

EDITORIAL

Is High-Dose Vitamin C Beneficial for Patients With Sepsis?

Emily B. Brant, MD; Derek C. Angus, MD, MPH

For centuries, scurvy was the scourge of naval exploration. Then, in 1747, in arguably the first clinical trial, James Lind assigned sailors sick with scurvy to alternative acid treatments, and noted that those who received citrus fruits, as sources of ascorbic acid (vitamin C), were the only ones who recovered.¹ Of note,

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scurvy was not eradicated immediately: Lind had not randomized the sailors and questioned whether their good outcomes were predestined rather than due to the ascorbic acid.² Decades elapsed before the British Navy began to routinely load fresh citrus on all voyages.

Today, scurvy is rare and easily treated. What remains unclear, however, is the role of vitamin C in clinical situations other than overt scurvy or severe vitamin C deficiency, such as with infection. Although unproven, common folklore holds that orange juice and lemons speed recovery from both influenza and the common cold, apparently due to a boost to the immune system. Linus Pauling, winner of 2 Nobel prizes, claimed a beneficial role for vitamin C across a wide range of diseases, including terminal cancer.³ More recently, researchers and clinicians have begun advocating for vitamin C as treatment for various critical illnesses, most notably sepsis.⁴

There are several explanations for the growing scientific enthusiasm. First, vitamin C deficiency, albeit less profound than that seen in classic scurvy in which plasma concentrations are less than 11 $\mu\text{mol/L}$, is common in patients with sepsis.⁵ Observational studies have estimated that nearly 40% of patients with sepsis are vitamin C deficient, defined as plasma concentrations less than 23 $\mu\text{mol/L}$ (to convert values to mg/dL , divide by 56.78).^{4,6} Furthermore, mean plasma vitamin C concentrations are significantly lower in patients with septic shock compared with patients without sepsis and approached scurvy levels in one study (mean [SD], 15.3 [7.9] $\mu\text{mol/L}$).⁶ Second, plasma concentrations of vitamin C in early sepsis are inversely correlated with measures of multiorgan dysfunction.⁵ Third, vitamin C plays a critical role in many physiologic processes typically deranged in sepsis. For example, vitamin C is crucial for endogenous synthesis of catecholamines.⁷ Moreover, preclinical work has demonstrated that vitamin C ameliorates cytokine surges implicated in sepsis-induced organ dysfunction.⁸ Fourth, small trials and retrospective cohort studies of vitamin C in humans have demonstrated promising results among patients with burns, sepsis, and septic shock.^{4,9,10} Nevertheless, no rigorous clinical trial has demonstrated a clear reduction in mortality among patients with sepsis and sepsis-associated organ dysfunction.

In this issue of *JAMA*, Fowler and colleagues¹¹ report the results of the CITRIS-ALI randomized trial, a double-blind,

placebo-controlled multicenter clinical trial in which 167 adults with sepsis and acute respiratory distress syndrome (ARDS) were randomized to receive high-dose intravenous vitamin C (50 mg/kg) or placebo every 6 hours for 96 hours. The trial was conducted among patients in the intensive care unit (ICU) with sepsis who were receiving mechanical ventilation and developed ARDS within 24 hours of ICU admission. Sepsis was defined as suspected or proven infection with presence of at least 2 of 4 systemic inflammatory response syndrome criteria,¹² and ARDS was defined using the Berlin criteria.¹³ Mechanical ventilation and fluid management strategies were consistent with the NHLBI ARDS Network recommendations.^{14,15}

Based on prior work,¹⁰ the authors hypothesized that the administration of high-dose vitamin C may attenuate organ dysfunction, inflammation, and vascular injury. Thus, the primary outcomes were a change from baseline to 96 hours in the modified Sequential Organ Failure Assessment score (mSOFA) and changes from baseline to 168 hours in C-reactive protein (a marker of inflammation) and thrombomodulin (a marker of vascular injury). The authors also evaluated 43 secondary outcomes, including a variety of measures of inflammation and organ recovery.

The cohort was well-balanced between the groups, with features typical of sepsis and severe ARDS. Pneumonia and intra-abdominal sepsis were the most common sources of infection and most patients were in septic shock at enrollment. Baseline median vitamin C concentrations were similarly deficient in both groups (22 $\mu\text{mol/L}$ in both groups). Plasma vitamin C concentrations were significantly higher in the treatment group during the infusion (median vitamin C concentration at 96 hours, 169 $\mu\text{mol/L}$ [IQR, 87-412] vs 26 $\mu\text{mol/L}$ [IQR, 9-41]; $P < .001$) and remained higher after treatment completion (median vitamin C concentration at 168 hours, 46 $\mu\text{mol/L}$ [IQR, 19-66] vs 29 $\mu\text{mol/L}$ [IQR, 12-39]; $P < .002$).

None of the 3 primary outcomes was statistically significantly different between the treatment groups. At 96 hours, mSOFA scores decreased overall but were not significantly different between groups (difference, -0.1 [95% CI, -1.2 to 1.0]; $P = .86$). In addition, at 168 hours, plasma levels of C-reactive protein and thrombomodulin also were not significantly different between groups (C-reactive protein difference, 7.94 [95% CI, -8.23 to 24.11]; $P = .33$; thrombomodulin difference, 0.69, 95% CI, -2.8 to 4.2; $P = .70$).

In secondary analyses, 28-day all-cause mortality rates were 29.8% in the treatment group and 46.3% in the placebo group, with a hazard ratio of 0.55 (95% CI, 0.33-0.90) favoring the treatment group, which was statistically significant without adjusting for multiple comparisons ($P = .01$), an obvious concern in a study with so many secondary outcomes.

At 28 days, treatment was associated with more ICU-free days and more hospital-free days to day 60, although it is notable that mortality also contributes to these outcomes. None of the other secondary analyses, including ventilator-free days or vasopressor use, showed a statistically significant difference. There were no reported adverse events.

The study has considerable strengths. First, it represents the largest randomized, placebo-controlled clinical trial of vitamin C administration in patients with sepsis. Second, the trial was performed well, with excellent protocol adherence, strong evidence that the intervention raised circulating vitamin C concentrations, and only 2 patients were lost to follow-up across 7 centers. Third, the enrolled patients appear representative of the target population. Fourth, considerable clinical and biological data were collected.

The study also has several important limitations. First, the timing of vitamin C administration in this cohort may make the null primary outcomes difficult to interpret. CITRIS-ALI was designed using pilot data from a phase 1 trial of patients who received vitamin C in the very early phases of sepsis.¹⁰ Enrollment in the present study required that patients have both sepsis and ARDS, potentially delaying drug administration. It is not clear if the lack of effect on organ dysfunction and plasma biomarkers was because vitamin C was given too late. Second, the authors tested only a single relatively high dose, which led to supranormal plasma vitamin C concentrations that were consistent with those of other studies reporting a potential benefit.⁹⁻¹¹ Nonetheless, it

remains possible that the lack of effect on the primary outcomes was due to dosage.

Despite this rigorous clinical trial, the question remains: does administration of vitamin C in patients with sepsis with organ dysfunction confer benefit? The difference in mortality is tantalizing and likely to spur much debate. However, this outcome was one of many secondary outcomes, and although reported as statistically significant, that finding was without adjustment for multiple comparisons. It is also concerning that none of the other outcomes that might explain why mortality would differ, such as organ dysfunction, or the inflammatory and vascular injury markers, appeared to differ between groups. Perhaps this lack of correlation is because the wrong measures were chosen, and vitamin C works via alternative mechanisms. Alternatively, the difference in mortality rates could be due to chance alone. No adverse events were reported, vitamin C is generally considered safe, and it is not expensive.

For all these reasons, further evaluation in a larger trial seems warranted. Should such a study be conducted, it would be helpful to reconsider optimal dosing and timing, as well as the likelihood that any potential benefits may only accrue to subsets of patients, given the underlying heterogeneity of sepsis. The search for an effective therapy for sepsis has thus far proven elusive. If vitamin C does indeed confer important benefits for patients with sepsis, it seems prudent to remember James Lind's doubts over his own study design and take all efforts to ensure that poor study design does not obfuscate the path to truth.

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Conflict of Interest Disclosures: Dr Angus reported receiving consulting fees from Ferring Pharmaceuticals Inc, Bristol-Myers Squibb, and Beckman Coulter Inc; holding stock in Alung Technologies Inc; and having a patent pending for selepressin (compounds, compositions, and methods for treating sepsis) filed with Ferring BV and another patent pending for proteomic biomarkers of sepsis in elderly patients filed with the University of Pittsburgh. No other disclosures were reported.

REFERENCES

1. Lind J. A treatise of the scurvy in three parts. Containing an inquiry into the nature, causes and cure of that disease, together with a critical and chronological view of what has been published on the subject. Edinburgh, UK: A. Miller; 1753.
2. Hughes RE. James Lind and the cure of scurvy: an experimental approach. *Med Hist*. 1975;19(4):342-351. doi:10.1017/S0025727300020469

3. Pauling L. Evolution and the need for ascorbic acid. *Proc Natl Acad Sci U S A*. 1970;67(4):1643-1648. doi:10.1073/pnas.67.4.1643

4. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, vitamin C and thiamine for the treatment of severe sepsis and septic shock: a retrospective before-after study. *Chest*. 2017;151(6):1229-1238. doi:10.1016/j.chest.2016.11.036

5. Borrelli E, Roux-Lombard P, Grau GE, et al. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. *Crit Care Med*. 1996;24(3):392-397.

6. Carr AC, Rosengrave PC, Bayer S, et al. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care*. 2017;21(1):300. doi:10.1186/s13054-017-1891-y

7. Kuhn SO, Meissner K, Mayes LM, Bartels K. Vitamin C in sepsis. *Curr Opin Anaesthesiol*. 2018;31(1):55-60. doi:10.1097/ACO.0000000000000549

8. Fisher BJ, Seropian IM, Kraskauskas D, et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. *Crit Care Med*. 2011;39(6):1454-1460. doi:10.1097/CCM.0b013e3182120cb8

9. Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: a randomized, prospective study. *Arch Surg*. 2000;135(3):326-331. doi:10.1001/archsurg.135.3.326

10. Fowler AA III, Syed AA, Knowlson S, et al; Medical Respiratory Intensive Care Unit Nursing.

Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med*. 2014;12:32. doi:10.1186/1479-5876-12-32

11. Fowler AA, Truitt JD, Hite RD, et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial [published October 1, 2019]. *JAMA*. doi:10.1001/jama.2019.11825

12. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med*. 2015;372(17):1629-1638.

13. Ferguson ND, Fan E, Camporota L, et al. The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. *Intensive Care Med*. 2012;38(10):1573-1582.

14. Grissom CK, Hirshberg EL, Dickerson JB, et al; National Heart Lung and Blood Institute Acute Respiratory Distress Syndrome Clinical Trials Network. Fluid management with a simplified conservative protocol for the acute respiratory distress syndrome. *Crit Care Med*. 2015;43(2):288-295. doi:10.1097/CCM.0000000000000715

15. Brower RG, Matthay MA, Morris A, et al; Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. 2000;342(18):1301-1308. doi:10.1056/NEJM200005043421801

Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure

The CITRIS-ALI Randomized Clinical Trial

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IMPORTANCE Experimental data suggest that intravenous vitamin C may attenuate inflammation and vascular injury associated with sepsis and acute respiratory distress syndrome (ARDS).

OBJECTIVE To determine the effect of intravenous vitamin C infusion on organ failure scores and biological markers of inflammation and vascular injury in patients with sepsis and ARDS.

DESIGN, SETTING, AND PARTICIPANTS The CITRIS-ALI trial was a randomized, double-blind, placebo-controlled, multicenter trial conducted in 7 medical intensive care units in the United States, enrolling patients (N = 167) with sepsis and ARDS present for less than 24 hours. The study was conducted from September 2014 to November 2017, and final follow-up was January 2018.

INTERVENTIONS Patients were randomly assigned to receive intravenous infusion of vitamin C (50 mg/kg in dextrose 5% in water, n = 84) or placebo (dextrose 5% in water only, n = 83) every 6 hours for 96 hours.

MAIN OUTCOMES AND MEASURES The primary outcomes were change in organ failure as assessed by a modified Sequential Organ Failure Assessment score (range, 0-20, with higher scores indicating more dysfunction) from baseline to 96 hours, and plasma biomarkers of inflammation (C-reactive protein levels) and vascular injury (thrombomodulin levels) measured at 0, 48, 96, and 168 hours.

RESULTS Among 167 randomized patients (mean [SD] age, 54.8 years [16.7]; 90 men [54%]), 103 (62%) completed the study to day 60. There were no significant differences between the vitamin C and placebo groups in the primary end points of change in mean modified Sequential Organ Failure Assessment score from baseline to 96 hours (from 9.8 to 6.8 in the vitamin C group [3 points] and from 10.3 to 6.8 in the placebo group [3.5 points]; difference, -0.10; 95% CI, -1.23 to 1.03; $P = .86$) or in C-reactive protein levels (54.1 vs 46.1 $\mu\text{g/mL}$; difference, 7.94 $\mu\text{g/mL}$; 95% CI, -8.2 to 24.11; $P = .33$) and thrombomodulin levels (14.5 vs 13.8 ng/mL; difference, 0.69 ng/mL; 95% CI, -2.8 to 4.2; $P = .70$) at 168 hours.

CONCLUSIONS AND RELEVANCE In this preliminary study of patients with sepsis and ARDS, a 96-hour infusion of vitamin C compared with placebo did not significantly improve organ dysfunction scores or alter markers of inflammation and vascular injury. Further research is needed to evaluate the potential role of vitamin C for other outcomes in sepsis and ARDS.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT02106975

JAMA. 2019;322(13):1261-1270. doi:10.1001/jama.2019.11825

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Acute respiratory distress syndrome (ARDS) is a common sepsis-associated organ injury and results in significant mortality¹⁻⁴; for instance, the LUNG SAFE study reported 34% to 45% mortality.⁵ Despite advances in understanding mechanisms that lead to ARDS,⁶ disease-modifying strategies have not improved outcomes.⁷⁻¹² Previous research found that vitamin C attenuates systemic inflammation, corrects sepsis-induced coagulopathy, and attenuates vascular injury.¹³⁻¹⁵ The CITRIS-ALI randomized trial examined whether high-dose vitamin C would reduce organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and ARDS.

Methods

Study Oversight

The Food and Drug Administration provided oversight for this trial and issued Investigational New Drug 113856 for its performance. The study protocol, including the complete statistical analysis plan, is available in Supplement 1. The CITRIS-ALI protocol was approved by the institutional review boards at each of 7 participating medical centers. Written informed consent was obtained from patients or patients' legally authorized representatives. Patients were enrolled from September 2014 through November 2017, with last patient follow-up in January 2018.

Study Patients

After intensive care unit (ICU) admission for sepsis, patients were followed up for development of acute respiratory failure. They were included in CITRIS-ALI if they were undergoing mechanical ventilation through an endotracheal tube, had a Pao_2 to Fio_2 ratio less than 300 mm Hg, had bilateral opacities by chest radiography within 1 week of known clinical insult, had new or worsening respiratory symptoms without evidence of left atrial hypertension,¹⁶ had suspected or proven infection, and met 2 of 4 systemic inflammatory response criteria.¹⁷ All criteria had to be met within a 24-hour period. Patients were excluded if they had a known allergy to vitamin C; there was no ability to obtain informed consent; they were younger than 18 years, non-English speaking, or a ward of the state; more than 48 hours had elapsed since they met ARDS criteria (ie, informed consent was required to occur within 48 hours of the patients' meeting ARDS criteria); they did not have a patient surrogate or physician committed to full support; they were pregnant or breastfeeding; they were moribund and not expected to survive 24 hours; they required home mechanical ventilation (via tracheostomy or noninvasively); they were receiving home oxygen greater than 2 L/min; or they had interstitial lung disease, diffuse alveolar hemorrhage, diabetic ketoacidosis, or an active kidney stone.

Patients' race or ethnicity was determined by either a researcher or legal next of kin by open-ended questions. No patient was excluded because of race, ethnicity, or sex. Inclusion of race/ethnicity in this study was required by the funding agency.

Key Points

Question Can intravenous administration of high-dose vitamin C reduce organ failure scores and biomarkers of inflammation and vascular injury among patients with sepsis and acute respiratory distress syndrome (ARDS)?

Findings In this randomized clinical trial that included 167 patients in the intensive care unit, intravenous infusion of high-dose vitamin C vs placebo for 96 hours resulted in no significant differences in the modified Sequential Organ Failure Assessment score at 96 hours, or in levels of C-reactive protein and thrombomodulin at 168 hours.

Meaning Among patients with sepsis and ARDS, high-dose vitamin C infusion compared with placebo did not significantly reduce organ failure scores at 96 hours or improve biomarker levels at 168 hours.

Randomization, Masking, and Study Drug Administration

Patients were randomized 1:1 to receive vitamin C or placebo (Figure 1), using randomization generated by the VCU Health Investigational Drug Service by means of Research Randomizer at <http://www.randomizer.org>. Blinding was maintained by the investigational pharmacy at each institution. Investigators were blinded from onset of enrollment to completed analysis of primary and secondary outcomes. Patients were randomized to receive vitamin C or placebo. Vitamin C was intravenously infused in the treatment group at 50 mg/kg actual body weight every 6 hours for 96 hours. Infusion bags (50 mL) containing the calculated vitamin C dosage in dextrose 5% in water or placebo (dextrose 5% in water alone) were prepared at study site investigational pharmacies, light protected with amber covers, and stored refrigerated at 2°C to 8°C for up to 24 hours.

At infusion, ICU nursing infused the hooded study agent through light-protected tubing during 30 minutes. Dose 1 of the study drug was administered within 6 hours of randomization or at earliest available times after clinically indicated procedures (eg, imaging) requiring patients to be out of the ICU. Study drug infusion was stopped when the final dosage was administered (ie, hour 96) or at ICU discharge, discharge from the study hospital, study withdrawal, or death, whichever occurred first.

Vitamin C for injection (Ascor) was supplied by McGuff Pharmaceuticals in amber 50-mL vials containing pH-balanced L-ascorbic acid for injection at 500 mg/mL (Supplement 2).

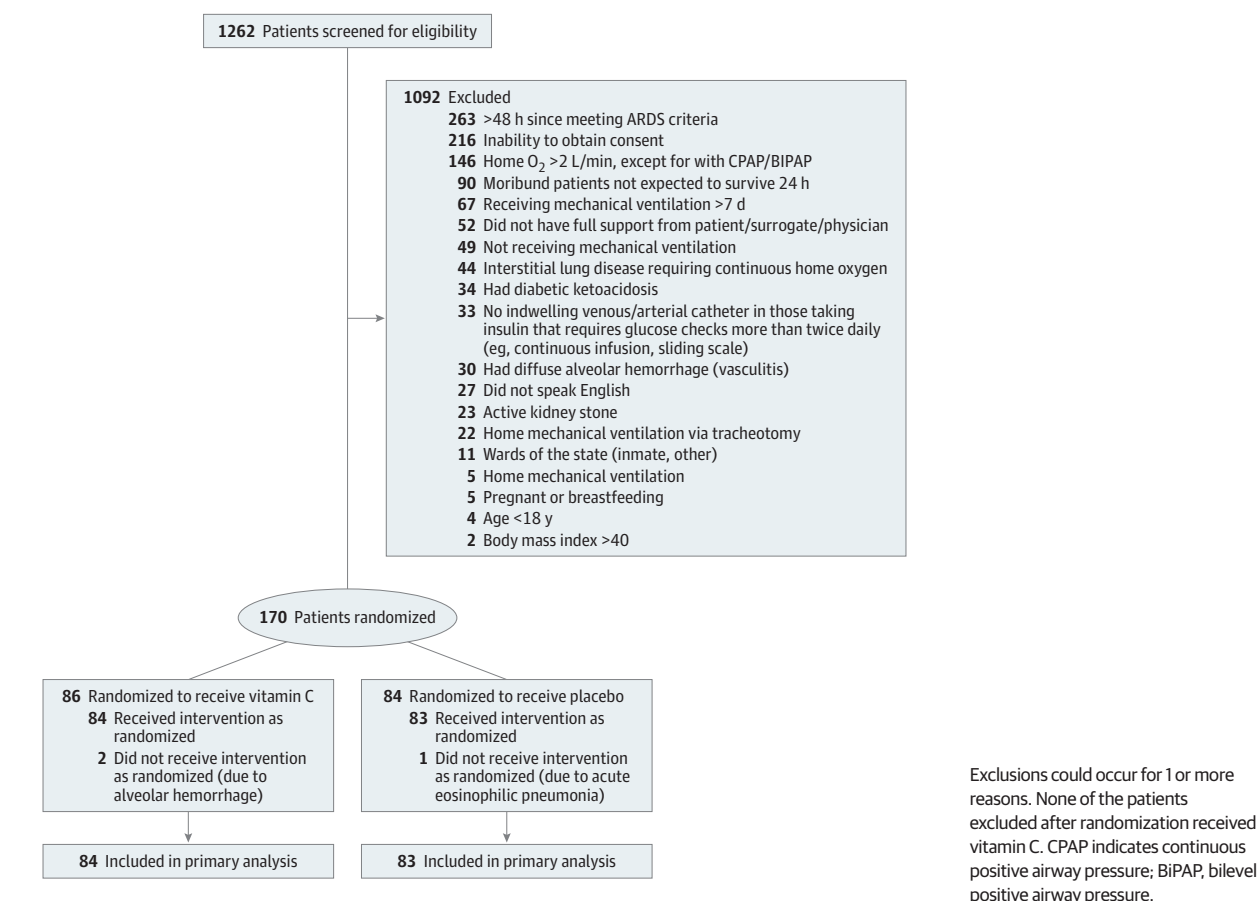
Ventilator Procedures and Fluid Administration

Mechanical ventilation was performed with ARDS Network tidal volume settings.¹⁸ A conservative fluid administration protocol was used as described in the ARDSNet FACTT Lite trial.¹⁹ FACTT Lite was instituted within the first 4 hours of randomization of patients and was continued until unassisted breathing or study day 7, whichever occurred first.

Outcomes

The primary outcomes were modified Sequential Organ Failure Assessment (mSOFA) scores at 96 hours and plasma

Figure 1. Flow of Patients Through the CITRIS-ALI Trial in Sepsis and Acute Respiratory Distress Syndrome



biomarker levels (C-reactive protein and thrombomodulin) at 168 hours. The selection of the primary outcomes was based on a previously performed phase 1 trial.¹⁵ There were 46 pre-specified secondary outcomes, including all-cause mortality at day 28, ventilator-free days to day 28, ICU-free days to day 28, and hospital-free days at day 60.²⁰ Ventilator-free days were defined as the number of days a patient was extubated from mechanical ventilation, after ICU admission to day 28. Days requiring reintubation were subtracted from the total days to day 28. If the patient died in the hospital postextubation, a value of zero was assigned. ICU-free days began the moment the patient was transferred out of the ICU to day 28. If the patient was readmitted to the ICU, the days were subtracted from the total ICU-free days. Hospital- and ICU-free days were calculated similarly.

Additional secondary outcomes at study hours 0, 48, 96, and 168 were oxygenation index ($F_{iO_2} \times \text{mean airway pressure}/P_{aO_2}$) (if ventilated), VE-40 (minute ventilation, L/min) ($\text{vent RR} [\text{respiratory rate}] \times \text{tidal volume/weight}$) $\times (P_{aCO_2}/40)$ (if intubated), and SOFA score components (ie, P_{aO_2} to F_{iO_2} ratio, Sp_{O_2} to F_{iO_2} ratio, platelet counts, total bilirubin, vasopressor use, Glasgow Coma Scale score, creatinine level, and biomarkers [angiotensin 2, procalcitonin, receptor for advanced glycation end products, tissue factor pathway inhibitor, and plasma ascorbate concentrations]).

Study Measurements and Procedures

For organ system failure, the SOFA score was modified, eliminating bilirubin during the blinded analysis period as a result of many missing values because clinicians determined that bilirubin measurement was not clinically indicated (mSOFA, range from 0 [normal organ function] to 20 [worst organ function]). In previous research, elimination of a SOFA component did not affect its predictive ability.²¹⁻²³ mSOFA score was measured at hour 0 and then at hours 48 and 96.²⁴

Plasma was obtained for quantification of vitamin C levels at enrollment and then at hours 48, 96, and 168. High-pressure liquid chromatography was used to quantify plasma vitamin C (Supplement 2). Blood was obtained at hours 0, 48, 96, and 168 for biomarker analysis. Tubes were placed on ice and centrifuged (1000g) within 30 minutes of collection. Aliquoted plasma was stored at -80°C for batch analysis by Luminex technology (Supplement 2).

Statistical Analysis

Sample size was calculated with means, variances, and correlations for SOFA, C-reactive protein, and thrombomodulin data from another trial.¹⁵ Simulations (1000) were performed at sample sizes of 75 to 100 per group for each of 3 outcome variables and empirical power was calculated according to

Table 1. Baseline and Follow-up Characteristics of All Patients

Variable	Vitamin C (n = 84)	Placebo (n = 83)
Demographic data, No. (%)		
Age, median (IQR), y	54 (39-67)	57 (44-70)
Men	45 (54)	45 (54)
Women	39 (46)	38 (46)
Non-Hispanic white	68 (81)	60 (72)
Non-Hispanic black	13 (15)	19 (23)
Hispanic/Asian/ Pacific Islander	3 (4)	4 (5)
Sepsis etiology, No. (%)		
Thorax	69 (82)	58 (70)
Abdomen	6 (7)	13 (16)
Urinary tract	3 (4)	2 (2)
Central nervous system	1 (1)	3 (4)
Central venous catheter	0	1 (1)
Unknown/other	5 (6)	6 (7)
Admission source, No. (%)		
Emergency department	39 (46)	36 (43)
Outside hospital transfer	26 (31)	28 (34)
Inpatient ward transfer	17 (20)	18 (22)
Operating room	1 (1)	1 (1)
Direct admission	1 (1)	0
Kidney failure, No. (%)		
Acute kidney failure, No. (%)	21 (25)	26 (31)
Chronic kidney failure/ dialysis, No. (%)	7 (8)	8 (10)
Respiratory, mean (SD)		
Tidal volume, mL	423.7 (88.4)	418.3 (85.5)
Pao ₂ /Fio ₂ ratio at baseline	189.3 (95.9)	214.5 (182.8)
PEEP, cm H ₂ O	9.9 (4.0)	9.6 (4.0)
Oxygenation index, ^a mean (SD)	10.7 (7.4)	10.1 (6.3)
Incidence of shock, No. (%)		
At baseline, vasopressor in use	57 (68)	60 (72)
mSOFA scores, ^b mean (SD)		
At randomization	9.8 (3.2)	10.3 (3.1)
At 96 h	8.02 (4.2)	6.96 (3.5)
Corticosteroid use during study, No. (%)	56 (67)	54 (65)
IV fluids, mL/kg/24 h		
Day 1, mean (SD)	40 (28.5)	42.6 (35.5)
Day 7, mean (SD)	32.8 (19.6)	33.9 (16.8)
Day 1, median (IQR)	35.1 (21.2-50.3)	33.9 (20.2-55.3)
Day 7, median (IQR)	26.5 (19.7-40.9)	26.8 (16.7-38.3)
Urine output, mL/kg/24 h		
Day 1, mean (SD)	14.1 (14.5)	10.5 (11.7)
Day 7, mean (SD)	24.4 (24.9)	24.6 (22.9)
Day 1, median (IQR)	9.9 (3.9-20)	6.7 (1.7-15)
Day 7, median (IQR)	18.2 (1.5-36)	20.9 (6.1-34.4)

Abbreviations: IQR, interquartile range; PEEP, positive end-expiratory pressure; mSOFA, modified Sequential Organ Failure Assessment; IV, intravenous.

^a Oxygenation index = mean airway pressure × Fio₂/PaO₂.

^b mSOFA score range, 0 to 20 (values >8 indicate severe illness).

means and sample sizes. In simulations (18 000), a mixed linear model was used to assess the effect of treatment on outcome and to fit a repeated-measures analysis of variance. The model included 1 between-participant factor (group [placebo or vitamin C]), 1 within-participant factor (time [0, 12, 24, 36, 48, 72, and 96 hours, and 7 days]), and the interaction between group and time, testing the hypothesis that differences between treatment groups are the same over time. Group difference at 96 hours for each of the 3 outcomes was calculated with simulations.

Power estimates for the various sample sizes were derived according to the difference at 96 hours in the 3 co-primary end points (SOFA, C-reactive protein, and thrombomodulin). Significance was set at $\alpha = .05$ and a Holm-Bonferroni correction for 3 co-primary end points was conducted. Only if the smallest *P* value was less than .02, the second smallest less than .03, and the largest less than .05 was simulation considered a success. Simulations and calculations resulted in the empirical power based on various sample sizes (eTable 5 in Supplement 2).

In accordance with these calculations, CITRIS-ALI enrolled 170 patients (85 per group) to allow for 10% dropouts, providing a statistical power of 80%, with an $\alpha < .05$. Testing was 2 sided. Effects are reported with a point estimate and 95% CIs in addition to *P* values. Distributions of measures were examined to identify outliers.

The primary end points were analyzed with a mixed linear model and fit to repeated-measures analysis of variance as described earlier. All-cause mortality to day 28 was estimated with a Kaplan-Meier analysis, and survival curves were compared with the Wilcoxon test because of its greater sensitivity to earlier survival differences. Nonlongitudinal data were analyzed with linear regressions, using a random effect for study site and *P* < .05. Testing was 2 sided. Missing data patterns were examined, revealing monotone missing data, whose pattern provides evidence that assumptions of missing completely at random and missing at random may be violated. Multiple imputation with a pattern-mixture model with control-based pattern imputation was used, with 50 imputed data sets created and analyzed to examine the effect of monotone missing data.

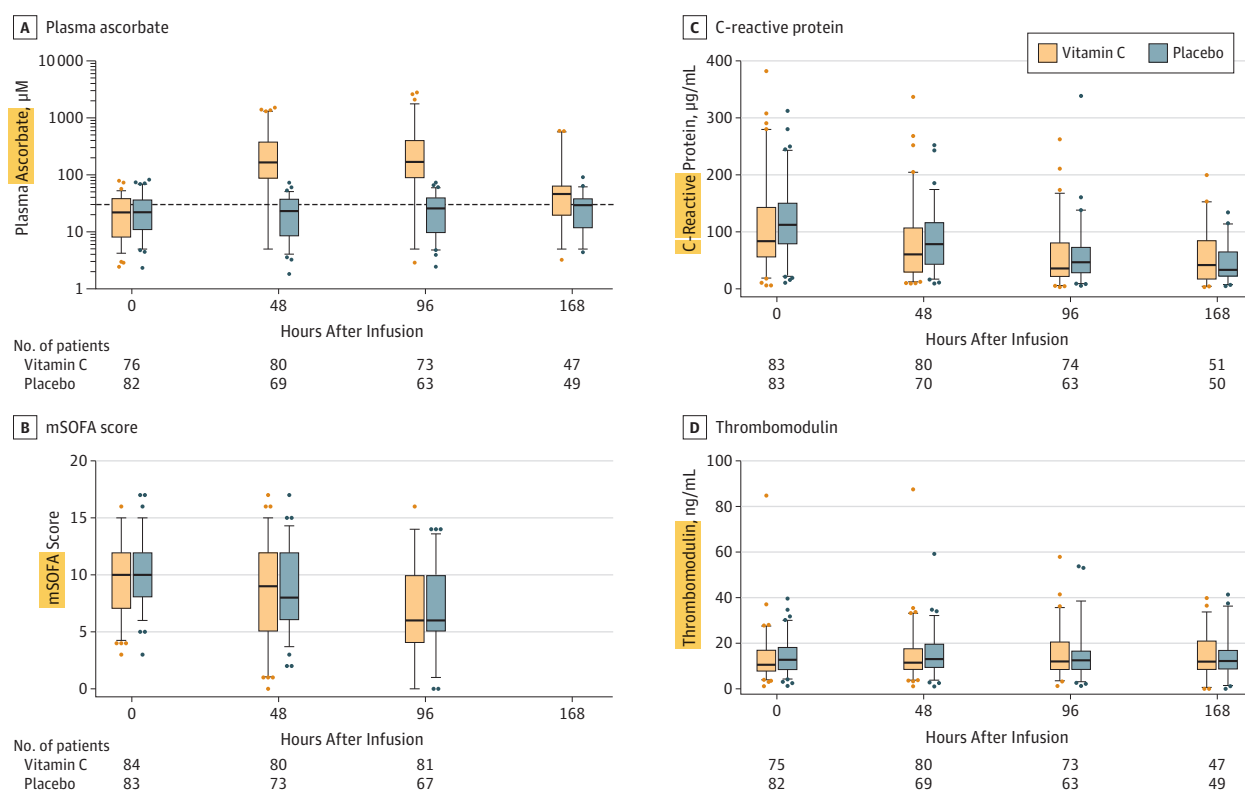
Because of a potential for type I error caused by multiple comparisons, findings for analyses of secondary end points should be interpreted as exploratory. Secondary end points and the primary outcomes were analyzed similarly, with exclusion of the multiple imputations. Statistical analysis was performed with SAS version 9.4, Stata version 15.1, and GraphPad Prism version 7.00.

Results

Characteristics of the Patients

A total of 1262 eligible patients were identified, of whom 1062 were excluded because of the diagnoses listed in Figure 1. Three patients were excluded after randomization because of a subsequent diagnosis of diffuse alveolar hemorrhage (*n* = 2) and acute eosinophilic pneumonia (*n* = 1). None

Figure 2. Plasma Ascorbate Concentrations, Modified Sequential Organ Failure Assessment Score, and Plasma Biomarkers



Median values are shown by the horizontal line inside the box, interquartile range (IQR) by the top and bottom of the boxes, 95% CI by the whiskers, and values were outside the 5th and 95th percentiles by circles. Box plots have been offset to avoid superimposition.

A, Plasma ascorbate levels at enrollment were marginally deficient (dotted line, $<28 \mu\text{M}$) in both groups. After initiation of treatment, plasma ascorbate levels were significantly greater in vitamin C patients compared with placebo patients (median at baseline, 22 vs $22 \mu\text{M}$ [IQR, 8 - 39 vs 11 - 37], $P = .68$). At 48 hours, median was $166 \mu\text{M}$ [IQR, 88 - 376] for vitamin C vs $23 \mu\text{M}$ for placebo [9-37], $P < .001$. At 96 hours, median was $169 \mu\text{M}$ [IQR, 87 - 412] for vitamin C vs $26 \mu\text{M}$ for placebo [9-41], $P < .001$. At 168 hours, median was $46 \mu\text{M}$ [IQR, 19 - 66] for

vitamin C vs $29 \mu\text{M}$ for placebo [12-39], $P < .002$. After cessation of vitamin C infusion, plasma levels in the vitamin C cohort decreased below $100 \mu\text{M}$ but remained significantly higher than plasma ascorbate levels in placebo patients.

B, There was no difference in the initial modified Sequential Organ Failure Assessment (mSOFA) scores of the 2 groups at baseline (vitamin C vs placebo, median, 10 [IQR, 7 - 12] vs $10 \mu\text{M}$ [8-12]). During the next 96 hours, mSOFA scores decreased but were not different between the 2 groups.

C and D, Plasma levels of C-reactive protein and thrombomodulin were measured at baseline and at 48, 96, and 168 hours. At no time were the levels of these significantly different between the 2 groups.

of these patients received vitamin C. One hundred sixty-seven patients were randomized, with 83 assigned to placebo and 84 to vitamin C. Baseline demographic data (eg, sex, race) were similar between the 2 groups (Table 1). The presumed etiology of sepsis most commonly resulted from pneumonia (Table 1). Other etiologies included central line sepsis, urosepsis, typhilitis in leukemia patients, cellulitis, and leukopenia in bone marrow transplant patients.

Primary Outcomes

There was no statistically significant difference in mSOFA scores between placebo and the vitamin C-infused patients from enrollment to 96 hours (Figure 2). The mean mSOFA score from baseline to 96 hours decreased from 9.8 to 6.8 in the vitamin C group (3 points) and from 10.3 to 6.8 in the placebo group (3.5 points) (difference, -0.10 ; 95% CI, -1.23 to 1.03 ; $P = .86$). There were no significant differences between the vitamin C group and placebo group in the C-reactive protein levels (54.1 vs $46.1 \mu\text{g/mL}$; difference,

7.94 ; 95% CI, -8.23 to 24.1 ; $P = .33$) or thrombomodulin levels (14.5 vs 13.8 ng/mL ; difference, 0.69 ; 95% CI, -2.8 to 4.2 ; $P = .70$) assessed at 168 hours. Two patients were lost to follow-up.

Secondary Outcomes

Forty-three of the 46 prespecified secondary outcomes were not significantly different between the vitamin C group and the placebo group (Table 2). In exploratory analyses that did not adjust for multiple comparisons, 3 secondary outcomes were significantly different between groups. At day 28, mortality was 46.3% (38/82) in the placebo group vs 29.8% (25/84) in the vitamin C group ($\chi^2 = 4.84$; $P = .03$; between-group difference, 16.58% [95% CI, 2% to 31.1%]) (Figure 3). The Kaplan-Meier survival curves for the 2 groups were significantly different by the Wilcoxon test ($\chi^2_1 = 6.5$; $P = .01$).

The number of ventilator-free days was 13.1 in the vitamin C group vs 10.6 in the placebo group (mean difference, 2.47;

Table 2. Secondary Outcomes in a Trial of Vitamin C Infusion in Patients With Sepsis and Severe Acute Respiratory Failure

Variable	Hour	Vitamin C			Placebo			Difference, Coefficient (95% CI)	P Value
		No.	Median or %	IQR	No.	Median or %	IQR		
Angiotensin-converting enzyme-2, median (IQR), ng/mL ^a	0	83	8.8	14.9	83	11.6	17.5	-5.1 (-10.9 to 0.6)	.08
	48	80	6.9	11.5	70	9.7	16.9	-2.4 (-9.0 to 4.2)	.48
	96	74	5.7	9.5	63	5.2	7.3	3.8 (-2.5 to 10.0)	.24
	168	53	5.3	6.4	51	3.9	5.5	2.9 (-2.0 to 7.9)	.24
Procalcitonin, median (IQR), ng/mL ^b	0	83	2.4	8.5	83	3.7	19.7	-14.3 (-30.0 to 1.5)	.07
	48	80	1.1	5.2	70	1.7	5.5	-1.3 (-7.4 to 4.8)	.68
	96	74	0.7	2.5	63	0.7	2.2	-1.0 (-3.6 to 1.6)	.44
	168	53	0.5	1.6	51	0.5	1.3	-1.5 (-4.6 to 1.7)	.36
RAGE, median (IQR), ng/mL ^c	0	83	4.0	5.2	83	5.0	6.2	-0.9 (-2.5 to 0.7)	.26
	48	80	2.8	4.2	70	3.3	4.0	0.3 (-0.9 to 1.4)	.67
	96	74	2.1	4.0	63	2.0	3.4	0.8 (-0.3 to 1.8)	.15
	168	53	1.7	2.9	51	1.5	3.2	0.3 (-0.6 to 1.1)	.55
TFPI, median (IQR), ng/mL ^d	0	83	36.7	52.4	83	36.3	33.7	3.4 (-6.2 to 13.0)	.48
	48	80	31.3	34.5	70	34.3	33.4	-0.5 (-10.8 to 9.8)	.92
	96	74	36.2	39.0	63	36.7	30.6	-2.0 (-11.2 to 7.2)	.66
	168	53	30.5	51.0	51	30.2	31.7	1.6 (-11.1 to 14.2)	.81
Vasopressor use, %	0	84	64.3		83	71.1		-6.8	.35
	48	82	54.9		72	50.0		4.9	.55
	96	80	30.0		65	27.7		2.3	.76
	168	72	22.2		59	10.2		10	.07
Oxygenation index, ^e median (IQR)	0	76	0.082	0.090	80	0.089	.077	0.129 (-0.096 to 0.353)	.26
	48	53	0.058	0.062	57	0.056	.054	0.004 (-0.016 to 0.023)	.71
	96	42	0.079	0.084	33	0.045	.040	0.016 (-0.017 to 0.049)	.33
	168	28	0.052	0.051	16	0.074	.066	-0.003 (-0.050 to 0.044)	.90
VE-40 ^f to median (IQR)	0	78	0.126	0.044	81	0.115	.058	0.013 (-0.002 to 0.028)	.09
	48	59	0.109	0.049	60	0.110	.061	0.001 (-0.017 to 0.019)	.94
	96	46	0.129	0.060	39	0.105	.047	0.036 (-0.015 to 0.086)	.16
	168	29	0.114	0.043	17	0.118	.057	-0.020 (-0.055 to 0.015)	.26
Net fluid balance to median (IQR), mL	0	83	1604	2927	79	1901	3034	-3759 (-1123 to 373)	.32
	48	81	768	2471	73	473	1797	545 (-255 to 1345)	.18
	96	76	134	2168	66	-659	2560	792 (208 to 1376)	.01
	168	57	190	2076	54	-380	2213	496 (-206 to 1198)	.16
All-cause mortality to day 28, %			84	29.8	38	82	46.3	-0.17	.03
Ventilator-free days to day 28, median (IQR), d		84	17	24	82	8	22	2.5 (-0.9 to 5.9)	.15
ICU-free days to day 28, median (IQR), d		83	11	21	82	0	18	3.2 (0.3 to 6.0)	.03
Hospital-free days, to day 60, median (IQR), d		82	22	46	80	0	39	7.0 (0.3 to 13.8)	.04
Bilirubin, total, median (IQR), mg/dL	0	77	0.7	2.8	79	1.2	1.7	0 (-1.3 to 1.2)	.96
	48	72	0.8	2.0	68	0.8	2.3	0.1 (-1.3 to 1.5)	.87
	96	62	0.8	2.5	64	0.8	1.7	0.6 (-1.1 to 2.2)	.50
	168	45	0.6	1.0	42	0.8	1.0	0.6 (-1.6 to 2.7)	.60
Creatinine, median (IQR), mg/dL	0	84	1.4	1.3	83	1.7	1.8	-0.1 (-0.6 to 0.3)	.49
	48	82	1.6	1.4	73	1.2	1.0	0.2 (-0.3 to 0.6)	.41
	96	78	1.1	1.1	65	1.1	.9	0.1 (-0.2 to 0.5)	.47
	168	64	1.0	1.2	55	1.2	1.6	0.1 (-0.5 to 0.6)	.82
Platelets, median (IQR), ×10 ³ /μL	0	83	147	174	83	160	137	-5.1 (-45.0 to 34.8)	.80
	48	82	137	166	73	116	153	23.8 (-15.9 to 63.4)	.24
	96	77	144	183	63	146	175	15.2 (-29.5 to 59.9)	.50
	168	64	183	209	55	219	149	-2.4 (-59.9 to 55.0)	.93

(continued)

Table 2. Secondary Outcomes in a Trial of Vitamin C Infusion in Patients With Sepsis and Severe Acute Respiratory Failure (continued)

Variable	Hour	Vitamin C			Placebo			Difference, Coefficient (95% CI)	P Value
		No.	Median or %	IQR	No.	Median or %	IQR		
Glasgow Coma Scale score, median (IQR)	0	84	8	4	83	7	5	0.7 (−0.3 to 1.6)	.15
	48	80	9	5	72	9	4	0.6 (−0.5 to 1.7)	.30
	96	75	10	5	66	10	3	−0.4 (−1.7 to 0.8)	.49
	168	56	10	6	53	12	6	−1.5 (−3.0 to −0.1)	.04
SOFA component, cardiovascular, median (IQR)	0	84	3.0	4.0	83	3.0	3.0	−0.2 (−0.8 to 0.3)	.35
	48	80	3.0	4.0	73	3.0	4.0	0.1 (−0.5 to 0.6)	.82
	96	71	0	3.0	61	0	3.0	0.2 (−0.3 to 0.8)	.41
	168	51	0	3.0	48	0	1.0	0.8 (0.2 to 1.3)	.01
SOFA component, hematologic, median (IQR)	0	82	1.0	1.0	74	1.0	1.0	0.1 (−0.1 to 0.3)	.40
	48	75	1.0	1.0	65	0	1.0	0 (−0.2 to 0.2)	.91
	96	56	0	1.0	49	0	1.0	0.1 (−0.2 to 0.4)	.43
	168	84	0	2.0	83	0	2.0	0.2 (−0.1 to 0.5)	.19
SOFA component, liver, median (IQR)	0	82	0	2.0	74	0	2.0	−0.2 (−0.5 to 0.2)	.39
	48	72	0	1.0	64	0	2.0	−0.1 (−0.5 to 0.3)	.70
	96	53	0	0	49	0	1.0	0 (−0.5 to 0.4)	.87
	168	84	3.0	1.0	83	3.0	2.0	0 (−0.4 to 0.5)	.95
SOFA component, neurologic, median (IQR)	0	82	3.0	1.0	75	3.0	1.0	−0.2 (−0.5 to 0.1)	.21
	48	72	2.5	1.0	66	2.0	1.0	−0.2 (−0.6 to 0.2)	.32
	96	54	2.0	1.0	51	2.0	3.0	0.1 (−0.3 to 0.5)	.71
	168	82	1.0	1.0	74	1.0	1.0	0.4 (−0.1 to 0.9)	.08
SOFA component, kidney, median (IQR)	0	84	1.0	2.0	83	1.0	2.0	−0.1 (−0.5 to 0.2)	.45
	48	82	1.0	2.0	72	1.0	1.0	0.1 (−0.2 to 0.4)	.52
	96	72	0	1.5	64	0	1.0	0.1 (−0.2 to 0.5)	.43
	168	56	1.0	2.0	50	1.0	2.0	−0.1 (−0.5 to 0.4)	.80
SOFA component, respiratory, median (IQR)	0	80	3.0	1.0	82	3.0	1.0	0.1 (−0.2 to 0.4)	.41
	48	64	2.0	1.5	66	2.0	2.0	0.1 (−0.3 to 0.5)	.48
	96	53	2.0	2.0	50	2.0	2.0	0.3 (−0.1 to 0.7)	.16
	168	35	2.0	2.0	27	2.0	2.0	0.1 (−0.6 to 0.8)	.78

Abbreviations: ICU, intensive care unit; IQR, interquartile range; RAGE, receptor for advanced glycation end products; TFPI, tissue factor pathway inhibitor; VE-40, minute ventilation 40 L/min; SOFA, Sequential Organ Failure Assessment. SI conversion factors: To convert creatinine to $\mu\text{mol/L}$, multiply values by 88.4.

^a Angiopoietin (range of normal, 1.1–4.4 ng/mL).

^b Procalcitonin (range of normal, <0.05 ng/mL).

^c Receptor for advanced glycation end products (range of normal, 0.4–4.3 ng/mL).

^d Tissue factor pathway inhibitor (range of normal, 13.0–44.9 ng/mL).

^e $\text{FiO}_2 \times \text{mean airway pressure} / \text{Po}_2 \times 100$.

^f $\text{VE-40 vent RR (respiratory rate)} \times \text{tidal volume/weight} \times (\text{Paco}_2/40)$. SOFA score range, 0 to 24, with higher values indicating greater severity of organ failure. Individual SOFA components range from 0 to 4, with higher component values indicating greater severity on score values. *P* values calculated by linear regression for continuous variables and logistic regression for binary variables.

95% CI, −0.90 to 5.85; *P* = .15) (eFigure 1 in Supplement 2). The number of ICU-free days to day 28 was 10.7 in the vitamin C group vs 7.7 in the placebo group (mean difference, 3.2; 95% CI, 0.3 to 5.9; *P* = .03) (eFigure 1 in Supplement 2). Transfer out of the ICU by hour 168 or less occurred in 25% of patients in the vitamin C group (21/84) vs 12.5% in the placebo group (10/83) ($\chi^2 = 4.63$; *P* = .03; difference, 12.95% [95% CI, 1.16% to 24.73%; *P* = .31]). The number of hospital-free days in the vitamin C group vs the placebo group was 22.6 vs 15.5, respectively (mean difference, 6.69; 95% CI, 0.3 to 13.8; *P* = .04) (eFigure 1 in Supplement 2).

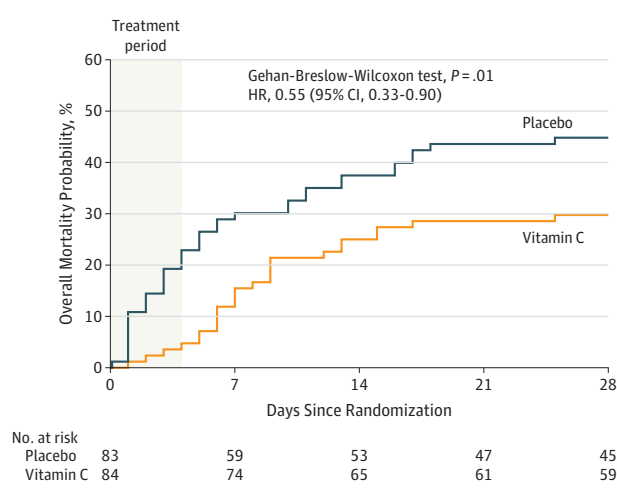
Adverse Events

Patients were followed up for adverse events. No unexpected study-related adverse events occurred during the trial.

Vitamin C Pharmacokinetic Findings

Plasma vitamin C levels in all patients were subnormal at enrollment (<28 $\mu\text{mol/L}$), with no significant difference between groups (median for vitamin C-infused patients vs placebo, 22 vs 22 $\mu\text{mol/L}$ [interquartile range {IQR}, 8–39 vs 11–37]; *P* = .49). (To convert vitamin C to mg/dL, divide values by 56.78.) Plasma vitamin C levels sampled at trough periods before infusion had increased significantly in vitamin C-infused patients by hours 48 (median, 166 $\mu\text{mol/L}$; IQR, 88–376) and 96 (median, 169 $\mu\text{mol/L}$; IQR, 87–412) compared with placebo patients by hours 48 (median, 23 $\mu\text{mol/L}$; IQR, 9–37) and 96 (median, 26 $\mu\text{mol/L}$; IQR, 9–41) (Figure 3). At hour 168, plasma vitamin C level in placebo patients remained low (median, 29 $\mu\text{mol/L}$; IQR, 12–39). After cessation of vitamin C infusion at 96 hours, vitamin C

Figure 3. All-Cause Mortality From Randomization (Day 0) to Day 28 Among Patients With Sepsis-Associated Acute Respiratory Distress Syndrome



Vitamin C-infused patients exhibited a significant reduction in 28-day all-cause mortality, although the P value was not adjusted for multiple comparisons. The median observation time was 28 days (interquartile range, 15-28 days) for the vitamin C group and 28 days (interquartile range, 5-28 days) for the placebo group.

levels declined but remained significantly elevated at hour 168 (median, 46 $\mu\text{mol/L}$; IQR, 19-66) compared with placebo (Figure 3).

Discussion

In this preliminary study of patients with sepsis and severe ARDS, a 96-hour infusion of vitamin C compared with placebo did **not improve organ dysfunction (as measured by the mSOFA score at 96 hours) or levels of biomarkers indicating inflammation (C-reactive protein) or vascular injury (thrombomodulin) by 168 hours**. The inability of vitamin C to affect C-reactive protein and thrombomodulin levels in this trial possibly resulted from the advanced stages of sepsis that were present before the development of ARDS. **Delayed quantification of these biomarkers in CITRIS-ALI compared with early vitamin C infusion in severe sepsis may explain results discordant with those of previous studies of vitamin C in severe sepsis.**¹⁵ Preclinical research in early sepsis revealed that **vitamin C prevented sepsis-induced cytokine surges that activate and sequester neutrophils in lung, thus damaging alveolar capillaries.**^{13,14} **Vitamin C increased alveolar fluid clearance by preventing activated neutrophil accumulation in alveolar spaces, limiting alveolar epithelial water-channel damage, and promoting their increased expression.**¹⁴ In addition, **vitamin C prevented neutrophil extracellular trap formation, a biological event in activated neutrophils that promotes vascular injury.**²⁵

Among the **46 secondary outcomes** that were examined in this trial, **43 showed no significant differences between the vitamin C group and the placebo group, although vita-**

min C compared with placebo was associated with a significant reduction in 28-day all-cause mortality, and with significantly increased ICU-free days to day 28 and hospital-free days to day 60. However, these findings were based on analyses that did **not account for multiple comparisons and therefore must be considered exploratory.** It is possible that these observations represent the effects of vitamin C on underlying sepsis-induced biological abnormalities that are not reflected in the biomarker analysis, a **hypothesis supported by 3 findings: early deaths in the placebo group, the proportion of patients in the vitamin C-infused cohort who left the ICU before 168 hours, and the survival curve parallel to that of placebo after cessation of vitamin C infusion.** However, these observations and hypotheses would require further evaluation.

Patients with sepsis have a high mortality and accounted for \$23.7 billion in US hospital costs in 2013.²⁶ Patients with sepsis and acute respiratory distress syndrome have worse clinical outcomes than those without sepsis and ARDS, with higher 30- and 60-day mortality and with fewer ventilator- and ICU-free days.²⁷ A report from US hospitals revealed declining ARDS mortality, likely because of both improved supportive care and regimented approaches to mechanical ventilation.⁴ However, in the **LUNG SAFE study**, which included more than **3000 patients with ARDS in 50 countries**, all-cause **mortality** remained high, varying from **34% to 46%**, depending on ARDS severity.⁵ Given these facts, a new approach in the form of a disease-modifying therapy is needed. The exploratory findings from the CITRIS-ALI trial suggest that further research may be warranted to determine whether vitamin C has a role in the care of patients with sepsis and ARDS.

Limitations

This study has several limitations. First, CITRIS-ALI as proposed was based on a previously performed phase 1 safety trial of vitamin C administered to patients in the very early stages of severe sepsis, not ARDS.¹⁵ CITRIS-ALI enrollment required fully developed ARDS with endotracheal intubation, which could have delayed vitamin C administration in the treatment group and possibly limited the ability to detect an effect on mSOFA scores and biomarkers. Second, CITRIS-ALI may have been underpowered to detect a difference in mSOFA scores and biomarker levels. Third, differences in baseline characteristics of this necessarily heterogeneous population may have influenced mortality. Fourth, the dosage of vitamin C used in this trial (50 mg/kg every 6 hours for 96 hours) may be insufficient for optimal care of sepsis-associated ARDS. Higher vitamin C dosages or longer administration times may have produced different results. Fifth, given the absence of previous clinical data, this proof-of-concept trial was designed to measure the effect of ascorbic acid on organ failure and laboratory measures of inflammation in patients with severe sepsis rather than mortality. Sixth, death and ICU graduation rates between the 2 groups were dissimilar, thus rendering the results susceptible to internal selection bias. The mortality data from this trial were intended for use in the design of future trials.

Conclusions

In this preliminary study of patients with sepsis and ARDS, a 96-hour infusion of vitamin C compared with

placebo did not significantly improve organ dysfunction scores or alter markers of inflammation and vascular injury. Further research would be needed to evaluate the potential role of vitamin C for other outcomes in sepsis and ARDS.

ARTICLE INFORMATION

Accepted for Publication: July 22, 2019.

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Author Contributions: Drs Fowler and Truwit had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Statistical analysis: Fowler, Truwit, Hite, Morris, Thacker, Kashiouris.

Obtained funding: Fowler.

Administrative, technical, or material support: Fowler, DeWilde, Priday, Fisher, Natarajan, Brophy, Sculthorpe, Nanchal, Syed, Sturgill, Martin, Hamman, Egan, Hastings, Tench, Mehkri, Bindas, Duggal, Graf, Cassity, Halquist.

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Other (data acquisition): Hollrith.

Conflict of Interest Disclosures: Dr Fowler reports receiving an honorarium from Virginia Tech School of Medicine; grants from the National Heart, Lung, and Blood Institute (NHLBI); and study materials from McGuff Pharmaceuticals during the conduct of the study. Dr Truwit reports receiving grants from the National Institutes of Health (NIH) during the conduct of the study. Dr Hite reports receiving grants from NHLBI during the conduct of the study, grants from the Marcus Foundation, and grants from NHLBI outside the submitted work. Dr Morris reports receiving grants from NIH during the conduct of the study. Dr Thacker reports receiving grants from NIH during the conduct of the study. Dr Brophy reports receiving grants from NIH during the conduct of the study. Dr Sturgill reports

receiving grants from NIH during the conduct of the study. Dr Martin reports receiving grants from NHLBI during the conduct of the study.

Dr Sevransky reports receiving grants from NIH during the conduct of the study and grants from the Marcus Foundation outside the submitted work.

Ms Egan reports receiving grants from Emory University during the conduct of the study.

Dr Duggal reports receiving grants from NIH during the conduct of the study. Ms Graf reports receiving grants from Virginia Commonwealth University during the conduct of the study.

Ms Zellner reports receiving grants from Virginia Commonwealth University during the conduct of the study.

Ms Yanny reports receiving grants from the Medical College of Wisconsin during the conduct of the study.

Ms McPolin reports receiving grants from the Medical College of Wisconsin during the conduct of the study.

Ms Hollrith reports receiving grants from the Medical College of Wisconsin during the conduct of the study.

Dr Damm reports receiving grants from the Medical College of Wisconsin during the conduct of the study.

Mr Cassity reports receiving grants from NIH during the conduct of the study.

No other disclosures were reported.

Funding/Support: The study was supported by the National Heart, Lung, and Blood Institute (UM1HL116885), National Center for Advancing Translational Sciences, VCU Wright Center for Translational Science Award (CTSA)

(UL1TR000058, NIH P50 AR072000), VCU Investigational Drug Services, and McGuff Pharmaceuticals, who supplied the vitamin C

at no charge. Funds from the NHLBI were distributed to the participating institutions on a per-patient-enrolled basis.

The principal investigator at each site determined how the funds were distributed.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study;

collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Meeting Presentation: Presented in part at the international conference of the American Thoracic Society, San Diego, California, May 18-23, 2018; and the European Society of Intensive Care Medicine, Berlin, Germany, October 2, 2019.

Data Sharing Statement: See [Supplement 3](#).

Additional Contributions: We thank the individuals who consented for their family members to participate in this trial.

We acknowledge support from John R. Owen, BSME, MS; Steven Kates, MD; and Evan Fowler, BS, Virginia Commonwealth University, who assisted in the comprehensive biomarker analysis.

They did not receive compensation for their role in the study. We thank critical care nursing staff, respiratory therapists, critical care physicians, and investigational pharmacists at the trial sites who cared for patients enrolled in this trial and strictly maintained the blind. In addition, we thank the members of the data and safety monitoring board

(Claude Piantadosi, MD, Duke University; Lorraine Ware, MD, Vanderbilt University; Mary Faith Marshall, PhD, University of Virginia; and Mark Conaway, PhD, University of Virginia) who monitored the performance of the trial.

The VCU Johnson Center for Critical Care and Pulmonary Research served both as clinical coordinating center and study site. Trial monitoring was performed by SPRI Clinical Trials (Pittsboro, North Carolina).

REFERENCES

1. Erickson SE, Martin GS, Davis JL, Matthay MA, Eisner MD; NIH NHLBI ARDS Network. Recent trends in acute lung injury mortality: 1996-2005. *Crit Care Med*. 2009;37(5):1574-1579. doi:10.1097/CCM.0b013e31819fefdf
2. Estenssoro E, Dubin A, Laffaire E, et al. Incidence, clinical course, and outcome in 217 patients with acute respiratory distress syndrome. *Crit Care Med*. 2002;30(11):2450-2456. doi:10.1097/00003246-200211000-00008
3. Roupie E, Lepage E, Wysocki M, et al; SRLF Collaborative Group on Mechanical Ventilation; Société de Réanimation de Langue Française. Prevalence, etiologies and outcome of the acute respiratory distress syndrome among hypoxemic ventilated patients. *Intensive Care Med*. 1999;25(9):920-929. doi:10.1007/s001340050983
4. Cochi SE, Kempker JA, Annangi S, Kramer MR, Martin GS. Mortality trends of acute respiratory distress syndrome in the United States from 1999 to 2013. *Ann Am Thorac Soc*. 2016;13(10):1742-1751. doi:10.1513/AnnalsATS.201512-8410C
5. Bellani G, Laffey JG, Pham T, et al; LUNG SAFE Investigators; ESICM Trials Group. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. *JAMA*. 2016;315(8):788-800. doi:10.1001/jama.2016.0291
6. Iba T, Levy JH. Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *J Thromb Haemost*. 2018;16(2):231-241. doi:10.1111/jth.13911
7. Truwit JD, Bernard GR, Steingrub J, et al; National Heart, Lung, and Blood Institute ARDS Clinical Trials Network. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. *N Engl J Med*. 2014;370(23):2191-2200. doi:10.1056/NEJMoa1401520
8. Gibbison B, López-López JA, Higgins JP, et al. Corticosteroids in septic shock: a systematic review and network meta-analysis. *Crit Care*. 2017;21(1):78. doi:10.1186/s13054-017-1659-4
9. Venkatesh B, Finfer S, Cohen J, et al; ADRENAL Trial Investigators and the Australian-New Zealand Intensive Care Society Clinical Trials Group. Adjunctive glucocorticoid therapy in patients with septic shock. *N Engl J Med*. 2018;378(9):797-808. doi:10.1056/NEJMoa1705835

10. Reinhart K, Wiegand-Löhnert C, Grimminger F, et al. Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody-fragment, MAK 195F, in patients with sepsis and septic shock: a multicenter, randomized, placebo-controlled, dose-ranging study. *Crit Care Med*. 1996;24(5):733-742. doi:10.1097/00003246-199605000-00003
11. Greenberg RN, Wilson KM, Kunz AY, Wedel NJ, Gorelick KJ. Randomized, double-blind phase II study of anti-endotoxin antibody (E5) as adjuvant therapy in humans with serious gram-negative infections. *Prog Clin Biol Res*. 1991;367(1):179-186.
12. Fisher CJ Jr, Dhainaut JF, Opal SM, et al; Phase III rhIL-1ra Sepsis Syndrome Study Group. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized, double-blind, placebo-controlled trial. *JAMA*. 1994; 271(23):1836-1843. doi:10.1001/jama.1994. 03510470040032
13. Fisher BJ, Kraskauskas D, Martin EJ, et al. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am J Physiol Lung Cell Mol Physiol*. 2012;303(1):L20-L32. doi:10.1152/ajplung.00300.2011
14. Fisher BJ, Seropian IM, Kraskauskas D, et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. *Crit Care Med*. 2011;39 (6):1454-1460. doi:10.1097/CCM.0b013e3182120cb8
15. Fowler AA III, Syed AA, Knowlson S, et al; Medical Respiratory Intensive Care Unit Nursing. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med*. 2014;12: 32. doi:10.1186/1479-5876-12-32
16. Ferguson ND, Fan E, Camporota L, et al. The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. *Intensive Care Med*. 2012;38(10):1573-1582. doi:10.1007/ s00134-012-2682-1
17. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med*. 2015;372(17):1629-1638. doi:10.1056/ NEJMoa1415236
18. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A; Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. 2000; 342(18):1301-1308. doi:10.1056/ NEJM200005043421801
19. Grissom CK, Hirshberg EL, Dickerson JB, et al; National Heart Lung and Blood Institute Acute Respiratory Distress Syndrome Clinical Trials Network. Fluid management with a simplified conservative protocol for the acute respiratory distress syndrome. *Crit Care Med*. 2015;43(2):288-295. doi:10.1097/CCM.0000000000000715
20. Hendrickson CM, Abbott J, Zhuo H, Liu KD, Calfee CS, Matthay MA; NHLBI ARDS Network. Higher mini-BAL total protein concentration in early ARDS predicts faster resolution of lung injury measured by more ventilator-free days. *Am J Physiol Lung Cell Mol Physiol*. 2017;312(5):L579-L585. doi:10.1152/ajplung.00381.2016
21. Javed A, Guirgis FW, Sterling SA, et al. Clinical predictors of early death from sepsis. *J Crit Care*. 2017;42(1):30-34. doi:10.1016/j.jcrc.2017.06.024
22. Vasilevskis EE, Pandharipande PP, Graves AJ, et al. Validity of a modified Sequential Organ Failure Assessment score using the Richmond Agitation-Sedation Scale. *Crit Care Med*. 2016;44(1): 138-146. doi:10.1097/CCM.0000000000001375
23. Rahmatinejad Z, Reihani H, Tohidinezhad F, et al. Predictive performance of the SOFA and mSOFA scoring systems for predicting in-hospital mortality in the emergency department. *Am J Emerg Med*. 2019;37(7):1237-1241. doi:10.1016/j. ajem.2018.09.011
24. Kajdacsy-Balla Amaral AC, Andrade FM, Moreno R, Artigas A, Cantraine F, Vincent JL. Use of the Sequential Organ Failure Assessment score as a severity score. *Intensive Care Med*. 2005;31(2):243-249. doi:10.1007/s00134-004-2528-6
25. Mohammed BM, Fisher BJ, Kraskauskas D, et al. Vitamin C: a novel regulator of neutrophil extracellular trap formation. *Nutrients*. 2013;5(8): 3131-3151. doi:10.3390/nu5083131
26. Torio C, Moore B. *National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2013: Statistical Brief #204*. Rockville, MD: Agency for Healthcare Research & Quality; 2006-2016.
27. Sheu CC, Gong MN, Zhai R, et al. Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. *Chest*. 2010;138(3):559-567. doi:10.1378/chest.09-2933