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Temporal Changes of Protein Biomarkers in Sepsis-Induced Acute Respiratory Distress Syndrome

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Temporal Changes of Protein Biomarkers in Sepsis-Induced Acute Respiratory Distress Syndrome

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Abstract

Temporal Changes of Protein Biomarkers
in Sepsis-Induced Acute Respiratory Distress Syndrome

By Philip Yang

Introduction: Protein biomarkers including soluble receptor for advanced glycation end-products (sRAGE), angiopoietin-2 (Ang-2), and surfactant protein-D (SP-D) have been studied for diagnosis and prognostication in the acute respiratory distress syndrome (ARDS). However, prior studies of ARDS biomarkers often included heterogeneous populations or did not assess longitudinal changes of the biomarkers. The aim of this study was to compare the differences in sRAGE, Ang-2, and SP-D levels and their changes over time between patients with sepsis who developed ARDS vs. those who did not develop ARDS.

Methods: This was a prospective observational cohort study that enrolled adult patients admitted to the medical intensive care unit within 72 hours of sepsis or septic shock diagnosis. Serial plasma samples were collected for three consecutive days after enrollment, and were analyzed for sRAGE, Ang-2, and SP-D levels. Patients were followed for ARDS development and other outcomes. The biomarker levels and their changes over the three-day period were compared between ARDS and non-ARDS patients, and between non-survivors and survivors. Logistic regression was performed to examine the association between the biomarker levels and important binary clinical outcomes.

Results: 111 patients were included, of whom 21 patients (18.9%) developed ARDS. ARDS patients had higher levels of sRAGE and SP-D compared to non-ARDS patients, though the mean differences were not statistically significant. Non-survivors had significantly higher levels of sRAGE, Ang-2, and SP-D compared to survivors across multiple days. The changes of the three biomarker levels over time were not different between ARDS vs. non-ARDS patients, and between non-survivors vs. survivors. In logistic regression analyses, absolute SP-D level on day 1 was significantly associated with ARDS development (odds ratio [OR] 1.83, 95% confidence interval [CI] 1.06-3.15, p=0.03) and mortality (OR 1.52, 95% CI 1.03-2.24, p=0.03).

Conclusions: Among critically ill patients with sepsis, sRAGE, Ang-2, and SP-D levels were not different between ARDS and non-ARDS patients, but were higher in non-survivors compared to survivors. The changes of the biomarker levels over time were not different between the outcome groups. Absolute SP-D level on day 1 was associated with ARDS development and mortality in multivariable logistic regression models.

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INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a severe form of acute, diffuse inflammatory lung injury associated with high mortality rates up to 46% in severe cases[1]. ARDS is a markedly heterogeneous syndrome, with a wide variety of predisposing conditions that result in different phenotypes of ARDS[2]. The heterogeneity is highlighted by the fact that only 45% of patients meeting the clinical criteria for ARDS had the characteristic histopathologic finding of diffuse alveolar damage, with the remainder of patients demonstrating a variety of other pathologic findings[3]. This clinical and pathologic heterogeneity of ARDS is thought to contribute to the current lack of a reliable diagnostic test or a specific pharmacologic therapy for ARDS despite decades of research[2, 4]. In order to address this problem, protein biomarkers have been studied as a method to diagnose, prognosticate, and phenotype ARDS. Protein biomarkers, which can be measured from various body compartments, can provide clues about the pathophysiologic mechanisms in ARDS, and can help in the prediction, diagnosis, and management of ARDS[5]. In particular, elevated levels of soluble receptor for advanced glycation end-products (sRAGE) have been correlated with the presence and severity of ARDS, as well as increased mortality and worse clinical outcomes from ARDS[6-9]. Angiopoietin-2 (Ang-2) and surfactant protein D (SP-D) have also been shown to correlate with ARDS diagnosis and prognosis, as well as to be helpful in distinguishing different subtypes of ARDS[10-13].

However, most prior studies did not differentiate patients based on the underlying etiologies of ARDS, despite the highly heterogeneous nature of ARDS. In particular, patients with sepsis-induced ARDS behave differently and have worse clinical outcomes compared to those with ARDS from other causes[14], but the data regarding the ARDS biomarkers specifically in patients with sepsis is scant. In addition, many prior studies measured the biomarker levels

only at a single time point, and only a few studies monitored the longitudinal changes of the biomarkers prospectively. However, monitoring the changes in biomarker levels over time can provide useful information about the dynamic changes in acute disease processes such as ARDS. For example, secondary analyses of specimens from ARDS clinical trials showed that patients receiving low tidal volume ventilation had a greater decline in plasma sRAGE levels[8] and smaller increase in SP-D levels[15] over time compared to those receiving high tidal volume ventilation. Therefore, serial measurements of ARDS biomarkers can help monitor the development and progression of ARDS as well as the responses to interventions.

The objective of this study was to address the limitations in prior studies by performing serial measurements of ARDS-related protein biomarkers in a narrower population consisting of critically ill patients with sepsis. We conducted a prospective observational cohort study in order to determine the differences in sRAGE, Ang-2, and SP-D levels and their changes over time between patients with sepsis who developed ARDS vs. those who did not develop ARDS. The hypothesis was that patients who developed ARDS will have higher absolute biomarker levels, and have greater increases in the biomarker levels over time, compared to patients who did not develop ARDS.

BACKGROUND

While a large number of protein biomarkers have been studied in various contexts related to ARDS, no single biomarker has been shown to perform consistently better than any other, and there is currently no proven role for these protein biomarkers in the clinical management of ARDS[4]. Among the protein biomarkers that were previously studied in ARDS, we chose to examine three biomarkers that were thought to be relevant for our study population and aims:

soluble receptor for advanced glycation end-products (sRAGE), angiopoietin-2 (Ang-2), and surfactant protein-D (SP-D).

sRAGE is the extracellular domain of a multiligand receptor expressed on alveolar type 1 cells and is a marker of lung epithelial injury[7]. sRAGE may be involved in the causal pathway in the pathophysiology of sepsis-induced ARDS[16], and is therefore pertinent to the patient population of interest for this study. In a study by Jabaudon et al., plasma sRAGE levels were found to be elevated in patients with acute lung injury or ARDS, and correlated with clinical and radiographic severity of disease[7]. Another study by Fremont et al. also found that plasma levels of sRAGE, along with several other biomarkers, were significantly elevated in trauma patients who developed acute lung injury/ARDS compared to controls[17]. sRAGE levels were also associated with increased severity of acute lung injury, increased mortality, and fewer ventilator-free and organ-failure-free days in prior studies of ARDS[8].

Ang-2 is a molecule that leads to impairment of lung endothelial barrier function and serves as a marker of lung endothelial injury[10]. Ang-2 may distinguish ARDS due to indirect lung injury[13], and could potentially identify specific subsets of sepsis-induced ARDS. Elevated plasma level of Ang-2 in critically ill patients receiving mechanical ventilation was shown to be predictive of ARDS and to correlate with severity of disease[10]. Another study found that elevated Ang-2 levels were strongly associated with increased development of acute lung injury in critically ill patients[11], and the aforementioned study in a trauma ICU population by Fremont et al. also found that Ang-2 levels were significantly elevated in acute lung injury/ARDS patients compared to controls[17].

Finally, SP-D is one of the surfactant-associated proteins that are mainly synthesized in alveolar type 2 cells, and is thought to be a marker of lung epithelial injury and inflammation[12].

SP-D may distinguish ARDS due to direct lung injury[13], and is correlated with various clinical outcomes in ARDS, such as mortality, number of days on the ventilator or ventilator-free days, and length of stay in the hospital[15, 18]. In addition, one study showed a smaller increase in SP-D level over time in ARDS patients ventilated with lung-protective strategy compared to those ventilated with the conventional strategy, indicating that SP-D may be a marker of ventilator-induced lung injury (VILI) in ARDS[15]. This also supported the rationale for using serial biomarker measurements to monitor the development or progression of ARDS in our project.

METHODS

Study Design and Characteristics

This was a prospective observational cohort study of patients who were admitted to the medical ICU at Grady Memorial Hospital, Atlanta, Georgia, with a primary diagnosis of sepsis or septic shock between September 16, 2020 and November 8, 2021. This study was approved by the Institutional Review Board (IRB) at Emory University, Atlanta, Georgia, and by the Research Oversight Committee (ROC) at Grady.

Study Participant Screening and Enrollment

The patient list for the medical ICU at Grady was screened daily for eligible patients by one of the trained study team members. Patients were eligible if they were admitted to the medical ICU at Grady Memorial Hospital ≤72 hours of diagnosis of sepsis or septic shock, as defined by the Sepsis-3 definition[19]. Briefly, sepsis was defined as an acute change in the total sequential organ failure assessment (SOFA) score of ≥2 points from baseline, in the setting of a confirmed or strongly suspected infection; septic shock was defined as sepsis plus persistent

hypotension requiring vasopressors to maintain mean arterial pressure ≥65 mmHg and having a serum lactate level >2 mmol/L. Patients were excluded if they were under 18 years of age, pregnant, or incarcerated; already had ARDS at the time of screening; were not candidates for full resuscitation (specifically endotracheal intubation) or were pursuing comfort measures only; or declined participation in the study.

Informed consent for study participation was obtained from the patients meeting enrollment criteria or their legally authorized representatives. For eligible patients who were unable to consent for themselves and whose legally authorized representatives could not be reached after multiple attempts, a waiver of informed consent was permitted by the Emory University IRB and Grady ROC given minimal risk to the participants.

Protocol for Blood Sample and Data Collection

After obtaining informed consent, serial blood samples were collected from each participant once daily on days 1, 2, and 3 of study enrollment (i.e. first blood sample on the day of enrollment as soon as possible after obtaining or waiving informed consent, then 24 and 48 ± 3 hours after the first blood sample collection). Blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes, then centrifuged to isolate the plasma. Plasma was separated into cryotubes, then frozen and stored at -80°C until analysis. Levels of sRAGE, Ang-2, and SP-D were measured from each of the plasma samples using commercially available enzyme-linked immunosorbent assay (ELISA) kits (sRAGE – BioVendor, Asheville, NC; Ang-2 and SP-D – R&D Systems, Minneapolis, MN).

Participants were followed for up to 28 days for important clinical outcomes as detailed below. Additional clinical information including demographics, medical comorbidities, SOFA

scores, primary and secondary sources of infection, ventilator settings, duration of mechanical ventilation, ICU and hospital length of stays, and the final disposition status were recorded from the medical charts. In order to avoid potential bias, the investigators assessing the ARDS diagnosis and other clinical outcomes were blinded to the biomarker measurements until completion of all clinical data entry, and the investigators performing the biomarker measurements were blinded to the clinical information until completion of all biomarker measurements.

Outcomes

The primary outcome was development of ARDS according to the Berlin definition[20]: severe acute respiratory failure occurring within 7 days of inciting event (sepsis or septic shock), with bilateral infiltrates on chest imaging that were not explained by cardiogenic pulmonary edema, and the ratio of partial pressure arterial oxygen to fraction of inspired oxygen (P/F ratio) ≤300 mmHg and positive end-expiratory pressure (PEEP) ≥5 cmH_2O. For patients without P/F ratio available, ratio of peripheral oxygen saturation to fraction of inspired oxygen (S/F ratio) was used as needed. For participants receiving oxygen support with heated and humidified high-flow nasal cannula (HFNC), the fraction of inspired oxygen (FiO2) setting on the HFNC was used to calculate the P/F or S/F ratios, but they were not considered to meet the Berlin definition of ARDS unless they subsequently satisfied the PEEP criterion by requiring either non-invasive positive-pressure ventilation (NIPPV) or invasive mechanical ventilation (IMV). For those subsequently requiring NIPPV or IMV, a new P/F or S/F ratio after initiation of positive-pressure ventilation was used as the qualifying measure for ARDS. The ARDS diagnosis was determined

from chart review by the primary investigator (P.Y.), with the senior investigator (A.M.E.) also reviewing the cases that were deemed equivocal for ARDS diagnosis.

Secondary outcomes included all-cause in-hospital mortality (including in-hospital death and discharge to hospice), 28-day ventilator-free days (the number of days that the patient remained alive and off invasive mechanical ventilation in the first 28 days of study enrollment), and 28-day ICU-free days (the number of days that the patient remained alive and not admitted in an ICU in the first 28 days of study enrollment).

Statistical Analysis and Analytical Methods

Based on preliminary data from a prior internal study of sRAGE, the difference in the biomarker levels between ARDS and non-ARDS patients of 2822 pg/mL and standard deviation of 3468 pg/mL was used for sample size calculation. With expected ARDS incidence of 20% resulting in 1:4 enrollment ratio of ARDS to non-ARDS patients, significance level of 0.05, and power of 0.80, the calculated sample size needed was 75 participants. This sample size calculation was extrapolated to Ang-2, SP-D, and for serial measurements, given lack of preliminary data related to these aspects of the study.

Simple descriptive statistics were used for comparisons of baseline demographic characteristics and clinical data between ARDS and non-ARDS patients. Two-sample independent t-test was used for comparing normally-distributed continuous variables; Wilcoxon rank-sum test, for comparing non-normally-distributed continuous variables; and chi-square or Fisher's exact test, for comparing categorical variables.

The absolute sRAGE, Ang-2, and SP-D levels were found to be non-normally-distributed, and were log-transformed to approximate a normal distribution. The log-transformed

absolute sRAGE, Ang-2, and SP-D levels were compared between the outcome groups (ARDS vs. non-ARDS and survivors vs. non-survivors) using two-sample t-test. The changes in the sRAGE, Ang-2, and SP-D levels from day 1 to days 2 and 3 were calculated ([biomarker level day 2] – [biomarker level day 1] and [biomarker level day 3] – [biomarker level day 1]), but these values were not log-transformed, and were compared between the outcome groups listed above using Wilcoxon rank-sum test.

Multivariable logistic regression models were constructed to examine the adjusted association between the biomarker levels and the outcomes. The absolute levels of sRAGE, Ang-2, and SP-D on day 1 and the changes of the sRAGE, Ang-2, and SP-D levels from day 1 to days 2 and 3 were the main exposure variables of interest. Due to significant correlation between the three biomarker levels, each of the biomarker variables were input individually into separate logistic regression models. Age, sex, and race were included as covariates by convention. The following covariates were considered for inclusion in the model: primary source of infection (re-coded as Coronavirus disease-2019 [COVID-19] vs. pulmonary [pneumonia or aspiration pneumonia] vs. non-pulmonary [all other sources of infection]), vasopressor requirement, renal replacement therapy requirement, tidal volume per ideal body weight (TV/IBW), positive end-expiratory pressure (PEEP), and ARDS diagnosis (when modeling mortality as the outcome). From these potential covariates, clinical reasoning and likelihood ratio tests for sequential addition of the covariates to the model were used to select covariates that were both clinically relevant and improved the model fit. The final model for ARDS included age, sex, race, primary source of infection, TV/IBW, and PEEP in addition to the biomarker variable. The final model for mortality included age, sex, race, and vasopressor use, in addition to the biomarker variable.

Significance level of $\alpha = 0.05$ was used for all statistical tests, without adjustment for multiple testing. All data analyses and statistical tests were performed in SAS v9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Patient Characteristics

A total of 111 critically ill patients with sepsis were enrolled between September 16, 2020 and November 8, 2021. Of these, 91 patients (82.0%) met sepsis definition within 3 hours from initial presentation to the emergency room or the hospital. The primary outcome of ARDS developed in 21 patients (18.9%), whereas 90 patients (81.1%) did not develop ARDS. ARDS and non-ARDS patients were similar with regard to their demographics, chronic medical comorbidities, and baseline severity of illness (as determined by the SOFA score) at the time of enrollment (Table 1). ARDS patients had a higher proportion of pulmonary sources of infection, including pneumonia, aspiration pneumonia, and COVID-19, whereas non-ARDS patients had a higher proportion of non-pulmonary sources of infection (Table 1). A higher proportion of ARDS patients required IMV compared to non-ARDS patients (n=20 [95.2%] in ARDS group vs. n=59 [65.6%] in non-ARDS group, p=0.007); one ARDS patient met ARDS criteria while receiving NIPPV, but did not require IMV. Overall mortality was not significantly different between ARDS vs. non-ARDS patients (n=10 [47.6%] in ARDS group vs. n=35 [38.9%] in non-ARDS group, p=0.46), but ARDS patients had significantly fewer 28-day ventilator-free days (median [interquartile range (IQR)] 8 [0-22] days vs. 20.5 [6-28] days, p=0.02) and fewer 28-day ICU-free days (1 [0-21] days vs. 16.5 [3-24] days, p=0.02) (Table 2).

Sample Collections

All 111 patients had plasma samples collected on day 1, with the median time elapsed from sepsis onset to first sample collection of 21 (IQR 15-31) hours. 100 patients (90.1%, 18 ARDS and 82 non-ARDS patients) had samples collected on day 2, and 83 patients (74.8%, 16 ARDS and 67 non-ARDS patients) had samples collected on day 3. The reasons for incomplete serial collections included: discharge from ICU (n=15), death or transfer to hospice (n=7), logistical issues (n=5), and withdrawal of participation (n=1).

Protein Biomarker Analysis by ARDS Diagnosis

The comparisons of the absolute biomarker levels on each day and the changes of the biomarkers over time between ARDS vs. non-ARDS patients are summarized in Table 3 and Figure 1. The absolute sRAGE and SP-D levels were higher in ARDS patients than in non-ARDS patients, but the mean differences were not statistically significant due to significant overlap between the groups. The differences in the absolute sRAGE and SP-D levels were greater on day 1, and the differences became smaller on subsequent days. ARDS patients had a greater change in Ang-2 level from day 1 to day 2 compared to non-ARDS patients, but there was a significant overlap between the groups; the changes in sRAGE or SP-D levels over time were not significantly different between ARDS patients and non-ARDS patients.

Protein Biomarker Analysis by Mortality Status

The comparisons of the absolute biomarker levels on each day and the changes of the biomarkers over time between non-survivors (patients who died or were discharged to hospice) vs. survivors are summarized in Table 4 and Figure 2. Non-survivors had significantly higher

absolute levels of sRAGE on days 1 and 2, higher absolute levels of Ang-2 across all three days, and higher absolute SP-D on day 1. The changes of the biomarker levels over time were not significantly different between non-survivors and survivors regardless of the time points.

Protein Biomarker Analysis in Subgroups

Within the subgroup of patients with pulmonary sepsis (sepsis as a result of pneumonia, aspiration pneumonia, or COVID-19), sRAGE level was significantly higher in ARDS patients compared to non-ARDS patients on day 1 (mean ± standard deviation [std] 1.135 ± 0.981 log[ng/mL] in ARDS vs. 0.577 ± 0.928 log[ng/mL] in non-ARDS, p=0.04), but the differences became smaller and were not significant on days 2 and 3.

Within the subgroup of patients with septic shock (those requiring vasopressors), non-survivors had higher absolute sRAGE levels on day 1 (1.037 ± 0.979 log[ng/mL] in non-survivors vs. 0.498 ± 1.025 log[ng/mL] in survivors, p=0.02) and day 2 (0.882 ± 0.908 log[ng/mL] vs. 0.414 ± 1.086 log[ng/mL], p=0.049) and higher absolute Ang-2 levels on day 2 (2.077 ± 0.672 log[ng/mL] vs. 1.732 ± 0.765 log[ng/mL], p=0.04) and day 3 (2.024 ± 0.664 log[ng/mL] vs. 1.631 ± 0.683 log[ng/mL], p=0.03), compared to survivors.

Notably, the temporal changes of any of the biomarker levels over time were not significantly different between ARDS vs. non-ARDS patients, and between non-survivors vs. survivors in the above subgroup analyses.

Multivariable Logistic Regression Analyses

Results of the multivariable logistic regression analyses are summarized in Table 5. Briefly, absolute SP-D level on day 1 was significantly associated with ARDS development

(adjusted odds ratio [aOR] 1.83, 95% confidence interval [CI] 1.06-3.15, p=0.03) after adjusting for age, sex, race, primary source of infection, TV/IBW, and PEEP. Absolute SP-D level on day 1 was also significantly associated with mortality (aOR 1.52, 95% CI 1.03-2.24, p=0.03) after adjusting for age, sex, race, and vasopressor requirement. Absolute day 1 levels of sRAGE and Ang-2 and the changes of any of the biomarkers over time were not significantly associated with ARDS development or mortality.

DISCUSSION AND CONCLUSIONS

In this prospective observational cohort study of critically ill patients with sepsis, sRAGE and SP-D levels had a signal toward being higher in patients who developed ARDS compared to those who did not develop ARDS, though the mean differences were not statistically significant likely due to the overlap of the biomarker levels between the outcome groups. The differences in the biomarker levels were more pronounced when considering mortality as the outcome: sRAGE, Ang-2, and SP-D levels were higher in non-survivors compared to survivors, and the differences remained significant in some of the subgroup analyses and logistic regression models. However, the temporal changes of the three biomarker levels over time were not significantly different between ARDS patients compared to non-ARDS patients, and between non-survivors compared to survivors.

Although not statistically significant, the comparisons of the absolute biomarker levels nonetheless showed a trend toward higher levels of sRAGE and SP-D in sepsis patients who developed ARDS, and the SP-D level on day 1 was significantly associated with ARDS development after adjusting for potential confounders in a multivariable model. The results demonstrate, to an extent, the feasibility of using these protein biomarkers in a prospective

setting to identify sepsis patients at high risk of developing ARDS. This study also found that the differences in the biomarker levels tended to be greater on the first day than on subsequent days, suggesting that it may be important to measure these biomarkers early in the course of sepsis in order to maximize their diagnostic utility. These findings may be useful as pilot data for designing future studies and/or clinical implementation of these biomarkers.

This study also demonstrated some of the challenges associated with the protein biomarkers, as there was a significant overlap of the biomarker levels between ARDS vs. non-ARDS patients, and measuring the changes of the biomarker levels over time were not useful for distinguishing the outcomes of interest. There are several possible explanations for these findings. First, many non-ARDS patients in the analysis required IMV and had P/F ratios <300, suggesting that these patients may have had severe lung injury. This could have led to elevated biomarker levels in some non-ARDS patients, even though they did not meet the clinical definition of ARDS. Furthermore, majority of patients requiring IMV received low tidal volume ventilation regardless of ARDS status, which could have further attenuated the differences in biomarker levels between the outcome groups[8]. Second, the majority of patients met the sepsis criteria within less than 3 hours of initial presentation to the emergency room or the hospital, suggesting that many of these patients could have already had sepsis for an unknown period of time prior to admission. Our screening protocol also identified a substantial number of patients (n=34) who were excluded from the study because they had already developed ARDS at the time of screening. It is possible that the timing of our patient screening and biospecimen collection was later than ideal, and that earlier sampling may be necessary to better delineate the temporal changes of biomarker levels early in the course of sepsis. Third, this study may not have sufficiently controlled for the heterogeneity of sepsis itself, especially with the significant

proportion of COVID-19 patients included in the study. Lastly, plasma may not accurately reflect the localized pathology within the lungs in ARDS, and biospecimen sampling from the lungs or the alveolar spaces (such as bronchoalveolar lavage, tracheal aspirate, or exhaled breath condensate) could be considered for a more direct examination of ARDS pathophysiology.

The differences in the biomarkers were more pronounced and more significant when examining mortality as the outcome, as non-survivors had significantly higher levels of the biomarkers compared to survivors across multiple days. The differences also persisted in several of the subgroup and adjusted analyses, suggesting that these biomarkers may be associated with adverse outcomes such as mortality in patients with sepsis. However, the results must be interpreted with caution, since mortality was not the primary outcome of this study, and sRAGE, Ang-2, and SP-D have not been studied extensively in the context of *sepsis*-related mortality (as opposed to *ARDS*-related mortality). In addition, the changes of the biomarker levels over time were still not significantly different between non-survivors vs. survivors, which again raises the concern that the timing of the plasma sampling may have been suboptimal.

This study has several additional limitations. First, this was a single-center study conducted at an urban safety net hospital, consisting predominantly of African-American patients. Generalizability to other geographic regions, practice settings, and patient populations is limited. Second, the overall sample size was small, and the sample size calculation was extrapolated from prior data examining one-time measurement of sRAGE. Therefore, the statistical power was likely limited for Ang-2 and SP-D measurements, serial measurements of the biomarkers, and subgroup analyses. Third, ARDS frequently developed before the serial sample collections were completed, so it is difficult to interpret the results in the context of predicting ARDS development in sepsis patients. Lastly, our statistical tests did not correct for

multiple comparisons resulting from examining three different biomarker levels over three days and multiple logistic regression models, increasing the probability of false discovery.

In conclusion, in this prospective observational cohort study of critically ill patients with sepsis, sRAGE, Ang-2, and SP-D levels and their changes over the first three days of study enrollment were not different between patients who developed ARDS vs. those who did not develop ARDS. However, there was a trend toward higher sRAGE and SP-D levels in ARDS patients, and the absolute SP-D level on day 1 was associated with ARDS development in the adjusted model. Higher levels of the three biomarkers may be associated with mortality in critically ill patients with sepsis, though this was not the original intent of this study. Larger sample size, narrower populations of patients with sepsis, and/or alternative timing and sources of biospecimen sampling may be needed to better understand the role of protein biomarkers in the clinical management of sepsis-induced ARDS.

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TABLES AND FIGURES

Table 1. Baseline characteristics of the study participants at the time of study enrollment

	Total (n=111)	**ARDS (n=21, 18.9%)**	**Non-ARDS (n=90, 81.1%)**	**p-value**
Age (years), median (IQR)	65 (55-74)	62 (52-71)	65 (55-75)	0.44*
Sex, n (%)				
Male	67 (60.4%)	14 (66.7%)	53 (58.9%)	0.51#
Race, n (%)				
Black	88 (79.3%)	13 (61.9c%)	75 (83.3%)	0.07#
White	16 (14.4%)	5 (23.8%)	11 (12.2%)	
Other	7 (6.3%)	3 (14.3%)	4 (4.44%)	
BMI (kg/m^2), median (IQR)	25.4 (21.8-30.0)	24.4 (22.4-31.0)	25.4 (21.5-29.9)	0.66*
SOFA score at enrollment, median (IQR)	8 (6-11)	8 (6-11)	8 (6-11)	0.77*
Medical comorbidities, n (%)				
Dementia	20 (18.0%)	3 (14.3%)	17 (18.9%)	>0.05§
Stroke	25 (22.5%)	4 (19.1%)	21 (23.3%)	
Congestive heart failure	29 (26.1%)	2 (9.5%)	27 (30.0%)	
CAD and/or MI	11 (9.9%)	0 (0.0%)	11 (12.2%)	
Atrial fibrillation	19 (17.1%)	1 (4.8%)	18 (20.0%)	
Hypertension	63 (56.8%)	11 (52.4%)	52 (57.8%)	
Chronic lung disease	31 (27.9%)	5 (23.8%)	26 (28.9%)	
Cirrhosis	6 (5.4%)	1 (4.8%)	5 (5.6%)	
Chronic kidney disease	23 (20.7%)	3 (14.3%)	20 (22.2%)	
End-stage renal disease	8 (7.2%)	1 (4.8%)	7 (7.8%)	
Diabetes mellitus	42 (37.8%)	10 (47.6%)	32 (35.6%)	
Malignancy	11 (9.9%)	2 (9.5%)	9 (10.0%)	
HIV	7 (6.3%)	2 (9.5%)	5 (5.6%)	
Primary infection, n (%)				
Pneumonia	26 (23.4%)	5 (23.8%)	21 (23.3%)	0.02§
Aspiration	14 (12.6%)	5 (23.8%)	9 (10.0%)	
COVID-19	19 (17.1%)	8 (38.1%)	11 (12.2%)	
Urine	24 (21.6%)	2 (9.5%)	22 (24.4%)	
GI/abdominal	5 (4.5%)	0 (0.0%)	5 (5.6%)	
Skin/soft tissue	14 (12.6%)	0 (0.0%)	14 (15.6%)	
Other	9 (8.1%)	1 (4.8%)	8 (8.9%)	

Abbreviations: ARDS = acute respiratory distress syndrome, IQR = interquartile range, BMI = body mass index, SOFA = sequential organ failure assessment, CAD = coronary artery disease, MI = myocardial infarction, HIV = human immunodeficiency virus, COVID-19 = novel coronavirus disease-2019, GI = gastrointestinal.

*Wilcoxon rank-sum test, #Chi-square test, and §Fisher's exact test were used to calculate the p-values.

Table 2. Clinical course and outcomes of the study participants

	Total (n=111)	**ARDS (n=21, 18.9%)**	**Non-ARDS (n=90, 81.1%)**	**p-value**
Vasopressor requirement, n (%)	84 (75.7%)	17 (81.0%)	67 (74.4%)	0.53#
Renal replacement therapy, n (%)	27 (24.3%)	3 (14.3%)	24 (26.7%)	0.23#
Invasive mechanical ventilation, n (%)	79 (71.2%)	20 (95.2%)	59 (65.6%)	0.007#
Initial TV/IBW (mL/kg), median (IQR)	6.21 (5.85-6.95)	5.88 (5.43-6.53)	6.26 (5.92-7.04)	0.08*
Initial PEEP (cm H_2O), median (IQR)	8 (8-8)	8 (8-12)	8 (8-8)	0.01*
Worst P/F ratio, median (IQR)	132 (181-250)	118 (78-166)	202 (143-262)	<0.001*
Mortality, n (%)	45 (40.5%)	10 (47.6%)	35 (38.9%)	0.46#
28-day ventilator-free days (days), median (IQR)	19 (2-26)	8 (0-22)	20.5 (6-28)	0.02*
28-day ICU-free days (days), median (IQR)	15 (0-24)	1 (0-21)	16.5 (3-24)	0.02*

Abbreviations: ARDS = acute respiratory distress syndrome, TV/IBW = tidal volume per ideal body weight, IQR = interquartile range, PEEP = positive end-expiratory pressure, P/F = partial pressure of arterial oxygen to fraction of inspired oxygen.
#Chi-square test and *Wilcoxon rank-sum test were used to calculate the p-values.

Table 3. Comparison of biomarker levels by ARDS diagnosis

	ARDS (n=21, 18.9%)	**Non-ARDS (n=90, 81.1%)**	**p-value***
sRAGE levels			
Day 1 (log[ng/mL])	1.042 ± 1.002	0.629 ± 0.980	0.09
Day 2 (log[ng/mL])	0.853 ± 0.994	0.535 ± 1.003	0.22
Day 3 (log[ng/mL])	0.608 ± 1.027	0.480 ±1.082	0.67
Δ day 1 to 2 (ng/mL)	-0.120 (-1.240 – 0.102)	-0.076 (-0.367 – 0.284)	0.23
Δ day 1 to 3 (ng/mL)	-0.514 (-1.552 – -0.075)	-0.153 (-0.800 – 0.359)	0.06
Ang-2 levels			
Day 1 (log[ng/mL])	1.825 ± 0.616	1.893 ± 0.722	0.69
Day 2 (log[ng/mL])	1.836 ± 0.683	1.745 ± 0.748	0.64
Day 3 (log[ng/mL])	1.720 ± 0.631	1.672 ± 0.706	0.80
Δ day 1 to 2 (ng/mL)	0.380 (-1.117 – 1.898)	-0.511 (-1.979 – 0.190)	0.049
Δ day 1 to 3 (ng/mL)	-0.440 (-1.324 – 0.558)	-1.005 (-2.965 – 0.016)	0.19
SP-D levels			
Day 1 (log[ng/mL])	1.921 ± 1.244	1.361 ± 1.211	0.06
Day 2 (log[ng/mL])	2.009 ± 1.258	1.456 ± 1.286	0.10
Day 3 (log[ng/mL])	1.900 ± 1.089	1.606 ± 1.325	0.41
Δ day 1 to 2 (ng/mL)	0.856 (-0.250 – 2.468)	0.214 (-1.025 – 2.363)	0.32
Δ day 1 to 3 (ng/mL)	1.084 (-1.923 – 3.948)	0.556 (-0.938 – 3.866)	0.68

Abbreviations: ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiopoietin-2, SP-D = surfactant protein-D.
Absolute biomarker levels on each day were log-transformed to approximate a normal distribution, and presented as mean ± standard deviation. Changes of the biomarker levels over time (denoted by the symbol Δ) are not log-transformed, and presented as median (interquartile range).
*Two-sample t-test was used for calculating the p-values when comparing the means of the absolute biomarker levels, and Wilcoxon rank-sum test was used for calculating the p-values when comparing the medians of the changes of the biomarker levels over time.

Table 4. Comparison of biomarker levels by mortality status

	Non-survivors (n=45, 40.5%)	**Survivors (n=66, 59.5%)**	**p-value***
sRAGE levels			
Day 1 (log[ng/mL])	0.999 ± 0.964	0.508 ± 0.970	0.01
Day 2 (log[ng/mL])	0.848 ± 0.876	0.428 ± 1.052	0.04
Day 3 (log[ng/mL])	0.736 ± 0.898	0.367 ± 1.141	0.13
Δ day 1 to 2 (ng/mL)	-0.005 (-0.642 – 0.595)	-0.112 (-0.412 – 0.098)	0.27
Δ day 1 to 3 (ng/mL)	-0.096 (-1.281 – 0.389)	-0.234 (-0.792 – 0.207)	0.90
Ang-2 levels			
Day 1 (log[ng/mL])	2.065 ± 0.673	1.754 ± 0.697	0.02
Day 2 (log[ng/mL])	2.044 ± 0.652	1.581 ± 0.732	0.002
Day 3 (log[ng/mL])	1.973 ± 0.652	1.508 ± 0.656	0.002
Δ day 1 to 2 (ng/mL)	0.050 (-2.051 – 1.898)	-0.563 (-1.535 – 0.062)	0.12
Δ day 1 to 3 (ng/mL)	-0.829 (-3.856 – 0.381)	-0.744 (-2.424 – -0.020)	0.91
SP-D levels			
Day 1 (log[ng/mL])	1.792 ± 1.287	1.245 ± 1.150	0.02
Day 2 (log[ng/mL])	1.772 ± 1.321	1.418 ± 1.265	0.18
Day 3 (log[ng/mL])	1.975 ± 1.182	1.477 ±1.314	0.08
Δ day 1 to 2 (ng/mL)	-0.093 (-1.913 – 2.468)	0.303 (-0.542 – 2.370)	0.26
Δ day 1 to 3 (ng/mL)	0.657 (-3.188 – 6.681)	0.676 (-0.661 – 3.231)	0.37

Abbreviations: sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiopoietin-2, SP-D = surfactant protein-D.

Absolute biomarker levels on each day were log-transformed to approximate a normal distribution, and presented as mean ± standard deviation. Changes of the biomarker levels over time (denoted by the symbol Δ) are not log-transformed, and presented as median (interquartile range).

*Two-sample t-test was used for calculating the p-values when comparing the means of the absolute biomarker levels, and Wilcoxon rank-sum test was used for calculating the p-values when comparing the medians of the changes of the biomarker levels over time.

Table 5. Results from multivariable logistic regression analyses

Biomarker Variable	**Adjusted OR for ARDS Development***	**95% CI**	**p-value**
Absolute sRAGE level, day 1	1.22	0.57-2.61	0.60
Δ sRAGE, day 1 to 2	0.78	0.36-1.71	0.54
Δ sRAGE, day 1 to 3	0.81	0.42-1.56	0.53
Absolute Ang-2 level, day 1	1.56	0.60-4.10	0.37
Δ Ang-2, day 1 to 2	1.06	0.93-1.21	0.38
Δ Ang-2, day 1 to 3	1.01	0.88-1.16	0.84
Absolute SP-D level, day 1	1.83	1.06-3.15	0.03
Δ SP-D, day 1 to 2	1.08	0.96-1.22	0.19
Δ SP-D, day 1 to 3	1.03	0.96-1.13	0.35
Biomarker Variable	**Adjusted OR for Mortality#**	**95% CI**	**p-value**
Absolute sRAGE level, day 1	1.57	0.99-2.48	0.06
Δ sRAGE, day 1 to 2	1.04	0.58-1.88	0.90
Δ sRAGE, day 1 to 3	0.94	0.57-1.55	0.81
Absolute Ang-2 level, day 1	1.65	0.85-3.20	0.14
Δ Ang-2, day 1 to 2	1.08	0.96-1.21	0.18
Δ Ang-2, day 1 to 3	1.04	0.95-1.14	0.41
Absolute SP-D level, day 1	1.52	1.03-2.24	0.03
Δ SP-D, day 1 to 2	0.96	0.91-1.02	0.23
Δ SP-D, day 1 to 3	0.96	0.91-1.02	0.21

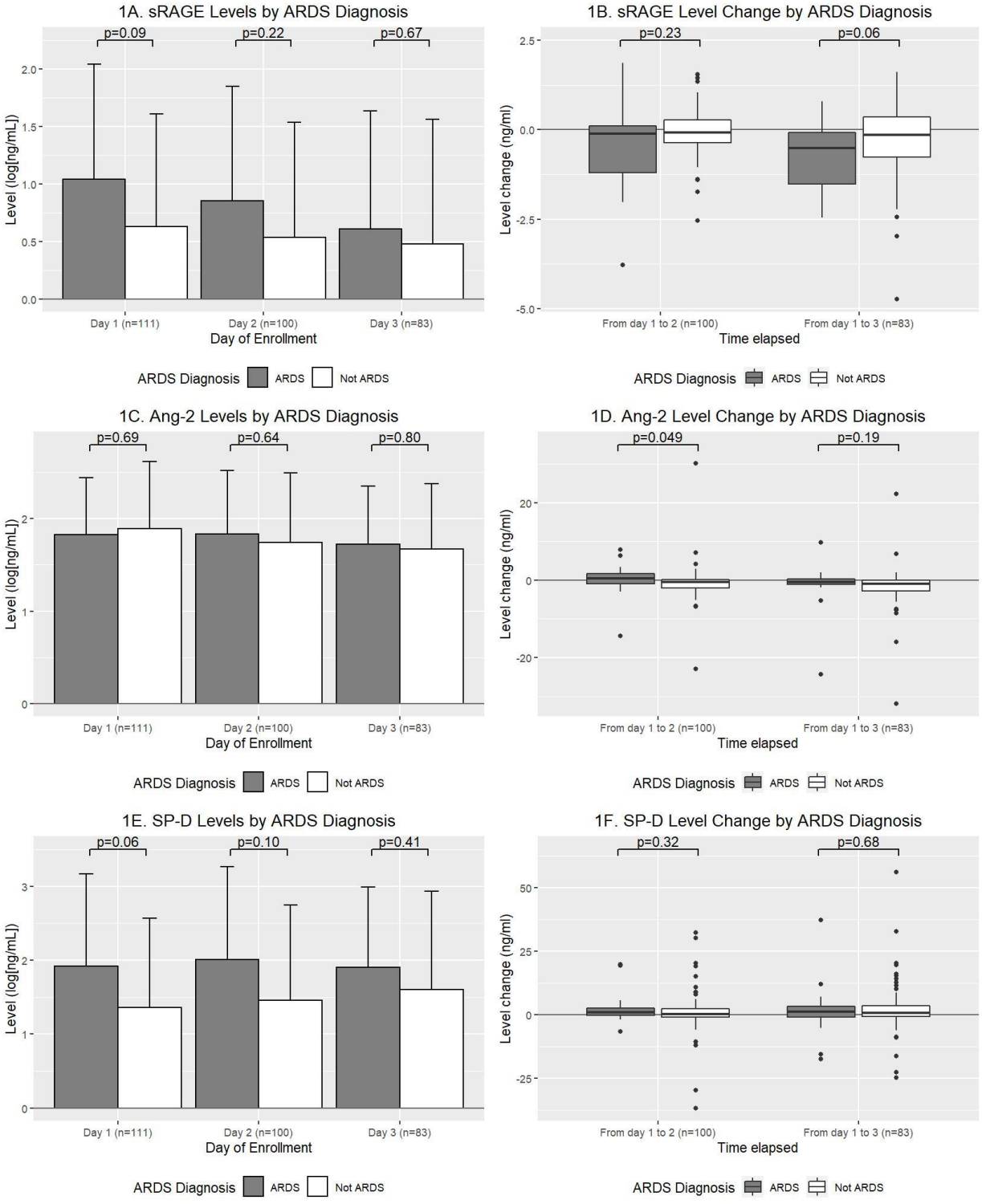
Abbreviations: OR = odds ratio, CI = confidence interval, ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiopoietin-2, SP-D = surfactant protein-D.

Each row of the table represents separate logistic regression models, each adjusting for the biomarker-related variable in that row only, plus the covariates detailed below. Only the results for the biomarker-related variable from each model is presented in the table.

*Each logistic regression model for ARDS development adjusts for age (continuous), sex (male or female), race (black, white, or other), primary source of infection (Coronavirus disease-19, pulmonary infection [pneumonia or aspiration pneumonia], or other [all other sources of infection]), tidal volume per ideal body weight (continuous), and positive end-expiratory pressure (continuous), in addition to the biomarker-related variable in that row.

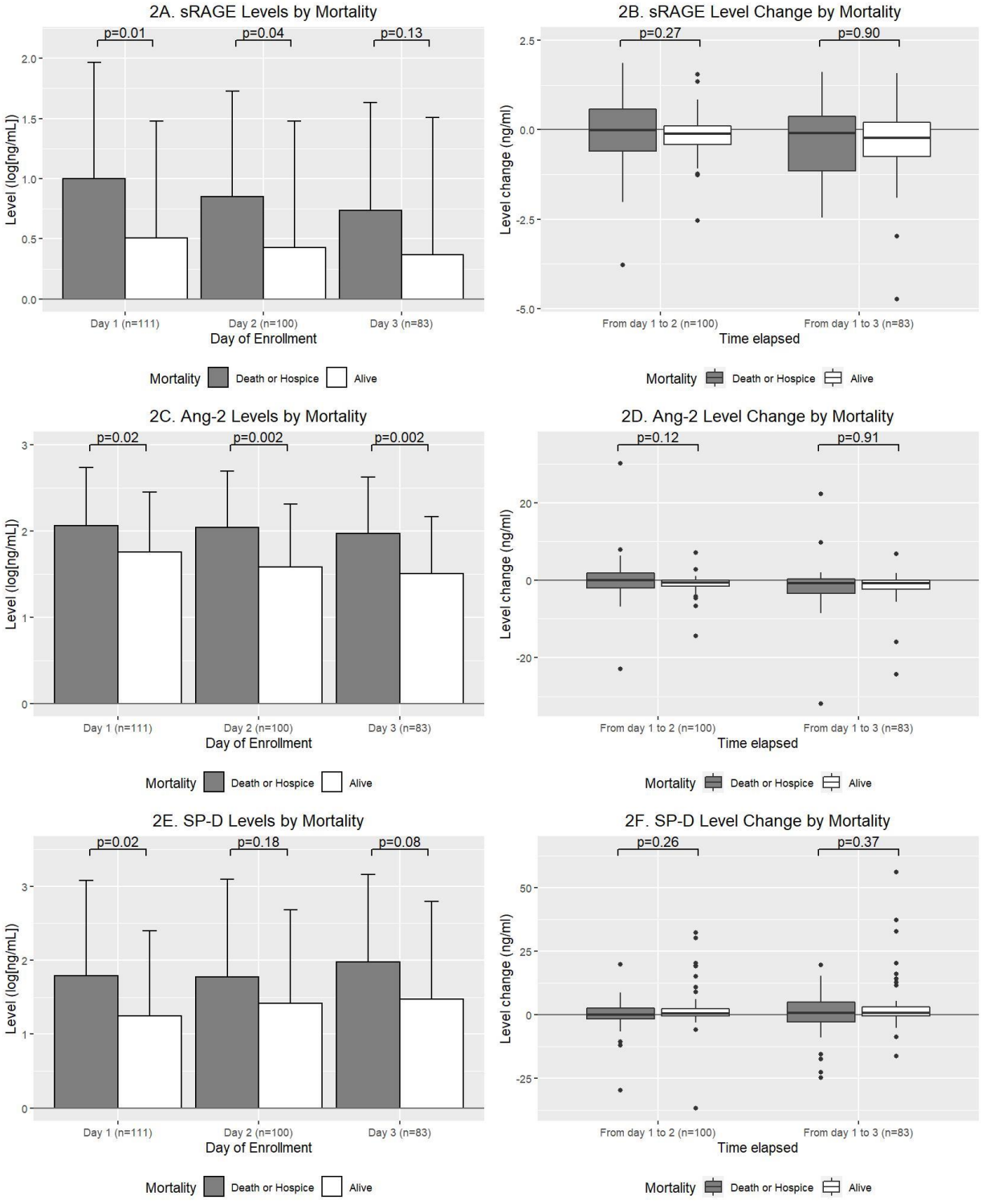
#Each logistic regression model for mortality adjusts for age (continuous), sex (male or female), race (black, white, or other), and vasopressor use (yes or no), in addition to the biomarker-related variable in that row.

Figure 1. Biomarker levels and their changes over time by ARDS diagnosis



Abbreviations: ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiopoietin-2, SP-D = surfactant protein D. Number of patients for each day was: 111 on day 1 (21 ARDS vs. 90 non-ARDS), 100 on day 2 (18 ARDS vs. 82 non-ARDS), and 83 on day 3 (16 ARDS vs. 67 non-ARDS).

Figure 2. Biomarker levels and their changes over time by mortality status



Abbreviations: ARDS = acute respiratory distress syndrome, sRAGE = soluble receptor for advanced glycation end-products, Ang-2 = angiopoietin-2, SP-D = surfactant protein D. Number of patients for each day was: 111 on day 1 (21 ARDS vs. 90 non-ARDS), 100 on day 2 (18 ARDS vs. 82 non-ARDS), and 83 on day 3 (16 ARDS vs. 67 non-ARDS).