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The circulating renin-angiotensin system and mortality among patients hospitalized for COVID-19: a mechanistic substudy of the ACTIV-4 Host Tissue trials

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Abstract

SARS-CoV-2 targets angiotensin-converting enzyme-2 (ACE2), a key peptidase of the renin-angiotensin system (RAS), which regulates the balance of the vasoconstrictor/inflammatory peptide Ang II and the vasodilator/anti-inflammatory peptide Ang-(1–7). Few studies have quantified the circulating elements of the RAS longitudinally in SARS-CoV-2 infection and their association with COVID-19 outcomes. Thus, we evaluated the association of circulating RAS enzymes and peptides with mortality among patients with COVID-19. Blood samples were collected from 111 patients with COVID-19 and new-onset hypoxemia during the delta and omicron waves at 19 hospitals in the United States. Circulating RAS components were quantified via radioimmunoassay or ELISA at 0 (baseline), 1, 3, and 5 days after randomization. We used multivariable Cox regression to estimate the association of baseline and longitudinal RAS concentrations with 90-day mortality. Participants were aged 18–90 (means [SD]: 55 [14]) yr and 62% were male. There were 22 (20%) deaths over 90 days of follow-up. ACE2 levels above the sample median (≥4.9 pM; adjusted HR [95% CI]: 0.10 [0.02, 0.43]) and ACE2/ACE ratio (≥6.0 × 10^{-3}; adjusted HR: 0.08 [0.02, 0.39]) were associated with significantly lower mortality. Similarly, when analyzed as continuous, $\log_2$-normalized, time-varying predictors from *day 0* to *day 5*, twofold increments of ACE2 and ACE2/ACE ratio over this period were associated with lower mortality (adjusted HR: 0.79 [0.65, 0.97] and 0.78 [0.63, 0.97], respectively). Circulating Ang II, Ang-(1–7), and ACE levels were not associated with mortality. These results suggest higher circulating ACE2 protein in hospitalized patients with COVID-19 is associated with reduced mortality.

NEW & NOTEWORTHY We measured circulating components of the renin-angiotensin system (RAS) longitudinally over 5 days among patients hospitalized with COVID-19 and new-onset hypoxemia. We found that higher serum angiotensin-converting enzyme (ACE)-2 protein and ACE2/ACE ratio, both at baseline and when analyzed as time-varying, repeated measures, were associated with lower 90-day mortality. Results suggest a role for circulating ACE2 as a biomarker

of adverse outcomes and could inform treatment strategies targeting the RAS in severe COVID-19 illness.

Keywords

COVID-19; critical care; hypoxemia; pulmonary; renin-angiotensin system

INTRODUCTION

Two major discoveries over the past 20 years have garnered heightened interest in the role of the renin-angiotensin system (RAS) in mediating clinical outcomes from SARS coronavirus infections. First, both SARS-CoV-1 and SARS-CoV-2 were found to gain cellular entry by binding to and promoting internalization of the membrane-bound form of the angiotensin-converting enzyme (ACE)-2 viral complex (1, 2). Second, ACE2 constitutes an important nexus between the classical ACE-Ang II-angiotensin type 1 receptor (AT_1R) axis of the RAS and the alternative ACE2-Ang-(1–7)-Mas receptor arm that generally antagonizes the proinflammatory and profibrotic actions of the classical axis (3). Thus, the pathological actions of acute SARS-CoV-2 infection may encompass a significant alteration in the overall balance of the RAS favoring the ACE-Ang II-AT_1R axis, which may exacerbate disease severity in patients with COVID-19 (4).

Despite the view of an activated ACE-Ang II-AT_1R pathway in patients with COVID-19 and subsequent clinical trials to block the AT_1 receptor or augment Ang-(1–7) levels during the COVID-19 pandemic (5–7), many questions remain about the influence of the RAS on outcomes from COVID-19. Since relatively few studies have robustly quantified circulating elements of the RAS, either at single time points or longitudinally, and their association with clinical outcomes among hospitalized patients with COVID-19, we evaluated the association of key circulating RAS components with mortality in adults hospitalized with COVID-19 and new hypoxemia.

MATERIALS AND METHODS

Study Design

This study was nested within the fourth Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV-4) (8) Host Tissue trials (NCT04924660) (9), a randomized, shared-placebo platform evaluating multiple therapies in patients hospitalized for COVID-19 with new-onset hypoxemia. The first RAS-targeting agents studied were TXA-127 (synthetic human Ang-[1–7] [Asp-Arg-Val-Tyr-Ile-His-Pro]) and TRV-027 (AT_1R biased agonist, [Sar-Arg-Val-Tyr-Ile-His-Pro-Ala or [Sar_1,Ala^8]-Ang II) (8). Interventions were administered intravenously over the first 5 days of the study post-randomization (TXA-127: 0.5-mg/kg infusion once daily or placebo; TRV-027: 12-mg/h continuous infusion or placebo). Both trials met prespecified early stopping criteria for low probability of efficacy (8).

Patient Sample

This study included all participants randomized to the shared placebo arm of the parent trials with at least one circulating RAS peptide or peptidase measurement over the first 5 study days since randomization: baseline (*day 0*); *day 1* ± 1 day and *day 3* ± 1 day (on study treatment); and 2–16 h after final inpatient study treatment on *day 5*. These participants were enrolled between July 22, 2021, and April 20, 2022, when the SARS-CoV-2 delta strain was predominant. Participants were recruited from 19 hospitals across the United States. Briefly, inclusion criteria (8) included: age ≥18 yr; hospitalized for COVID-19 with positive SARS-CoV-2 molecular or antigen test; and diagnosed with new-onset hypoxemia defined as oxygen saturation measured by pulse oximetry (Sp_{O_2}) <92% on room air, use of supplemental oxygen to maintain an Sp_{O_2} >92%, or an increase in oxygen support for patients who were receiving supplemental oxygen before COVID-19. All participants provided written informed consent. The study was approved by the institutional review board of Vanderbilt University Medical Center.

A total of 111 participants from the parent study placebo arm with at least one RAS peptide or peptidase measurement were included in this study, including 87 with *day 0* measurements, 95 with *day 1* measurements, 70 with *day 3* measurements, and 65 with *day 5* measurements. Participants self-reported their age, sex, race, and ethnicity. The baseline level of oxygen support was determined according to the 8-level World Health Organization (WHO) COVID-19 clinical progression ordinal scale; patients meeting criteria for *levels 4* through *7* were eligible for the parent trials (10). Medical history and comorbidity status including obesity, hypertension, diabetes, chronic kidney disease, and RAS inhibitor medication use before COVID-19 (i.e., ACE inhibitors or angiotensin receptor blockers) were obtained from the medical record of each participant. Participants who received any RAS modulators during hospitalization (e.g., Ang II as a vasopressor) were excluded from the analysis.

Biospecimen Collection

Whole blood was collected into 10 mL EDTA-containing tubes pretreated with protease inhibitors for peptide measurements (11) and separate untreated 6 mL tubes for serum peptidases at the four time points described earlier. EDTA-inhibitor tubes were inverted five times and centrifuged at 2,000 *g* at 4°C for 15 min within 3 h of collection. Serum tubes were clotted upright at room temperature for ~60 min before centrifugation at 2,000 *g* for 15 min at 4°C. Plasma was then aliquoted into 15 mL polypropylene tubes, serum aliquoted into cryovials, and samples were stored at −80°C then shipped to the ACTIV-4 Biospecimen Central Laboratory at the University of Vermont (Burlington, VT) for storage. Blinded, deidentified patient samples were sent to Wake Forest University School of Medicine (Winston-Salem, NC) for processing in the Biomarker Core laboratory.

RAS Quantification

Plasma samples for peptide levels were thawed on ice, extracted on Sep-Pak C18 mini-columns (Waters Corp., Milford, MA), and quantified by radioimmunoassays (RIAs) for Ang II and Ang-(1–7). Values were corrected for overall recovery by the addition of

^{125}I-Ang II to each sample prior to extraction and expressed as pg/mL of plasma. Serum peptidase levels were measured by separate human ACE and ACE2 ELISAs (RayBiotech, Peachtree Corners, GA) with detection limits of 84 pg/mL and 25 pg/mL, respectively, and expressed as ng/mL of serum.

Statistical Analysis

Analyses were completed in R 4.2.3 (2023) with a two-sided α of 0.05. The association of baseline RAS values with 90-day all-cause mortality was estimated using Cox proportional hazards regression with adjustment for age, sex, race/ethnicity, and baseline level of oxygen support. Baseline RAS components were assessed as binary variables by sample median split due to skewed distributions and as $\log_2$-normalized continuous variables. To evaluate the longitudinal association of the RAS with mortality, RAS components were modeled via time-varying Cox regression as continuous, $\log_2$-normalized repeated measures with robust variance and inverse probability of censoring (IPC) weighting to account for missing time points or death prior to *day 5* (12, 13). Stabilized IPC weights were generated by logistic regression conditioned on the aforementioned covariates. In exploratory analyses, we examined the consistency of associations across age groups (18–30, 31–64, and ≥65 yr) and baseline level of oxygen support (WHO ordinal scale *level 4* vs. *levels 5–7*) using interaction terms and a two-sided α of 0.10. Finally, we investigated the curvilinearity of associations between baseline RAS measures and mortality using restricted cubic splines and compared the goodness of fit for nested models with first-order, quadratic, and cubic terms via likelihood ratio test.

RESULTS

The 111 placebo-treated participants included in this study were aged 18–90 (mean [SD]: 54.6 [13.9]) yr; were 62% male, 58% non-Hispanic White, and had 58% and 48% prevalence of obesity and hypertension, respectively (Table 1). Only 33% of enrolled participants had been vaccinated for SARS-CoV-2. At study entry, 66% of participants were receiving supplemental oxygen by nasal cannula or mask (*level 4* on the WHO COVID-19 ordinal scale), 31% high-flow nasal oxygen or non-invasive positive pressure ventilation (*level 5*), and 4% were mechanically ventilated (*level 6* or *7*; Table 1). There were 22 (20%) deaths over 90 days of follow-up (median [IQR] time-to-event: 16 [9–29] days), and 18 (21%) deaths among 87 participants with a baseline (*day 0*) RAS measurement.

There were no associations of baseline Ang II, Ang-(1–7), or ACE levels with mortality, either as continuous measures or compared with those below their respective medians (Table 2). However, those with higher circulating levels of ACE2 relative to the median (≥0.56 ng/mL [4.9 pM]) had lower mortality, even after adjustments for covariates (adjusted HR [95% CI]: 0.10 [0.02, 0.43]; Table 2). Accordingly, a higher ACE2/ACE ratio relative to the median ($\geq 6.0 \times 10^{-3}$) was also associated with lower mortality (adjusted HR [95% CI]: 0.08 [0.02, 0.39]; Table 2). Likewise, both higher baseline $\log_2$-transformed ACE2 (adjusted HR [95% CI]: 0.81 [0.67, 0.98] per twofold increment) and ACE2/ACE ratio (adjusted HR [95% CI]: 0.77 [0.63, 0.94] per twofold increment) were associated with lower mortality (Table 2).

Restricted cubic splines (Fig. 1, *A* and *B*) and comparisons of nested models including first-order, quadratic, and cubic baseline ACE2 terms by likelihood ratio tests supported cubic curvilinearity of the association with mortality (cubic vs. first-order models: $P = 0.041$; vs. quadratic models: $P = 0.034$). Nested models for the ACE2/ACE ratio were similar (cubic vs. first-order: $P = 0.036$; vs. quadratic: $P = 0.017$).

Associations of the longitudinal RAS over the first 5 study days with 90-day mortality were consistent with those of baseline RAS measures (Table 3). For example, the weighted adjusted HR (95% CI) for longitudinal ACE2 and ACE2/ACE ratio were 0.79 (0.65, 0.97) and 0.78 (0.63, 0.97), respectively, whereas longitudinal Ang II or Ang-(1–7) were not associated with mortality (Table 3). Associations did not differ by age group or baseline level of oxygen support (all interactions $P > 0.10$).

DISCUSSION

In this multicenter study of hospitalized patients with COVID-19 and new-onset hypoxemia requiring supplemental oxygen, we report that higher baseline and longitudinal circulating levels of ACE2 protein, and a higher ACE2/ACE ratio, were associated with lower 90-day mortality independent of baseline level of oxygen support. These associations were moderately curvilinear, with risk of death appearing to plateau in the highest and lowest quartiles of ACE2 and ACE/ACE2 ratio, respectively. In contrast, circulating ACE and the RAS peptides Ang II and Ang-(1–7) were not associated with mortality, and associations did not differ by age group or oxygen support level. Collectively, these results suggest a potential role for ACE2 as a biomarker to identify patients at higher risk for adverse outcomes in severe COVID-19 illness.

The cellular entry of SARS-CoV-2 to pulmonary, vascular, myocardial, and renal tissues via membrane-bound ACE2 is a critical step in COVID-19 pathology (2). This process, resulting in the internalization of the ACE2-viral complex, may inhibit the conversion of Ang II to Ang-(1–7) and promote Ang II-mediated acute lung injury, as preclinical models strongly suggest (3, 14, 15). Furthermore, during hypoxemia, Ang II-mediated pulmonary vasoconstriction potentially enhances fibrosis and inflammation, both of which are ameliorated by concomitant upregulation of ACE2 (4, 14, 16). Bourgonje et al. (14) propose that under similar hypoxemic conditions, SARS-CoV-2-induced ACE2 suppression impairs the clearance of Ang II and further aggravates tissue damage. Indeed, our findings suggest higher serum levels of ACE2 reflect greater tissue expression of the peptidase that is constitutively shed into the circulation. Thus, coupled with potential mitigation of COVID-19-related microvascular dysfunction (17), higher ACE2 may be a plausible protective mechanism among patients with SARS-CoV-2-induced hypoxemia, though additional studies are needed to determine if it may modify the disease course.

There remains a considerable degree of contradictory results on the status of the RAS in patients with COVID-19, as well as the contribution of this system to disease severity (18). These issues may reflect the use of various approaches to quantify RAS components, particularly the measurement of Ang II and Ang-(1–7) using non-specific assays; heterogenous patient populations regarding co-morbidities and prior treatment with

RAS blockers; differing collection times and sample handling procedures, presence of endogenous ACE2 inhibitors and ACE2 isoforms, or autoantibodies that may modify activity; as well as emerging new strains of the virus over the past 5 yr (19–21). However, the current results are consistent with those of Díaz-Troyano et al. (22), who found lower ACE2 content to be associated with increased mortality and hospitalization of patients with COVID-19 using an ACE2 ELISA identical to the present study. Moreover, Robertson et al. (23) report lower circulating ACE2 was associated with disease severity and higher inflammatory markers in male patients with COVID-19.

In contrast, other studies suggest that higher ACE2 is predictive of disease severity in patients with COVID-19 and other pathologies including acute respiratory distress syndrome (ARDS), heart failure, and other respiratory distress (24, 25). Wang et al. (26) found that soluble ACE2 content was elevated among hospitalized patients with COVID-19 compared with healthy controls and that a raw increase in ACE2 from admission to 7 days was associated with increased mortality. Importantly, our longitudinal analysis assessed overall levels rather than raw increases in ACE2, which could be a compensatory response to worsening progression of the disease. Moreover, pre-existing comorbidities, including cardiovascular disease, have a considerable influence on ACE2 expression and disease severity in patients with COVID-19 (27). We did not previously observe effect modification by hypertension status (28), or obesity or diabetes status (unpublished observations), but future studies of the RAS in COVID-19 should assess the impact of prevalent cardiovascular diseases in more detail.

Although we found that higher circulating ACE2 values were associated with lower mortality in patients with COVID-19, there was no relationship between soluble ACE2 and circulating levels of Ang II or Ang-(1–7). In an earlier study that assessed the circulating RAS in patients with ARDS and COVID-19, we also reported no association of ACE2 with plasma levels of Ang II or Ang-(1–7) (11). This may reflect the dissociation between tissue-bound and soluble levels of ACE2 in the regulation of Ang II and Ang-(1–7) (11, 21). Preclinical studies have questioned the extent that ACE2 contributes to the balance of Ang II and Ang-(1–7) in the circulation and that other peptidases such as prolyloligopeptidase or neprilysin may have a more relevant role in Ang II metabolism or Ang-(1–7) formation (29, 30). In this regard, the benefit of higher circulating levels of ACE2 in patients with COVID-19 may entail the sequestration of the virus and prevent the cellular internalization of the viral-ACE2 complex. Although Yeung et al. (31) reported the interaction of soluble ACE2, vasopressin, and the vasopressin B1 receptor to promote viral infection in HK2 proximal tubule cells, this unusual pathway for viral entry was not confirmed by other investigators in the same cells (32). Moreover, a clinical study that administered vasopressin in patients with COVID-19 failed to show any effect on circulating levels of the virus or soluble ACE2 (33). Alternatively, alterations in circulating ACE2 may reflect higher tissue levels of the peptidase locally influencing the balance of Ang II and Ang-(1–7). Apart from the circulating system, it is well recognized that multiple organs express a local or tissue RAS with autocrine or paracrine actions (29, 34). Although these tissue or local systems present considerable challenges to fully characterize in humans, the ultimate tissue source of circulating ACE2 in patients with COVID-19 remains unclear. The presence of ACE2 autoantibodies detected in patients with COVID-19 (35–37) represents another

potential confounding source, as whether they inhibit, stimulate, or have a null effect on overall ACE2 activity is unknown. Indeed, Arthur et al. (35) report that the serum of all patients with COVID-19 in their cohort enhanced human ACE2 activity despite differences in autoantibody function. Thus, the impact of circulating factors that influence ACE2 in patients with COVID-19 should be further explored in future studies.

Major strengths of this study include its prospective, multicenter design; detailed assessment of the circulating RAS over four time points; the use of inhibitors to prevent ex vivo degradation of RAS peptides; and robust repeated-measures survival analysis. Some limitations should also be considered. Foremost, this study was designed to characterize the longitudinal circulating RAS in COVID-19 by collecting blood samples from an ongoing clinical trial. Thus, it represents a subset of the overall trial population and was not designed to detect all potentially meaningful associations. Furthermore, the parent trials were not designed to evaluate the influence of comorbidity burden, including prevalent cardiovascular disease, on COVID-19-related outcomes. Second, blood collection over multiple time points occurred only in hospitalized patients, so the ability to explore the circulating RAS longitudinally during recovery is limited. Moreover, some study participants may have presented to the hospital earlier in the disease course than others, which cannot be directly accounted for. Patients with lung injury progress and resolve at different rates, making this an inherent study challenge. Third, clinically meaningful thresholds for RAS markers in COVID-19 have not yet been established, though the sample median of ACE2 in our study was similar to the optimal value shown to predict hospital admission in a previous study (22). This approach may be useful for comparing distributions across different studies and settings. However, we note the use of the median RAS value as a reference point in statistical analysis, although common among recent RAS biomarker studies in critical illness settings including COVID-19 (26, 38, 39), is arbitrary and should be interpreted with caution and in specific contexts. For this reason, we additionally examined baseline RAS levels as continuous variables to complement analyses using the sample median to define groups (Table 2). Nevertheless, we strongly encourage more studies to propose and validate clinically meaningful cut-points for RAS biomarkers in the setting of severe COVID-19 and to better standardize methods for measuring the RAS. Lastly, our study involves the evaluation of only a subset of RAS enzymes and peptides.

Conclusions

Higher circulating levels of ACE2 and a higher ACE2/ACE ratio were associated with markedly lower mortality among patients hospitalized for COVID-19 with new-onset hypoxemia requiring supplemental oxygen support. A better understanding of the temporal course of the RAS response in severe COVID-19 illness may facilitate identifying patients at greater risk for adverse outcomes and inform treatment strategies targeting the RAS.

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DISCLOSURES

Dr. Rice reports receiving grant support from NHLBI, NCATS, CDC, Department of Defense, and PCORI, and consulting fees from Cumberland Pharmaceuticals, Inc. and Cytovale, Inc. Dr. Rice also serves as a DSMB member for Sanofi, Inc.

Dr. Collins reports receiving grant support from NCATS to his institution outside of the present work and consulting fees from Encanta Pharmaceuticals outside of the present work.

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DATA AVAILABILITY

Datasets analyzed in this study are available from the BioData Catalyst repository (https://biodatacatalyst.nhlbi.nih.gov) under study identifier phs003708.

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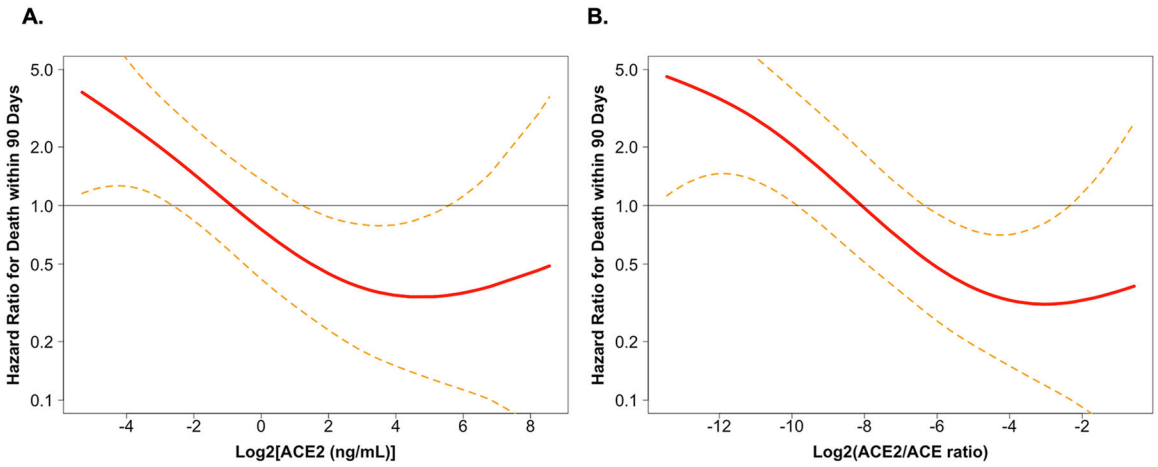

Figure 1.

Restricted cubic splines of the association of $\log_2$-transformed baseline circulating ACE2 levels (*A*) and ACE2 to ACE ratio (*B*) with 90-day mortality among 87 patients hospitalized for COVID-19 with new-onset hypoxemia. Dashed lines represent the 95% confidence interval; the horizontal line represents the null hazard ratio value (1.0). *Y*-axis is on the log scale. Median values serve as the referent. ACE, angiotensin-converting enzyme.

Table 1.

Baseline characteristics of 111 patients from the shared placebo arm of the ACTIV-4 Host Tissue trials with measured circulating renin-angiotensin system components.

Age, years, mean (SD)	54.6 (13.9)
Age group, n (%)	
18–30 years	6 (5.4)
31–64 years	80 (72.1)
≥65 years	25 (22.5)
Sex assigned at birth, n (%)	
Female	42 (37.8)
Male	69 (62.2)
Race/ethnicity, n (%)	
Non-Hispanic White	64 (57.7)
Non-Hispanic Black	17 (15.3)
Hispanic of any race	18 (16.2)
Other or prefer not to answer	12 (10.8)
Obesity (body mass index ≥30 kg/m^2), n (%)	64 (57.7)
Hypertension, n (%)	53 (47.7)
Diabetes, n (%)	33 (29.7)
Chronic kidney disease (not undergoing kidney replacement therapy), n (%)	7 (6.3)
Medication use prior to COVID-19, n (%)	
Angiotensin converting enzyme inhibitor	6 (5.4)
Angiotensin receptor blocker	8 (7.2)
Vasopressor use within 7 days of hospitalization,[a] n (%)	14 (12.6)
Time from hospital admission to randomization, days, median [25%, 75%]	1 [1, 2]
COVID-19 characteristics, n (%)	
Predominant SARS-CoV-2 variant in the US	
Delta (enrolled prior to and including Dec 26, 2021)	104 (93.7)
Omicron (enrolled after Dec 26, 2021)	7 (6.3)
Receipt of ≥1 vaccine dose	37 (33.3)
Baseline level of O_2 support according to World Health Organization COVID-19 clinical progression scale,[b] assessed at randomization	
Level 4: hospitalized and receiving supplemental O_2 by nasal prongs or mask	73 (65.8)
Level 5: hospitalized and receiving high-flow nasal O_2 or non-invasive ventilation	34 (30.6)
Level 6 or 7: hospitalized and receiving invasive mechanical ventilation alone or with other organ support	4 (3.6)
Death within 90 days of randomization, n (%)	22 (19.8)
Days to death, median [25%, 75%]	16 [9, 29]
Circulating renin-angiotensin system concentrations, median [25%, 75%]	
Angiotensin (Ang) II, pg/mL	11.4 [7.9, 17.2]
Ang-(1–7), pg/mL	17.3 [11.8, 31.0]

Ang II to Ang-(1–7) ratio	0.57 [0.39, 1.14]
Angiotensin converting enzyme (ACE), ng/mL	239.2 [125.6, 264.7]
ACE2, ng/mL	0.56 [0.08, 6.06]
ACE2 to ACE ratio	0.006 [0.0004, 0.041]

[a] Includes any vasopressors or inotropes (e.g., dobutamine, dopamine, epinephrine, milrinone, norepinephrine, phenylephrine, and vasopressin).

[b] Patients meeting criteria for Levels 4–7 of the 8-level ordinal scale were eligible for this study. Other levels included: Level 1, ambulatory and not hospitalized with no limitation in activities; Level 2, ambulatory and not hospitalized with some limitation of activities or receiving home O_2 therapy; Level 3, hospitalized with mild disease and not receiving supplemental O_2; level 8, dead.

Table 2.

Multivariable associations of baseline (Study Day 0) renin-angiotensin system levels with mortality over 90 days among 87 hospitalized COVID-19 patients with new-onset hypoxemia randomized to the shared placebo arm of the ACTIV-4 Host Tissue trials.

	Hazard Ratio (95% CI)	
	Unadjusted	Adjusted[a]
Study Day 0 RAS Measures	**Referent: <median**	
Ang II	1.06 (0.40, 2.82)	1.24 (0.39, 3.99)
Ang-(1–7)	0.91 (0.33, 2.50)	1.32 (0.33, 5.35)
Ang II to Ang-(1–7) ratio	0.90 (0.32, 2.53)	1.18 (0.37, 3.80)
ACE	1.41 (0.54, 3.70)	1.73 (0.64, 4.67)
ACE2	0.31 (0.11, 0.88)	0.10 (0.02, 0.43)
ACE2 to ACE ratio	0.35 (0.13, 0.94)	0.08 (0.02, 0.39)
	Per twofold increment in continuous RAS measure	
Ang II	1.16 (0.71, 1.89)	1.08 (0.59, 1.96)
Ang-(1–7)	1.17 (0.79, 1.73)	1.35 (0.81, 2.27)
Ang II to Ang-(1–7) ratio	0.99 (0.72, 1.35)	0.92 (0.64, 1.32)
ACE	1.16 (0.73, 1.83)	1.25 (0.76, 2.05)
ACE2	0.92 (0.80, 1.05)	0.81 (0.67, 0.98)
ACE2 to ACE ratio	0.90 (0.79, 1.03)	0.77 (0.63, 0.94)

Ang, angiotensin; ACE, angiotensin converting enzyme.

[a]Adjusted for age, sex assigned at birth, race/ethnicity, and baseline level of oxygen support according to World Health Organization COVID-19 ordinal severity scale.

Table 3.

Weighted multivariable associations of time-varying renin-angiotensin system levels over Study Day 0 to Day 5 with 90-day mortality among 111 hospitalized COVID-19 patients with new-onset hypoxemia randomized to the shared placebo arm of the ACTIV-4 Host Tissue trials.

	Hazard ratio[a] (95% CI) per twofold increment in time-varying RAS measure (Study Day 0 to Day 5)	
	Unadjusted	**Adjusted[b]**
Ang II	1.15 (0.79, 1.68)	1.31 (0.83, 2.06)
Ang-(1–7)	1.15 (0.87, 1.52)	1.01 (0.75, 1.37)
Ang II to Ang-(1–7) ratio	1.01 (0.79, 1.30)	1.14 (0.85, 1.53)
ACE	1.14 (0.77, 1.69)	1.08 (0.66, 1.78)
ACE2	0.92 (0.82, 1.04)	0.79 (0.65, 0.97)
ACE2 to ACE ratio	0.91 (0.81, 1.02)	0.78 (0.63, 0.97)

Ang, angiotensin; ACE, angiotensin converting enzyme; CI, confidence interval.

[a]Estimates are inverse probability of censoring-weighted based on death before Study Day 5 or lacking complete RAS timecourse data.

[b]Adjusted for age, sex assigned at birth, race/ethnicity, and baseline level of O_2 support according to World Health Organization COVID-19 ordinal severity scale.