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Circulating Biomarkers of Endothelial Dysfunction Associated With Ventilatory Ratio and Mortality in ARDS Resulting From SARS-CoV-2 Infection Treated With Antiinflammatory Therapies

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Abstract

BACKGROUND: The association of plasma biomarkers and clinical outcomes in ARDS resulting from SARS-CoV-2 infection predate the evidence-based use of immunomodulators.

RESEARCH QUESTION: Which plasma biomarkers are associated with clinical outcomes in patients with ARDS resulting from SARS-CoV-2 infection treated routinely with immunomodulators?

STUDY DESIGN AND METHODS: We collected plasma from patients with ARDS resulting from SARS-CoV-2 infection within 24 h of admission to the ICU between December 2020 and March 2021 (N = 69). We associated 16 total biomarkers of inflammation (eg, IL-6), coagulation (eg, D-dimer), epithelial injury (eg, surfactant protein D), and endothelial injury (eg, angiopoietin-2) with the primary outcome of in-hospital mortality and secondary outcome of ventilatory ratio (at baseline and day 3).

RESULTS: Thirty patients (43.5%) died within 60 days. All patients received corticosteroids and 6% also received tocilizumab. Compared with survivors, nonsurvivors demonstrated a higher baseline modified Sequential Organ Failure Assessment score (median, 8.5 [interquartile range (IQR), 7–9] vs 7 [IQR, 5–8]); $P = .004$), lower Pao_2 to F_{IO_2} ratio (median, 153 [IQR, 118–182] vs 184 [IQR, 142–247]; $P = .04$), and higher ventilatory ratio (median, 2.0 [IQR, 1.9–2.3] vs 1.5 [IQR, 1.4–1.9]; $P < .001$). No difference was found in inflammatory, coagulation, or epithelial biomarkers between groups. Nonsurvivors showed higher median neural precursor cell expressed, developmentally down-regulated 9 (NEDD9) levels (median, 8.4 ng/mL [IQR, 7.0–11.2 ng/mL] vs 6.9 ng/mL [IQR, 5.5–8.0 ng/mL]; $P = .0025$), von Willebrand factor domain A2 levels (8.7 ng/mL [IQR, 7.9–9.7 ng/mL] vs 6.5 ng/mL [IQR, 5.7–8.7 ng/mL]; $P = .007$), angiopoietin-2 levels (9.0 ng/mL [IQR, 7.9–14.1 ng/mL] vs 7.0 ng/mL [IQR, 5.6–10.6 ng/mL]; $P = .01$), and syndecan-1 levels (15.9 ng/mL [IQR, 14.5–17.5 ng/mL] vs 12.6 ng/mL [IQR, 10.5–16.1 ng/mL]; $P = .01$). Only NEDD9 level met the adjusted threshold for significance ($P < .003$). Plasma NEDD9 level was associated with 60-day mortality (adjusted OR, 9.7; 95% CI, 1.6–60.4; $P = .015$). Syndecan-1 level correlated with both baseline ($\rho = 0.4$; $P = .001$) and day 3 ventilatory ratio ($\rho = 0.5$; $P < .001$).

INTERPRETATION: Biomarkers of inflammation, coagulation, and epithelial injury were not associated with clinical outcomes in a small cohort of patients with ARDS uniformly treated with immunomodulators. However, endothelial biomarkers, including plasma NEDD9, were associated with 60-day mortality.

Keywords

ARDS; biomarkers; NEDD9; SARS-CoV-2 infection; Syndecan-1; ventilatory ratio

Circulating biomarkers of inflammation, coagulation, and endothelial and epithelial injury are associated with morbidity and mortality in ARDS,[1–3] including ARDS resulting from SARS-CoV-2 infection.[4–7] Distinct patterns of biomarkers are observed depending on the direct (eg, pneumonia) or indirect (eg, sepsis) mechanism of acute lung injury and time elapsed from the onset of lung injury.[4,8] In particular, markers of systemic inflammation, including IL-6 and IL-8, and markers of coagulation, including D-dimer, are associated with poor outcomes in patients with severe SARS-CoV-2 infection.[9–12] However, these associations are drawn largely from cohort studies predating the evidence-based use of corticosteroids and targeted immunomodulators for the treatment of ARDS resulting from SARS-CoV-2 infection.[13–15] Indeed, early cohort studies demonstrate that < 25% of patients with ARDS resulting from SARS-CoV-2 admitted to the ICU between March 11 and May 31, 2020, received immunomodulatory therapy with systemic corticosteroids alone or in combination with tocilizumab.[4,16]

In this study, our primary objective was to determine which plasma biomarkers were associated with clinical outcomes in a prospectively enrolled cohort of patients with ARDS resulting from SARS-CoV-2 infection who routinely received immunomodulation. Our primary outcome was in-hospital mortality. Our secondary outcome was ventilatory ratio, a validated noninvasive estimate of dead space ventilation, which is associated with extent of alveolar capillary injury and time to death in ARDS resulting from SARS-CoV-2 infection.[17,18] Additionally, an increase in ventilatory ratio from baseline to day 3 is associated with mortality.[19,20] Thus, ventilatory ratio represents a practical, noninvasive way to phenotype ARDS at the bedside. We hypothesized that in patients receiving antiinflammatory therapies, inflammatory biomarkers may not be associated with clinical outcomes.

Study Design and Methods

Study Design

We prospectively enrolled an observational cohort of patients consecutively admitted to the ICU of an urban, academic hospital in Boston, Massachusetts, from December 31, 2020, through March 31, 2021. Patients were aged ≥ 18 years, showed confirmed SARS-CoV-2 infection by positive polymerase chain reaction test results, and demonstrated acute hypoxemic respiratory failure. A diagnosis of ARDS was adjudicated by board-certified pulmonary and critical care physicians (J. W. A., G. A. A.) according to the Berlin definition.[21] Patients were excluded if they were transferred from an outside hospital or had life-sustaining treatment withdrawn within the first week of admission. The Mass General Brigham institutional review board approved the study (Identifier: 2015P001650) and informed consent was waived.

Data Collection

We collected plasma samples from excess clinical blood draws on the first day of ICU admission (within 24 h of intubation for mechanical ventilation, considered the baseline value), and ethylenediami netetraacetic acid-treated plasma aliquots were stored at −80 °C until analysis. We recorded clinical data from the electronic medical record, including

demographics, comorbidities, modified Sequential Organ Failure Assessment (mSOFA) score, hospital and ICU length of stay, and ventilator days and followed the clinical course for 60 days after enrollment or until hospital discharge or death. The nadir Pao_2 to F_{IO_2} ratio was determined based on the lowest Pao_2 (and corresponding F_{IO_2}) within the first 24 h after intubation and admission to the ICU. To calculate our secondary outcome, the ventilatory ratio, a validated estimate of dead space ventilation,[17] we used the $PaCO_2$ (in mm Hg) from arterial blood gas measured within the first 24 h of intubation and admission to the ICU matched with the corresponding tidal volume (in mL) and respiratory rate (in breaths/min) set on the ventilator to calculate the minute ventilation (in mL/min). Predicted body weight (PBW) was calculated using the ARDS Network PBW calculator using the formula PBW (kg)=50+2.3 (height in inches)−60 for male participants or PBW (kg)=45.5+2.3 (height in inches)−60 for female participants.[22] We calculated ventilatory ratio at baseline and on day 3 because temporal worsening in ventilatory ratio is prognostic and is observed in lethal ARDS resulting from SARS-CoV-2 infection.[18–20]

Plasma Biomarker Assays

We used a neural precursor cell expressed, developmentally downregulated 9 (NEDD9) enzyme-linked immunosorbent assay (Aviva Systems Biology) to quantify NEDD9 level (in ng/mL) and a multiplexed Luminex Discovery Assay (R&D Systems) to assess additional plasma protein levels, including: inflammatory markers (IL-6 and IL-8 [in pg/mL]); coagulation markers (tissue factor [in pg/mL], thrombomodulin [in ng/mL], plasminogen activator inhibitor [in ng/mL], intercellular adhesion molecule-1 [in ng/mL], P-selectin [in ng/mL], a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [in ng/mL], and von Willebrand factor domain A2 [vWF-A2; in ng/mL]); endothelial markers (vascular endothelial growth factor [in pg/mL], angiopoietin-2 [in ng/mL], soluble receptor for advanced glycation end-products [in ng/mL], and syndecan-1 [in ng/mL]); and the epithelial marker surfactant protein D (in ng/mL) (e-Table 1). D-dimer (in ng/mL), erythrocyte sedimentation rate (in mm/h), and C-reactive protein (in mg/L) were analyzed by the hospital core laboratory and values were abstracted from the electronic medical record.

Statistical Analysis

We performed summary statistics of patient demographics, clinical characteristics, and 60-day outcomes. Differences between groups were compared using the χ^2 or Fisher exact test for categorical variables, as appropriate, and the Wilcoxon rank-sum test for continuous variables. Differences between ventilatory ratio quartiles were compared using the Kruskal-Wallis test. A P value of $< .003$ was considered significant after adjusting for multiple comparisons by Bonferroni correction. We log2-transformed the plasma biomarkers and performed multivariable logistic regression model adjusting for age, sex, and severity of illness (using mSOFA) to obtain adjusted ORs (aORs) and 95% CIs. We performed a sensitivity analysis in which individuals treated with tocilizumab (n = 4) were excluded from analyses involving plasma IL-6, given the known effect of tocilizumab administration on IL-6 levels.[23] All analyses were performed using Stata SE version 18.0 software (StataCorp).

Results

Baseline Characteristics

Of 100 patients consecutively admitted to the ICU with acute hypoxemic respiratory failure resulting from SARS-CoV-2 infection, 74 patients (74%) were intubated for mechanical ventilation, all of whom met the Berlin definition of ARDS. Of these, 69 patients (93%) had a plasma sample available from the first day of ICU admission. Thus, our final analytic sample included the 69 patients intubated for ARDS who had available plasma samples (Fig 1). The baseline characteristics of the cohort are summarized in Table 1. The mean ± SD age was 62 ± 15 years; 31 patients (45%) were female; and 56 patients (81%) were White, four patients (6%) were Black, six patients (9%) were Asian, and 19 patients (28%) were Hispanic. The mean BMI was 31 ± 8 kg/m^2, median mSOFA score was 7 (interquartile range [IQR], 6–9), median duration of mechanical ventilation was 17 days (IQR, 11–25 days), median ICU length of stay was 19 days (IQR, 12–32 days), and median hospital length of stay was 26 days (IQR, 19–33 days). The median nadir Pao_2 to F_{IO_2} ratio was 174 (IQR, 132–235), consistent with moderate ARDS.[21] Patients received lung protective ventilation with a median tidal volume of 6 mL/kg PBW (IQR, 5–6 mL/kg PBW), median positive end-expiratory pressure of 10 mm Hg (IQR, 8–14 mm Hg), and median plateau pressure of 21 mm Hg (IQR, 19–25 mm Hg). The calculated median ventilatory ratio was 1.7 (IQR, 1.4–1.9) within 24 h of ICU admission (baseline) and 1.7 (IQR, 1.5–2.0) on day 3 of ICU admission. A total of 69 patients (100%) received systemic corticosteroids, 48 patients (70%) received remdesivir, and four patients (6%) received tocilizumab. At the time of hospital admission, 21 patients (30%) were prescribed antiplatelet therapies and eight patients (12%) were prescribed therapeutic anticoagulation. While admitted, 69 patients (100%) received prophylactic heparin and 31 patients (45%) received therapeutic heparin. Nineteen cases (28%) of acute thromboembolism were confirmed, including 13 cases (19%) of acute pulmonary embolism, DVT, or both and six cases (9%) acute myocardial infarction. By 30 days, 26 patients (38%) died, and by 60 days, 30 patients (44%) died.

Association of Baseline Characteristics and 60-Day Mortality in Univariable Analysis

Compared with survivors, nonsurvivors at 60 days were disproportionately male (73% vs 41%; $P = .007$), more severely ill based on baseline mSOFA score (median, 8.5 [IQR, 7–9] vs 7 [IQR, 5–8]; $P = .004$), and had more severe baseline hypoxemia based on a lower median Pao_2 to F_{IO_2} ratio (153 [IQR, 118–182] vs 184 [IQR, 142–247]; $P = .04$) (Table 2). No difference was found in 60-day mortality based on age, race, ethnicity, and BMI in the cohort. Further, no difference was found in 60-day mortality based on receipt of antiviral medication, preadmission prescription of antiplatelet or anticoagulant therapy, or the administration of therapeutic heparin during hospitalization.

Association of Plasma Biomarkers and 60-Day Mortality in Univariable and Multivariable Analyses

In total, 16 plasma analytes were measured (Table 3, e-Table 1) in the 69 patients with ARDS resulting from SARS-CoV-2 infection whose plasma was available from the first day of ICU admission. In univariable analysis, compared with survivors, nonsurvivors at 60 days showed higher baseline plasma NEDD9 (median, 8.4 ng/mL [IQR, 7.0–11.2 ng/mL] vs

6.9 ng/mL [IQR, 5.5–8.0 ng/mL]; $P = .0025$), vWF-A2 (median, 8.7 ng/mL [IQR, 7.9–9.7 ng/mL] vs 6.5 ng/mL [IQR, 5.7–8.7 ng/mL]; $P = .007$), angiopoitein-2 (median, 9.0 ng/mL [IQR, 7.9–14.1 ng/mL] vs 7.0 ng/mL [IQR, 5.6–10.6 ng/mL]; $P = .01$), and syndecan-1 (median, 15.9 ng/mL [IQR, 14.5–17.5 ng/mL] vs 12.6 ng/mL [IQR, 10.5–16.1 ng/mL]; $P = .01$), all of which are endothelial biomarkers (Fig 2). However, only plasma NEDD9 met the Bonferroni-adjusted threshold for significance ($P < .003$). No statistically significant differences were found in the other plasma biomarkers, including IL-6 ($P = .2$), IL-8 ($P = .9$), or D-dimer ($P = .2$) (Table 3). Compared with patients not treated with tocilizumab (n = 65), we observed a higher median plasma IL-6 level in patients treated with tocilizumab (n = 4) (median, 562 pg/mL [IQR, 328–748 pg/mL] vs 113 pg/mL [IQR, 95–143 pg/mL]; $P = .04$) (e-Table 2). When the patients treated with tocilizumab were excluded, we again did not observe a difference in plasma IL-6 level between the survivors and nonsurvivors ($P = .4$) (e-Table 3).

In multivariable regression analyses adjusted for age, sex, and mSOFA score, log2-transformed plasma NEDD9 was associated with 60-day mortality (aOR, 9.7; 95% CI, 1.6–60.4; $P = .015$) (Table 4). Log2-transformed plasma vWF-A2, syndecan-1, and angiopoietin-2 levels also remained significant in adjusted analyses with decreased estimated effect sizes (aOR, 8.6 [95% CI, 1.5–49.6; $P = .017$], 8.0 [95% CI, 1.7–38.0; $P = .009$] and 3.2 [95% CI, 1.1–9.2; $P = .033$], respectively).

Association of Ventilatory Ratio With 60-Day Mortality and Circulating Plasma Biomarkers

Compared with survivors, nonsurvivors demonstrated a higher baseline and day 3 ventilatory ratio (baseline: median, 1.5 [IQR, 1.4–1.9] vs 2.0 [IQR, 1.9–2.3], $P < .001$; and day 3: median, 1.5 [IQR, 1.4–1.9] vs 1.9 [IQR, 1.6–2.1], $P = .001$; respectively) (Table 2), with a notable stepwise increase in 60-day mortality for every increased quartile of ventilatory ratio both at baseline and on day 3 (Fig 3A, 3B). At baseline, no difference in NEDD9 level was found across ventilatory ratio quartiles ($P = .09$), but a statistically significant increase in plasma vWF-A2 and syndecan-1 levels were found across ventilatory ratio quartiles ($P = .004$ and $P = .006$, respectively) (Fig 4A–4C). Although D-dimer was not associated with mortality in this sample, previously it was associated with ventilatory ratio in ARDS resulting from COVID-19[12]; here, we similarly observed a statistically significant increase in D-dimer across baseline ventilatory ratio quartiles (Fig 4D). When stratifying by quartiles of ventilatory ratio on day 3, no significant difference was found in plasma vWF-A2 ($P = .3$) or D-dimer ($P = .5$) levels (Fig 4E, 4F), but a significant difference in plasma syndecan-1 level remained ($P = .005$) (Fig 4G). A moderate correlation was found between plasma syndecan-1 level and both baseline ventilatory ratio ($\rho = 0.4$; $P = .001$) and day 3 ventilatory ratio (r ¼ 0.5; $P < .001$), but no correlation was seen with the other plasma biomarkers (e-Fig 1). No association was found between baseline and day 3 ventilatory ratio for the remaining plasma biomarkers.

Discussion

In this prospective study of patients with ARDS resulting from COVID-19 uniformly treated with antiinflammatory therapies, we did not observe significant associations between

the circulating inflammatory, epithelial, or coagulation proteins and mortality. In contrast, four endothelial biomarkers—NEDD9, vWF-A2, syndecan-1, and angiopoietin-2—were associated with 60-day mortality after adjusting for age, sex, and severity of illness. Additionally, we observed that ventilatory ratio, a validated estimate for dead space ventilation shown to be associated with mortality independently in patients with ARDS,[17] was associated with 60-day mortality both at baseline and on day 3.

Several studies have investigated the diagnostic and prognostic role of circulating biomarkers in ARDS—encompassing several protein biomarkers of coagulation, inflammation, epithelial injury, and endothelial injury—with the goal of parsing the heterogeneity of ARDS to identify treatable traits.[1–3,7,24–27] Indeed, combinations of biomarkers reproducibly identify a hyperinflammatory subphenotype of ARDS with increased mortality, decreased ventilator-free days, and differential response to treatments.[28,29] Notably, the greatest differences distinguishing the hyperinflammatory and hypoinflammatory subphenotypes are IL-6 and IL-8 levels.[28] At the onset of the COVID-19 pandemic, longitudinal analyses by Leisman et al[4] demonstrated that alveolar injury markers increase first, followed by endothelial markers, and that the latter are associated with acute kidney injury and 28-day mortality. However, most studies profiling circulating biomarkers in ARDS resulting from COVID-19 occurred early in the pandemic, before the widespread, evidence-based use of systemic steroids and other immunomodulators.[13–15] In the aforementioned longitudinal analysis, only nine of 74 patients (12%) undergoing mechanical ventilation received steroids.[4] In our study of patients uniformly receiving antiinflammatory therapies, we observed no association with clinical outcomes between inflammatory and epithelial biomarkers. One hypothesis is that immunomodulators could attenuate the association between inflammatory biomarkers with clinical outcomes, thereby changing the prognostic value of circulating biomarkers in patients with ARDS treated with immunomodulators. However, our small sample size precludes any definitive conclusions regarding any negative associations, and we cannot assess the impact of antiinflammatory therapy on the plasma biomarkers studied, given our single time point analysis and the absence of a compactor group. However, with an increasing recognition of the beneficial effects of antiinflammatory in acute lung injury more broadly,[30,31] understanding the implications on circulating biomarkers used in subphenotyping ARDS is critical and warrants prospective validation.

To our knowledge, circulating NEDD9 has not been characterized in ARDS before. NEDD9 is a noncatalytic scaffolding protein that contains docking sites for proteins involved in multiple signal transduction pathways relevant to cellular adhesion, motility, proliferation, and survival.[32] NEDD9 is expressed widely in humans. In bulk tissue RNA sequencing accessible through the publicly available Genotype-Tissue Expression Portal,[33] NEDD9 is expressed most highly in the human lung, and within the lung, single-cell RNA sequencing demonstrates that NEDD9 is expressed most highly in B cells and lung vascular endothelial cells. Pulmonary artery endothelial NEDD9 is a hypoxia-regulated endothelial protein that mediates platelet-endothelial adhesion through an interaction with P-selectin on activated platelets[34] and is upregulated in the pulmonary arteriolar endothelium of patients with lethal ARDS resulting from SARS-CoV-2 infection not receiving immunomodulatory therapy.[35] Importantly, in patients with pulmonary vascular disease, plasma levels are associated with

adverse invasive pulmonary hemodynamics and clinical outcomes.[36] Here, we observed that, of all plasma biomarkers quantified, NEDD9 showed the greatest estimated effect size in its association with mortality in this cohort, comparable with that of established endothelial biomarkers like angiopoietin-2 and syndecan-1.

Pulmonary artery endothelial NEDD9 expression is increased in ARDS, both that resulting from SARS-CoV-2 infection and other causes not treated with antiinflammatory therapy, and colocalizes with intraluminal microthombi.[35] However, we did not observe a correlation between plasma NEDD9 and circulating coagulation proteins in this sample (e-Fig 2). Hypoxia upregulates NEDD9 in human pulmonary artery endothelial cells via hypoxia inducible factor 1-α,[34] but we did not observe a significant correlation between indexes of hypoxemia, such as the lowest Pao_2 or Pao_2 to F_{IO_2} ratio within 24 h of ICU admission (e-Fig 3). However, most patients showed a Pao_2 level in the normal physiologic range because of the high F_{IO_2} administered clinically, and systemic measures of oxygenation do not reflect local pulmonary endothelial hypoxia in poorly ventilated lung units. Separately, IL-6 also upregulates NEDD9 in human pulmonary artery endothelial cells in vitro,[34] but we did not observe a significant correlation between plasma NEDD9 and IL-6 levels in this sample (e-Fig 4). The mechanism underpinning our observation that circulating NEDD9 is increased in ARDS remains an area of active investigation. However, plasma NEDD9 is a compelling biomarker to study in both acute and postacute lung injury, because it differentiates patients with persistent abnormal pulmonary vascular perfusion after nonsevere COVID-19,[37] potentially offering insight into the long-term pulmonary endothelial recovery after ARDS.

This study further underscores the association between elevations in estimated dead space ventilation and adverse clinical outcomes in ARDS, even in the presence of immunomodulation. In a prospective study of autopsy lung specimens from patients with ARDS that combined detailed radiologic, histopathologic, and morphometric analyses to quantify the degree of pulmonary vascular congestion of the alveolar septae on lung histologic examination, ARDS resulting from COVID-19 showed the greatest degree of vascular congestion compared with alternative causes of ARDS, suggesting more prominent pulmonary capillary injury.[18] Notably, a correlation was found between the degree of vascular congestion and the ventilatory ratio, suggesting a direct link between pulmonary vascular injury and dead space fraction in ARDS. Importantly, the degree of alveolar septal congestion associated with time to death in both the cohorts of patients who died of ARDS resulting from COVID-19 and those who died of ARDS not caused by COVID-19, providing compelling evidence that pulmonary vascular injury is an important driver of respiratory impairment in fatal ARDS in the presence or absence of immune suppression. Ventilatory ratio represents a promising noninvasive bedside measurement to improve subphenotyping of ARDS and potentially to guide targeted therapies of pulmonary endothelial dysfunction.[38] Interestingly, only syndecan-1 was associated with ventilatory ratio both at baseline and on day 3, suggesting that circulating measures of endothelial injury and glycocalyx degradation may provide useful information about longitudinal ventilatory impairment in ARDS resulting from SARS-CoV-2 infection. Syndecan-1 is a heparan sulfate proteoglycan component of both the epithelial and endothelial glycocalyx that is shed during

acute lung injury and is associated with organ dysfunction and risk of ARDS in patients with nonpulmonary sepsis.[39]

Our study has several important limitations. The chief limitation is the small sample size, which substantially limits the power of the study and increases the risk of type 2 error. Although this was a prospective study, all participants were enrolled from a single center, thus limiting generalizability. Although all plasma samples were obtained within the first 24 h of intubation for mechanical ventilation, patients were at different phases of acute lung injury, and longitudinal analyses for temporal trends were not performed. Finally, we did not directly compare biomarker profiles with patients with ARDS resulting from COVID-19 before the universal adoption of systemic corticosteroids and targeted immunomodulatory therapy, nor did we compare plasma biomarkers before and after antiinflammatory therapy in this cohort.

Interpretation

In a cohort of patients with ARDS resulting from COVID-19 uniformly treated with immunomodulatory therapies, endothelial proteins best associate with clinical outcomes. Notably, plasma NEDD9 is, to our knowledge, a heretofore uncharacterized endothelial biomarker in ARDS that is associated independently with 60-day mortality, whereas syndecan-1 correlates with ventilatory ratio, a prognostically relevant estimate of dead space ventilation. The prognostic association of endothelial biomarkers underscores the importance of advancing endothelial-targeted therapies in acute lung injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

aOR	adjusted OR
IQR	interquartile range
NEDD9	neural precursor cell expressed, developmentally down-regulated 9
PBW	predicted body weight
mSOFA	modified Sequential Organ Failure Assessment
vWF-A2	von Willebrand factor domain A2

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Take-home Points

Study Question:

Which plasma biomarkers are associated with clinical outcomes in patients with ARDS resulting from SARS-CoV-2 infection routinely treated with immunomodulators?

Results:

In a cohort of patients with ARDS resulting from SARS-CoV-2 infection uniformly treated with immunomodulators, plasma biomarkers of inflammation, coagulation, and epithelial injury were not associated with clinical outcomes, but endothelial biomarkers remained prognostic.

Interpretations:

Our findings underscore the association of endothelial biomarkers with clinical outcomes in patients with ARDS treated with immunomodulators and warrant prospective validation, given the increasing evidence-based use of antiinflammatory therapy in acute lung injury.

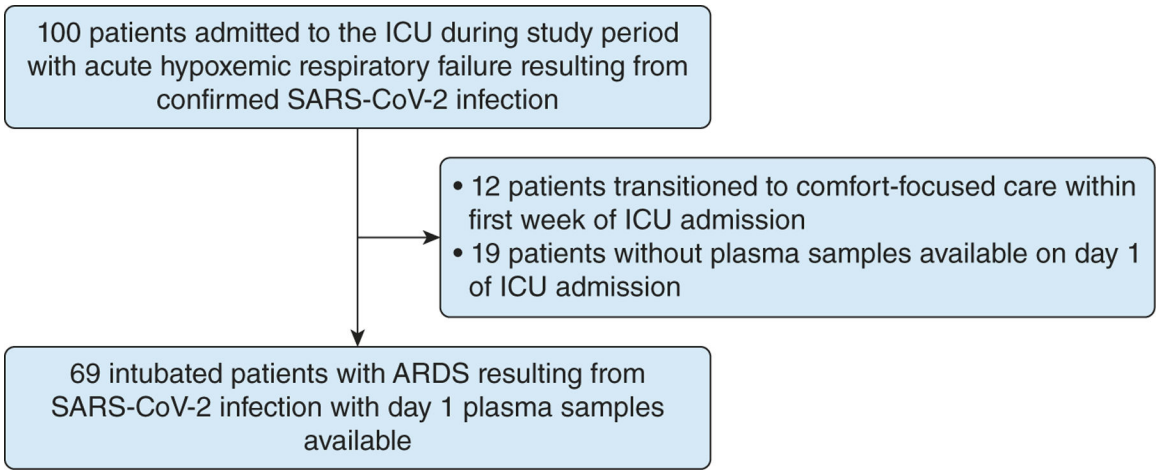

Figure 1 –.
Flow diagram showing study sample selection.

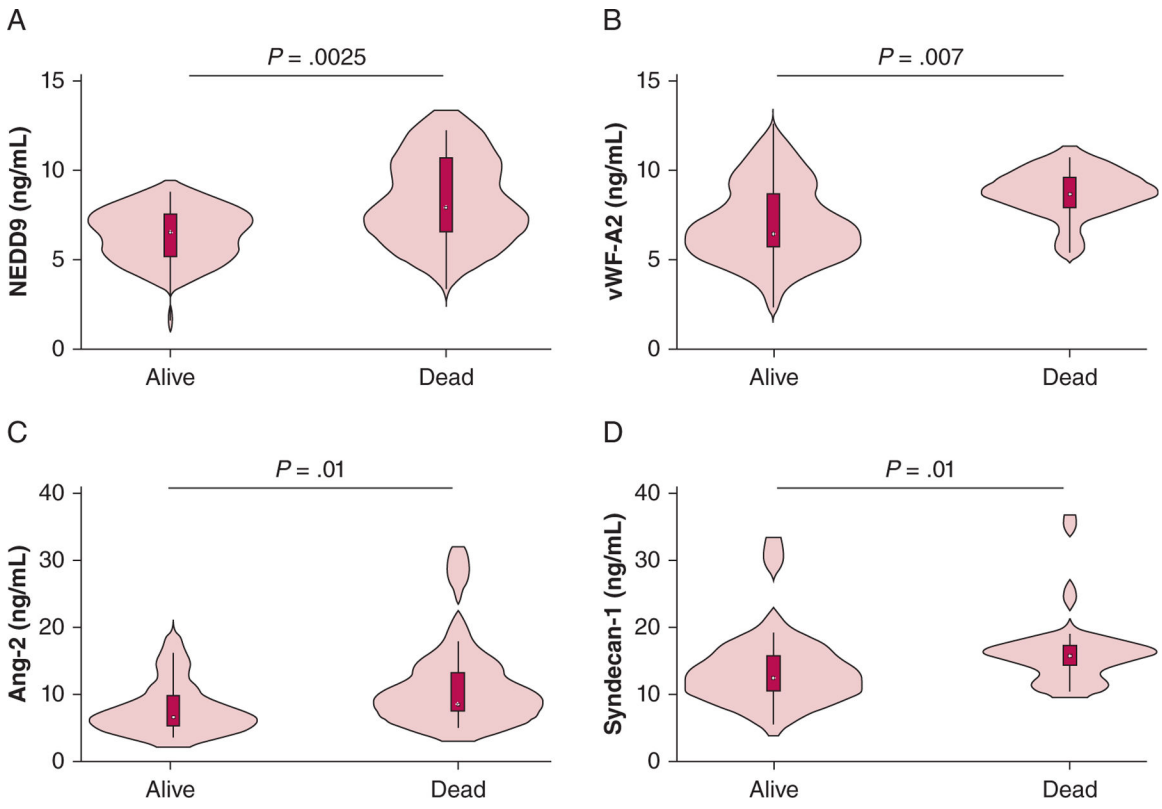

Figure 2 –.

A-D, Graphs showing plasma biomarkers stratified by 60-day mortality: compared with survivors, nonsurvivors at 60 days showed increased baseline plasma NEDD9 (A), vWF-A2 (B), angiopoitein-2 (C), and syndecan-1 (D). However, only plasma NEDD9 met the Bonferroni-adjusted threshold for significance (P < .003). NEDD9 = neural precursor cell expressed, developmentally down-regulated 9; vWF-A2 = von Willebrand factor A2.

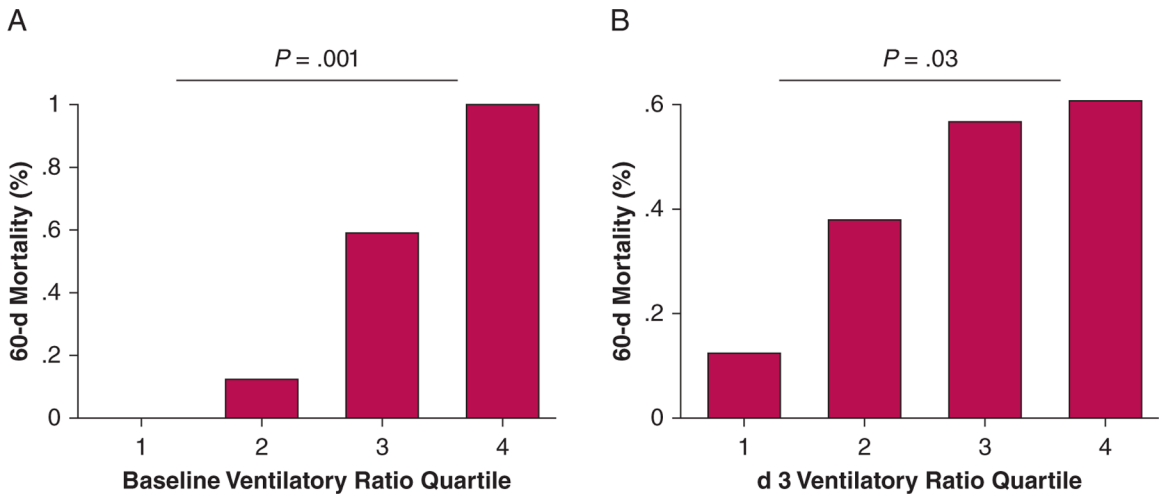

Figure 3 –.

A, B, Bar graphs showing ventilatory ratio and 60-day mortality: when ventilatory ratio is stratified by quartile, a stepwise increase in 60-day mortality is present for every increased quartile of ventilatory ratio when it is measured at baseline (A) and on day 3 (B).

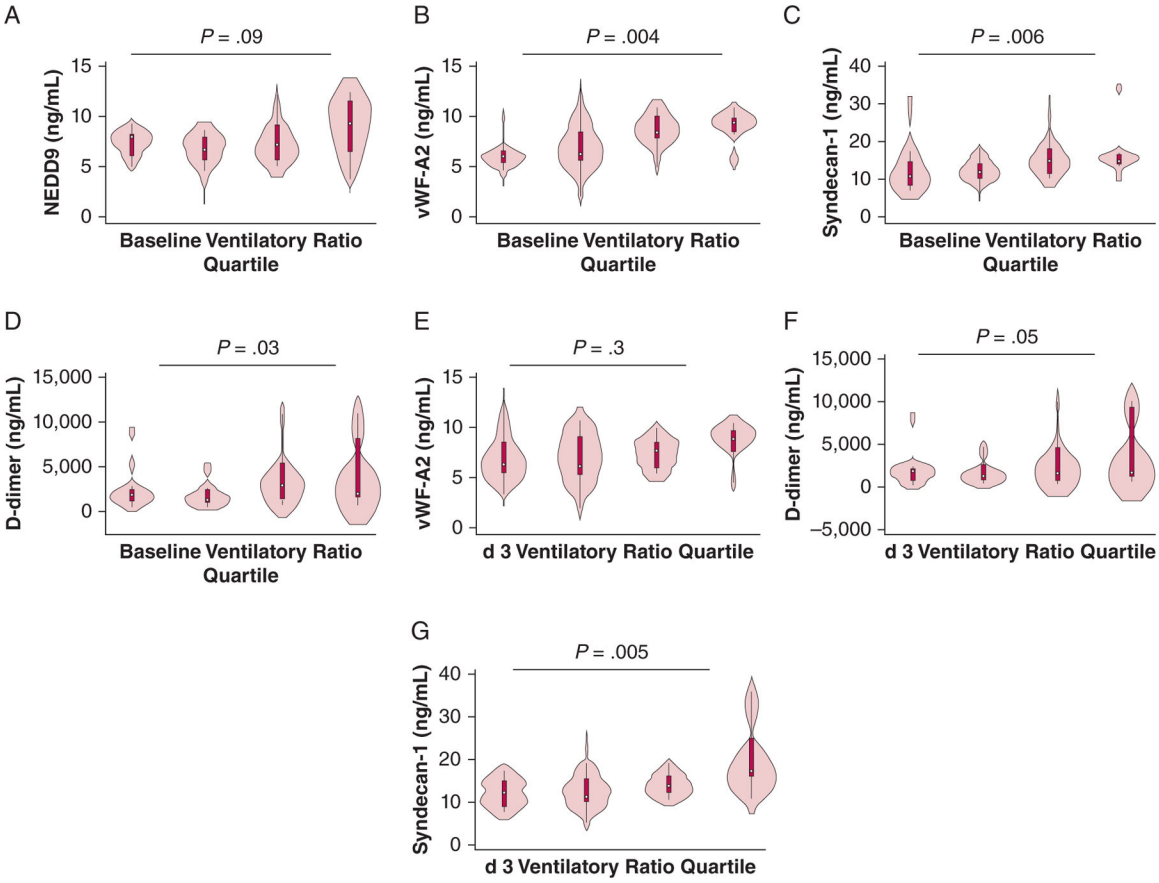

Figure 4 –.

Graphs showing plasma biomarkers and ventilatory ratio. A-C, When analyzing baseline ventilatory ratio stratified by quartile, no difference in NEDD9 level across ventilatory ratio quartiles was found (A), but a statistically significant increase in plasma vWF-A2 (B) and plasma syndecan-1 (C) levels across ventilatory ratio quartiles was found. D, A statistically significant increase in D-dimer was found across baseline ventilatory ratio quartiles. E-G, When analyzing day 3 ventilatory ratio stratified by quartile, no significant difference in plasma vWF-A2 (E) or D-dimer (F) was found, but a statistically significant difference in plasma syndecan-1 level remained (G). NEDD9 = neural precursor cell expressed, developmentally down-regulated 9; vWF-A2 = von Willebrand factor domain A2.

TABLE 1]

Cohort Baseline Characteristics

Variable	Total Cohort (N = 69)
Age, y	61.7 ± 15.2
Female sex	31 (45%)
Race	
White	56 (81)
Black	4 (5.8)
Asian	6 (8.7)
Other	3 (4.5)
Ethnicity	
Hispanic	19 (27.5)
BMI, kg/m^2	30.6 ± 8.1
mSOFA score	7 (6–9)
Length of stay	
ICU	19 (12–32)
Hospital	26 (19–33)
Ventilatory parameters	
Tidal volume, mL/kg PBW	5.7 (5.2–6.1)
PEEP	10 (8–14)
Plateau pressure	21 (19–25)
Pao_2 to FIO_2 ratio	167 (132–235)
Ventilatory ratio	
Baseline[a]	1.7 (1.4–1.9)
Day 3[b]	1.7 (1.5–2.0)
Ventilator duration, d	17 (11–25)
Therapies	
Steroids	69 (100)
Remdesivir	48 (70)
Tocilizumab	4 (6)
Preadmission anticoagulants	8 (12)
Preadmission antiplatelets	21 (30)
Anticoagulation	
Prophylactic	69 (100)
Therapeutic	31 (45)
Acute thromboembolic disease	
All confirmed causes	19 (28)
PE, DVT, or both	13 (19)
Acute myocardial infarction	6 (9)

Data are presented as No. (%), mean ± SD, or median (interquartile range). Msofa = modified Sequential Organ Failure Assessment; PBW = predicted body weight; PE = pulmonary embolism; PEEP = positive end-expiratory pressure; VV-ECMO, venovenous extracorporeal membrane oxygenation.

[a] Two patients not included (receiving VV-ECMO)

[b] Six patients not included (receiving VV-ECMO or died).

TABLE 2]

Cohort Stratified by 60-Day Mortality

Variable	Alive (n = 39)	Dead (n = 30)	*P* Value
Age, y	60.8 13.9	62.7 16.9	.6
Female sex	23 (59)	8 (27)	**.007**
Race			
White	29 (74)	27 (90)	.1
Black	3 (8)	1 (3)	.4
Asian	5 (13)	1 (3)	.2
Ethnicity			
Hispanic	9 (23)	10 (33)	.3
BMI, kg/m^2	29.6 ± 8.9	31.8 ± 6.9	.1
mSOFA score	7 (5–8)	8.5 (7–9)	**.004**
Ventilatory parameters			
Tidal volume, mL/kg PBW	5.8 (5.1–6.2)	5.6 (5.4–6.0)	.9
PEEP	10 (8–14)	10.5 (10–14)	.2
Plateau pressure	20 (18.5–24)	23 (19–25)	.3
Pao_2 to FIO_2 ratio	184 (142–247)	153 (118–182)	**.04**
Ventilatory ratio			
Baseline[a]	1.5 (1.2–1.6)	2.0 (1.9–2.3)	< **.001**
Day 3[b]	1.5 (1.4–1.9)	1.9 (1.6–2.1)	**.001**
Ventilator duration, d	16 (11–27)	17 (11–24)	.8
Therapy			
Steroids	39 (100)	30 (100)	...
Remdesivir	30 (77)	18 (60)	.1
Tocilizumab	2 (5)	2 (7)	.8
Baseline			
Anticoagulants	3 (8)	5 (17)	.2
Antiplatelets	15 (38)	6 (20)	.1
Admission heparin			
Prophylactic	39 (100)	30 (100)	...

Variable	Alive (n = 39)	Dead (n = 30)	*P* Value
Therapeutic	14 (36)	17 (57)	.09

Data are presented as No. (%), mean ± SD, or median (interquartile range), unless otherwise indicated. Boldface values indicate significance (*P* value < .05). mSOFA = modified Sequential Organ Failure Assessment; PBW = predicted body weight; PE = pulmonary embolism; PEEP = positive end-expiratory pressure; VV-ECMO, venovenous extracorporeal membrane oxygenation.

[a]Two patients not included (receiving VV-ECMO).

[b]Six patients not included (receiving VV-ECMO or died).

TABLE 3]

Plasma Biomarkers Stratified by 60-Day Mortality

Biomarker	Alive (n = 39)	Dead (n = 30)	*P* Value[a]
D-dimer, ng/mL	1,580.0 (883.0–2322.0)	1,967.0 (1316.0–4873.0)	.2
IL-6, pg/mL	109.3 (89.3–143.3)	118.0 (97.6–210.6)	.2
IL-8, pg/mL	29.8 (7.5–79.4)	15.9 (7.9–84.3)	.9
Tissue factor, pg/mL	181.5 (146.9–215.7)	185.4 (166.1–233.8)	.3
Thrombomodulin, ng/mL	8.3 (7.0–10.6)	10.0 (7.5–13.8)	.08
PAI-1, ng/mL	2.2 (1.3–5.1)	2.3 (1.5–6.9)	.4
ICAM-1, ng/mL	898.4 (724.5–1155.4)	1,194.0 (855.8–1641.6)	.1
NEDD9, ng/mL	6.9 (5.5–8.0)	8.4 (7.0–11.2)	**.0025** [b]
VEGF, pg/mL	212.7 (33.5–428.3)	50.7 (30.3–276.7)	.1
vWF-A2, ng/mL	6.5 (5.7–8.7)	8.7 (7.9–9.7)	**.007**
Angiopoietin-2, ng/mL	7.0 (5.6–10.6)	9.0 (7.9–14.1)	**.01**
P-selectin, ng/mL	50.1 (34.0–72.5)	47.5 (36.6–70.5)	.8
ADAMTS13, ng/mL	844.2 (709.2–972.7)	830.0 (720.7–1054.7)	.9
sRAGE, ng/mL	8.4 (4.6–16.9)	11.7 (6.7–16.3)	.3
Syndecan-1, ng/mL	12.6 (10.5–16.1)	15.9 (14.5–17.5)	**.01**
Surfactant protein D, ng/mL	13.9 (5.7–27.6)	13.0 (5.9–23.5)	.9

Data are presented as median (interquartile range), unless otherwise indicated. Boldface values indicate significance (*P* value < .05). ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ICAM-1 = intercellular adhesion molecule-1; NEDD9 = neural precursor cell expressed developmentally down-regulated protein 9; PAI-1 = plasminogen activator inhibitor-1; RAGE = receptor for advanced glycation end-products; sRAGE = soluble receptor for advanced glycation end-products; VEGF = vascular endothelial growth factor; vWF-A2 = von Willebrand factor domain A2.

[a]Wilcoxon rank-sum test.

[b]*P* < .003, Bonferroni-adjusted threshold for multiple comparisons.

TABLE 4]

Multivariable Logistic Regression of Log2-Transformed Plasma Biomarkers and 60-Day Mortality

Biomarker	OR	95% CI	*P* Value	Adjusted OR	95% CI	*P* Value
NEDD9, ng/mL	12.5	2.3–68.6	**.004**	9.7	1.6–60.4	**.015**
vWF-A2, ng/mL	8.9	1.8–43.5	**.007**	8.6	1.5–49.6	**.017**
Syndecan-1, ng/mL	4.1	1.2–14.7	**.03**	8.0	1.7–38.0	**.009**
Angiopoietin-2, ng/mL	2.8	1.2–6.5	**.015**	3.2	1.1–9.2	**.033**

Boldface values indicate significance (*P* value < .05). NEDD9 = neural precursor cell expressed, developmentally down-regulated 9; vWF-A2 = von Willebrand factor domain A2.

[a]All models adjusted for age, sex, and severity of illness (modified Sequential Organ Failure Assessment score).