OPEN

Association of Active Renin Content With Mortality in Critically Ill Patients: A Post hoc Analysis of the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) Trial*

Laurence W. Busse, MD, FCCM, MBA[1,2]

Christopher L. Schaich, PhD[3]

Mark C. Chappell, PhD, FAHA[3]

Michael T. McCurdy, MD, FCCM[4]

Erin M. Staples, MD[5]

Caitlin C. Ten Lohuis, ACNP-BC[2]

Jeremiah S. Hinson, MD, PhD[6]

Jonathan E. Sevransky, MD, MHS[1,2]

Richard E. Rothman, MD, PhD[7]

David W. Wright, MD, FACEP[8,9]

Greg S. Martin, MD, MSc[1,2]

Ashish K. Khanna, MD, MS, FCCP, FCCM, FASA[10–12]

on behalf of the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) Investigators

OBJECTIVE: Sepsis is a leading cause of mortality. Predicting outcomes is challenging and few biomarkers perform well. Defects in the renin–angiotensin system (RAS) can predict clinical outcomes in sepsis and may outperform traditional biomarkers. We postulated that RAS dysfunction (elevated active renin, angiotensin 1-7 [Ang-(1-7)], and angiotensin-converting enzyme 2 (ACE2) activity with depressed Ang-II and ACE activity) would be associated with mortality in a cohort of septic patients.

DESIGN: Post hoc analysis of patients enrolled in the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) randomized controlled trial.

SETTING: Forty-three hospitals across the United States.

PATIENTS: Biorepository samples of 103 patients.

INTERVENTIONS: We analyzed day 0 (within 24 hr of respiratory failure, septic shock, or both) and day 3 samples ($n = 103$ and 95, respectively) for assessment of the RAS. The association of RAS values with 30-day mortality was determined using Cox proportional hazards regression with multivariable adjustments for age, sex, VICTAS treatment arm, systolic blood pressure, Sequential Organ Failure Assessment Score, and vasopressor use.

MEASUREMENTS AND MAIN RESULTS: High baseline active renin values were associated with higher 30-day mortality when dichotomized to the median of 188.7 pg/mL (hazard ratio [HR] = 2.84 [95% CI, 1.10–7.33], $p = 0.031$) or stratified into quartiles (Q1 = ref, $HR_{Q2} = 2.01$ [0.37–11.04], $HR_{Q3} = 3.22$ [0.64–16.28], $HR_{Q4} = 5.58$ [1.18–26.32], p for linear trend = 0.023). A 1-SD (593.6 pg/mL) increase in renin from day 0 to day 3 was associated with increased mortality (HR = 3.75 [95% CI, 1.94–7.22], $p < 0.001$), and patients whose renin decreased had improved survival compared with those whose renin increased (HR 0.22 [95% CI, 0.08–0.60], $p = 0.003$). Ang-(1-7), ACE2 activity, Ang-II and ACE activity did not show this association. Mortality was attenuated in patients with renin over the median on day 0 who received the VICTAS intervention, but not on day 3 (p interaction 0.020 and 0.137, respectively). There were no additional consistent patterns of mortality on the RAS from the VICTAS intervention.

CONCLUSIONS: Baseline serum active renin levels were strongly associated with mortality in critically ill patients with sepsis. Furthermore, a greater relative activation in circulating renin from day 0 to day 3 was associated with a higher risk of death.

KEYWORDS: angiotensin; biomarker; renin–angiotensin system; shock; vitamin C; Vitamin C, Thiamine, and Steroids in Sepsis Trial

***See also p. 509.**

DOI: 10.1097/CCM.0000000000006095

KEY POINTS

Question: Is RAS dysfunction (elevated active renin, Ang-(1-7) and ACE2 activity with depressed Ang-II and ACE activity) associated with mortality in septic patients?

Findings: Baseline serum active renin levels were strongly associated with mortality in critically ill patients with sepsis, as was the trend over time of active renin levels.

Meaning: Active renin content can predict clinical outcomes in sepsis.

Nationally 1.7 million patients per year develop sepsis, which has an estimated mortality rate of 10–50% (1, 2). Circulating biomarkers can potentially distinguish those patients at risk for poor outcomes early in the course of the disease, providing an opportunity to intervene to modify outcomes. However, currently used sepsis biomarkers including WBC count, lactate, and blood cultures have variable impacts on prognosis and/or management. For example, WBC count has not been shown to correlate with disease, and blood cultures are negative in up to 47% cases (3, 4). Additionally, although serum lactate correlates with disease, it may become elevated only after the onset of detrimental tissue hypoperfusion (5). Recent evidence suggests that circulating renin is more predictive of worse outcomes in septic shock than lactate and that elevated renin may be a more relevant biomarker for mortality (6, 7).

Renin is initially synthesized as an inactive precursor (prorenin) and converted into active renin by hydrolysis of the pro-segment within juxtaglomerular cells of the kidney (**Fig. 1**) (8). Renin release is the obligatory enzymatic event to activate the renin–angiotensin system (RAS) cascade that generates the bioactive peptides angiotensin (Ang) II and Ang-(1-7). Angiotensin-converting enzyme (ACE) hydrolyzes the renin product Ang-I to Ang-II, whereas ACE2 converts Ang-II to Ang-(1-7). The ACE–Ang-II–AT_1 receptor (AT_1R) axis comprises the classic arm of the RAS that maintains blood pressure through multiple peripheral and central mechanisms. Conversely, the ACE2–Ang-(1-7)–Mas receptor (MasR) axis constitutes the nonclassical or alternative RAS that lowers blood pressure and reduces inflammation and fibrosis through stimulation of nitric oxide-mediated pathways. Although ACE is the primary pathway for the generation of Ang-II, the peptidase also plays a key role in the metabolism of Ang-(1-7) to Ang-(1-5) (9).

In critical illness, a dysfunctional RAS reflects reduced ACE activity, lower Ang-II levels, and an attenuated AT_1 receptor response to Ang-II, and it predicts mortality (10–15). Additionally, elevated serum levels of renin have been associated with mortality (16, 17). Elevated levels of circulating renin in critically ill patients likely reflect the disinhibition of renin release from the kidney by reduced Ang-II. The increase in renin and lower ACE levels may concurrently result in higher circulating levels of Ang-(1-7) through reduced metabolism of the peptide and a shift in the processing of Ang-I to Ang-(1-7). Activation of the ACE2–Ang-(1-7)–MasR axis may further contribute to RAS dysfunction by antagonizing the actions of the classical Ang-II pathway. Given this complex RAS response, the current study interrogated the enzymatic and peptidergic components of both classical and nonclassical arms of the RAS in a subset of patients with sepsis-induced respiratory and/or circulatory failure enrolled in the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) trial (18). Specifically, we addressed whether the differential regulation of the RAS, including elevated renin, ACE2, and Ang-(1-7), but lower ACE and Ang-II are associated with worse survival in septic patients. We also assessed whether the VICTAS interventional cocktail of vitamin C, thiamine, and steroids would impact the expression of RAS components and their association with mortality.

MATERIAL AND METHODS

The VICTAS trial was a randomized, double-blind, adaptive-sample size, placebo-controlled trial conducted at 43 hospitals across the United States, which randomized patients to IV vitamin C (1.5 g), thiamine hydrochloride (100 mg), and hydrocortisone sodium succinate (50 mg) or matching placebos every 6 hours for 4 days. The full protocol is described elsewhere (19). Enrollment was stopped before meeting any prespecified criterion after funding was withheld due to a change in the funder's priorities, but the trial failed to demonstrate significant benefit of the treatment intervention to patients. Plasma and serum samples were obtained

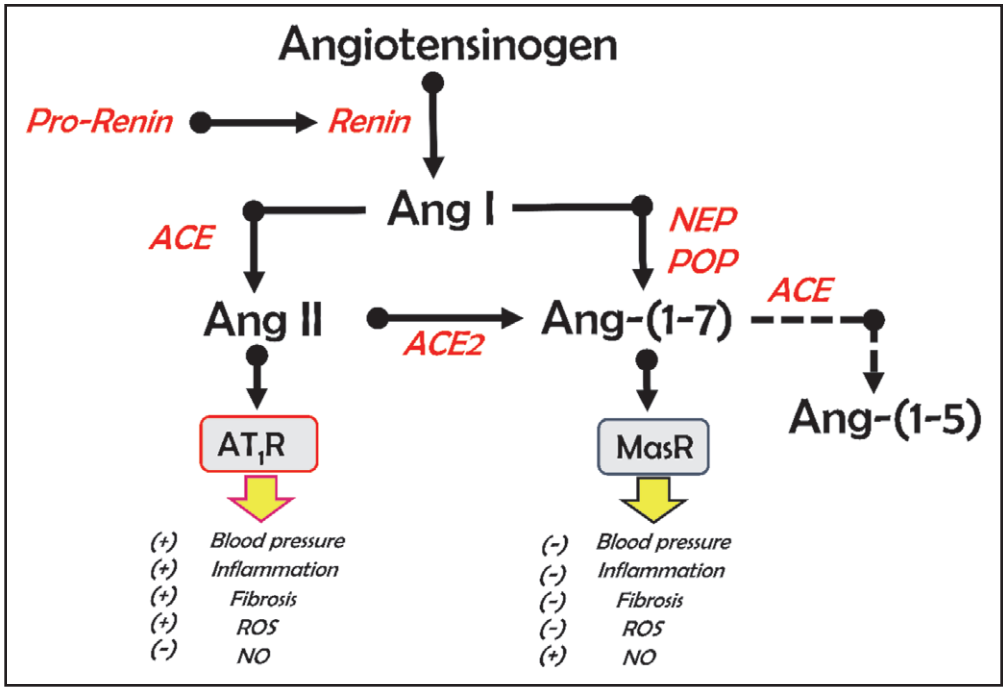

Figure 1. Classical and alternative arms of the renin–angiotensin system. Prorenin is converted to active renin within the kidney and secreted renin hydrolyzes angiotensinogen to angiotensin I (Ang-I). Angiotensin-converting enzyme (ACE) generates Ang-II that binds the AT_1 receptor (AT_1R) to facilitate an increase in blood pressure. In situations of ACE inhibition or dysfunction, Ang-I is converted to Ang-(1-7) by endopeptidases including neprilysin (NEP) and prolyl oligopeptidase (POP). Metabolism of Ang-(1-7) to Ang-(1-5) by ACE is also inhibited by ACE dysfunction. Vasodilatory effects of ACE dysfunction may reflect greater generation and reduced metabolism of Ang-(1-7), as well as lower levels of Ang-II that contribute to a decrease in blood pressure. Reproduced with permission [Chappell MC: Renin-angiotensin system and sex differences in COVID-19: A critical assessment. *Circ Res* 2023; 132:1320-1337.]. MasR = Mas receptor, NO = nitric oxide, ROS = reactive oxygen species.

from the VICTAS biorepository (Johns Hopkins School of Medicine, Baltimore, MD) and transported overnight on dry ice to the Wake Forest University School of Medicine (Winston-Salem, NC) investigator. RAS measurements were made at day 0 (per the VICTAS protocol, within 24 hours of the onset of respiratory failure, septic shock, or both) and day 3. Demographic and clinical characteristics were extrapolated from a deidentified dataset from the VICTAS study. The current study was reviewed and approved in April of 2021 with a waiver of consent by the Wake Forest University School of Medicine institutional review board (00073908) and all procedures were followed in accordance with local ethics standards and the Helsinki Declaration of 1975.

RAS Analyses

The active form of renin was determined in stored serum samples that were immediately thawed at 37°C to minimize the cryoactivation of prorenin, the major form of renin in the circulation. Serum renin content was then assayed at room temperature with an active renin enzyme-linked immunosorbent assay (ELISA) kit (assay sensitivity of 1 pg/mL of sample; DRG International, Fisher Scientific, Waltham MA).

Plasma samples for Ang-II and Ang-(1-7) levels were thawed on ice, extracted on Sep-Pak C18 mini-columns (Waters Corp., Milford, MA), and quantified by radioimmunoassays for Ang-II (IBL America, Minneapolis, MN; sensitivity of 2.0 pg/mL with intra-assay and inter-assay coefficients of variation of 8% and 20%, respectively), and Ang-(1-7) (custom radioimmunoassay, 2.5 pg/mL sensitivity with intra-assay and inter-assay coefficients of variation of 12% and 22%, respectively). The peptide values were corrected for overall recovery by addition of ^{125}I-Ang-II to each sample before extraction and the peptide content expressed as picograms per milliliter plasma as described (20).

ACE and ACE2 activities were measured in serum samples in duplicate using quenched fluorescent substrates methyl coumarin acetate (MCA)-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys-DNP and MCA-Ala-Pro-Lys-DNP, respectively, at a final concentration of 10 µM (Enzo Life Sciences, VWR, Atlanta, GA). Specific activities for the ACE and ACE2 assays were determined by adding the ACE inhibitor lisinopril (Enzo Life Sciences) or the ACE2 inhibitor MLN4760 (MedChem Express, Monmouth Junction, NJ), respectively, at a final concentration of 10 µM in parallel samples as described (21). For ACE, 10 µL of serum was added to a final volume of 200 µL, 25 mM of hydroxyl ethylpi perazine ethane sulfonic acid (HEPES)

(pH 7.4), 125 mM NaCl, and 10 μM $ZnCl_2$ and incubated for 1 hour at 37°C. For ACE2, 10 μL of serum was added to a final volume of 200 μL 25 mM HEPES buffer pH 7.0 and 10 μM $ZnCl_2$ was incubated for 18 hours at 37°C. Serum samples were read in 96-well black plates with clear bottom wells using a SpectraMax plate reader set (Molecular Devices, San Jose, CA) at an excitation wavelength λ = 320 nm and emission wavelength λ = 405 nm (Molecular Devices, San Jose, CA). Peptidase activities were expressed as the rate of the MCA product generated in relative fluorescent unit per hour per milliliter of serum.

Statistical Analysis

We compared baseline (day 0) demographic and clinical characteristics of the treatment groups using Student *t* tests for normally distributed continuous variables (presented as mean [SD]), Wilcoxon rank sum tests for non-normally distributed continuous variables (presented as median [25%–75%]), and Pearson chi-square tests for proportions. To assess the association of RAS components with 30-day mortality, we used Cox proportional hazards regression with multivariable adjustments for age, sex, VICTAS treatment arm, systolic blood pressure, day 0 Sequential Organ Failure Assessment (SOFA) score, and day 0 vasopressor use. We assessed RAS components as continuous variables normalized by $\log_2$ transformation, and as ordered categorical variables (above or below median values and by quartiles). We expressed estimates as hazard ratios (HRs) with robust 95% CIs per two-fold increment in RAS component (for $\log_2$-normalized values), or relative to below-median or lowest quartile (Q1) values. We supplemented HRs for categorically assessed RAS values with the log-rank test and differences in restricted mean survival time to aid interpretation due to possibility of invalid Cox model assumptions (22, 23). Further, we investigated possible nonlinearity of associations using restricted cubic splines and likelihood ratio tests for linearity. To assess the consistency of associations between treatment groups, we included a treatment-by-RAS interaction term and stratified results by significant interactions. We then evaluated treatment effects on changes in RAS values from day 0 to day 3 using analysis of covariance with adjustment for day 0 value, sex, and systolic blood pressure. Finally, to assess whether the magnitude of changes in RAS components were associated with mortality, we used Cox proportional hazards models adjusted for day 0 values, age, sex, treatment group, SOFA score, and vasopressors use, and expressed these estimates as HR (95% CI) per SD increment of day 0 value. All analyses were conducted in R 4.1.2 (2022) with a two-sided significance criterion of *p* value of less than 0.05.

RESULTS

Day 0 samples were collected from 103 patients, including 49 in the VICTAS control arm and 54 in the active comparator arm. Of these, day 3 samples were collected from 95 patients, including 46 in the VICTAS control arm and 49 in the active comparator arm. **Supplementary Table 1-S** (http://links.lww.com/CCM/H445) shows baseline demographics and clinical parameters by VICTAS treatment arm. Serum renin content, ACE and ACE2 activities, as well as plasma levels of Ang-II and Ang-(1-7) did not differ significantly between the control and comparator groups. Consistent with the results of the VICTAS trial, 30-day mortality did not differ between groups. **Table 1** shows the same baseline demographics and clinical parameters stratified by median renin level (N = 95). When stratified by baseline renin, patients had well-matched baseline demographics, medical history, and clinical parameters aside from serum creatinine (p = 0.045). Similar percentages of patients required vasopressor support, mechanical ventilation, or both (p = 0.679). Serum renin content, plasma levels of Ang-II and Ang-(1-7) differed significantly between the control and comparator groups, but ACE and ACE2 activities did not. When stratified by renin level, 30-day mortality differed between groups (p = 0.049).

Baseline Renin Response

Serum renin at day 0 risk stratified critically ill patients at high risk of death. When dichotomizing by the median serum renin value (normal and historical values presented as molar values in **Supplemental Table 4-S**, http://links.lww.com/CCM/H445) for all patients (188.7 pg/mL, n = 103), those above the median (> 188.7 pg/mL) had significantly higher mortality than those below the median renin value (HR = 2.84 [95% CI, 1.10–7.33], p = 0.031) (**Supplemental Fig. 1**, http://links.lww.com/CCM/H445). Higher quartiles of

TABLE 1.
Baseline Characteristics, Stratified by Median Renin Concentration

	Renin, < 188.7 pg/mL	Renin, ≥ 188.7 pg/mL	*p*
n	49.0	49.0	
Age, y	61.1 (17.4)	61.0 (14.4)	0.965
Women, %	46.9	46.9	0.999
White race, %	61.2	51.0	0.416
Systolic blood pressure, mm Hg	103.7 (22.3)	103.2 (24.2)	0.921
Diastolic blood pressure, mm Hg	63.3 (14.2)	57.9 (12.8)	0.052
Mean arterial pressure, mm Hg	75.8 (14.2)	71.9 (14.8)	0.189
Heart rate, beats/min	95.1 (23.8)	97.6 (21.5)	0.594
Body mass index, kg/m^2	27.1 (10.3)	29.2 (9.5)	0.311
Diabetes, %	20.4	30.6	0.354
Cardiovascular disease, %	57.1	49.0	0.544
Neurologic disease, %	24.5	20.4	0.809
Respiratory disease, %	14.3	32.7	0.057
Respiratory failure at enrollment, %	69.4	67.3	0.999
Cardiovascular failure at enrollment, %	77.6	71.4	0.643
WBC	14.5 (11.5)	15.9 (10.2)	0.514
Platelets	207.7 (116.4)	213.8 (155.2)	0.824
Hemoglobin	10.4 (2.1)	11.0 (2.4)	0.206
Lactic acid	3.2 (2.8)	4.0 (3.6)	0.226
Creatinine	1.6 (1.0)	2.2 (1.6)	0.045
Acute Physiology And Chronic Health Evaluation 2	26.4 (9.3)	26.7 (7.2)	0.846
Sequential Organ Failure Assessment	8.3 (3.4)	9.5 (3.2)	0.094
Renin–angiotensin system, day 0, median (25%–75%)			
Renin, pg/mL	82.3 (27.7–129.7)	547.5 (189.9–1,072.1)	<0.001
Ang-II, pg/mL	10.7 (7.0–13.0)	14.0 (9.7–22.8)	0.002
Ang-(1-7), pg/mL	19.2 (13.7–38.6)	49.4 (28.6–92.7)	<0.001
Ang-II/Ang-(1-7) ratio	0.46 (0.28–0.75)	0.32 (0.15–0.45)	0.015
ACE activity, ×10^5	10.9 (7.0–13.4)	10.3 (6.4–13.4)	0.864
ACE2 activity, ×10^3	9.1 (5.9–14.6)	11.2 (7.7–17.5)	0.277
Ventilator- and vasopressor-free days	29 (23–30)	23 (1–29)	0.025
Death within 30 d, %	12.2	30.6	0.049
Time to death, d, median (25%–75%)	14.5 (7.5–20.8)	11.0 (6.0–15.0)	0.412
Time to vent, high-flow nasal cannula, or VP start from first Vitamin C, Thiamine, and Steroids in Sepsis[a] dose, hr	9.5 (7.6–15.5)	14.8 (9.6–20.9)	0.056
Ventilator, VP, or both, %			0.679
Ventilator	22.4	28.5	
VP	30.6	32.7	
Both	46.9	38.8	

ACE = angiotensin-converting enzyme, Ang = angiotensin, HFNC = high-flow nasal cannula, VP = vasopressor.
[a]Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) cocktail (referring to 1.5 g IV vitamin C, 100 mg thiamine hydrochloride, and 50 mg hydrocortisone sodium succinate or matching placebos).

serum renin at day 0 were associated with progressively higher mortality (Q1 = ref, HR_{Q2} = 2.01 (0.37–11.04), HR_{Q3} = 3.22 (0.64–16.28), HR_{Q4} = 5.58 (1.18–26.32), p for linear trend = 0.023) (**Fig. 2**). A restricted cubic spline curve suggests that the association of serum renin with 30-day mortality is broadly linear (p for linearity = 0.012) (**Fig. 3**).

Renin Response by Day 3

Serum renin at day 3 also risk stratified critically ill patients at high risk of death. When dichotomized to the day 3 median renin values of 148.3 pg/mL, those patients whose renin levels exceeded the day 3 median remained at higher likelihood for death (HR = 2.89 [95% CI, 1.12–7.44], p = 0.028) (Supplemental Fig. 1, http://links.lww.com/CCM/H445). Sequential serum renin values were also associated with patient outcomes. An increase in serum renin values of 1 SD (593.6 pg/mL) from day 0 to day 3 was associated with a significant increase in mortality among all patients irrespective of treatment (HR = 3.75 [95% CI, 1.94–7.22], p < 0.001). **(Supplementary Table 2-S**, http://links.lww.com/CCM/H445) Additionally, patients whose renin levels remained persistently above the study median at day 0 and at day 3 were at a greater risk of death than those whose renin levels exceeded the day 0 study median and then fell to below the median on day 3 (HR = 2.89 [95% CI, 1.23–6.82], p = 0.015) (**Supplemental Fig. 2**, http://links.lww.com/CCM/H445). Furthermore, patients who experienced a decrease in renin had a lower risk of death than those who experienced an increase (HR = 0.22 [95% CI, 0.08–0.60], p = 0.003). Patients whose initial

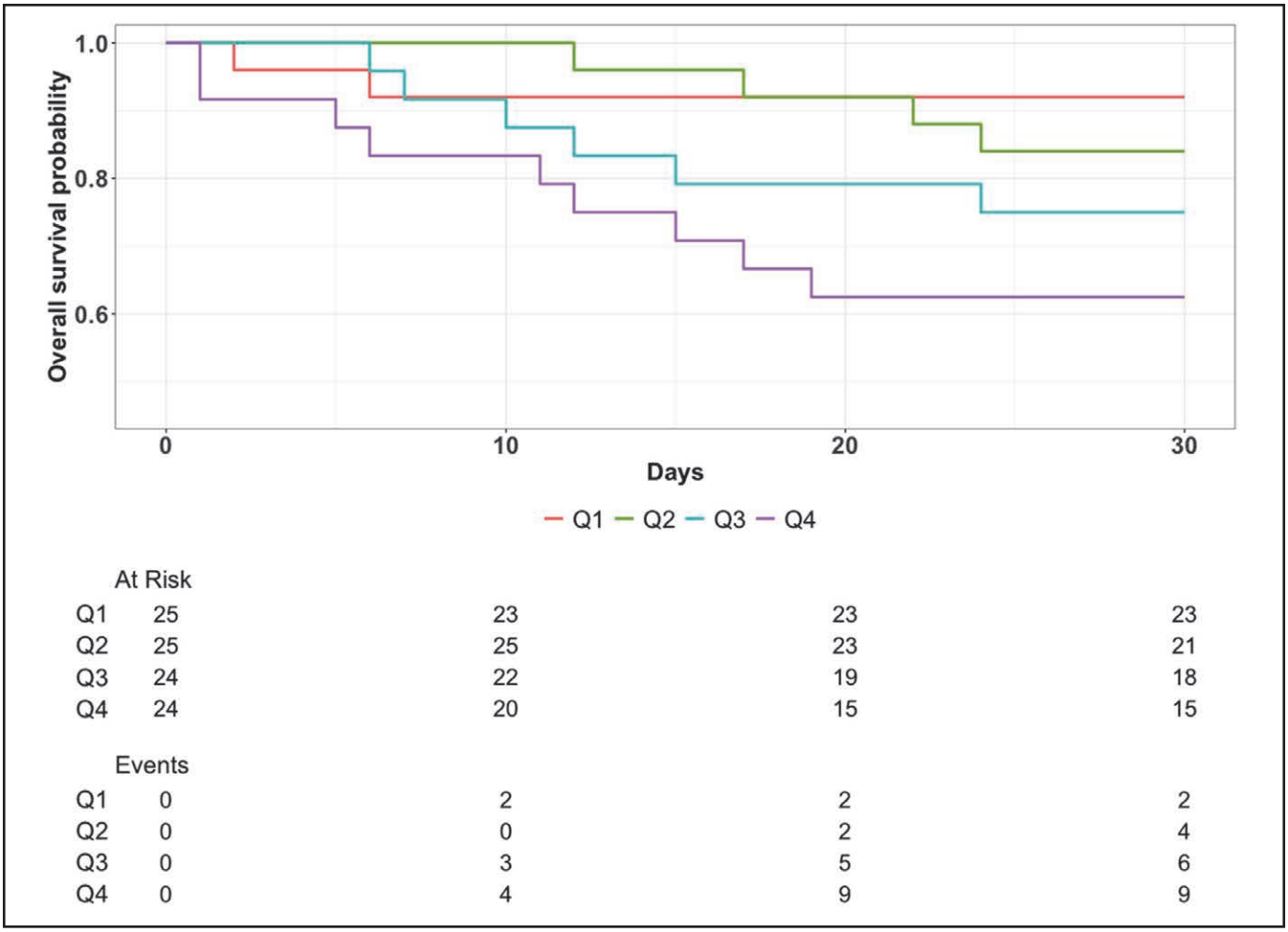

Figure 2. Kaplan-Meier estimate of survival by quartiles of renin. Cumulative survival to day 30 shown, stratified by renin quartiles for the population (Log-rank p = 0.057). Hazard ratios (HRs) per quartile, adjusted for age, sex, treatment, systolic blood pressure, Sequential Organ Failure Assessment, and cardiovascular failure at enrollment (vasopressor support): HR_{Q1} = ref, HR_{Q2} = 2.01 (0.37–11.04), HR_{Q3} = 3.22 (0.64–16.28), HR_{Q4} = 5.58 (1.18–26.32), p for linear trend = 0.023.

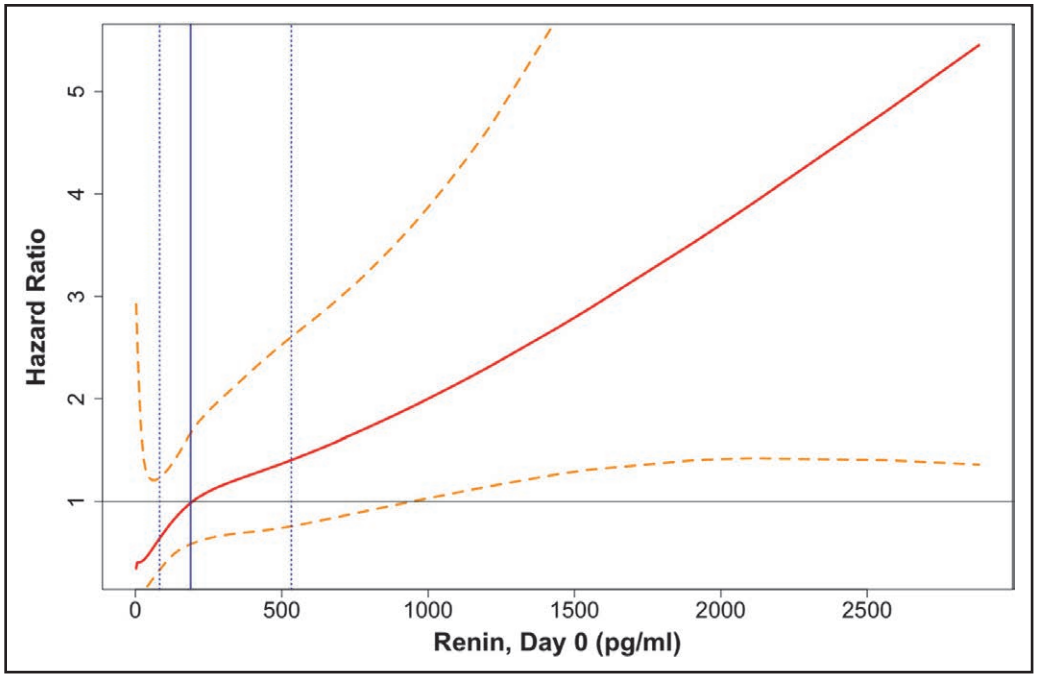

Figure 3. Restricted cubic spline of renin hazard ratio (HR). HR (*solid red line*) is calculated for day 0 renin vs 30-day mortality. *Orange dashed lines* represent the 95% CI. The *horizontal solid black line* represents the HR null value (1). The *vertical blue solid line* marks the median renin value (188.7 pg/mL), with the *vertical blue dotted lines* marking the 25th and 75th percentile of renin values. Likelihood ratio test for linearity: $p = 0.012$. This analysis suggests that the association of renin with mortality is broadly linear.

renin was below the day 0 median and had lower subsequent renin levels fared the best, whereas those with initial serum renin above the day 0 median that further increased fared the worst (log-rank $p < 0.001$) (**Supplemental Fig. 3**, http://links.lww.com/CCM/H445).

Angiotensins and Peptidase Activities

We evaluated the plasma levels of Ang-II and Ang-(1-7), as well as serum ACE and ACE2 activities (normal and historical values converted to molar concentrations are presented in Supplementary Table 4-S, http://links.lww.com/CCM/H445). In contrast to renin, a day 0 two-fold increase in values for Ang-II, Ang-(1-7), and the Ang-II to Ang-(1-7) ratio, as well as ACE and ACE2 activities, were not associated with 30-day mortality (all HR $p > 0.05$). At day 3, a two-fold increase in the plasma levels of both Ang-II and Ang-(1-7) was associated with a higher risk for death, but neither ACE nor ACE2 activities were statistically significant for this outcome (**Table 2**). When looking at RAS value changes over time, a 1-SD increase from day 0 to day 3 of Ang-II, Ang-(1-7), ACE and ACE2 were not associated with increased mortality (all p values > 0.05), in contrast to renin (Supplementary Table 2-S, http://links.lww.com/CCM/H445). In aggregate, although changes in serum renin were associated with mortality via all analyses, the same cannot be said for the other RAS elements, which showed an inconsistent signal.

VICTAS Treatment Effects

Stratifying the patient groups by treatment and the day 0 median renin value revealed that the VICTAS intervention appeared to be associated with lower mortality in patients above the renin median (> 188.7 pg/mL, interaction $p = 0.020$) (**Supplementary Table 3-S**, http://links.lww.com/CCM/H445). Conversely, for patients below the median, the VICTAS intervention was not associated with mortality (**Supplemental Fig. 4*A***, http://links.lww.com/CCM/H445). In contrast, patients with day 3 renin above the median (> 148.3 pg/mL) did not have a reduction in mortality (interaction $p = 0.137$) (Supplementary Table 3-S, http://links.lww.com/CCM/H445). Likewise, we did not see a mortality association for those patients with consistently high renin values (> 188.7 pg/mL at both day 0 and day 3) (**Supplemental Fig. 4**, http://links.lww.com/CCM/H445). When evaluating changes in RAS components by treatment arm, renin, Ang-II, and Ang-(1-7) were lower on day 3 on average irrespective of treatment. However, serum ACE and ACE2 activities were significantly lower (10% and 20%, respectively) only in those patients receiving the VICTAS intervention (**Supplemental Fig. 5**, http://links.lww.com/CCM/H445). When examining the cumulative treatment effects of the VICTAS intervention on components of the RAS, we found no discernable pattern of benefit or detriment.

TABLE 2.
Renin–Angiotensin System Levels and Death Within 30 Days (Via Cox Proportional Hazard)

Renin–Angiotensin System Assay	Unadjusted HR	Adjusted HR	HR, *p*	Treatment Interaction, *p*
Day 0 values				
Renin	1.34 (1.06–1.69)	1.36 (1.06–1.75)	0.015	0.011
Ang-II	1.05 (0.69–1.58)	0.95 (0.60–1.49)	0.815	0.086
Ang-(1-7)	1.15 (0.84–1.57)	1.09 (0.77–1.56)	0.625	0.538
Ang-II/Ang-(1-7) ratio	0.72 (0.48–1.07)	0.72 (0.48–1.08)	0.110	0.946
ACE activity	0.92 (0.66–1.28)	0.92 (0.65–1.29)	0.615	0.476
ACE2 activity	0.97 (0.61–1.55)	0.94 (0.58–1.54)	0.818	0.504
> Median renin	2.84 (1.10–7.33)	2.59 (0.99–6.77)	0.052	0.020
Day 3 values				
Renin	1.67 (1.26–2.21)	1.77 (1.30–2.42)	< 0.001	0.007
Ang-II	1.54 (1.09–2.17)	1.54 (1.05–2.24)	0.025	0.023
Ang-(1-7)	1.62 (1.15–2.26)	1.60 (1.10–2.33)	0.015	0.098
Ang-II/Ang-(1-7) ratio	0.65 (0.40–1.06)	0.65 (0.38–1.09)	0.100	0.654
ACE activity	0.83 (0.59–1.18)	0.76 (0.50–1.17)	0.212	0.224
ACE2 activity	1.14 (0.73–1.80)	1.10 (0.66–1.84)	0.706	0.200
> Median renin	2.89 (1.12–7.44)	2.73 (1.03–7.20)	0.043	0.137

ACE = angiotensin-converting enzyme, Ang = angiotensin, HR = hazard ratio, VP = vasopressor.
HR (95% CI) per two-fold increment in renin–angiotensin system peptide concentration or enzyme activity. Adjusted HR adjusted for age, sex, treatment with Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) cocktail (referring to 1.5 g IV vitamin C, 100 mg thiamine hydrochloride, and 50 mg hydrocortisone sodium succinate or matching placebos). Sequential Organ Failure Assessment, systolic blood pressure, and presence of cardiovascular failure at enrollment (vasopressors).

DISCUSSION

In this post hoc analysis of patients enrolled in the VICTAS study, we showed that baseline, day 3, and trends in serum renin were associated with mortality, whereas the angiotensin peptides and ACE activity did not show this consistent signal. When measured early during sepsis with organ dysfunction (within 24 hr of the onset of respiratory failure, septic shock, or both), a median serum renin of 188.7 pg/mL (5.1 pM) was able to delineate survival. These results support prior analyses in which a median renin concentration of 172.7 pg/mL (4.7 pM) was found to be predictive, and further suggest that a median renin between 170 and 190 pg/mL (4.6–5.1 pM) is an appropriate value for risk stratification (16). Furthermore, we showed that serum renin was also able to risk-stratify patients later in the course of disease, as evidenced by the day 3 serum renin of 148.3 pg/mL (4.0 pM), which was equally effective in predicting mortality. Importantly, serum renin concentrations, regardless of whether above or below the median, correlate linearly with mortality, as seen by the increasing HR for mortality by quartile, as well as the restricted cubic splines analysis. Finally, we demonstrated that the temporal trend in serum renin identified patients at high risk of death. Patients with persistently high renin or an increase in renin over time fared worse than those whose initial renin started and stayed low or trended lower over time. This analysis supports the value of serial renin levels to predict 30-day mortality in critical illness.

RAS dysregulation is well-described in critically ill patients. Critical illness-related hyperreninemic hypoaldosteronism was first described over 40 years ago (24), and since then it has been implicated in poor outcomes. Multiple studies have reported an elevated plasma renin activity or content in critically ill patients, all associated with higher mortality (25, 26). Lung injury, common in critically ill patients, may be a contributory factor. ACE is predominant in the

pulmonary capillary endothelium, and its activity is altered in lung injury commensurate with the extent of the pathology (13). Alterations in the circulating elements of the RAS are also associated with shock, including plasma renin activity (27). Reduced levels of ACE, or down-regulation of receptor pathways commonly seen in sepsis may result in diversion of the RAS pathway, specifically conversion of Ang-I to Ang-(1-7), which is vasodilatory, in contrast to the predominantly vasoconstrictor effects of Ang-II. However, our analysis of several components of the RAS not previously reported in clinical sepsis models revealed inconsistent association of ACE, ACE2, Ang-II, or Ang-(1-7) with overall risk. We acknowledge that additional studies are required to identify possible pathways within the RAS that may account for the present findings.

RAS biomarkers may be superior to currently available predictive models in sepsis. Low levels of both ACE and Ang-II levels have been shown to be better predictive of sepsis outcomes than Acute Physiology and Chronic Health Evaluation or SOFA scores (15). Additionally, renin kinetics may be superior to lactate kinetics in predicting in-hospital mortality (7). Further analyses demonstrated that the change in serum renin was a better predictor of tissue perfusion and mortality than a change in lactate, and that serum renin was not influenced by diurnal variation, renal replacement therapy, or drugs that influence the RAS pathway (6). Our study supports the concept of renin as a prognosticator of outcomes in the critically ill.

Our analysis has several strengths. This post hoc analysis was conducted using data from a well-executed randomized controlled trial. As such, patient-level clinical characteristics and demographics and biorepository sample collection were subject to rigorous study procedures. Additionally, enrollment criteria into VICTAS were pragmatic, with few exclusion criteria based on type of sepsis or underlying medical history. Therefore, we believe the results presented herein are generalizable to a broad definition of septic patients. Furthermore, our hypothesis is rooted in previously described and validated physiologic responses to sepsis, which include RAS dysfunction (notably an increase in renin), although we are the first to describe RAS trends and trajectories and association with mortality. Finally, our results are consistent with other similar analyses of renin values and mortality, which adds credence to our conclusions.

The current study is not prospective and preplanned, but rather a post hoc, retrospective analysis. As such, our results may be affected by unknown confounders and we were not powered to detect all potentially meaningful effects. However, we controlled for many important covariables in our statistical analysis which yielded similar unadjusted and adjusted results. We acknowledge that our analysis is limited in size and lacks a validation cohort, and therefore may be considered hypothesis-generating. Furthermore, any associations of RAS peptides with mortality should not be considered causal. We did not have adequate plasma volume in the VICTAS samples to measure other RAS peptides (i.e., Ang-I, Ang-[1-9], or Ang-[1-5]), so our conclusions cannot be applied definitively to all aspects of the RAS. Additionally, some RAS molecules are inherently unstable in vitro at room temperature and under certain conditions, and we cannot be sure if any metabolic or catabolic processes took place before stabilization of biorepository samples. However, recent data suggest sufficient stability exists to measure serum renin levels (28). Additionally, Ang-II, Ang-(1-7) are stable in EDTA-collected plasma that is stored at –80°C. The chelating agent EDTA inhibits a variety of metallopeptidases that metabolize Ang-II (aminopeptidases, endopeptidases [neprilysin], carboxypeptidases [ACE2]) or metabolize Ang-(1-7) (ACE) (8, 9). EDTA does not block the serine protease renin that may lead to exaggerated levels of Ang-I; however, we did not include this peptide in the current peptide measurements due to the absence of a renin inhibitor in the plasma samples (8). The main concern with peptide lability is the collection of serum without EDTA which may result in variability of values for Ang-II and Ang-(1-7), particularly when assayed by direct ELISAs (8, 29). To our knowledge, policies and procedures for collection, processing, and storage of VICTAS biorepository samples were well-established and followed sufficiently, minimizing this risk. Limited data prevented us from examining other potentially interesting endpoints such as vasopressor utilization or the effect of certain premorbid medications, including ACE inhibitors (ACEis) and angiotensin receptor blockers (ARBs) (as these agents may affect RAS levels of certain metabolites). However, it should be noted that others have concluded that ACEi/ARB therapy does not appear to affect renin levels (6). Even with the knowledge that Ang-II activation by itself may trigger

downstream inflammation, fibrotic, coagulation, and capillary permeability, this work is unable to determine the balance of physiologically normal versus abnormal RAS response with complete certainty. Although we do posit that some degree of RAS dysfunction is shown in this analysis (**Supplemental Fig. 6**, http://links.lww.com/CCM/H445), future investigations are needed to more fully elucidate the interdependence of RAS perturbations as well as the possible therapeutic effects of RAS manipulation. Finally, although we hoped at the outset to ascertain whether the VICTAS intervention exhibited any effect on RAS in this cohort of septic patients, the VICTAS trial was stopped administratively, rendering this analysis challenging. Because of the early termination of the study, our analysis of VICTAS arm versus control arm patients yielded results based on sample sizes that were small, and hypothesis-generating at best. This said, there may be plausible explanations for our observations. Ang-II is known to stimulate the formation of reactive oxygen species and the antioxidant properties of vitamin C may counteract this effect (30). This could possibly explain why patients with high renin levels that exhibit a two-fold increase in Ang-II have better outcomes when assigned to the VICTAS treatment arm than the control arm (Supplemental Fig. 4**, all panels**, http://links.lww.com/CCM/H445). Elevated levels of renin may also bind and stimulate the (pro)renin receptor that is directly linked to oxidative stress and fibrosis (31). Thus, it is possible that vitamin C may counteract this maladaptive response by reducing oxidative stress during the hyperreninergic response in septic shock. Be this as it may, analyses of vitamin C have consistently demonstrated that lack of clinical utility for sepsis treatment, so the effect of vitamin C on the RAS in this septic cohort may require additional analysis.

In summary, both increased content and patterns of changes of active serum renin appear to be strongly associated with 30-day mortality in this population of septic patients. Additionally, the level and pattern of change in renin is not associated with a commensurate increase in Ang-II, which may be indicative of RAS dysfunction. High Ang-II at day 3 is associated with increased mortality whereas a relative change in Ang-II and other metabolites do not demonstrate this association. The serial measurement of active renin may help to stratify those patients at high risk of death, and such a determination may support appropriate resources to manage these patients. Further research is needed to validate the circulating levels of active renin prospectively, particularly in relation to other RAS components including angiotensin metabolites, as well as explore possible disease-modifying treatment options based on RAS dysfunction.

1 *Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Emory University, Atlanta, GA.*

2 *Emory Critical Care Center, Emory Healthcare, Atlanta, GA.*

3 *Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, NC.*

4 *Division of Pulmonary and Critical Care Medicine, University of Maryland School of Medicine, Baltimore, MD.*

5 *Department of Anesthesiology, Section on Critical Care Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.*

6 *Department of Emergency Medicine, Johns Hopkins School of Medicine, Baltimore, MD.*

7 *Department of Emergency Medicine, Johns Hopkins University, The Johns Hopkins Hospital, Baltimore, MD.*

8 *Department of Emergency Medicine, Emory University School of Medicine, Atlanta, GA.*

9 *Grady Marcus Trauma and Emergency Care Center, Atlanta, GA.*

10 *Department of Anesthesiology, Section of Critical Care Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.*

11 *Outcomes Research Consortium, Cleveland, OH.*

12 *Perioperative Outcomes and Informatics Collaborative, Atrium Health Wake Forest Baptist Medical Center, Winston-Salem, NC.*

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccmjournal).

Drs. Busse and Schaich shared in the primary authorship of this article.

Funding for the trial was provided by the Marcus Foundation to Emory University.

Dr. Schaich received funding from the National Institute on Aging (K01AG073581) and an internal institutional pilot grant. Dr. Chappell's institution received funding from the National Heart, Lung, and Blood Institute (R01HL146818); he received support for article research from the National Institutes of Health (NIH). Dr. McCurdy's institution received funding from the Maryland Innovation Initiative; he disclosed he is chief medical officer for BOA Biomedical. Drs. Sevransky, Rothman, and Wright's institutions received funding from the Marcus Foundation. Dr. Sevransky's institution received funding from the Centers for Disease Control and Prevention and the Department of Health and Human Services; He disclosed he is an associate editor for Critical Care Medicine. Dr. Rothman disclosed use of Vit C, thiamine and steroid for treatment of sepsis. Dr. Wright's institution received funding from the NIH; he received funding from the

Neurotrauma Sciences Advisory Board and Astrocyte Pharma; he disclosed he was an expert witness for multiple different legal firms; he received support for article research from the Marcus Foundation. Dr. Khanna received grant funding from Hypertension and Vascular Research Center at the Wake Forest University School of Medicine (AG073581) to study mechanisms of renin dysregulation in shock. The remaining authors have disclosed that they do not have any potential conflicts of interest.

For information regarding this article, E-mail: laurence.w.busse@emory.edu

REFERENCES

1. Cascella M, Rajnik M, Cuomo A, et al: Features, evaluation and treatment coronavirus (COVID-19). In: *StatPearls* [Internet]. Treasure Island, FL, StatPearls Publishing, 2023
2. Pavon A, Binquet C, Kara F, et al; EPIdemiology of Septic Shock (EPISS) Study Group: Profile of the risk of death after septic shock in the present era: an epidemiologic study. *Crit Care Med* 2013; 41:2600–2609
3. Gupta S, Sakhuja A, Kumar G, et al: Culture-negative severe sepsis: Nationwide trends and outcomes. *Chest* 2016; 150:1251–1259
4. Seigel TA, Cocchi MN, Salciccioli J, et al: Inadequacy of temperature and white blood cell count in predicting bacteremia in patients with suspected infection. *J Emerg Med* 2012; 42:254–259
5. Nichol AD, Egi M, Pettila V, et al: Relative hyperlactatemia and hospital mortality in critically ill patients: A retrospective multicentre study. *Crit Care* 2010; 14:R25
6. Gleeson PJ, Crippa IA, Mongkolpun W, et al: Renin as a marker of tissue-perfusion and prognosis in critically ill patients. *Crit Care Med* 2019; 47:152–158
7. Jeyaraju M, McCurdy MT, Levine AR, et al: Renin kinetics are superior to lactate kinetics for predicting in-hospital mortality in hypotensive critically ill patients. *Crit Care Med* 2022; 50:50–60
8. Chappell MC: Biochemical evaluation of the renin-angiotensin system: The good, bad, and absolute? *Am J Physiol Heart Circ Physiol* 2016; 310:H137–H152
9. Chappell MC, Pirro NT, Sykes A, et al: Metabolism of angiotensin-(1-7) by angiotensin-converting enzyme. *Hypertension (Dallas, Tex)* 1998; 31(1 Pt 2):362–367
10. Bitker L, Burrell LM: Classic and nonclassic renin-angiotensin systems in the critically ill. *Crit Care Clin* 2019; 35:213–227
11. Chawla LS, Busse LW, Brasha-Mitchell E, et al: The use of angiotensin II in distributive shock. *Crit Care* 2016; 20:137
12. Chawla LS, Chen S, Bellomo R, et al: Angiotensin converting enzyme defects in shock: Implications for future therapy. *Crit Care* 2018; 22:274
13. Orfanos SE, Armaganidis A, Glynos C, et al: Pulmonary capillary endothelium-bound angiotensin-converting enzyme activity in acute lung injury. *Circulation* 2000; 102:2011–2018
14. Rice CL, Kohler JP, Casey L, et al: Angiotensin-converting enzyme (ACE) in sepsis. *Circ Shock* 1983; 11:59–63
15. Zhang W, Chen X, Huang L, et al: Severe sepsis: Low expression of the renin-angiotensin system is associated with poor prognosis. *Exp Ther Med* 2014; 7:1342–1348
16. Bellomo R, Forni LG, Busse LW, et al: Renin and survival in patients given Angiotensin II for catecholamine-resistant vasodilatory shock. A clinical trial. *Am J Respir Crit Care Med* 2020; 202:1253–1261
17. Khanna A, English SW, Wang XS, et al; ATHOS-3 Investigators: Angiotensin II for the treatment of vasodilatory shock. *N Engl J Med* 2017; 377:419–430
18. Sevransky JE, Rothman RE, Hager DN, et al; VICTAS Investigators: Effect of vitamin C, thiamine, and hydrocortisone on ventilator- and vasopressor-free days in patients with sepsis: The VICTAS randomized clinical trial. *JAMA* 2021; 325:742–750
19. Hager DN, Hooper MH, Bernard GR, et al: The vitamin C, thiamine and steroids in sepsis (VICTAS) protocol: A prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial. *Trials* 2019; 20:197
20. Brosnihan KB, Chappell MC: Measurement of angiotensin peptides: HPLC-RIA. *Methods Mol Biol* 2017; 1527:81–99
21. South AM, Nixon PA, Chappell MC, et al: Association between preterm birth and the renin-angiotensin system in adolescence: Influence of sex and obesity. *J Hypertens* 2018; 36:2092–2101
22. Pak K, Uno H, Kim DH, et al: Interpretability of cancer clinical trial results using restricted mean survival time as an alternative to the hazard ratio. *JAMA Oncol* 2017; 3:1692–1696
23. Stensrud MJ, Hernán MA: Why test for proportional hazards? *JAMA* 2020; 323:1401–1402
24. Zipser RD, Davenport MW, Martin KL, et al: Hyperreninemic hypoaldosteronism in the critically ill: A new entity. *J Clin Endocrinol Metab* 1981; 53:867–873
25. Chung KS, Song JH, Jung WJ, et al: Implications of plasma renin activity and plasma aldosterone concentration in critically ill patients with septic shock. *Korean J Crit Care Med* 2017; 32:142–153
26. Findling JW, Waters VO, Raff H: The dissociation of renin and aldosterone during critical illness. *J Clin Endocrinol Metab* 1987; 64:592–595
27. Nguyen M, Denimal D, Dargent A, et al: Plasma renin concentration is associated with hemodynamic deficiency and adverse renal outcome in septic shock. *Shock (Augusta, Ga)* 2019; 52:e22–e30
28. Hepburn S, Munday C, Taylor K, et al: Stability of direct renin concentration and plasma renin activity in EDTA whole blood and plasma at ambient and refrigerated temperatures from 0 to 72 hours. *Clin Chem Lab Med* 2022; 60:1384–1392
29. Chappell MC, Pirro NT, South AM, et al: Concerns on the specificity of commercial ELISAs for the measurement of angiotensin (1-7) and angiotensin II in human plasma. *Hypertension (Dallas, Tex. : 1979)* 2021; 77:e29–e31
30. Lee SH, Fujioka S, Takahashi R, et al: Angiotensin II-induced oxidative stress in human endothelial cells: Modification of cellular molecules through lipid peroxidation. *Chem Res Toxicol* 2019; 32:1412–1422
31. Fang H, Yang T, Zhou B, et al: (Pro)renin receptor decoy peptide PRO20 protects against oxidative renal damage induced by advanced oxidation protein products. *Molecules* 2023; 28:3017