OBSERVATIONAL STUDY

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Serial Measurements of Protein Biomarkers in Sepsis-Induced Acute Respiratory Distress Syndrome

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IMPORTANCE: The role of early, serial measurements of protein biomarkers in sepsis-induced acute respiratory distress syndrome (ARDS) is not clear.

OBJECTIVES: To determine the differences in soluble receptor for advanced glycation end-products (sRAGEs), angiopoietin-2, and surfactant protein-D (SP-D) levels and their changes over time between sepsis patients with and without ARDS.

DESIGN, SETTING, AND PARTICIPANTS: Prospective observational cohort study of adult patients admitted to the medical ICU at Grady Memorial Hospital within 72 hours of sepsis diagnosis.

MAIN OUTCOMES AND MEASURES: Plasma sRAGE, angiopoietin-2, and SP-D levels were measured for 3 consecutive days after enrollment. The primary outcome was ARDS development, and the secondary outcome of 28-day mortality. The biomarker levels and their changes over time were compared between ARDS and non-ARDS patients and between nonsurvivors and survivors.

RESULTS: We enrolled 111 patients, and 21 patients (18.9%) developed ARDS. The three biomarker levels were not significantly different between ARDS and non-ARDS patients on all 3 days of measurement. Nonsurvivors had higher levels of all three biomarkers than did survivors on multiple days. The changes of the biomarker levels over time were not different between the outcome groups. Logistic regression analyses showed association between day 1 SP-D level and mortality (odds ratio, 1.52; 95% CI, 1.03–2.24; $p = 0.03$), and generalized estimating equation analyses showed association between angiopoietin-2 levels and mortality (estimate 0.0002; SE 0.0001; $p = 0.04$).

CONCLUSIONS AND RELEVANCE: Among critically ill patients with sepsis, sRAGE, angiopoietin-2, and SP-D levels were not significantly different between ARDS and non-ARDS patients but were higher in nonsurvivors compared with survivors. The trend toward higher levels of sRAGE and SP-D, but not of angiopoietin-2, in ARDS patients may indicate the importance of epithelial injury in sepsis-induced ARDS. Changes of the biomarker levels over time were not different between the outcome groups.

KEY WORDS: acute respiratory distress syndrome; biomarkers; sepsis

The acute respiratory distress syndrome (ARDS) is a severe form of acute inflammatory lung injury associated with high mortality (1). ARDS is a markedly heterogeneous syndrome, with a wide variety of predisposing conditions that result in different clinical phenotypes (2). The heterogeneity of ARDS is thought to contribute to the lack of a reliable diagnostic test or a specific pharmacologic therapy for ARDS despite decades of research (2, 3). In order to address these problems, protein biomarkers have been used to help understand ARDS heterogeneity and phenotypes. Protein biomarkers can be measured from various body compartments such as plasma and the lungs

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KEY POINTS

- **Question:** Are there differences in protein biomarkers and their changes over time between sepsis patients who develop ARDS and those who do not?
- **Findings:** The levels of soluble receptor for advanced glycation end-products (sRAGE), angiopoietin-2, and surfactant protein D (SP-D) and their changes over the first three days of enrollment were similar between ARDS and non-ARDS patients. Higher levels of these biomarkers, especially Ang-2 and SP-D, were associated with mortality in patients with sepsis.
- **Meaning:** Among sepsis patients, levels of sRAGE, angiopoietin-2, and SP-D were not significantly different between ARDS and non-ARDS patients, but larger studies and deeper mechanistic understanding are needed.

and can be used to help understand the pathophysiologic mechanisms in the development and progression of ARDS (4). In particular, sRAGE is thought to be a causal intermediate in sepsis-induced ARDS (5). Previous studies have demonstrated a correlation between sRAGE and the presence and severity of ARDS, as well as increased mortality (6–10). Angiopoietin-2 and SP-D are additional biomarkers that can also help distinguish different subtypes of ARDS (11–16).

However, several methodological limitations exist in prior studies of ARDS biomarkers. First, many prior studies were retrospective, often using biospecimens and data from prior ARDS clinical trials. Second, many prior studies did not differentiate patients based on the heterogeneous etiologies of ARDS. In particular, patients with sepsis-induced ARDS have worse clinical outcomes and demonstrate different biomarker profiles compared with those with ARDS from other causes (17, 18), suggesting differences in pathophysiology that warrant targeted investigation. Third, 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. Monitoring the changes in biomarker levels over time can provide useful information about the dynamic changes and responses to treatment interventions in sepsis (19) and ARDS (8, 15).

The objective of this study was to determine the differences in plasma sRAGE, angiopoietin-2, and SP-D levels and their changes over time between sepsis patients with and without ARDS, in order to examine the potential biological differences between the two groups. We sought to address the methodologic limitations in prior studies by conducting a prospective cohort study consisting only of patients with sepsis, and performing serial measurements of sRAGE, angiopoietin-2, and SP-D over the first 3 days of enrollment. The hypothesis was that the ARDS patients will have higher absolute sRAGE, angiopoietin-2, and SP-D levels and have greater increases in the biomarker levels over time, compared with the non-ARDS patients.

MATERIALS AND METHODS

Study Information

This was a prospective observational cohort study conducted in the medical ICU (MICU) at Grady Memorial Hospital, Atlanta, GA, between September 16, 2020, and November 8, 2021. This study was reviewed and approved by the Institutional Review Board (IRB) at Emory University, Atlanta, GA (study title: "Biomarkers and Metabolomics in Sepsis-Induced ARDS", approval number: "STUDY00001060", approval date: July 10, 2020) and by the Research Oversight Committee (ROC) at Grady (study title: "Examining the Association between Plasma Biomarkers and Metabolic Profiles and ARDS Development in Patients with Sepsis", approval number: "#000-1060", approval date: September 9, 2020). Informed consent was obtained from each participant or their legally authorized representatives. For eligible patients who were unable to consent and whose legally authorized representatives could not be reached, a waiver of informed consent was also permitted by the Emory IRB and Grady ROC given minimal risk to the participants. The study procedures were followed in accordance with the ethical standards of the Emory IRB and Grady ROC and with the Helsinki Declaration of 1975.

Screening and Enrollment

The Grady MICU was screened daily for eligible patients. Patients were eligible if they were admitted to the MICU within 72 hours of diagnosis of sepsis or septic shock, as defined by the Sepsis-3 definition (20).

There was no time limit on being admitted to the MICU as long as the patient was within 72 hours of a new diagnosis of sepsis or septic shock. Patients were excluded if they were under 18 years old, pregnant, or incarcerated; already had ARDS at the time of screening; were not candidates for full resuscitation or pursuing comfort measures only; or declined participation in the study.

Study Protocol

Serial blood samples were collected from each participant once daily on days 1, 2, and 3 of study enrollment (first blood sample on the day of enrollment as soon as possible after obtaining or waiving informed consent and then 24 ± 3 and 48 ± 3 hr after the first blood sample collection). The timing of the blood sample collections was chosen in order to capture the biomarker levels before or around the time of ARDS onset, based on literature reporting that the majority of ARDS cases develop within the first 2–3 days of acute hypoxic respiratory failure or hospital admission (1, 21). Blood was centrifuged to isolate the plasma, which was frozen and stored at –80°C until analysis. Levels of sRAGE, angiopoietin-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; angiopoietin-2 and SP-D: R&D Systems, Minneapolis, MN) (**Supplemental Table S1**, http://links.lww.com/CCX/B76).

Participants were followed for up to 28 days for the primary outcome of ARDS development according to the Berlin definition (22), with specific criteria for participants receiving oxygen support with heated and humidified high-flow nasal cannula (HFNC) and additional diagnostic considerations outlined in Supplemental Table S1 (http://links.lww.com/CCX/B76). The ARDS diagnosis was first determined by the primary investigator with experience in ARDS research (P.Y.). Any patient who received mechanical ventilation required verification of ARDS diagnosis by the senior investigator (A.M.E.). Secondary outcomes included 28-day all-cause in-hospital mortality (including in-hospital death and discharge to hospice), ventilator-free days, and ICU-free days.

Additional clinical information including demographics, medical comorbidities, severity of illness scores (Sequential Organ Failure Assessment [23] and Acute Physiology and Chronic Health Evaluation-II [24] 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. In order to reduce bias, the investigators assessing the ARDS diagnosis and clinical outcomes were blinded to the biomarker measurements until completion of clinical data entry, and the investigators performing the biomarker measurements were blinded to the clinical information until completion of biomarker measurements.

Statistical Analysis and Analytical Methods

Based on preliminary data from an internal study showing a difference in sRAGE level between ARDS and non-ARDS patients of 2,822 pg/mL and SD of 3,468 pg/mL, expected ARDS occurrence rate 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. This calculation was extrapolated to angiopoietin-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 demographics 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 nonnormally distributed continuous variables; and chi-square or Fisher exact test, for comparing categorical variables. The absolute sRAGE, angiopoietin-2, and SP-D levels were found to be nonnormally distributed and were log-transformed to approximate a normal distribution and then compared using two-sample *t* test. The absolute changes in the sRAGE, angiopoietin-2, and SP-D levels from day 1 to days 2 and 3 were calculated. The changes in the biomarker levels were also nonnormally distributed, but these values were not log-transformed and were compared as-is using the Wilcoxon rank-sum test.

Multivariable logistic regression (LR) and generalized estimating equation (GEE) models were used to examine the association between the biomarker levels and the outcome variables, adjusting for potential confounders. For LR models, the main exposure variables of interest were the absolute sRAGE, angiopoietin-2, and SP-D levels on day 1 and the changes of the three biomarker levels from day 1 to days 2 and 3. Due to significant correlation between the three biomarker

levels, each of the biomarker variables were input individually into separate LR models. For GEE models, the sRAGE, angiopoietin-2, and SP-D levels over the 3 days were analyzed as repeated measurements within each subject to account for the correlation from longitudinal sampling.

For covariate selection, age, sex, and race were included by convention. The following covariates were also considered: primary source of infection (COVID-19 vs pulmonary [pneumonia or aspiration pneumonia] vs nonpulmonary [all other sources of infection]), vasopressor requirement, renal replacement therapy requirement, tidal volume per ideal body weight, positive end-expiratory pressure, and ARDS diagnosis (when modeling mortality as the outcome). From this list, covariates for inclusion in the final model were selected based on clinical reasoning, results of the univariate analyses, likelihood ratio tests for sequential addition of the covariates to the model, and model fit considerations. The final model for ARDS included age, sex, race, and primary source of infection, in addition to the biomarker variable. The final model for mortality included age, sex, race, and vasopressor use as covariates, in addition to the biomarker variable. For the GEE models, the time variable indicating the day of sample collection and the interaction term between the biomarker levels and the time variable were also considered. However, these terms were not significant with estimates and SEs rounding to 0.0000 and were not included the final models.

Significance level of α equals to 0.05 was used for all statistical tests. All data analyses and statistical tests were performed in SAS v9.4 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

A total of 111 critically ill patients with sepsis were enrolled between September 16, 2020, and November 8, 2021 (**Supplemental Fig. S1**, http://links.lww.com/CCX/B76). The primary outcome of ARDS developed in 21 patients (18.9%), with median time from day 1 blood draw to ARDS onset of 24 hours (interquartile range [IQR], 8–42 hr). ARDS and non-ARDS patients were similar with regard to their demographics, chronic medical comorbidities, and severity of illness scores 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 (Table 1). A higher proportion of ARDS patients required invasive mechanical ventilation (IMV) compared with non-ARDS patients (n = 20 [95.2%] in ARDS vs n = 59 [65.6%] in non-ARDS, p = 0.007). One ARDS patient fulfilled the Berlin criteria while receiving noninvasive ventilation, but did not require IMV. Overall mortality was not significantly different between ARDS versus 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 [IQR] 8 [0–22] vs 20.5 [6–28] d; p = 0.02) and 28-day ICU-free days (median [IQR] 1 [0–21] vs 16.5 [3–24] d; p = 0.02) (**Table 2**).

Protein Biomarker Analysis by ARDS Diagnosis

The absolute levels of sRAGE, angiopoietin-2, and SP-D were not significantly different between ARDS and non-ARDS patients on all 3 days of measurement (**Fig. 1**; and **Supplemental Table S2**, http://links.lww.com/CCX/B76). The absolute sRAGE and SP-D levels trended higher in ARDS patients than in non-ARDS patients, but there was a significant overlap between the groups. The mean differences in the absolute sRAGE and SP-D levels between ARDS versus non-ARDS patients were greater on day 1 and became smaller on subsequent days. ARDS patients had a greater change in angiopoietin-2 level from day 1 to day 2 compared with 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 (**Supplemental Fig. S2**, http://links.lww.com/CCX/B76).

Protein Biomarker Analysis by Mortality Status

Nonsurvivors had significantly higher absolute levels of sRAGE on days 1 and 2, higher absolute levels of angiopoietin-2 on all 3 days, and higher absolute SP-D on day 1 (**Fig. 2**; and **Supplemental Table S3**, http://links.lww.com/CCX/B76). In particular, angiopoietin-2 levels, which were comparable between ARDS versus non-ARDS patients, showed a significant difference between nonsurvivors versus survivors that became more pronounced with time. The changes of the biomarker levels over time were not significantly

TABLE 1.
Baseline Characteristics of the Study Participants at the Time of Study Enrollment

Characteristic	Total (*N* = 111)	ARDS (*N* = 21; 18.9%)	Non-ARDS (*N* = 90; 81.1%)	*p*
Age (yr), median (IQR)	65 (55–74)	62 (52–71)	65 (55–75)	0.44[a]
Sex, *n* (%)				0.51[b]
Male	67 (60.4)	14 (66.7)	53 (58.9)	
Race, *n* (%)				0.07[b]
Black	88 (79.3)	13 (61.9)	75 (83.3)	
White	16 (14.4)	5 (23.8)	11 (12.2)	
Other	7 (6.3)	3 (14.3)	4 (4.44)	
Body mass index (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[a]
Sequential Organ Failure Assessment score, median (IQR)	8 (6–11)	8 (6–11)	8 (6–11)	0.77[a]
Acute Physiology and Chronic Health Evaluation-II score, median (IQR)	21 (16–26)	22 (16–24)	21 (17–26)	0.79[a]
Medical comorbidities, *n* (%)				> 0.05[c]
Dementia	20 (18.0)	3 (14.3)	17 (18.9)	
Stroke	25 (22.5)	4 (19.1)	21 (23.3)	
Congestive heart failure	29 (26.1)	2 (9.5)	27 (30.0)	
Coronary artery disease and/or myocardial infarction	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* (%)				0.02[c]
Pneumonia	26 (23.4)	5 (23.8)	21 (23.3)	
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)	
Gastrointestinal /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)	

ARDS = acute respiratory distress syndrome, IQR = interquartile range.

[a]Wilcoxon rank-sum test.

[b]χ^2.

[c]Fisher exact test were used to calculate the *p* values.

TABLE 2.
Clinical Course and Outcomes of the Study Participants

Outcome	Total (*N* = 111)	ARDS (*N* = 21; 18.9%)	Non-ARDS (*N* = 90; 81.1%)	*p*
Vasopressor requirement, *n* (%)	84 (75.7)	17 (81.0)	67 (74.4)	0.53[a]
Renal replacement therapy, *n* (%)	27 (24.3)	3 (14.3)	24 (26.7)	0.23[a]
Invasive mechanical ventilation, *n* (%)	79 (71.2)	20 (95.2)	59 (65.6)	0.007[a]
Initial tidal volume per ideal body weight (mL/kg), median (IQR)	6.21 (5.85–6.95)	5.88 (5.43–6.53)	6.26 (5.92–7.04)	0.08[b]
Initial positive end-expiratory pressure (cm H_2O), median (IQR)	8 (8)	8 (8–12)	8 (8)	0.01[b]
Worst Pao_2/Fio_2 ratio, median (IQR)	132 (181–250)	118 (78–166)	202 (143–262)	< 0.001[b]
Mortality, *n* (%)	45 (40.5)	10 (47.6)	35 (38.9)	0.46[a]
28-d ventilator-free days (d), median (IQR)	19 (2–26)	8 (0–22)	20.5 (6–28)	0.02[b]
28-d ICU-free days (days), median (IQR)	15 (0–24)	1 (0–21)	16.5 (3–24)	0.02[b]

ARDS = acute respiratory distress syndrome, IQR = interquartile range.
[a]χ^2.
[b]Wilcoxon rank-sum test were used to calculate the *p* values.

different between nonsurvivors and survivors regardless of the time points (**Supplemental Fig. S3**, http://links.lww.com/CCX/B76).

Additional Analyses

There were two patients in the non-ARDS group who were not considered to have ARDS solely because they received HFNC without subsequently requiring positive-pressure ventilation. When these two patients were reclassified into the ARDS group, the differences in the absolute SP-D level on day 1 (mean ± SD 1.979 ± 1.229 log [ng/mL] in ARDS vs 1.333 ± 1.203 log [ng/mL] in non-ARDS; $p = 0.02$) and day 2 (mean ± SD 2.120 ± 1.266 vs 1.415 ± 1.267 log [ng/mL]; $p = 0.03$) as well as the change in angiopoietin-2 from day 1 to 2 (median [IQR] 0.321 [–0.881 to 1.457] vs –0.609 [–2.006 to 0.146] ng/mL; $p = 0.02$) were statistically significant.

In subgroup analyses examining patients with COVID-19 as the primary source of infection ($n = 19$; 8 ARDS and 11 non-ARDS), none of the absolute biomarker levels or the changes of the biomarker levels over time were significantly different between ARDS and non-ARDS patients (**Supplemental Table S4**, http://links.lww.com/CCX/B76). Analyzing the percent changes of the biomarker levels rather than the absolute changes yielded similar results.

Multivariable Analyses

In LR analyses for the overall cohort, absolute SP-D level on day 1 was significantly associated with mortality (adjusted odds ratio, 1.52; 95% CI, 1.03–2.24; $p = 0.03$) after adjusting for age, sex, race, and vasopressor requirement (**Table 3**). The other biomarker variables were not significantly associated with ARDS development or mortality in LR analyses. All LR models had good fit by Hosmer-Lemeshow goodness-of-fit test ($p > 0.05$ for all models).

In GEE analyses, the time variable indicating the day of sample collection and the interaction term between the biomarker levels and the time variable were not significant and were not included in the final models. In the final GEE models, angiopoietin-2 levels were weakly associated with mortality (estimate 0.0002; SE 0.0001; $p = 0.04$), but none of the biomarker levels were significantly associated with ARDS development (**Table 4**).

DISCUSSION

In this prospective observational cohort study of critically ill patients with sepsis, sRAGE, angiopoietin-2, and SP-D levels were not significantly different between patients who developed ARDS and those who did not develop ARDS. The sRAGE, angiopoietin-2,

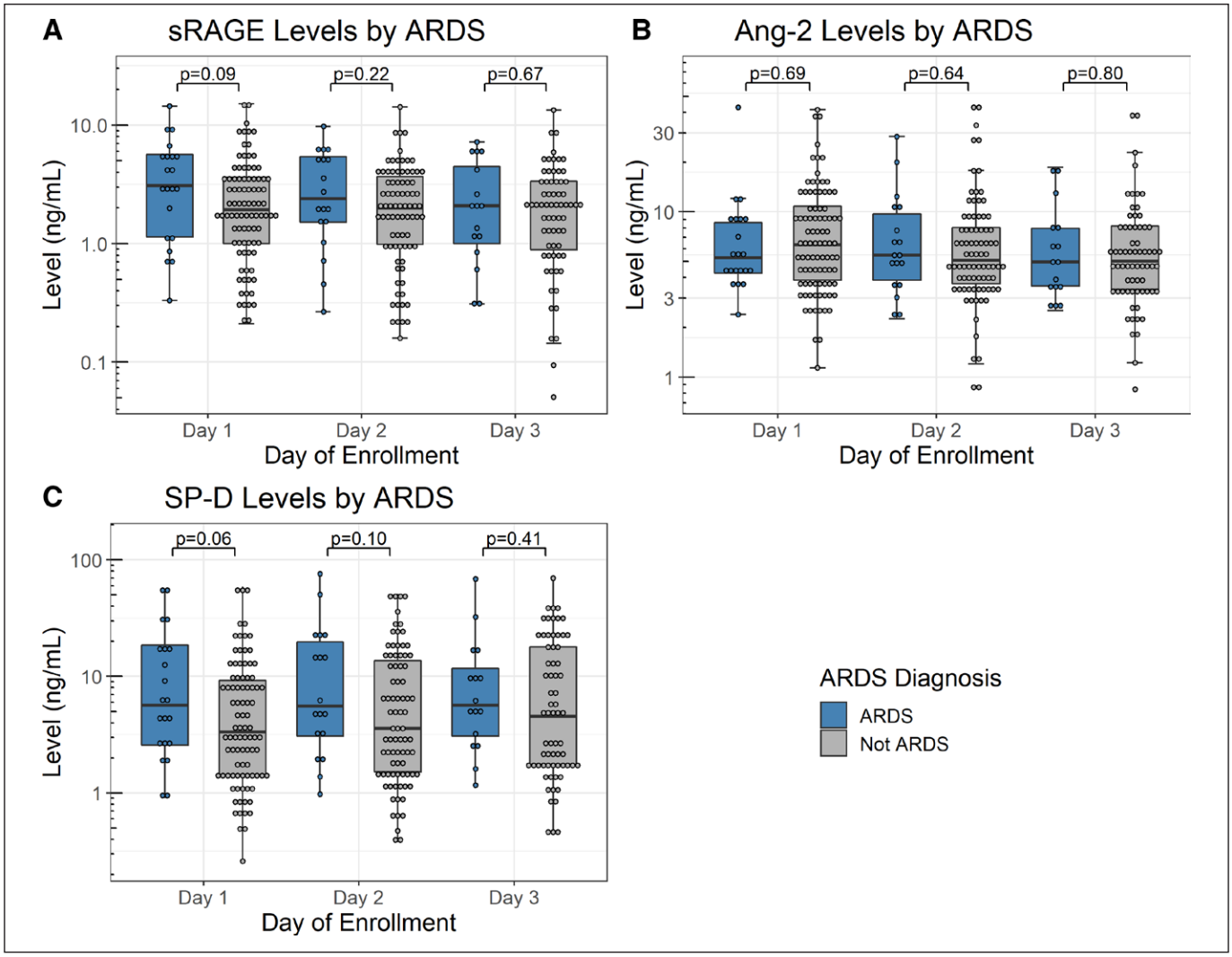

Figure 1. Levels of soluble receptor for advanced glycation end-products (sRAGE) (**A**), angiopoietin-2 (Ang-2) (**B**), and surfactant protein-D (SP-D) (**C**) on each day by acute respiratory distress syndrome (ARDS) diagnosis. Each panel shows the absolute levels and distribution of the three biomarkers on each day of collection. Number of patients for each day was as follows: 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).

and SP-D levels were significantly higher in nonsurvivors compared with survivors during the first 3 days of enrollment, and multivariable models showed associations between SP-D and angiopoietin-2 levels and mortality. However, the temporal changes of the three biomarker levels over time were not significantly different between ARDS patients compared with non-ARDS patients and between nonsurvivors compared with survivors.

Although the biomarker levels were not significantly different between ARDS and non-ARDS patients, some observations can be made from the results. sRAGE and SP-D, both markers of lung epithelial injury, trended higher in ARDS patients compared with non-ARDS patients, whereas angiopoietin-2, a marker of endothelial injury, was similar between ARDS and non-ARDS patients. Although it is possible that the high proportion of pulmonary sources of infection in the ARDS group contributed to the markers of epithelial injury being elevated in these patients (14), our results are similar to those of a prior study of sepsis patients by Ware et al (18): when compared with patients without ARDS, those with ARDS had higher levels of sRAGE, SP-D, and other markers of epithelial injury and inflammation, but not of angiopoietin-2. Interestingly, angiopoietin-2 level was significantly higher in nonsurvivors compared with survivors in our cohort on all three days of measurement. Prior studies (25, 26) reported the association of higher angiopoietin-2 levels with mortality and pulmonary dysfunction in

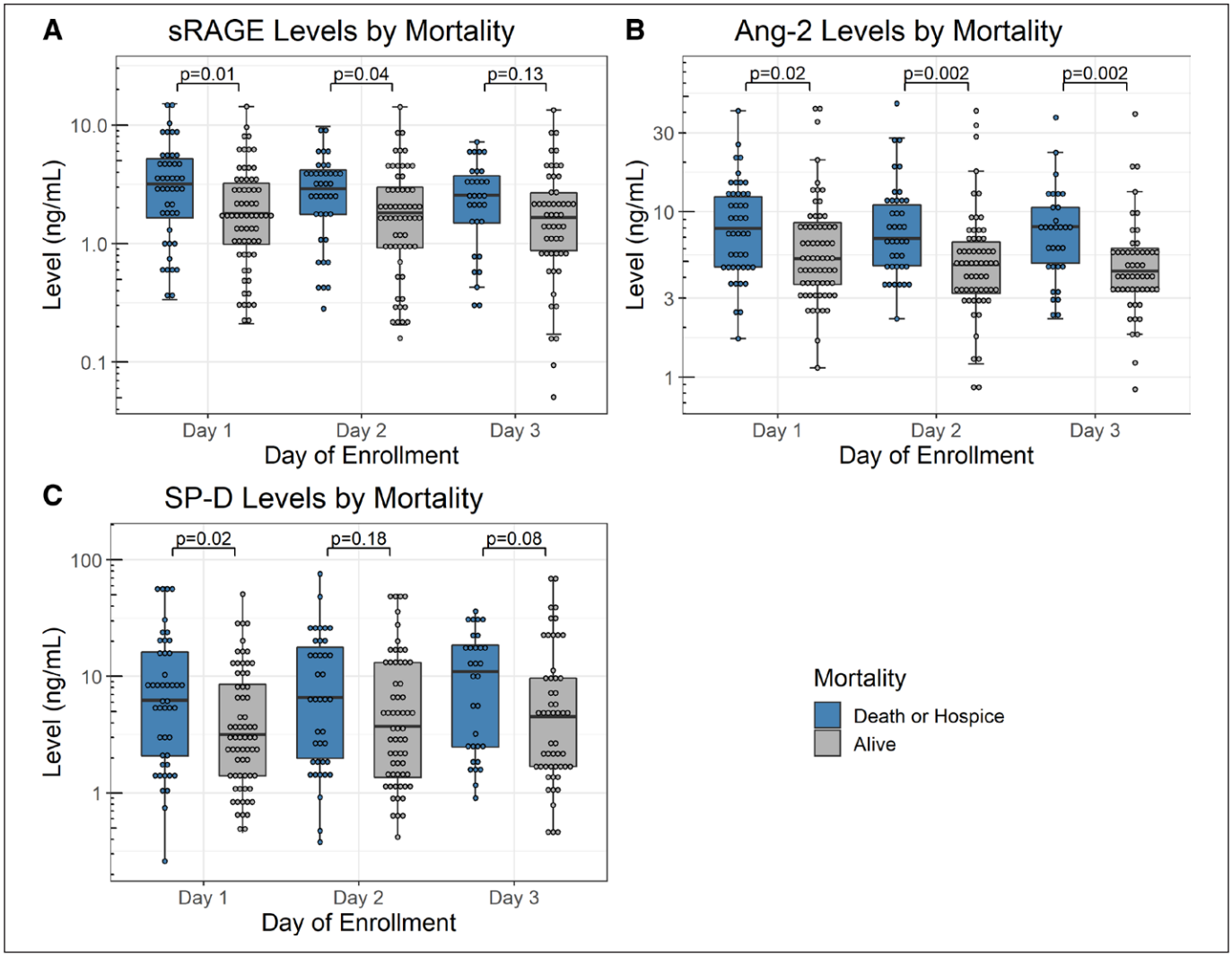

Figure 2. Levels of soluble receptor for advanced glycation end-products (sRAGE) (**A**), angiopoietin-2 (Ang-2) (**B**), and surfactant protein-D (SP-D) (**C**) on each day by mortality status. Each panel shows the absolute levels and distribution of the three biomarkers on each day of collection. Number of patients for each day was as follows: 111 on day 1 (45 death/hospice vs 66 alive), 100 on day 2 (39 death/hospice vs 61 alive) and 83 on day 3 (31 death/hospice vs 52 alive).

sepsis, although these studies did not specifically analyze the angiopoietin-2 levels based on the presence of ARDS. Another study by van der Heijden et al (11) reported that higher angiopoietin-2 levels correlated with ARDS and mortality, albeit in a mixed population of both sepsis and nonsepsis patients, whereas Calfee et al (27) has reported that the prognostic performance of angiopoietin-2 for clinical outcomes was weaker in infection-related acute lung injury (ALI) than in non-infection-related ALI. Taken together, these results suggest that endothelial injury is a hallmark of sepsis that is present regardless of ARDS status and contributes to sepsis-related mortality, but concomitant lung epithelial injury may play a more prominent and direct role in the progression from sepsis to sepsis-induced ARDS development. Although these results must be interpreted with caution given the lack of statistical significance and the observational nature of our study, they may serve as pilot data for future studies to better characterize the biological differences between sepsis patients with and without ARDS. Further investigation with larger sample size and more sophisticated analyses of biomarkers (such as multiomics) may help understand the pathophysiologic mechanisms in the development of sepsis-induced ARDS, as well as their implications for therapeutic targets.

In addition, the differences in sRAGE and SP-D levels between ARDS versus non-ARDS patients were the greatest on the first day than on subsequent days, and examining the changes of the biomarker levels over

TABLE 3.
Results From Multivariable Logistic Regression Analyses, With Separate Models for Each Biomarker Variable

Biomarker Variables	Adjusted OR for Acute Respiratory Distress Syndrome Development[a]	95% CI	*p*
log (sRAGE level), day 1	1.55	0.81–2.94	0.18
Δ sRAGE, day 1 to 2	0.73	0.36–1.45	0.36
Δ sRAGE, day 1 to 3	0.80	0.46–1.40	0.43
log (Ang-2 level), day 1	1.21	0.53–2.74	0.65
Δ Ang-2, day 1 to 2	1.04	0.92–1.17	0.57
Δ Ang-2, day 1 to 3	0.99	0.88–1.11	0.82
log (SP-D level), day 1	1.53	0.97–2.42	0.07
Δ SP-D, day 1 to 2	1.04	0.97–1.11	0.32
Δ SP-D, day 1 to 3	1.01	0.96–1.07	0.72
Biomarker Variables	**Adjusted OR for Mortality[b]**	**95% CI**	*p*
log (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
log (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
log (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

Ang-2 = angiopoietin-2, OR = odds ratio, SP-D = surfactant protein-D, sRAGE = soluble receptor for advanced glycation end-products.
[a]Each logistic regression model for acute respiratory distress syndrome development adjusts for age (continuous), sex (male or female), race (Black, White, or other), and primary source of infection (COVID-19, pulmonary infection [pneumonia or aspiration pneumonia], or other [all other sources of infection]), in addition to the biomarker-related variable in that row.
[b]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.
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.

time was not useful for distinguishing the outcomes of interest. This suggests the importance of measuring these biomarkers early in the course of sepsis in order to maximize their diagnostic and prognostic utility. In fact, the timing of the biospecimen sampling may be a limitation in this study, as many patients satisfied the sepsis definition within the first 3 hours of initial presentation to the emergency department or hospital and likely 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 because they already had ARDS at the time of screening. It is possible that earlier initiation of biospecimen sampling is necessary to detect more significant differences in the absolute biomarker levels. On the contrary, some prior studies have used serial biospecimens collected as late as 28 days after enrollment (7), and a longer follow-up period than was used in this study may be necessary for a more complete understanding of the variability and the trajectories of the biomarkers over time.

There was also a significant overlap of the biomarker levels between ARDS versus non-ARDS patients. There are several possible explanations for these findings. First, many non-ARDS patients in the analysis required IMV and had Pao_2/Fio_2 ratios less than 300, suggesting that these patients may have had severe lung injury with elevated biomarker levels without meeting the ARDS definition.

TABLE 4.
Results From Generalized Estimating Equation Analyses, With Separate Models for Each Biomarker Variable

Biomarker Variables	Estimate (SE) for Acute Respiratory Distress Syndrome Development[a]	*p*
log (sRAGE levels)	0.0002 (0.0002)	0.26
log (Ang-2 levels)	0.0001 (0.0001)	0.32
log (SP-D levels)	0.0001 (0.0001)	0.13
Biomarker Variables	**Estimate (SE) for Mortality[b]**	*p*
log (sRAGE levels)	0.0004 (0.0002)	0.06
log (Ang-2 levels)	0.0002 (0.0001)	0.04
log (SP-D levels)	0.0001 (0.0001)	0.14

Ang-2 = angiopoietin-2, sRAGE = soluble receptor for advanced glycation end-products, SP-D = surfactant protein-D.
Each row of the table represents separate generalized estimating equation 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. Time variable indicating the day of sample collection and the interaction term between the biomarker levels and the time variable were not significant, and were not included in the final models.
[a]Each generalized estimating equation model for acute respiratory distress syndrome development adjusts for age (continuous), sex (male or female), race (Black, White, or other), and primary source of infection (COVID-19, pulmonary infection [pneumonia or aspiration pneumonia], or other [all other sources of infection]), in addition to the biomarker-related variable in that row.
[b]Each generalized estimating equation 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.

Furthermore, majority of patients requiring IMV received relatively low tidal volumes regardless of ARDS status. This could have further attenuated the differences in biomarker levels between the outcome groups, as prior studies have reported a greater decline or a smaller rise of biomarker levels over time with low tidal volume ventilation (8, 15, 16). Second, 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. Last, plasma may not accurately reflect the localized pathology within the lungs in ARDS, and biospecimen sampling from the lungs or the alveolar spaces could be considered for a more direct examination of ARDS pathophysiology.

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, and generalizability may be limited. Second, the overall sample size and the number of ARDS patients were both 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 angiopoietin-2 and SP-D measurements, serial measurements of the biomarkers, and multivariable models that resulted in somewhat variable results for angiopoietin-2 and SP-D. The small sample size also limited our ability to perform subgroup analyses or other analytical methods to control for the heterogeneity within the cohort. Third, our analyses did not correct for multiple comparisons or for batch effects in the ELISA analyses. As discussed previously, a longer period of follow-up biomarker measurements may have allowed for a better understanding of the trajectories of biomarkers over time. Last, ARDS frequently developed before the serial sample collections were completed. Therefore, the ability to perform analyses incorporating time-to-event data and to interpret the results in the context of causal, prognostic, or predictive relationships with ARDS development was limited.

CONCLUSIONS

In conclusion, in this prospective observational cohort study of critically ill patients with sepsis, sRAGE, angiopoietin-2, and SP-D levels and their changes over the first 3 days of study enrollment were not different between ARDS versus non-ARDS patients. Higher levels of the three biomarkers were associated with mortality in critically ill patients with sepsis, although this was not the primary aim of the study. The results suggest different involvement of epithelial and endothelial injuries in ARDS development and mortality in sepsis, but further investigation is needed to better understand these pathophysiologic mechanisms as well as the role of protein biomarkers in the clinical management of sepsis-induced ARDS.

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