased morbidity from ALI in the 12 ml/kg ventilation group (Table 3). Specifically, for every one-log increase in plasma RAGE, ventilator-free days decreased by 1.57 (95% CI -2.57 to -0.56 days; \(p=0.002\)), and organ failure free days decreased by 2.44 (95% CI -3.44 to -1.44 days, \(p<0.001\)). Baseline plasma RAGE was not associated with increased morbidity in the 6 ml/kg group (Table 3).
<table>
<thead>
<tr>
<th>Model</th>
<th>Tidal Volume Group</th>
<th>Odds Ratio for Death at 180 Days (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>12 ml/kg</td>
<td>1.38 (1.13, 1.68)</td>
<td>0.002</td>
</tr>
<tr>
<td>Baseline plasma RAGE (one-log increment), multivariable model*</td>
<td>12 ml/kg</td>
<td>1.41 (1.12, 1.78)</td>
<td>0.004</td>
</tr>
<tr>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>6 ml/kg</td>
<td>1.07 (0.86, 1.32)</td>
<td>0.53</td>
</tr>
<tr>
<td>Baseline plasma RAGE (one-log increment), multivariable model*</td>
<td>6 ml/kg</td>
<td>1.03 (0.81, 1.31)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Multivariable model adjusts for age, gender, APACHE III score, and presence of sepsis or trauma as ALI risk factors. In 12 ml/kg model: Hosmer-Lemeshow p=0.24; linktest hatsq = 0.22. In 6 ml/kg model: Hosmer-Lemeshow p=0.35; linktest hatsq = 0.99. All transformations use natural logarithms.

* p-value for interaction between baseline RAGE and death in multivariable model = 0.06.
<table>
<thead>
<tr>
<th>Ventilator group</th>
<th>Model</th>
<th>Mean difference in ventilator-free days* (95% CI)</th>
<th>p-value</th>
<th>Mean difference in organ failure-free days** (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 ml/kg</td>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>-1.57 (-2.57, -0.56)</td>
<td>0.002</td>
<td>-2.44 (-3.44, -1.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Baseline plasma RAGE (one-log increment), multivariable model</td>
<td>-1.15 (-2.10, -0.20)</td>
<td>0.02</td>
<td>-1.98 (-2.94, -1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 ml/kg</td>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>-0.57 (-1.59, 0.45)</td>
<td>0.27</td>
<td>-1.01 (-2.06, 0.03)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Baseline plasma RAGE (one-log increment), multivariable model</td>
<td>-0.24 (-1.29, 0.80)</td>
<td>0.65</td>
<td>-0.72 (-1.73, 0.30)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Multivariable model adjusts for age, gender, APACHE III score, and presence of sepsis or trauma as ALI risk factors. Model checking performed using residual-based diagnostics. All transformations use natural logarithms.

* p-value for interaction between ventilator strategy and baseline RAGE in univariate model = 0.17; in multivariate model, p=0.12.
** p-value for interaction between ventilator strategy and baseline RAGE in univariate model = 0.05; in multivariate model, p=0.04.
**Multivariable Models**

The strong association between baseline plasma RAGE and poor clinical outcomes in the 12 ml/kg group persisted in multivariable models. After adjusting for age, gender, APACHE III score, and the presence of sepsis or trauma as a risk factor for ALI, higher baseline plasma RAGE remained strongly associated with an increased odds of death at 180 days in the 12 ml/kg group (Table 2). Additional adjustments for pre-existing diabetes and end-stage renal disease had no appreciable impact on the results of these regressions; similarly, including all precipitating risk factors for ALI in the model had no appreciable impact on the results. As with the mortality analyses, higher baseline plasma RAGE remained associated with a lower number of ventilator-free and organ failure-free days in multivariable linear models in the 12 ml/kg group, adjusting for the same clinical covariates (Table 3). Multivariable models revealed no association between baseline RAGE and clinical outcomes in the 6 ml/kg group (Table 3).

To further study the relationship of baseline plasma RAGE to clinical outcomes, the multivariable analyses were repeated using quartiles of RAGE, again controlling for the same clinical covariates (age, gender, APACHE III score, sepsis and trauma) and stratifying by ventilation strategy. In the 12 ml/kg group, this analysis demonstrated an increased risk of death for the highest quartile of RAGE, compared to the lowest quartile (Table 4; OR 2.73, 95% CI 1.31-5.68; overall p=0.003). In the 6 ml/kg group, RAGE by quartile was not significantly associated with mortality (data not shown). Linear regression models for ventilator-free days and organ-failure free days using quartiles of RAGE revealed similar findings (Table 4).

**Impact of Lower Tidal Volume Strategy**

The impact of the lower tidal volume ventilation strategy on the change in RAGE levels over the first 3 days of the study was measured using analysis of covariance. There were 599 patients with paired Day 0 and Day 3 samples available for analysis. In both the 6 ml/kg and the 12 ml/kg groups, plasma RAGE levels declined over the first 3 days; however, there was a 15% greater decline in the 6 ml/kg group compared to the 12 ml/kg group (p=0.02; 95% CI 2.4% to 25.0%; Figure 4).

Given the observed interaction between baseline plasma RAGE and ventilator strategy, we compared the effect of low tidal volume ventilation on mortality in the highest vs. the lowest quartile of baseline RAGE. In the highest quartile of baseline RAGE, the odds ratio for death in subjects treated with lower tidal volume ventilation was 0.36, compared with those treated with higher tidal volumes (95% CI 0.19 to 0.67; p=0.001). In comparison, in the lowest quartile of baseline RAGE, the odds ratio for death in subjects treated with lower tidal volumes was 0.52 (95% CI 0.26 to 1.00; p=0.05).
Table 4: Quartiles of RAGE and Clinical Outcomes in 12 ml/kg Group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Mean Effect, Compared to First Quartile (95% CI)</th>
<th>p-value for predictor</th>
<th>p-value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio for death at 180 days</td>
<td>Second quartile RAGE</td>
<td>0.77 (0.37, 1.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>0.86 (0.41, 1.80)</td>
<td>0.003</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>2.73 (1.31, 5.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilator-free days</td>
<td>Second quartile RAGE</td>
<td>0.1 (-3.0, 3.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>0.1 (-3.0, 3.4)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>-4.2 (-7.3, -1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ failure-free days</td>
<td>Second quartile RAGE</td>
<td>-2.4 (-5.5, 0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>-2.3 (-5.6, 1.0)</td>
<td>0.001</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>-6.4 (-9.5, -3.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All analyses adjusted for age, gender, APACHE III, sepsis and trauma.
DISCUSSION

In a large multicenter randomized controlled trial of ventilation strategy in ALI, higher baseline plasma RAGE levels were associated with worse clinical outcomes in patients randomized to higher tidal volumes, including increased mortality and fewer ventilator-free and organ failure-free days. These associations persisted even after adjusting for other important determinants of outcome, including age, etiology of ALI and severity of illness. In patients randomized to lower tidal volumes, plasma RAGE was not predictive of outcomes. Plasma RAGE was associated with measures of severity of lung injury including PaO₂:FiO₂, quasi-static compliance, and lung injury score. Further, lower tidal volume ventilation amplified the decline in RAGE levels over the first 3 days of the study, suggesting that ventilation with a 6 ml/kg strategy may cause less injury to alveolar type I cells than ventilation with higher tidal volumes.

Although RAGE is not specific for alveolar epithelial injury, several findings in this study and in prior work provide supportive evidence that the primary source of plasma RAGE in our patients is the alveolar type I cell. In our previous work on RAGE in animal models of ALI, both acid aspiration and lipopolysaccharide-induced lung injury resulted in RAGE release into the bronchoalveolar lavage fluid at much higher levels than in plasma, suggesting a pulmonary source for plasma RAGE in the setting of ALI.(11) A similar pattern was found in human edema fluid and plasma samples from subjects with ALI.(11) Likewise, in a recently published, isolated perfused human lung preparation in which the lung is the only possible source of RAGE, perfusate levels of RAGE were significantly and inversely associated with alveolar fluid clearance, suggesting that RAGE quantitatively reflects alveolar epithelial damage.(24) In the current study, the finding that plasma RAGE levels were associated with the severity of pulmonary physiologic disturbances (PaO₂/FiO₂ ratio, compliance, and plateau pressure) also supports an alveolar epithelial source of RAGE.

Despite this evidence, it is important to emphasize that non-pulmonary sources of RAGE may contribute to plasma levels in the setting of ALI, including release from non-pulmonary large vessel endothelial surfaces and systemic inflammation. Moreover, plasma RAGE levels may have been affected by the permeability of the alveolar-capillary interface, though this phenomenon would if anything strengthen its utility as a marker of lung injury. While the precise function of RAGE remains unclear, evidence is mounting that RAGE plays an important role in regulating inflammation. The primary ligands for RAGE are advanced glycation endproducts (AGE), non-enzymatically glycated adducts of endogenous proteins, lipids and nucleic acids whose formation is increased in the presence of hyperglycemia or oxidant stress.(12) In addition to AGE, RAGE has several alternative ligands, including pro-inflammatory molecules such as S100 and high mobility group box 1.(12) RAGE-ligand interaction mediates nuclear factor kappa B activation and seems thereby to play a role in propagating and sustaining systemic inflammatory responses.(25, 26) Thus, although we set out to study RAGE as a marker of alveolar epithelial injury, it is possible that the association between plasma RAGE and clinical outcomes of ALI observed in our study derives in part from RAGE’s role in an inflammatory cascade rather than purely from its release from alveolar type I cells. If the plasma RAGE levels measured in our study primarily reflect RAGE’s role in inflammation, then the interaction between RAGE and ventilator strategy lends further credence to the hypothesis that low tidal volume ventilation decreases systemic inflammation.(27, 28)

The finding of effect modification in this study—that is, that the predictive value of RAGE differed by tidal volume strategy—was particularly informative. If plasma RAGE primarily reflects alveolar epithelial injury, this interaction implies that patients with more severe alveolar epithelial injury are most susceptible to the additional ventilator-induced lung injury that may be generated by higher tidal volumes. This interpretation is reinforced by the finding that lower tidal volume ventilation preferentially amplified the decline in RAGE levels over the first 3 days of the study. Lower tidal volume ventilation preferentially affects the plasma levels of several biomarkers that have prognostic value in ALI/ARDS, including markers of inflammation...
interleukins 6 and 8), alveolar type II cell injury (surfactant protein D), and soluble tumor necrosis factor receptor 1, which may reflect neutrophil-endothelial interaction and/or epithelial injury. When taken in concert with the finding of effect modification by lower tidal volume ventilation, the amplified decline in RAGE levels over time in patients in the 6 ml/kg group provides important evidence that a lung-protective ventilation strategy is effective at least in part by decreasing injury to the alveolar epithelium.

The results of this study also suggest that the degree of injury to the alveolar type I cell is an important determinant of outcomes in human ALI. Type I cells cover the vast majority of the alveolar surface and form a key component of the alveolar capillary barrier. In addition to this role, type I cells have been traditionally thought to be primarily responsible for gas exchange and osmotically-driven water transport, whereas type II cells have been thought to be primarily responsible for surfactant production, ion transport, and alveolar fluid clearance. Recent research has suggested, however, that type I cells play a key role in ion and vectorial fluid transport as well, making their function all the more critically relevant to the pathogenesis and resolution of lung injury, since impaired alveolar fluid clearance is clearly linked to poor clinical outcomes in ALI. The specific association between alveolar type I cell damage and the severity of lung injury has been demonstrated in rat models of ALI, but the lack of a human marker of type I cell injury has precluded quantitative testing of this relationship (and the relationship of type I cell injury to clinical outcomes) in human studies until now.

The finding that plasma RAGE levels differed at baseline by gender was surprising and has not to our knowledge been reported in other clinical settings. Differences in lung mass among men and women; however, the magnitude of difference detected in this sample seems unlikely to be due to differences in lung mass. Although gender has not been associated with outcomes in most studies of ALI, we included gender as a covariate in all our multivariable models to account for any confounding that might have occurred due to this difference in baseline plasma RAGE levels.

This study has some limitations. First, plasma RAGE was not measured in all participants in the original trial, only in subjects for whom plasma samples were still available. Although our analyses revealed few differences between subjects who had plasma available and those who did not, it remains possible that systematic factors contributed in some way to plasma availability. Second, the selection of potential confounders for the multivariable models was limited to clinical data collected by the original ARDS Network studies. Several chronic diseases that may affect clinical outcomes may also impact RAGE levels; for instance, subjects with diabetes mellitus and end-stage renal disease have been shown to have elevated levels of plasma RAGE, whereas subjects with coronary artery disease, rheumatoid arthritis, Alzheimer’s disease, and essential hypertension have been shown to have decreased levels of plasma RAGE. Although we were able to control for diabetes mellitus and pre-existing dialysis-dependent renal failure in sensitivity analyses (and demonstrate that they had-no impact on our results), coronary artery disease, rheumatoid arthritis, Alzheimer’s disease, and hypertension were not recorded in the original dataset. Thus, it is possible that chronic diseases present in some study subjects are unmeasured confounders in our analyses.

Third, the finding of an interaction between baseline RAGE and ventilation strategy should, like all subgroup analyses, be interpreted with caution, particularly since the criteria used to determine potential importance of the interaction were less stringent than for other analyses. The use of a less stringent p-value for interaction analyses is common in the epidemiologic literature, though the optimum cut-off remains controversial. Moreover, when the data are analyzed without stratification to account for the interaction, baseline RAGE is significantly predictive of death and the other key clinical outcomes in the cohort as a whole in both unadjusted and multivariable models (data available in online supplement). Finally, plasma
samples used in this study had been stored for several years at -70° C; whether this extended storage has any effects on plasma biomarker levels remains unknown.

In conclusion, in this analysis of data from a large randomized controlled trial, baseline levels of plasma RAGE were independently associated with clinical outcomes in ALI/ARDS in patients randomized to traditional, higher tidal volumes. In this group, higher baseline plasma RAGE levels were associated with increased mortality and fewer ventilator-free and organ failure-free days. In addition, lower tidal volume ventilation amplified the decline in RAGE levels over time. These findings provide new evidence that lower tidal volume ventilation may be beneficial at least in part by decreasing injury to the alveolar epithelium.
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Please see supplemental material.

COMPETING INTERESTS
The authors declare that they have no competing interests to disclose.

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**Figure 1 Legend:**
Higher baseline plasma RAGE was associated with more severe disturbances in pulmonary physiology (n= 676 for PaO2/FiO2; n=522 for compliance). Graph depicts median and interquartile range. * p=0.008; † p=0.01

**Figure 2 Legend:**
Increasing clinical and radiographic lung injury scores (LIS), reflecting increasing severity of lung injury,(23) were associated with increasing baseline plasma RAGE (n=669). Graph depicts median and interquartile range. For clinical LIS: Kruskal-Wallis p=0.0008; test for trend p<0.001. For radiographic LIS: Kruskal-Wallis p=0.001; test for trend p<0.001.

**Figure 3 Legend:**
Baseline plasma RAGE by survival status, stratified by ventilation strategy (n=676). Baseline plasma RAGE was associated with death at 180 days only in the 12 ml/kg group. Graph shows median plasma RAGE in each group and interquartile range; comparisons made using Mann-Whitney (Wilcoxon) ranksum.

**Figure 4 Legend:**
Plasma RAGE levels declined in both the 6 ml/kg and 12 ml/kg groups over the first 3 days of the study (n=599); however, the decline was 14.6% greater in the lower tidal volume group (p=0.02; 95% CI 2.4%-25.0%). Note the y-axis scale differs between the two sides of the figure.
REFERENCES


Figure 1.
Figure 2.
Figure 3.

Median Plasma RAGE (pg/ml)

- 6 ml/kg
- 12 ml/kg

Alive
Dead

p=0.001
p=0.21
Figure 4.
## Table 1A: Baseline Plasma RAGE and Death at 180 Days among Patients with ALI/ARDS, Unstratified Analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds Ratio for Death at 180 Days (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>1.23 (1.06, 1.42)</td>
<td>0.006</td>
</tr>
<tr>
<td>Baseline plasma RAGE (one-log increment), multivariable model*</td>
<td>1.22 (1.03, 1.44)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Unadjusted model controls only for ventilator strategy. Multivariable model also adjusts for age, gender, APACHE III score, and presence of sepsis or trauma as ALI risk factors. In multivariable model: Hosmer-Lemeshow p=0.20; linktest hatsq = 0.77. All transformations use natural logarithms.
Table 2A: Baseline Plasma RAGE and Other Clinical Outcomes at 180 Days among Patients with ALI/ARDS, Unstratified Analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean difference in ventilator-free days* (95% CI)</th>
<th>p-value</th>
<th>Mean difference in organ failure-free days** (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline plasma RAGE (one-log increment), unadjusted model</td>
<td>-1.08 (-1.80, -0.37)</td>
<td>0.003</td>
<td>-1.76 (-2.48, -1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline plasma RAGE (one-log increment), multivariable model</td>
<td>-0.72 (-1.42, -0.02)</td>
<td>0.04</td>
<td>-1.35 (-2.04, -0.66)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Unadjusted model controls only for ventilator strategy. Multivariable model also adjusts for age, gender, APACHE III score, and presence of sepsis or trauma as ALI risk factors. Model checking performed using residual-based diagnostics.
Table 3A: Quartiles of RAGE and Clinical Outcomes, Unstratified Analysis

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Mean Effect, Compared to First Quartile (95% CI)</th>
<th>p-value for predictor</th>
<th>p-value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio for death at 180 days</td>
<td>Second quartile RAGE</td>
<td>0.84 (0.50, 1.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>1.34 (0.81, 2.22)</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>1.92 (1.14, 3.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilator-free days</td>
<td>Second quartile RAGE</td>
<td>1.20 (-0.96, 3.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>-0.53 (-2.71, 1.65)</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>-2.77 (-5.0, -0.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ failure-free days</td>
<td>Second quartile RAGE</td>
<td>-1.20 (-3.34, 0.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third quartile RAGE</td>
<td>-3.14 (-5.3, -0.99)</td>
<td>0.001</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Fourth quartile RAGE</td>
<td>-4.17 (-6.4, -1.94)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All analyses adjusted for age, gender, APACHE III, sepsis, trauma, and ventilation strategy.
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