CHEST

Additional Trials of Vitamin C in Septic Shock

A Bag of Mixed Fruit

Tomoko Fujii, MD, PhD
Andrew A. Udy, MB, ChB, PhD
Melbourne, VIC, Australia

As highlighted by a recent report on the worldwide burden of sepsis, life-threatening infection is a growing global health issue.[1,2] Indeed, it has been estimated that 11 million deaths in 2017 were sepsis related, with the burden being greater in developing regions.[2] As such, there is a clear need for more effective and affordable interventions for sepsis; a single-center before-after study that was published in the June 2017 issue of *CHEST* attracted substantial attention from the medical community.[3] This article suggested a strong association between combination therapy that consists of vitamin C 6 g/d, thiamine 400 mg/d, and hydrocortisone 200 mg/d and decreased mortality rates and more rapid liberation from vasopressors.[3] The reported effects were dramatic; hence, they prompted a number of randomized clinical trials (RCT).

Critically, any trial of combination therapy requires careful design, particularly when one of the therapeutic components has an established impact on the outcomes of interest. Otherwise, the effect of combination therapy may be over-estimated. In this circumstance, hydrocortisone is included as part of combination therapy, which has been demonstrated repeatedly to shorten the duration of vasopressor dependency in septic shock.[4] Thus, the design of clinical trials that assess the effect of vitamin C combination therapy ideally should account for such confounding, particularly the hemodynamic effects of hydrocortisone.[5]

FOR RELATED ARTICLE, SEE PAGES 164 AND 174

AFFILIATIONS: From ANZIC-RC, SPHPM, Monash University.

FINANCIAL/NONFINANCIAL DISCLOSURES: The authors have reported to *CHEST* the following: T. F. and A. A. U. are investigators of the VITAMINS trial.

CORRESPONDENCE TO: Tomoko Fujii, MD, PhD, ANZIC-RC, SPHPM, Monash University. 553 St Kilda Rd, Melbourne, VIC, 3004, Australia; e-mail: Tomoko.Fujii@monash.edu

DOI: https://doi.org/10.1016/j.chest.2020.03.030

In this issue of *CHEST*, results from two further RCTs of vitamin C combination therapy in sepsis, combined treatment with hydrocortisone, vitamin C, and thiamine for sepsis and septic shock (HYVCTTSSS) and outcomes of metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in the early treatment of sepsis (ORANGES), are reported.[6,7]

HYVCTTSSS was a single-center RCT conducted in an ICU in China.[6] The trial was designed to assess the effect of vitamin C combination therapy (for 7 days) compared with placebo in 140 patients with sepsis or septic shock. The trial was terminated after patients were enrolled because of a significantly higher incidence of severe hypernatremia in the intervention arm (13 patients vs 3 patients).

Data from these 80 patients did not demonstrate any benefit in 28-day mortality rate (27.5% vs 35.0%), duration of vasopressor therapy, duration of mechanical ventilation, ICU length of stay, clearance of lactate and procalcitonin, and newly diagnosed acute kidney injury.[6] The change in sepsis-related organ failure assessment (SOFA) score over 72 hours was statistically greater in the intervention group (3.5 vs 1.8); however, patients in the control group did not receive hydrocortisone. As such, it is uncertain whether the observed effect on SOFA scores is due to "combination" therapy.

Of particular interest is the early termination of a trial that investigated vitamin C therapy, in which this intervention has been considered widely to be safe. The investigators considered that the salt-retaining property of hydrocortisone might be responsible[6]; although because commercially available vitamin C products are prepared with the use of sodium ascorbate, high doses of vitamin C may also be implicated. Notably, this is not the first time that hypernatremia has been reported in patients who undergo vitamin C therapy.[8]

ORANGES was a double-blind RCT conducted in two ICUs in the United States, where 137 adult patients with sepsis or septic shock within 12 hours of ICU admission

were allocated randomly to vitamin C combination therapy (for 4 days) or placebo.[7] The investigators reported duration of vasopressor dependency and change in SOFA score (over 4 days) to be primary outcomes. Of the two outcomes, the duration of vasopressor dependency was significantly shorter in those who received vitamin C combination therapy (27 vs 53 hours), albeit what specifically constituted "shock resolution" is not well defined. Moreover, there was no significant difference in SOFA score (2.9 vs 1.9) and secondary outcomes; mortality rate, procalcitonin clearance, ICU and hospital length of stay, ventilator-free days, and acute kidney injury did not differ between the two groups.

The investigators conducted an additional analysis adjusting for hydrocortisone use in the control group (which occurred in 41%), whereby the beneficial effect of combination therapy on shock resolution persisted. However, the lack of any favorable effect on any of the other outcomes, including SOFA scores (where the cardiovascular component should reflect the observed hemodynamic effect) remains unexplained.

Of note, ORANGES was first registered on ClinicalTrials.gov (NCT 03422159) in January 2018, with recruitment commencing in February the same year. Recruitment appears to have stopped in April 2019; albeit in June 2019, the primary outcome was changed from hospital mortality rate to time-to-vasopressor independence and change in SOFA score.[9] Critically, hospital mortality rate is the most distant outcome measure reported in the article, and changing the primary outcome after completing patient follow up does raise some concerns. As such, one has to consider whether the primary study findings are based on a chance result, which has been over emphasized.

A recently published RCT assessed the effect of vitamin C combination therapy compared with hydrocortisone monotherapy in 216 patients with septic shock.[10] The primary outcome was time alive and free of vasopressors; secondary outcomes included death, organ dysfunction, artificial organ support, and ICU and hospital length of stay. The trial found that vitamin C combination therapy did not shorten the duration of septic shock. The trial reported a greater decrease in SOFA score with the intervention; however, no beneficial effect was seen in any of the other outcomes.

Inconsistent findings from these trials can be attributed to study design. Indeed, uncontrolled use of hydrocortisone in the comparator group of HYVCTTSSS and ORANGES confounds the interpretation of their results. As such, future research must be cognizant of including a valid comparison and should be conducted and reported in a transparent manner. In addition, all of the existing literature concerning vitamin C combination therapy is underpowered with respect to mortality rate and insufficient to prompt widespread practice change. Finally, although the clinical community is particularly interested in novel, inexpensive, and effective therapies for sepsis, the mixed findings from these trials remind clinicians of the importance of focusing on basic strategies, such as focused resuscitation, early antibiotic administration, and source control.

Several RCTs on vitamin C combination therapy are currently ongoing and will hopefully provide more conclusive answers. However, in an effort to avoid more inconclusive trials, future work must focus on patient-centered outcomes and be reported in combination with prepublished study protocols and statistical analysis plans.

References

1. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing sepsis as a global health priority - a WHO resolution. *N Engl J Med*. 2017;377(5):414-417.
2. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990 – 2017 : analysis for the Global Burden of Disease Study. *Lancet*. 2020;395(10219):200-211.
3. 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.
4. Rygård SL, Butler E, Granholm A, Møller MH, Cohen J, Finfer S, et al. Low-dose corticosteroids for adult patients with septic shock: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med*. 2018;44(7):1003-1016.
5. Fujii T, Udy AA, Venkatesh B. Comparing apples and oranges: the vasoactive effects of hydrocortisone and studies investigating high dose vitamin C combination therapy in septic shock. *Crit Care Resusc*. 2019;21(3):152-155.
6. Chang P, Liao Y, Guan J, et al. Combined treatment with hydrocortisone, vitamin C, and thiamine for sepsis and septic shock: a randomized controlled trial. *Chest*. 2020;158(1):174-182.
7. Iglesias J, Vassallo A, Patel VV, Sullivan JB, Cavanaugh J, Elbaga Y. Outcomes of metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in the early treatment of sepsis: the ORANGES trial. *Chest*. 2020;158(1):164-173.
8. Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. *Cancer Chemother Pharmacol*. 2013;72(1):139-146.
9. National Institutes of Health Clinical Center. Metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in sepsis. (ORANGES). NCT03422159. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2018. https://clinicaltrials.gov/ct2/show/NCT03422159. Updated September 3, 2019.
10. Fujii T, Luethi N, Young PJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. *JAMA*. 2020;323(5):423-431.

CHEST

Outcomes of Metabolic Resuscitation Using Ascorbic Acid, Thiamine, and Glucocorticoids in the Early Treatment of Sepsis

The ORANGES Trial

Jose Iglesias, DO; Andrew V. Vassallo, PharmD, BCPS, BCCCP; Vishal V. Patel, PharmD, BCCCP; Jesse B. Sullivan, PharmD, BCPS, BCCCP; Joseph Cavanaugh, PharmD, BCPS, BCCCP; and Yasmine Elbaga, PharmD, BCPS

BACKGROUND: Sepsis is a major public health burden resulting in 25% to 30% in-hospital mortality and accounting for over 20 billion dollars of US hospital costs.

RESEARCH QUESTION: Does hydrocortisone, ascorbic acid, thiamine (HAT) therapy improve clinical outcomes in sepsis and septic shock?

STUDY DESIGN AND METHODS: This was a randomized, double-blinded, placebo-controlled trial conducted from February 2018 to June 2019, assessing an HAT treatment bundle for the management of septic and septic shock patients admitted to an ICU. The primary outcomes were resolution of shock and change in Sequential Organ Failure Assessment (SOFA) score. Secondary outcomes included 28-day mortality, ICU mortality, hospital mortality, procalcitonin clearance (PCT-c), hospital length of stay (LOS), ICU LOS, and ventilator-free days.

RESULTS: One hundred thirty-seven patients were randomized to the treatment group (n = 68) and comparator group (n = 69), respectively, with no significant differences in baseline characteristics. A statistically significant difference was found in the time patients required vasopressors, indicating quicker reversal of shock in the HAT group compared with the comparator group (27 ± 22 vs 53 ± 38 hours, $P < .001$). No statistically significant change in SOFA score was found between groups 3 (1 - 6) vs 2 (0 - 4), $P = .17$. No significant differences were found between study arms in ICU and hospital mortality, ICU and hospital LOS, ventilator free days, and PCT-c.

INTERPRETATION: Our results suggest that the combination of IV ascorbic acid, thiamine, and hydrocortisone significantly reduced the time to resolution of shock. Additional studies are needed to confirm these findings and assess any potential mortality benefit from this treatment.

TRIAL REGISTRATION: ClinicalTrials.gov; No.: NCT03422159; URL: www.clinicaltrials.gov;

CHEST 2020; 158(1):164-173

KEY WORDS: ascorbic acid; HAT therapy; hydrocortisone; sepsis; septic shock; vitamin c; thiamine

FOR EDITORIAL COMMENT, SEE PAGE 13

ABBREVIATIONS: AA = ascorbic acid; AKI = acute kidney injury; ANCOVA = analysis of covariance; ANOVA = analysis of variance; HAT = hydrocortisone, ascorbic acid, thiamine; LOS = length of stay; PCT = procalcitonin; PCT-c = procalcitonin clearance; SCr = serum creatinine; SOFA = Sepsis-Related Organic Failure Assessment

AFFILIATIONS: From the Department of Critical Care, Department of Nephrology (Dr Iglesias) and the Department of Pharmacy (Drs Vassallo, Patel, and Cavanaugh), Community Medical Center, Toms River, NJ; Department of Nephrology, Jersey Shore University Medical Center, Hackensack Meridian School of Medicine at

Sepsis is a major public health burden resulting in 25% to 30% in-hospital mortality and accounting for over 20 billion dollars of US hospital costs.[1,2] It is defined as life-threatening organ dysfunction related to a dysregulated host response to infection.[1] Currently no treatments directly target the pathogenesis of sepsis; therefore, management relies on early identification and the rapid administration of antibiotics, IV fluids, and vasopressors when appropriate.[3]

Previous promising studies have demonstrated the potential benefit of co-administration of hydrocortisone, ascorbic acid (AA), and thiamine (known as HAT therapy), which may reverse shock organ dysfunction and reduce mortality.[4,5] Marik et al[5] performed a retrospective before-and-after analysis that identified a possible association between a vitamin C-based protocol and patient mortality.[5] The treatment protocol was associated with a 31.9% overall decrease in mortality and a 3-fold decrease in time to vasopressor discontinuation in patients presenting with severe sepsis and septic shock. Fowler et al[6] demonstrated that IV administration of AA decreased Sequential Organ Failure Assessment (SOFA) scores and pro-inflammatory biomarkers.[6] Currently, ClinicalTrials.gov has over half a dozen studies across the United States currently recruiting applicants or waiting to publish results on the use of a vitamin C-driven protocol on sepsis.[7] One such study published by Fujii et al[8] demonstrated that HAT therapy did not significantly improve the duration of time alive and free of vasopressor administration over 7 days.[8] To better understand the effect of HAT therapy on clinical outcomes in sepsis and septic shock, we conducted the ORANGES trial.

Materials and Methods

Study Design

This was a randomized, double-blinded, placebo-controlled trial assessing the utilization of an ascorbic acid, thiamine, and hydrocortisone treatment bundle for the management of septic and septic shock patients admitted to an ICU. This study was performed from February 2018 to June 2019 in two community nonteaching hospitals in the United States. The study was approved by the Community Medical Center Institutional Review Board (IRB # 17-004). All participants were provided with written informed consent. For patients who presented with altered mental status or requiring mechanical ventilation, consent was obtained from the patient's legally authorized representative. Patients were randomized to receive either ascorbic acid 1,500 mg q6h, thiamine 200 mg every 12 hours, and hydrocortisone 50 mg q6h or a matching saline placebo for a maximum of 4 days. Intensivists were allowed to order open-label corticosteroid therapy for patients as deemed necessary for their usual care (ie, for respiratory failure). Study medications were discontinued if patients were discharged from the ICU before 4 days. Before study therapy initiation, baseline ascorbic acid and thiamine levels were drawn and evaluated via liquid chromatography/mass spectrometry. Study randomization and blinding was performed by the main hospital pharmacy and maintained on a password-protected file. Patients were block randomized separately at each site into 70 sets of 2, which predetermined each patient's treatment group enrollment. Investigators were blinded up until termination of patient enrollment and both primary and secondary study outcomes were met.

Ethics Statement

All procedures performed in the study were in accordance with the 1964 Helsinki declaration and its later amendments. Patients' data were kept confidential, and no patients' identifiers were included in data files handled for the purposes of this study.

Population

Participants were adults (≥18 years of age) with a primary diagnosis of sepsis or septic shock according to the 2016 Surviving Sepsis Campaign definitions.[3] Additional inclusion criteria were diagnosis of sepsis or septic shock within 12 hours of admission to the ICU and compliance with the 3-hour sepsis bundle. Once consent was obtained, treatment was allowed to begin in the ED. Although there was an update in 2018 reducing the time of the bundle to 1 hour, the 3-hour time frame was maintained because patient enrollment had already begun.[9]

Exclusion criteria included patients under the age of 18, were pregnant, had a do not resuscitate or do not intubate order on admission, had a terminal end-stage disease (eg, stage IV cancer, end-stage heart failure), did not have a primary admitting diagnosis of sepsis or septic shock, required immediate surgery, had HIV and a CD4 < 50 mm^2, had known glucose-6 phosphate dehydrogenase deficiency, were transferred from another hospital, or presented with sepsis or septic shock more than 24 hours from admission.

Outcomes

The primary outcomes of the study were resolution of shock and change in SOFA score. Resolution of shock was defined as the time from starting blinded study medications to discontinuation of all vasopressor support. Change in SOFA score was defined as the initial SOFA score minus the day 4 SOFA score. A 4-day course was chosen to align with the maximum care provided with the study medications. SOFA scores were calculated daily, starting on the first day of admission to the ICU. This difference was calculated the same way even if the patient was discharged from the ICU before day 4. If the patient was discharged from the hospital before day 4, the last known SOFA score was carried forward. If a patient died

Seton Hall (Dr Iglesias), Neptune, NJ; Fairleigh Dickinson University School of Pharmacy & Health Sciences (Dr Sullivan), Teaneck, NJ; and the Department of Pharmacy (Dr Elbaga), Monmouth Medical Center Southern Campus, Lakewood, NJ.

FUNDING/SUPPORT: The authors have reported to *CHEST* that no funding was received for this study.

CORRESPONDENCE TO: Jose Iglesias, DO, Department of Critical Care, Department of Nephrology, Community Medical Center, 99 NJ-37, Toms River, NJ 08755; e-mail: jiglesias23@gmail.com

DOI: https://doi.org/10.1016/j.chest.2020.02.049

before day 4, their last SOFA score was also carried forward for assessment. For patients in whom the PaO_2/FIO_2 could not be obtained for SOFA score calculation, the peripheral capillary oxygen saturation/FIO_2 was used as an alternative.[10]

Secondary outcomes included ICU mortality, hospital mortality, procalcitonin clearance (PCT-c), hospital length of stay (LOS), ICU LOS, and ventilator-free days. PCT-c was calculated using the following formula: initial PCT minus PCT at 96 hours divided by the initial PCT multiplied by 100.[11,12] Ventilator-free days was calculated by the number of days free of mechanical ventilation up until day 28. Acute kidney injury (AKI) was defined on the basis of Kidney Disease: Improving Global Outcomes criteria; namely, an increase in serum creatinine (SCr) $>$ 0.3 mg/dL, a level $>$1.5 times the baseline value or the initiation of renal replacement therapy.[13] If baseline prehospitalization SCr was unknown or unavailable, we employed a prehospitalization estimated glomerular filtration rate of 75 mL/min/1.73 m^2 and "back-calculated" the SCr value using the simplified Modification of Diet in Renal Disease Equation for serial measurements of SCr. If patients required dialysis during the study or had end-stage renal disease, we arbitrarily assigned the patients an SCr of 5 mg/dL.[14] This assignment of a baseline SCr was performed only for the purpose of comparing baseline creatinine and not for the purpose of calculating acute kidney injury.

Primary safety outcomes included SCr, urine oxalate, and other reported adverse reactions documented by clinical staff. Safety evaluations included routine laboratory assessments and measurement of vital signs. Levels of urine oxalate were measured using a 24-hour urine collection on day 4 to assess for accumulation in the kidneys.

Statistics

Based on the results of the preliminary study of Marik et al,[5] we projected that the combination of ascorbic acid, thiamine, and hydrocortisone could reduce time to vasopressor discontinuation from 54 (±30 hours) vs 30 hours. For the additional primary outcome, we projected a greater change of SOFA score of 4 (±3) vs 2. Assuming a type 1 error of 5% (alpha of 0.05) and a power of 80%, this study would require a sample size of 94 patients. To account for dropouts and patients not requiring vasopressor therapy, we aimed for a sample size of 140 patients. Sample size was calculated based on both primary outcomes, and the larger of the two calculations was used.

The primary analysis was intention to treat. Summary statistics were computed for both study arms. Continuous variables were expressed as mean ± SD. Differences between HAT and comparator arms were compared by the Student *t* test or the Wilcoxon rank-sum test, as appropriate for non-normally distributed data. Variables that were serially measured during the study period such as procalcitonin levels, SOFA scores, vasopressor requirements, and laboratory parameters were compared by employing repeated-measures analysis of variance (ANOVA) with HAT therapy and the comparator being the between-subjects' factor. When the assumptions of the repeated-measures ANOVA were not met, a Student *t*-test with a Bonferroni correction was employed. Categorical values were compared with Pearson χ^2 test or Fischer χ^2 test when indicated. Significance was set at a *P* value of less than .05. Because 41% of patients in the comparator group received corticosteroids, any outcomes found to be significant were reanalyzed by adjusting for corticosteroid therapy use. Kaplan-Meier survival curves and log-rank analyses were employed to compare survival difference between HAT and comparator groups. Cox regression analysis was employed to compare differences in time with reversal of shock between groups.

Statistical analysis was performed using SPSS and R (IBM; R Foundation for Statistical Computing). We performed checks on the assumption of proportionality of hazards by evaluating Schoenfeld residuals and the Therneau, Grambsch global test on the summed Schoenfeld residuals.[15]

Results

Study Population

Between February 14th, 2018 and April 29th, 2019, 140 patients consented to participate in the study. Three patients were withdrawn from analysis after randomization because of a new diagnosis of terminal cancer. One hundred thirty-seven patients were randomized. Sixty-eight patients and 69 patients were randomized to the treatment arm (HAT) and comparator arms, respectively (Fig 1). Most of the patients received their first dose of study treatment between 3 and 14 hours (mean, 9.9 ± 4.5 hours) from presentation to the ED once enrolled and randomized. At the time of enrollment, there were no significant baseline differences in demographics, comorbidities, laboratory values, Acute Physiology and Chronic Health Evaluation II scores, SOFA scores, between the treatment arms (Table 1). This was a predominantly white patient population, representing 96% of the population. There were 43% male and 57% female participants in the study. The mean age of the participants was 69 ± 13 years. The major sources of infection were pulmonary 43%, urogenital 31%, primary bacteremia 14%, and GI/other 12%. There were 23 (17%) episodes of gram-negative bacteremia, 21 (15%) episodes of gram-positive bacteremia, and 1 (0.7%) episode of non-albicans candidemia. At time of enrollment 50% of the patients were on mechanical ventilation and 75% were on vasopressors. A total of 28 (41%) patients in the comparator arm received corticosteroids. The mean SOFA score was 8.1 ± 3.3, and the Apache II score was 24.5 ± 8.2, with an estimated mortality of 34% ± 2%, which is comparable to similar sepsis trials.[4,5,16] Hypovitaminosis, defined as an AA level of ≤23 μmol/L, was present in 50% of participants, and severe AA deficiency, defined as an AA level ≤ 11.3 μmol/L, was present in 14% of participants. Only one patient was discharged alive from the hospital before day 4.

Primary End points

A significant difference was seen in the time patients required vasopressors, indicating reversal of shock in the HAT arm compared with the comparator arm, 27 ± 22 vs 53 ± 38 hours, $P < .001$. Kaplan-Meier curves

comparing reversal of shock in HAT therapy, comparator arm without steroids, and comparator arm receiving open-label steroids showed a significant difference, log rank $P = .009$ (Fig 2). A Cox regression was performed with HAT therapy and corticosteroid therapy in the comparator group as factors. This identified an independent effect of HAT therapy on reversal of shock, $P = .007$, HR, 1.79, 95% CI, 1.17-2.75 (Fig 2).

To compare whether the effectiveness of HAT therapy on resolution of shock was not solely an effect of corticosteroid administration, we performed a one-way analysis of covariance (ANCOVA) adjusted for corticosteroid use as a covariate. The outcome was time to discontinuation of vasopressors. Preliminary analysis revealed that the assumptions of the ANCOVA test were not met. We therefore employed a nonparametric rank ANCOVA described by Quade.[17,18] In the rank ANCOVA, the dependent variable (time to reversal of shock/time to discontinuation of vasopressors) is rank transformed, and parametric analysis is performed on the rank values.[17,18]

Adjusting for corticosteroid use, HAT therapy remained significant in resolution of shock. The grand mean time to discontinuation of vasopressors and reversal of shock was 44 hours, with a mean time in HAT therapy being 34 hours compared with the control arm mean of 54 hours, demonstrating that patients in the control arm remained in shock 59% longer ($F_{1,84} = 28.6$, $P < .001$, adjusted $R^2 = 0.147$). Vasopressor dosage (norepinephrine equivalents) over time decreased; however, this difference did not meet traditional thresholds of statistical significance ($F_{1,19} = 4.28$, $P = .052$) (Fig 3).[19,20] These results suggest that HAT therapy has a significant effect on decreasing the time to reversal of shock, which is independent of corticosteroid effects.

During the study, no statistically significant change in SOFA score was seen between the HAT arm and the

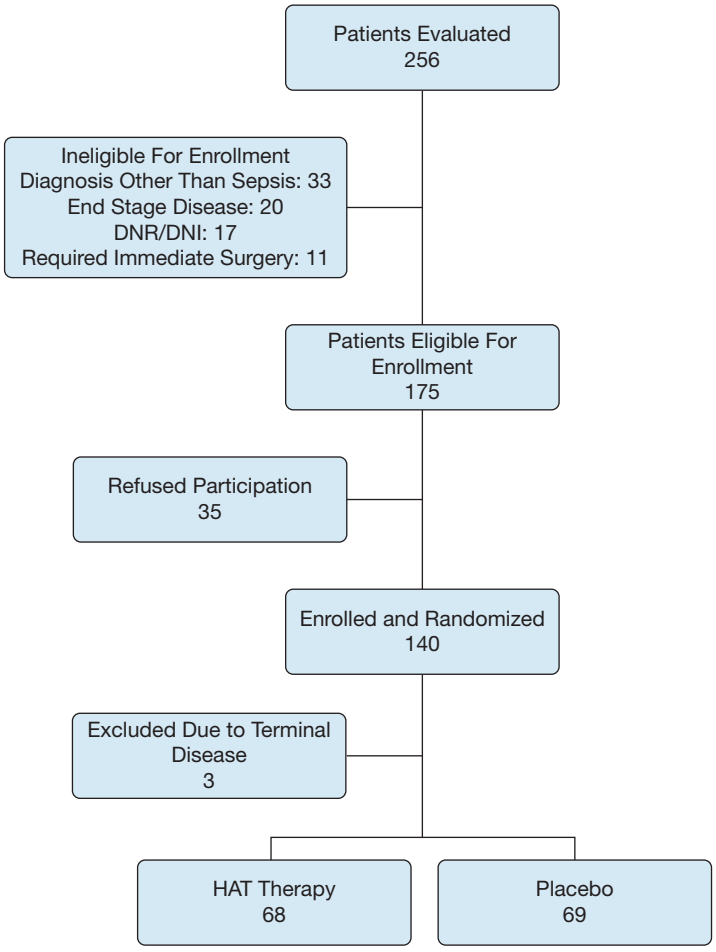


Figure 1 – *Flow diagram for patient enrollment.*

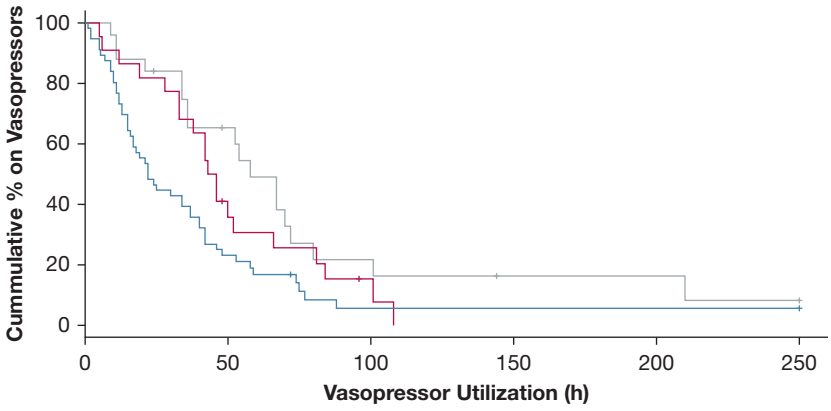
comparator arm, with decreases in SOFA of 3 (1-6) vs 2 (0-4), $P = .17$. Repeated-measures ANOVA demonstrated that there was no statistically significant change in SOFA score throughout the study (Fig 4) ($F_{3,103} = 1.3$, $P = .27$).

To account for patients who died before 72 hours or did not have values at each time period (24-72 hours) we also determined the mean change in SOFA score, the difference between the mean SOFA scores at 72 hours, and the mean SOFA score at baseline.

TABLE 1] Baseline Characteristics of HAT Therapy and Comparator Group

Characteristic	HAT Treatment (n = 68)	Comparator (n = 69)	*P*	OR	95% CI
Age	70 ± 12	67 ± 14	.17		
Race (white)	66 (97%)	65 (94%)	.68	0.49	0.2-2
Weight, kg	82 ± 27	82 ± 30	.37		
Sex (male)	32 (47%)	27 (39%)	.35	0.72	0.36-1.42
Comorbidities					
CAD	25 (37%)	21 (30%)	.43	0.75	0.37-1.5
Diabetes	24 (35%)	33 (48%)	.14	1.68	0.85-3.33
Dementia	7 (10 %)	4 (5.8%)	.33	0.53	0.15-1.9
Heart failure	18 (26%)	13 (19%)	.29	0.65	0.28-1.44
Malignancy	15 (22%)	11 (16%)	.36	0.67	0.30-1.6
COPD	23 (34%)	17 (25%)	.24	0.64	0.30-1.34
Cirrhosis	0 (0)	3 (2.2%)	.25	0.49	0.41-0.58
ESRD	3 (0.4%)	0 (0%)	1.2	0.48	0.40-0.57
CKD	10 (7%)	4 (2.9%)	.08	0.36	0.11-1.2
Morbid obesity (BMI > 40)	16 (23.5%)	13 (19%)	.5	0.75	0.33-1.71
Immunocompromised[a]	6 (8.8%)	5 (7.2%)	.73	0.87	0.23-2.8
Primary diagnosis					
Pneumonia	29 (43%)	30 (44%)	.92	1.03	0.53-2.03
Urosepsis	18 (26.5%)	25 (36%)	.21	1.58	0.76-3.3
Primary bacteremia	9 (13%)	11 (16%)	.65	1.24	0.48-3.23
GI/biliary	9 (13%)	8 (12%)	.8	0.66	0.31-2.4
Other	13 (19%)	9 (13%)	.33	0.63	0.25-1.6
Mechanical ventilation	34 (50%)	35 (51%)	.93	1.03	0.53-2
Vasopressors	56 (82%)	47 (68%)	.05	0.45	0.20-1.02
Acute kidney injury	54 (79%)	52 (75%)	.57	0.76	0.35-1.77
Positive blood cultures	22 (32%)	23 (33%)	.93	1.05	0.51-2.13
WBC × 10^9/L	16 ± 10	19 ± 9.7	.1		
Lactate (mM/L)	4.45 ± 3.5	4.8 ± 4.2	.49		
Creatinine (mg/dL)	2.1 ± 1.5	2 ± 1.51	.68		
Ascorbic acid level (mg/dL)[b]	0.52 ± 1	0.48 ± 0.4	.79		
Procalcitonin (ng/mL)	44 ± 72	23 ± 38	.61		
Thiamine (mg/dL)	193 ± 144	148 ± 53	.09		
Day 1 SOFA	8.3 ± 3	7.9 ± 3.5	.47		
APACHE II	24 ± 7.6	24.9 ± 8.7	.53		
APACHE IV	88 ± 28.3	87.5 ± 29.7	.84		
APACHE IV predicted mortality	34 ± 3	33.6 ± 2.6	.8		

APACHE = Acute Physiology and Chronic Health Evaluation; CAD = coronary artery disease; CKD = chronic kidney disease; ESRD = end stage renal disease; PLT = platelets; tBili = total bilirubin; SOFA = Sepsis-Related Organ Failure Assessment.
[a]HIV infection, neutropenia, posttransplantation, immunoglobulin deficiency etc.
[b]To convert ascorbic acid from mg/dL to μmol/L multiply by conversion factor 56.82.



No. at risk						
No steroids	25	12	4	2	2	1
Steroids	22	8	2	0	0	0
HAT	56	11	2	2	2	2

Figure 2 – *Cox proportional hazards and corresponding Kaplan–Meier survival curves (n = 103 patients with 3 factors; HAT therapy (blue line), comparator group patients who did (red line) and did not (gray line) receive corticosteroids; log rank P = .009. Cox proportional hazards analysis demonstrates an independent effect of HAT therapy on reversal of shock, P = .007 (Beta, 0.58, SE, 0.218, HR, 1.79, 95% CI, 1.17-2.75). HAT = Hydrocortisone, Ascorbic acid, Thiamine.*

There was no statistical difference found in the change in mean SOFA score (3.4 ± 4.4 vs 2.3 ± 5.2, $P = .18$).

Secondary End points

No significant differences in secondary end points and laboratory markers were obtained during the first 4 days of treatment between study arms (Table 2, Table 3). ICU mortality was 9% (6 patients) in the HAT arm and 14% (10 patients) in the comparator arm ($P = .37$, OR, 1.75, 95% CI, 0.59-5.1). Hospital mortality was 16.4% (11 patients) in the HAT arm and 19% (13 patients) in the comparator arm ($P = .65$. OR, 1.25, 95% CI, 0.5-2.97) (Figs 5, 6).

Renal Outcomes and Adverse Events

Renal outcomes were similar in both arms, with AKI occurring in 54 (79%) in the HAT arm and 52 patients (75%) in the comparator arm ($P = .68$, OR, 0.79, 95% CI, 0.35-1.77). Renal replacement therapy was required in 2 (3%) in the HAT arm and 8 patients (11%) in the comparator arm ($P = .098$, OR, 4.1, 95% CI, 0.84-20.3). Measurement of urinary oxalate on day 4 was not significant, with HAT arm 24-hour oxalate excretion 51 ± 35 mg/1.73 m^2 vs 40 ± 28 mg/1.73 m^2 in the

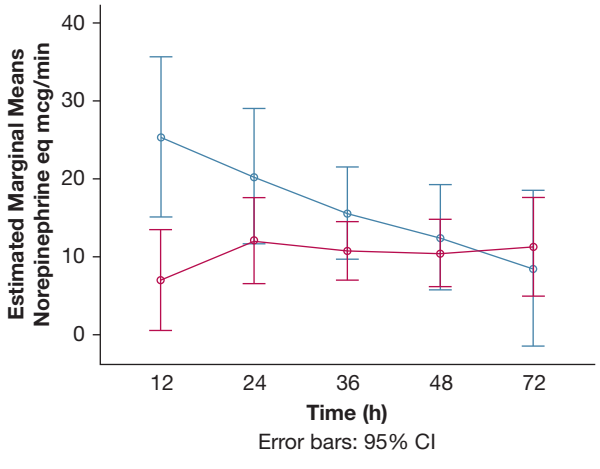


Figure 3 – *Graph displaying change in vasopressor dose in norepinephrine equivalents during the course of treatment in HAT arm (blue lines) and comparator arm (red lines) analysis of variance (ANOVA), F (1, 19 = 4.28, P = .052). SOFA = Sepsis-Related Organic Failure Assessment. See Figure 2 legend for expansion of other abbreviation.*

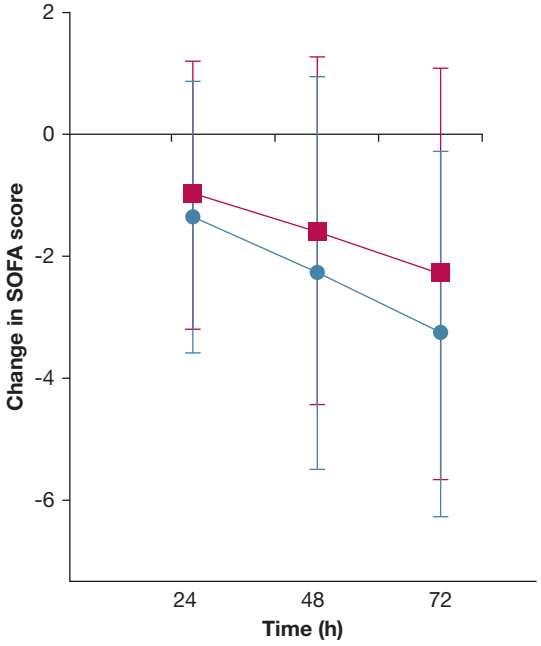


Figure 4 – *Graph of SOFA score kinetics during study period in HAT arm (blue line) and comparator arm (red line) analysis of variance (ANOVA), (F3, 103 = 1.3, P = .27). See Figure 2 and 3 legends for expansion of other abbreviations.*

TABLE 2] Laboratory Values and SOFA Score During Study Period

Laboratory Values	HAT Treatment (n = 68)		Comparator (n = 69)		*P*
WBC-initiation ($\times 10^9$/L)[a]	16 ± 10		19.0 ± 9.7		.18
WBC-24 h	16 ± 8.8		17.2 ± 8.2		.49
WBC-48 h	13.3 ± 7		14 ± 6.2		.61
WBC-72 h	12.8 ± 6		12.4 ± 6.4		.78
PLT-initiation ($\times 10^9$/L)[b]	233.41 ± 131.8		264.6 ± 147.15		.2
PLT-24 h	196.4 ± 127		216.36 ± 120.31		.31
PLT-48 h	172.12 ± 109.6		199.1 ± 112.6		.11
PLT-72 h	171.81 ± 103		193.5 ± 107.01		.1
Tbili-initiation (mg/dL)[c]	1.13 ± 1		1.44 ± 1.74		.32
Tbili-24 h	0.9 ± 0.6		1.24 ± 1.69		.21
Tbili-48 h	0.72 ± 0.6		0.9 ± 1.32		.52
Tbili-72 h	0.7 ± 0.72		0.71 ± 0.68		.58
PO/FiO-initiation[d]	267.2 ± 115.53		243.43 ± 127.35		.17
PO/FiO-24 h	287 ± 118.59		283.78 ± 132.6		.38
PO/FiO-48 h	288.54 ± 114.61		276.34 ± 119.22		.41
PO/FiO-72 h	265.42 ± 109.02		273.39 ± 127.46		.83
Lactate-initial[e]	4.45 ± 3.5		4.80 ± 4.2		.59
Lactate-24 h	2.39 ± 2.84		2.88 ± 3.87		.44
Lactate-48 h	2.5 ± 3.7		2.04 ± 2.34		.32
Lactate-72 h	2.01 ± 2.56		1.74 ± 2.57		.52
SOFA initial[f]	8.3 ± 3		7.9 ± 3.5		.34
SOFA-24 h	7.1 ± 3.35	n = 61	7 ± 3.38	n = 61	.62
SOFA-48 h	6.32 ± 3.82	n = 60	6.42 ± 3.6	n = 59	.83
SOFA-72 h	4.93 ± 3.14	n = 62	5.58 ± 3.78	n = 63	.51
SCr initial[g]	2.1 ± 1.5		2 ± 1.51		.82
SCr 24 h	1.74 ± 1.21		1.85 ± 1.6		.65
SCr 48 h	1.62 ± 1.32		1.8 ± 1.71		.53
SCr 72 h	1.47 ± 1.3		1.67 ± 1.71		.45
SCr at discharge	1.32 ± 1.13		1.37 ± 1.18		.78
Procalcitonin at enrollment	44 ± 72		23 ± 38		.61

See Table 1 legend for expansion of abbreviations.
[a-f]For repeated measurements, no statistically significant differences were found between groups by independent Student t test with Bonferroni correction. For SOFA calculations, "n" at each time interval includes patients alive and with all laboratory values available for calculation of SOFA score.

comparator arm, respectively ($P = .35$). No adverse events were noted that were deemed related to the study drug. One patient developed worsening hypoxia in the setting of severe COPD and gram-negative sepsis with mildly elevated methemoglobin levels with no evidence of hemolysis. This was reviewed by the adverse events committee and deemed unrelated to study treatment.

Discussion

This randomized double-blinded controlled study of HAT therapy demonstrated a marked acceleration in the reversal of shock. This effect remained significant after adjusting for corticosteroid administration in the comparator group, accounting for approximately 15% of the variability observed. This suggests both an independent and synergistic effect of AA in the reversal of shock and in augmenting the hemodynamic effects of corticosteroids.[5,21,22] This was in contrast to the recently published study by Fujii et al,[8] which showed no benefit. This may be due to differences in the patient population studied and trial design. Liberation from vasopressor support has numerous advantages, potentially preventing the immunosuppressive effects of catecholamines minimizing the risk of mesenteric, limb, and end-organ ischemia.[2,5,23]

TABLE 3] Treatment and Clinical Outcome

Treatments	HAT Treatment (n = 68)	Comparator (n = 69)	*P*	OR	95% CI
Days of HAT therapy or placebo	3.3 ± 0.8	3.25 ± 1	.94		
Fluid balance at 24 hours (mL/kg)	53 ± 26	46 ± 24.1	.09		
Fluid balance at 72 hours (mL/kg)	83 ± 97	80 ± 75	.82		
Vasopressors at time of enrollment	56 (82%)	47 (68%)	.05	0.45	0.2-1.02
Vasopressor initiated after study enrollment	4 (6%)	10 (14.5%)	.16	2.7	0.8-9.1
Renal replacement therapy for AKI	2 (3%)	8 (11.5%)	.1	4.1	0.84-20.3
Primary outcome					
Δ SOFA score at 72 hours	2.9 ± 3.3	1.93 ± 3.5	.1		
Duration of vasopressors, h	27 ± 22	53 ± 38	<.001		
Secondary outcomes					
Hospital mortality (%)	11 (16%)	13 (19.4)	.6	1.2	0.50-2.97
ICU mortality (%)	6 (9%)	10 (14%)	.3	1.7	0.59-2.63
Hospital LOS, d	11.5 ± 6.8	11 ± 6.2	.75		
ICU LOS, d	4.76 ± 4.3	4.66 ± 3.45	.88		
Procalcitonin clearance, %	63 ± 170	58 ± 66	.44		
Ventilator-free days	22 ± 6.2	22.4 ± 4.3	.63		
AKI	54 (79%)	52 (75%)	.57	0.76	0.35-1.77

AKI = acute kidney injury; LOS = length of stay. See Table 1 legend for expansion of other abbreviations.

AA possesses antioxidant, antiinflammatory, and immune-enhancing functions, while also serving as a co-factor in the synthesis of endogenous catecholamines, steroidogenesis, vasopressin synthesis, and enhancing adrenergic receptor activity.[24,25] Approximately 90% of septic shock patients have hypovitaminosis C, and 40% have AA deficiency. These rates are significantly higher than nonseptic critically ill patients.[26] The use of hydrocortisone in the treatment of septic shock has been controversial, with studies yielding mixed results.[3] Glucocorticoids and AA may synergistically protect against or reverse vascular endothelium dysfunction from damage due to endotoxins.[27]

In contrast to the Marik et al[5] and Fowler et al[6] studies, the current study did not demonstrate a difference in SOFA kinetics or PCT clearance. We postulate that this can potentially be attributed to less severity of AA hypovitaminosis (ORANGES = 21.7 ± 14.8 μmol/L, Marik et al[5] = 14.7 ± 11.8 μmol/L, Fowler et al[6] = 17.9

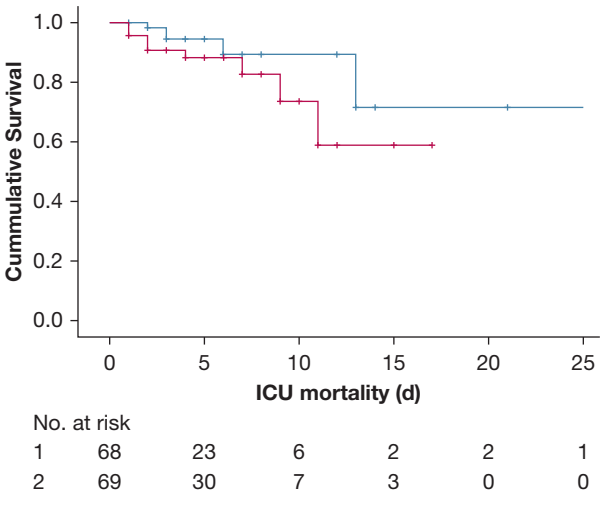


Figure 5 – *Kaplan-Meir survival curves of ICU mortality rate in days in HAT arm (blue lines) and comparator arm (red lines), P = .168. See Figure 2 legend for expansion of other abbreviation.*

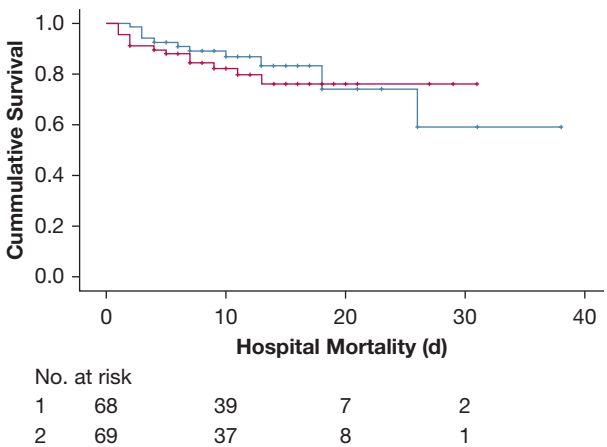


Figure 6 – *Kaplan-Meir survival curves of hospital mortality in days of HAT arm (blue lines) and comparator arm (red lines), P = .568.*

± 2.4 μmol/L) and shorter duration of HAT therapy in the current study.[5,6]

Administering AA is considered relatively safe; however, prolonged intake of high IV doses in the presence of impaired renal function increases the risk of oxalate kidney stones, resulting in nephropathy or death in rare cases.[24] Thiamine may reduce the risk of hyper-oxalosis because of its function as a cofactor in the oxidation of glyoxylate by the enzyme glyoxylate aminotransferase.[23] Additionally, correction of thiamine deficiency may help mitigate oxidative stress and inflammation, as shown in an animal model of sepsis.[28] Thiamine deficiency has been shown to occur in 10% to 70% of patients presenting with sepsis.[29] Although oxalate excretion was higher in the HAT therapy group, no significant differences were seen between groups or differences in the development of AKI. Therefore, short-term parenteral AA administration in patients with sepsis was safe from a renal standpoint.

The strengths of our study include that it was performed in a non-teaching community hospital setting with minimal resource utilization reflecting real-world clinical management. The relative weakness was its small, homogenous (primarily white) cohort size, limiting the ability to detect differences in hospital mortality and length of stay.

Conclusions

HAT therapy is safe and decreases the duration of shock in patients with sepsis. This effect appears to be due to the ascorbic acid component of HAT therapy rather than the mineralocorticoid effect of steroids alone. Further randomized trials are needed, with larger cohorts to determine whether HAT therapy translates to improved mortality or a decrease in ICU length of stay.

Acknowledgements

Author contributions: All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, including any adverse effects. All authors approved the final version to be published. A. V., J. I., V. P., J. S., Y. E., and J. C. drafted and revised the manuscript. All authors are guarantors of the paper.

Financial/nonfinancial disclosures: None declared.

Other contributions: The authors would like to thank all participants and their families for their involvement in this study. The authors would also like to acknowledge the hard work and time committed to this study by the ED and intensive care nurses, rapid response team, pharmacy residents, and pharmacy departments of Community Medical Center and Monmouth Medical Center Southern Campus.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

References

1. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
2. Fleischmann C, Scherag A, Adhikari NKJ, et al. Assessment of global incidence and mortality of hospital-treated sepsis: current estimates and limitations. *Am J Respir Crit Care Med*. 2015;193(3):259-272.
3. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2016. *Crit Care Med*. 2017;45(3):486.
4. Marik PE. Hydrocortisone, ascorbic acid and thiamine (HAT therapy) for the treatment of sepsis: focus on ascorbic acid. *Nutrients*. 2018;10(11).
5. 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.
6. Fowler AA, Syed AA, Knowlson S, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med*. 2014;12:32.
7. Search of: vitamin c | sepsis—List Results. ClinicalTrials.gov [Internet]. https://clinicaltrials.gov/ct2/results?cond=sepsis&term=vitamin+c&cntry=&state=&city=&dist=. Accessed August 1, 2019.
8. Fujii T, Luethi N, Young PJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. *JAMA*. 2020;323(5):423-431.
9. Levy MM, Evans LE, Rhodes A. The surviving sepsis campaign bundle: 2018 update. *Intensive Care Med*. 2018;44(6):925-928.
10. Pandharipande PP, Shintani AK, Hagerman HE, et al. Derivation and validation of Spo2/Fio2 ratio to impute for Pao2/Fio2 ratio in the respiratory component of the Sequential Organ Failure Assessment score. *Crit Care Med*. 2009;37(4):1317-1321.
11. Azevedo JRA de, Torres OJM, Beraldi RA, Ribas CAPM, Malafaia O. Prognostic evaluation of severe sepsis and septic shock: procalcitonin clearance vs Δ sequential organ failure assessment. *J Crit Care*. 2015;30(1). 219.e9-219.e12.
12. Ruiz-Rodríguez JC, Caballero J, Ruiz-Sanmartin A, et al. Usefulness of procalcitonin clearance as a prognostic biomarker in septic shock: a prospective pilot study. *Medicina Intensiva*. 2012;36(7):475-480.
13. Kellum JA, Lameire N; for the KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care*. 2013;17(1):204.
14. Pickering JW, Endre ZH. Back-calculating baseline creatinine with MDRD misclassifies acute kidney injury in the intensive care unit. *Clin J Am Soc Nephrol*. 2010;5(7):1165-1173.
15. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81:515-526.
16. Santhakumaran S, Gordon A, Prevost AT, O'Kane C, McAuley DF, Shankar-Hari M. Heterogeneity of treatment effect by baseline risk of mortality in critically ill patients: re-analysis of three recent sepsis and ARDS randomised controlled trials. *Crit Care*. 2019 May 3;23(1):156.
17. Quade D. Rank analysis of covariance. *J Am Stat Assoc*. 1967;62(320):1187-1200.
18. Conover WJ, Iman RL. Analysis of covariance using the rank transformation. *Biometrics*. 1982;38(3):715-724.

19. Mancl EE, Muzevich KM. Tolerability and safety of enteral nutrition in critically ill patients receiving intravenous vasopressor therapy. *JPEN J Parenter Enteral Nutr*. 2013;37(5):641-651.

20. Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med*. 2008;358(9):877-887.

21. Kim J, Arnaout L, Remick D. Hydrocortisone, ascorbic acid and thiamine (HAT) therapy decreases oxidative stress, improves cardiovascular function and improves survival in murine sepsis. *Shock*. 2020 Apr;53(4):460-467.

22. Wang Y, Lin H, Lin B-W, Lin J-D. Effects of different ascorbic acid doses on the mortality of critically ill patients: a meta-analysis. *Ann Intensive Care*. 2019;9(1):58.

23. Dalimonte MA, DeGrado JR, Anger KE. Vasoactive agents for adult septic shock: an update and review [published online ahead of print May 5, 2019]. *J Pharm Pract*. 2019. https://doi.org/10.1177/0897190019844124.

24. Marik PE. Vitamin C for the treatment of sepsis: the scientific rationale. *Pharmacol Ther*. 2018;189:63-70.

25. Carr AC, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care*. 2015;19:418.

26. Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrtens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care*. 2017;21(1):300.

27. Barabutis N, Khangoora V, Marik PE, Catravas JD. Hydrocortisone and ascorbic acid synergistically prevent and repair lipopolysaccharide-induced pulmonary endothelial barrier dysfunction. *Chest*. 2017;152(5):954-962.

28. de Andrade JAA, Gayer CRM, Nogueira NP de A, et al. The effect of thiamine deficiency on inflammation, oxidative stress and cellular migration in an experimental model of sepsis. *J Inflamm (Lond)*. 2014;11:11.

29. Donnino MW, Andersen LW, Chase M, et al. Randomized, double-blind, placebo-controlled trial of thiamine as a metabolic resuscitator in septic shock: a pilot study. *Crit Care Med*. 2016;44(2):360-367.

CHEST

Combined Treatment With Hydrocortisone, Vitamin C, and Thiamine for Sepsis and Septic Shock

A Randomized Controlled Trial

Ping Chang, MD, PhD; Yuping Liao, MD; Jianbin Guan, MD; Yuexun Guo, MD; Ming Zhao, MD, PhD; Jianmin Hu, MD, PhD; Jian Zhou, MD; Hua Wang, MD, PhD; Zhongran Cen, MD, PhD; Ying Tang, MD; and Zhanguo Liu, MD, PhD

BACKGROUND: Whether hydrocortisone, vitamin C, and thiamine treatment can reduce the mortality of patients with sepsis is controversial.

RESEARCH QUESTION: To evaluate the efficacy and safety of hydrocortisone, vitamin C, and thiamine combination treatment for patients with sepsis or septic shock (HYVCTTSSS).

STUDY DESIGN AND METHODS: This single-blind, randomized controlled trial evaluated treatment with hydrocortisone (50 mg every 6 h for 7 days), vitamin C (1.5 g every 6 h for 4 days), and thiamine (200 mg every 12 h for 4 days) vs placebo (normal saline) in patients with sepsis. The intention-to-treat analysis was used. Primary outcome was 28-day all-cause mortality, and secondary outcomes were organ protection, procalcitonin reduction, and adverse events related to hydrocortisone, vitamin C, and thiamine.

RESULTS: Eighty patients were randomized to receive combination treatment (n = 40) or normal saline (n = 40). No difference in 28-day all-cause mortality was observed (27.5% vs 35%, respectively; $P = .47$); however, treatment was associated with a significant improvement of 72-h change in Sequential Organ Failure Assessment score ($P = .02$). In adverse events analysis, the treatment group exhibited more incidents of hypernatremia ($P = .005$). In prespecified subgroup analysis, patients of the treatment subgroup diagnosed with sepsis within 48 h showed lower mortality than those in the control subgroup ($P = .02$). The study was terminated after the midterm analysis.

INTERPRETATION: Among patients with sepsis or septic shock, the combination of hydrocortisone, vitamin C, and thiamine did not reduce mortality compared with placebo.

TRIAL REGISTRY: ClinicalTrials.gov; No.: NCT03258684; URL: www.clinicaltrials.gov

CHEST 2020; 158(1):174-182

KEY WORDS: hydrocortisone; HYVCTTSSS; sepsis; thiamine; vitamin C

FOR EDITORIAL COMMENT, SEE PAGE 13

ABBREVIATIONS: LOS = length of stay; PCT = procalcitonin; RR = relative risk; SOFA = Sequential Organ Failure Assessment

AFFILIATIONS: From the Department of Critical Care Medicine (Drs Chang, Liao, Guan, Guo, Zhou, Wang, Cen, Tang, and Liu), Zhujiang Hospital, Southern Medical University, Guangzhou; the Department of Critical Care Medicine (Dr Liao), Dongguan People's Hospital, Dongguan; and the Department of Organ Transplant (Drs Zhao and Hu), Zhujiang Hospital, Southern Medical University, Guangzhou, China.

Drs Chang, Liao, Guan, and Guo contributed equally to this manuscript.

FUNDING/SUPPORT: This research was supported by the National Natural Science Foundation of China [Grant 81971859], the Science and Technology Planning Project of Guangdong Province [Grant 2014A020212203], and the Clinical Research Startup Program of Southern Medical University by the High-level University Construction Funding of Guangdong Provincial Department of Education [Grant LC2019ZD014].

CORRESPONDENCE TO: Zhanguo Liu, MD, PhD, Department of Critical Care Medicine, Zhujiang Hospital, Southern Medical University, 253 Gongye Rd, Guangzhou 510282, China; e-mail: zhguoliu@163.com

DOI: https://doi.org/10.1016/j.chest.2020.02.065

Sepsis rapidly progresses causing multiple organ dysfunction. In developed countries, approximately 2.8 million individuals die from sepsis annually; in most low-income countries, the mortality of sepsis and septic shock is twofold higher.[1-3] The World Health Organization recognizes sepsis as a primary health threat.[4] In the last 30 years, new therapeutic approaches for sepsis have been explored. However, there is insufficient evidence to support the effectiveness of therapies beyond basic treatment, such as the use of antibiotics, vasoactive drugs, and fluid resuscitation.[5] Commonly used adjuvant therapies are only weakly recommended by the 2016 guidelines for the management of sepsis.[6] Therefore, safe, effective, and inexpensive adjuvant treatments are required for sepsis.

Vitamin C levels rapidly decline in critically ill patients, and plasma vitamin C levels in patients with sepsis are lower than other critically ill patients.[7] Vitamin C is a strong antioxidant that prevents vascular endothelial damage and maintains microvascular integrity.[8] Moreover, it acts as a cofactor for catecholamine synthesis to help maintain vascular tone and cardiac output.[9,10] Furthermore, vitamin C promotes lymphocyte proliferation, thereby helping neutrophils kill bacteria and improving the chemotaxis of WBCs.[11] In a randomized, double-blind, placebo-controlled study of 24 patients, vitamin C reduced Sequential Organ Failure Assessment (SOFA) score, C reactive protein level, and procalcitonin (PCT) inflammatory markers. Moreover, the study confirmed the safety of a high dose (200 mg/kg/24 h) of IV vitamin C.[12] In another randomized, double-blind, placebo-controlled trial of vitamin C for the treatment of surgical septic shock involving 28 patients, 25 mg/kg IV vitamin C administered every 6 h for 3 days significantly reduced the dose of norepinephrine required and shortened the duration of administration.[13] However, there is insufficient evidence that vitamin C can reduce mortality. Glucocorticoids have widely been used in the treatment of sepsis for years. A study showed that hydrocortisone adjuvant therapy in patients with septic shock reduced time to shock relief and length of stay (LOS) in the ICU but not 90-day mortality.[14] The use of glucocorticoid combined with vitamin C maybe more effective. On the one hand, vitamin C contributes to the recovery of glucocorticoid receptor function,[15,16] whereas hydrocortisone promotes the expression of the vitamin C transporter SVCT2.[17-19] On the other hand, both vitamin C and hydrocortisone enhance endothelial barrier function.[20,21] Thiamine is an important cofactor involved in lipid, glucose, amino acid, and neurotransmitter metabolism.[22] Simultaneously, thiamine can promote oxalate decomposition, thereby reducing vitamin C metabolite oxalate deposition and crystallization in the kidneys.[23-25]

A study suggested that combined hydrocortisone, vitamin C, and thiamine treatment can reverse organ dysfunction in patients with sepsis and improve their prognosis.[26] This view was confirmed by a retrospective study by Marik et al.[27] The mortality of the treatment group was significantly lower than that of the control group ($P <$ .001), and SOFA score and requirement for vasopressor drugs decreased in patients in the treatment group ($P <$ .001). Considering these findings, these three affordable and readily available drugs offer a promising adjuvant treatment for sepsis. However, that was a retrospective study, and evidence from randomized controlled trials to evaluate the efficacy of the combination treatment is urgently required.[28] Therefore, to evaluate the efficacy and safety of hydrocortisone, vitamin C, and thiamine combination treatment for patients with sepsis or septic shock, we conducted a randomized controlled trial using the same regimen described by Marik et al.[27]

Methods

This single-center, single-blind, randomized, parallel, controlled trial was performed at Zhujiang Hospital of Southern Medical University in Guangdong Province, China. The protocol and statistical analysis were designed by the research initiators and revised according to the opinions of the clinical trial committee of Zhujiang Hospital. The study was conducted in accordance with the 1964 Declaration of Helsinki and relevant clinical research regulations in China. The protocol was approved by the Clinical Ethics Committee of Zhujiang Hospital of Southern Medical University (2017-ZZYXK-002) and was registered on ClinicalTrials.gov (NCT03258684). Informed consent was provided by all patients or their families.

The detailed methods of the study are described in the study protocol in e-Appendix 1. Briefly, we prospectively recruited patients with sepsis or septic shock using the following inclusion criteria: (1) meeting the diagnostic criteria for Sepsis-3 developed by the American Society of Critical Care Medicine/European Society of Intensive Care Medicine,[29] (2) $\geq$ 18 years of age, and (3) PCT $\geq$ 2 ng/mL when entering the ICU.[30] The exclusion criteria were pregnancy; limitations of care (families discontinued using treatment for sepsis); noninfectious factors, such as severe head injury, uncontrollable major bleeding, cardiogenic shock, advanced tumors, and paraquat poisoning, that may lead to death; and persistent infection sources that cannot be removed by puncture and drainage, debridement, or other surgical procedures.

After confirming eligibility, participants were randomly assigned to the treatment or control group. The treatment group was administered IV hydrocortisone (50 mg every 6 h for 7 days or until ICU discharge, whichever occurred first), vitamin C (1.5 g every 6 h for 4 days or

until ICU discharge, whichever occurred first), and IV thiamine (200 mg every 12 h for 4 days or until ICU discharge, whichever occurred first). The control group was administered the same frequency and volume of saline as the treatment group. Neither the patients nor their families knew what intervention was being administered. In addition, all patients were routinely monitored by attending physicians with reference to the 2016 international management of sepsis guidelines,[6] including early initial resuscitation, diagnosis of infection and early antimicrobial therapy, vasopressor strategy, mechanical ventilation, and renal replacement therapy.

The primary outcome was mortality from any cause within 28 days after randomization. Secondary outcomes included the duration of vasopressor use, ICU LOS, change in SOFA (ΔSOFA) score within 72 h after experimental intervention, and PCT clearance rate within 72 h after experimental intervention.[31,32] All vasopressor doses were converted to the norepinephrine equivalent dosage.[33] Baseline data collected included age; sex; site of infection; comorbidities; blood culture results; vasopressor and mechanical ventilation requirements; lactic acid, bilirubin, creatinine, and PCT levels; SOFA score; and Acute Physiology and Chronic Health Evaluation II score.

Statistical Analysis

According to the previous treatment of patients with sepsis in the research center, it is estimated that the 28-day mortality in the control group is 40%. The treatment group is expected to have the mortality reduced by 30% as observed in the study by Marik et al.[27] For a two-sided test, 114 patients (57 patients in each group) will provide 90% power to detect a 30% difference in mortality. Assuming that 20% of the patients would withdraw or be lost to follow-up during treatment, the sample size was calculated as 140 patients. The Pearson χ^2 test was used for the analysis of dichotomous variables (if it was not applicable, the Fisher exact test was used). For continuous outcome variables with a normal distribution, a two-sample t test was performed. Mann-Whitney U test was used for nonparametric data. The level of statistical significance was set at $P < .05$. Moreover, primary outcome was examined in three prespecified subgroups, which were defined according to the following indicators that may affect mortality risk: age $\geq$ 65 vs $<$ 65 years, Acute Physiology and Chronic Health Evaluation II score $\geq$ 25 vs $<$ 25, and the duration of sepsis at enrollment $>$ 48 vs $\leq$ 48 h. All tests were two-sided with no adjustment for the primary outcome. Survival of both groups was compared using the Kaplan-Meier (log-rank test) method, and the difference in survival was evaluated using a Cox proportional hazards model. SPSS 23 (IBM) was used to perform data analysis.

Interim Analysis and Early Termination

The statisticians conducted an interim analysis when the sample size reached one-half the determined size. The experiment was considered for early termination in case it reached the O'Brien-Fleming stopping boundary[34] (ie, $P < .005$ for primary end point or any incidence of adverse events that may affect the treatment of the patient). Interim analysis was completed under the supervision of the Clinical Ethics Committee of Zhujiang Hospital, which ultimately decided whether to proceed with the study.

Results

From September 25, 2017, to January 7, 2019, 159 suspected patients with sepsis were screened; 80 patients who were willing to participate in the study were eventually recruited in the trial (Fig 1). Of the 40 patients in the treatment group, two patients dropped out of the trial because of severe hypernatremia and GI bleeding. In the control group, 28 of 40 patients received only routine treatment with nonadministration of a placebo. The treating physicians of these patients thought that the extra use of normal saline may not be conducive to volume management of the patients. Hence, at the request of the treating physicians, these patients only received routine treatment as control treatment. The 28-day survival information was obtained and no patients were lost to follow-up. All comparisons are reported in the form of the treatment group vs the control group. The study was discontinued after interim analysis because of the high incidence of severe hypernatremia ($>$ 160 mmol/L) and ineffectiveness of the combined treatment protocol.

Baseline Characteristics

The intention-to-treat analysis included all 80 patients. Baseline characteristics of both groups were similar (Table 1). Pulmonary infection was the most common infection in both treatment and control groups (31 vs 27, respectively). Most patients in the two groups exhibited comorbidities, including diabetes (14 vs 15), hypertension (16 vs 16), and cerebrovascular accident (13 vs 9), when they entered the ICU, respectively. The number of patients requiring mechanical ventilation (30 vs 32), patients with acute kidney injury (17 vs 21), and patients requiring vasoactive drugs (22 vs 24) did not significantly differ between the two groups, respectively. There were no significant differences in WBC counts or in lactate, creatinine, or bilirubin levels. The similar SOFA scores (9.6 $\pm$ 4.5 vs 10.1 $\pm$ 4.0) and Acute Physiology and Chronic Health Evaluation II scores (22.1 $\pm$ 8.4 vs 23.8 $\pm$ 7.6) reflected similar organ function status and disease severity between the groups, respectively.

Primary Outcome and Secondary Outcomes

Table 2 shows the results of all primary and secondary outcomes. For primary outcome, on the 28th day after treatment, there was no difference in mortality between the treatment and control groups (relative risk [RR], 0.79; 95% CI, 0.41-1.52; $P = .47$). For secondary outcomes, median ICU LOS was 7.5 days (4-12.8) and 7.5 days (4-11.8) in the treatment and control groups, respectively, which was not a significant difference. Furthermore, there were no significant differences between the two groups in terms of the duration of vasoactive drug use (46 h; 23.8-102.5 vs 58.5 h; 28-104),

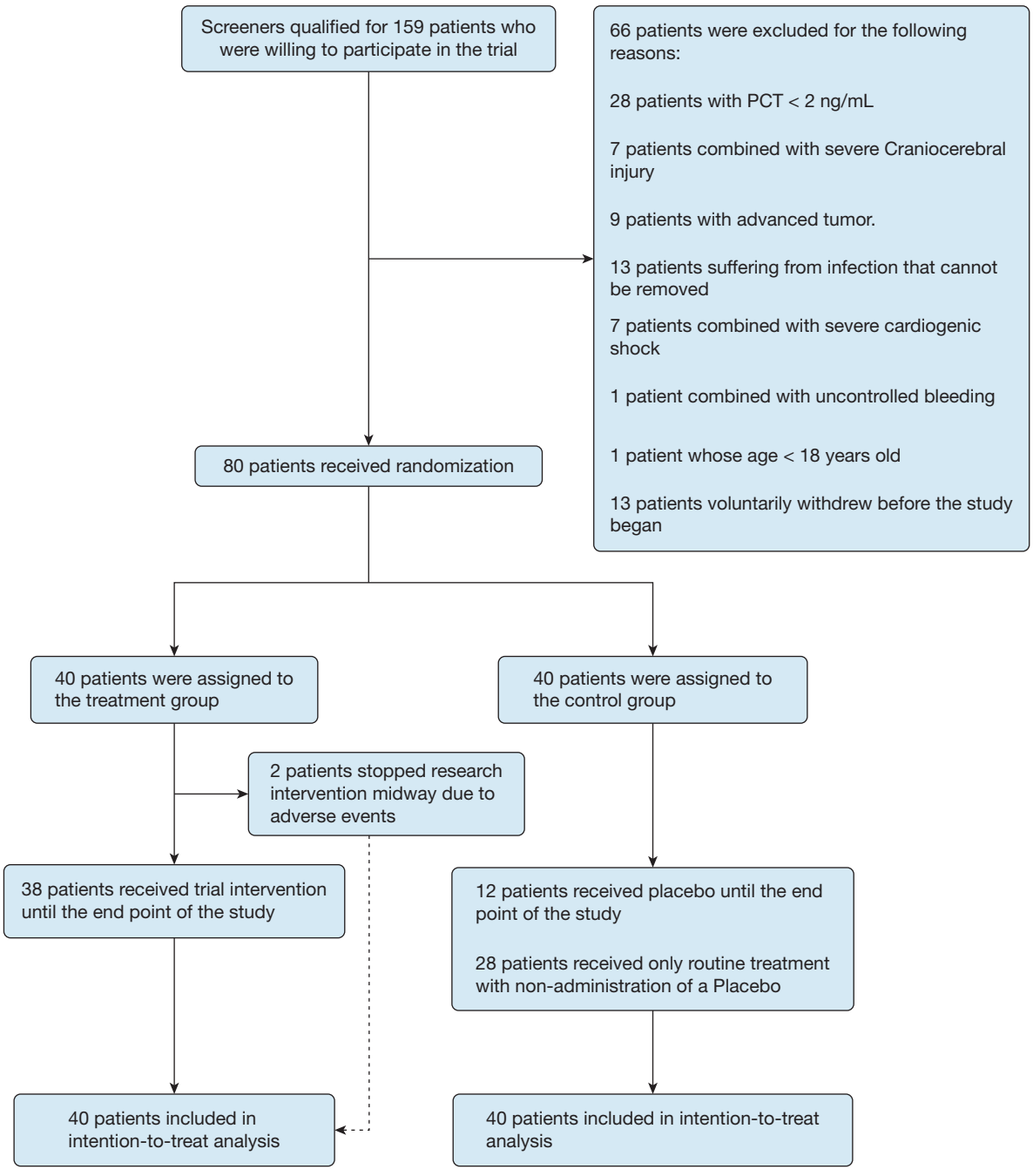


Figure 1 – *Trial flowchart. There were two patients in the treatment group who discontinued the intervention because of adverse events. One patient experienced hypernatremia and the physicians interpreted that the use of hydrocortisone made it challenging to manage the patient's sodium retention. One patient withdrew because of GI bleeding because hydrocortisone may aggravate bleeding.*

median duration of mechanical ventilation (126.5 h; 63.5-239.3 vs 94.5 h; 39.8-211), or median 72-h PCT clearance rate (75.8%; 62.2-86.4 vs 68.2%; 25.9-82.5; $P > .05$), respectively. Additional post hoc analysis revealed no significant differences in the proportion of a new acute kidney injury after entering the ICU (2.5% vs 5%) and median 72-h lactate clearance rate (21.3%; −49.7 to 44.2; vs 0%; −35.1 to 47.7) between the two groups. However, the ΔSOFA score within 72 h was slightly improved in the treatment group compared with that in the control group (3.5 ± 3.3 vs 1.8 ± 3.0, respectively; $P = .02$). Simultaneously, the Kaplan-Meier survival curve indicated that the 28-day survival was not significant between the treatment and control groups (hazard ratio, 0.71; 95% CI, 0.32-1.56; $P = .40$) (Fig 2).

TABLE 1] Baseline Characteristics of the Intention-to-Treat Population

Variable	Treatment Group (n = 40)	Control Group (n = 40)
Age, y	59.5 ± 15.0	63.7 ± 12.8
Sex, male	22 (57.5)	21 (52.5)
Primary diagnosis		
Pulmonary infection	31 (77.5)	27 (67.5)
Urinary infection	5 (12.5)	6 (15)
Digestive and abdominal infection	3 (7.5)	3 (7.5)
Skin and soft tissue infection	1 (2.5)	2 (5)
Unknown site	0 (0)	1 (2.5)
Comorbidities		
None	3 (7.5)	3 (7.5)
Diabetes	14 (35)	15 (37.5)
Heart failure	3 (7.5)	3 (7.5)
Hypertension	16 (40)	16 (40)
Cerebrovascular accident	13 (32.5)	9 (22.5)
CHD	0 (0)	2 (5)
Chronic renal failure	4 (10)	5 (12.5)
Acute kidney injury	17 (42.5)	21 (52.5)
Other	8 (20)	8 (20)
Organ function support		
Mechanical ventilation	30 (75)	32 (80)
Vasopressors	22 (55)	24 (60)
Laboratory examination		
Blood culture, positive	6 (15)	9 (22.5)
WBC count,[a] $\times 10^9$/L	13.0 (8.5-16.9)	13.1 (10.4-20.4)
Lactate, mmol/L	2.2 (1.6-3.2)	2.0 (1.2-3.1)
Creatinine,[b] μmol/L	112 (68.8-200.0)	136.4 (88.5-257)
Bilirubin, μmol/L	16.4 (8.2-32.9)	18.2 (8.9-30.8)
Procalcitonin, ng/mL	20.6 (4.2-35.9)	14.3 (4.8-38.4)
SOFA score	9.6 ± 4.5	10.1 ± 4.0
APACHE II score	22.1 ± 8.4	23.8 ± 7.6

Values are No. (%), mean ± SD, or median (interquartile range). APACHE = Acute Physiology and Chronic Health Evaluation; CHD = coronary heart disease; SOFA = Sequential Organ Failure Assessment.
[a]Excluding neutropenic patients.
[b]Excluding patients with chronic renal failure.

Subgroup Analysis

In the subgroup analysis of primary outcome, only the subgroup diagnosed with sepsis within 48 h at ICU admission showed an improvement in mortality in the treatment group (13.6% vs 47.6%; RR, 0.29; 95% CI, 0.09-0.90; $P = .02$). In the post hoc analysis of secondary outcome indicators for this subgroup, the median PCT clearance rate (75.6%; 62.3-92.0 vs 58.9%; 16.0-79.5, respectively; $P = .02$) was significantly higher in the treatment group than in the control group (e-Fig 1). For the median ICU retention time, median duration of vasoactive drug use, median 72-h lactate clearance rate, and 72-h ΔSOFA, the treatment group showed better outcomes than the control group, but they were not significant ($P > .05$) (e-Table 1). The primary outcomes of the other subgroups were not significant between the two groups (Fig 3).

Adverse Events Analysis

Adverse events were defined as side effects that occur after the trial intervention. As a result, the attending physicians and researchers recorded a total of 23 side effects (e-Table 2).

TABLE 2] Primary and Secondary Outcomes

Variable	Treatment Group (n = 40)	Control Group (n = 40)	Relative Risk or Difference (95% CI)	*P* Value
28-d mortality	11 (27.5)	14 (35)	0.79 (0.41-1.52)	.47
ICU LOS, d	7.5 (4-12.8)	7.5 (4-11.8)	…	.98
Duration of vasopressors,[a] h	46 (23.8-102.5)	58.5 (28-104)	…	.70
New AKI after entering ICU	1 (2.5)	2 (5)	0.50 (0.05-5.30)	> .99
△ SOFA score, 72 h	3.5 ± 3.3	1.8 ± 3.0	…	.02
Procalcitonin clearance, 72 h	75.8 (62.2-86.4)	68.2 (25.9-82.5)	…	.07
Duration mechanical ventilation,[b] h	126.5 (63.5-239.3)	94.5 (39.8-211)	…	.36
Lactate clearance, 72 h, %	21.3 (−49.7 to 44.2)	0 (−35.1 to 47.7)	…	.98

Values are No. (%), median (interquartile range), mean ± SD, or as otherwise indicated. Missing data for indicators were estimated using the last observation carried forward scheme. AKI = acute kidney injury; LOS = length of stay. See Table 1 legend for expansion of other abbreviation.
[a]Excluding patients without vasopressor support (18 patients in the treatment group vs 16 patients in the control group).
[b]Excluding patients without mechanical ventilation (10 patients in the treatment group vs eight patients in the control group).

Among them, 16 patients (13 in the treatment group vs three in the control group; RR, 4.33; 95% CI, 1.34-14.1; $P = .005$) were diagnosed with severe hypernatremia (> 160 mmol/L) (e-Figs 2-4, e-Table 3). In addition, five patients showed GI bleeding (three in the treatment group vs two in the control group). Furthermore, a new infection was reported in the treatment group. After consulting with the attending physicians, we initiated the necessary treatments, including the discontinuation of trial interventions (n = 2).

Discussion

In our study, we found that hydrocortisone, vitamin C, and thiamine did not significantly reduce the mortality of patients with sepsis and septic shock, which is consistent with the results of a retrospective study by Litwak et al.[35] In this retrospective analysis of real-world application, Litwak et al.[35] found that no significant difference in hospital mortality and secondary outcomes, including ICU mortality, requirement for renal replacement therapy for acute kidney injury, ICU LOS, hospital LOS, and time to vasopressor independence between the treatment and control groups.

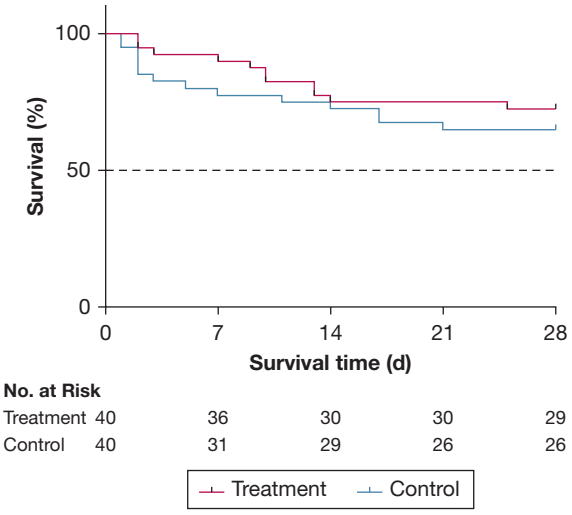


Figure 2 – *Kaplan-Meier estimates of survival rate distribution among patients in the treatment or control group. Log-rank (Mantel-Cox) test (P = .42) for intergroup differences in survival rate distribution. Hazard ratio for mortality is 0.71 (95% CI, 0.32-1.56; P = .40); P value was calculated using a Cox proportional hazards model that included the randomized trial group.*

The HYVCTTSSS study was performed in a large tertiary teaching hospital in Guangzhou, China. Most patients were referred from secondary hospitals, and patients were in all stages of sepsis when they were transferred to the hospital ICU. Therefore, there may be differences in the effects of intervention between patients at different stages of sepsis. In the prespecified subgroup of patients who were diagnosed with sepsis within 48 h, the treatment group showed a better therapeutic effect than the control group, which was reflected mainly in improvement in the 28-day mortality and 72-h PCT clearance rate. Moreover, the survival rate of the treatment group increased by 34% compared with the control group, which is extremely close to the 37.9% value reported by Marik et al.[27] Therefore, the efficacy of this combination therapy in the early stage of sepsis may still be worth exploring. Moreover, in the early stage of sepsis, the release of numerous cytokines and dysregulation of inflammatory response caused by damaged tissues can injure vascular endothelial cells, leading to acute organ dysfunction.[36] Therefore, restoring vascular endothelial integrity and capillary function and the early reduction of inflammatory reaction in sepsis are important targets for the treatment of sepsis. Together with the pharmacologic mechanisms

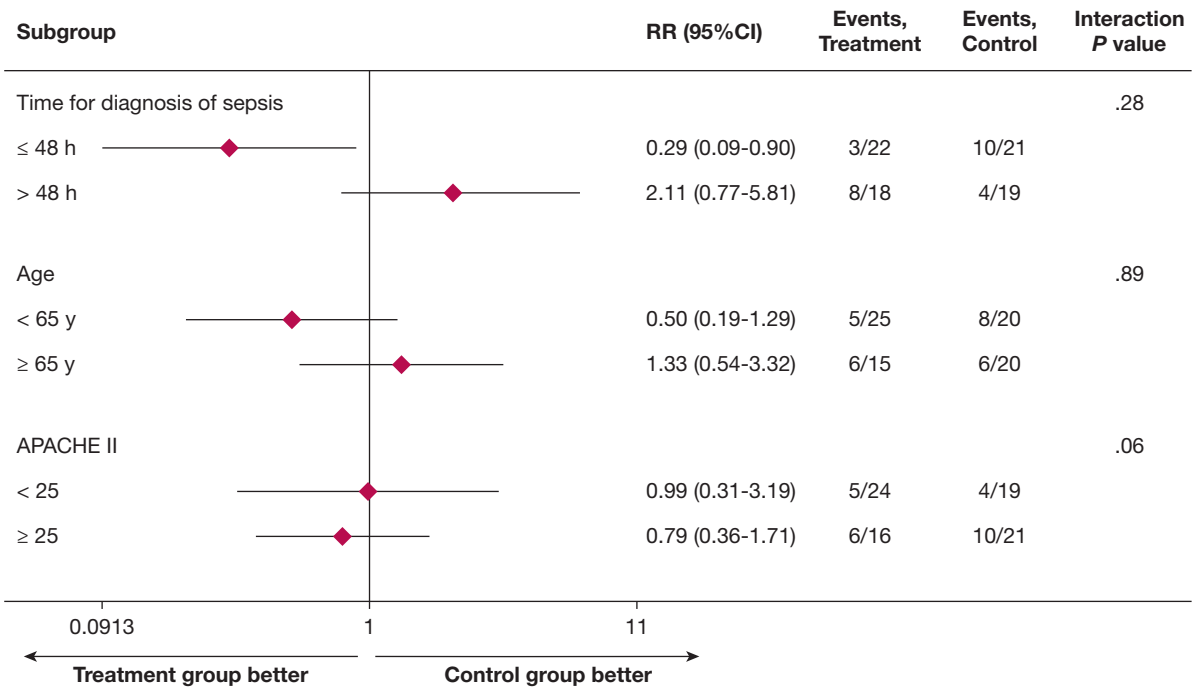


Figure 3 – *Subgroup analysis. Subgroup analysis of mortality at 28 d. The forest map shows the grouped variables of the subgroup analysis, RR, 95% CI in each subgroup, number of patients (denominator), and number of deaths (numerator) in each subgroup. APACHE = Acute Physiology and Chronic Health Evaluation; RR = relative risk.*

of hydrocortisone, vitamin C, and thiamine, and our results, we speculate that the early use of combination treatment may be meaningful but not for all patients at different stages of sepsis.

In addition, we observed that the treatment group showed a higher risk of severe hypernatremia compared with the control group, which may be related to the promotion of sodium retention by glucocorticoids. In a large randomized controlled study of hydrocortisone for the treatment of septic shock, the treatment group was administered hydrocortisone 50 mg every 6 h for a total of 5 days. The results showed that hydrocortisone increased the risk of hypernatremia (RR, 1.58; 95% CI, 1.13-2.22).[37] Therefore, we should also pay attention to side effects, such as severe hypernatremia.

In the interim analysis, the combination therapy did not show a significant improvement trend compared with placebo for patients with sepsis. Additionally, significant differences in severe hypernatremia between the two groups reached the threshold for termination as defined by the O'Brien-Fleming stopping boundary ($P < .005$). Considering these reasons, we terminated the experiment in advance according to the ethics committee.

Two recent trials have been published in *JAMA* on vitamin C protocol for the treatment of sepsis. The CITRIS-ALI trial found that high-dose vitamin C compared with placebo did not significantly improve organ dysfunction scores in patients with sepsis and ARDS, but exploratory analysis found a lower 28-day mortality in the vitamin C group.[38] The VITAMINS trial showed that the combination of vitamin C, hydrocortisone, and thiamine did not reduce time to shock relief over 7 days or 28-day mortality compared with hydrocortisone alone in patients with septic shock.[39] The difference in the results of the two trials suggests that more trials are needed to provide evidence for the efficacy of the vitamin C protocol. Our study could enrich the clinical evidence of vitamin C protocol for the treatment of sepsis. However, there remain some limitations that cannot be avoided. First, the trial is slightly underpowered to detect a minimal clinically important difference because of the early termination. Second, the sample size was small and this was a single-center, single-blind study design, which may lead to selective bias to some extent. Third, 28 patients in the control group received only routine treatment with nonadministration of a placebo, which may affect the exclusion of placebo effects from this combination protocol. Finally, our experimental therapeutic dosage was performed according to the recommended dosage by Marik et al.[27] In the future, there is a need to

determine the optimal therapeutic dosage for this treatment.

Interpretation

Hydrocortisone, vitamin C, and thiamine did not appear to reduce 28-day mortality compared with placebo in patients with sepsis or septic shock. Moreover, we must pay attention to side effects, such as severe hypernatremia. However, larger sample, multicenter, randomized controlled trials are required to validate the effectiveness and timing of this treatment.

Acknowledgments

Author contributions: Z. L. and P. C. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Z. L. and P. C. were the principal investigators and prepared the study design and protocol, which was approved by all authors. P. C. was responsible for supervising the implementation of the study. Y. T. and Z. C. were responsible for the screening and registration of patients. Z. L. performed the randomization of patients. Y. L., J. G., and Y. G. implemented the trial and conducted data collection and checked the database for accuracy. J. Z., M. Z., J. H., and H. W. performed the statistical data analysis and interpretation. P. C., Z. L., J. G., Y. L., and Y. G. were responsible for writing the manuscript. All authors have read, revised, and approved the manuscript.

Financial/nonfinancial disclosures: None declared.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Other contributions: We thank all ICU medical staff at Zhujiang Hospital, including the nurses, rehabilitation therapists, and attending physicians of the trial patients.

Additional information: The e-Appendix, e-Figures, and e-Tables can be found in the Supplemental Materials section of the online article.

References

1. Gizem P, Anil UR, Elif C, Halici Z. Sepsis and septic shock: current treatment strategies and new approaches. *Eurasian J Med*. 2017;49(1):53-58.
2. Machado FR, Cavalcanti AB, Bozza FA, et al. The epidemiology of sepsis in Brazilian intensive care units (the Sepsis PREvalence Assessment Database, SPREAD): an observational study. *Lancet Infect Dis*. 2017;17(11):1180-1189.
3. Rhee C, Dantes R, Epstein L, et al. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. *JAMA*. 2017;318(13):1241-1249.
4. Reinhart K, Daniels R, Kissoon N, et al. Recognizing sepsis as a global health priority - a WHO resolution. *N Engl J Med*. 2017;377(5):414-417.
5. Artenstein AW, Higgins TL, Opal SM. Sepsis and scientific revolutions. *Crit Care Med*. 2013;41(12):2770-2772.
6. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Crit Care Med*. 2017;45(3):486-552.
7. Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrtens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care*. 2017;21(1):300.
8. Koekkoek WA, van Zanten AR. Antioxidant vitamins and trace elements in critical illness. *Nutr Clin Pract*. 2016;31(4):457-474.
9. Treschan TA, Peters J. The vasopressin system: physiology and clinical strategies. *Anesthesiology*. 2006;105(3):599-612.
10. May JM, Qu ZC, Meredith ME. Mechanisms of ascorbic acid stimulation of norepinephrine synthesis in neuronal cells. *Biochem Biophys Res Commun*. 2012;426(1):148-152.
11. Ströhle A, Hahn A. Vitamin C and immune function. *Med Monatsschr Pharm*. 2009;32(2):49-54.
12. Syed AA, Knowlson S, Sculthorpe R, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med*. 2014;12(1):32.
13. Zabet MH, Mohammadi M, Ramezani M, Khalili H. Effect of high-dose ascorbic acid on vasopressor's requirement in septic shock. *J Res Pharm Pract*. 2016;5(2):94-100.
14. Venkatesh B, Finfer S, Cohen J, et al. Adjunctive glucocorticoid therapy in patients with septic shock. *N Engl J Med*. 2018;378(9):797-808.
15. Okamoto K, Tanaka H, Ogawa H, et al. Redox-dependent regulation of nuclear import of the glucocorticoid receptor. *J Biol Chem*. 1999;274(15):10363-10371.
16. Okamoto K, Tanaka H, Makino Y, et al. Restoration of the glucocorticoid receptor function by the phosphodiester compound of vitamins C and E, EPC-K1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl hydrogen phosphate] potassium salt), via a redox-dependent Mechanism. *Biochem Pharmacol*. 1998;56(1):79-86.
17. Bürzle M, Hediger MA. Functional and physiological role of vitamin C transporters. *Curr Top Membr*. 2012;70:357-375.
18. Seno T, Inoue N, Matsui K, et al. Functional expression of sodium-dependent vitamin C transporter 2 in human endothelial cells. *J Vasc Res*. 2004;41(4):345-351.
19. Fujita I, Hirano J, Itoh N, Nakanishi T, Tanaka K. Dexamethasone induces sodium-dependant vitamin C transporter in a mouse osteoblastic cell line MC3T3-E1. *Br J Nutr*. 2001;86(2):145-149.
20. Han M, Pendem S, Teh SL, Sukumaran DK, Wu F, Wilson JX. Ascorbate protects endothelial barrier function during septic insult: role of protein phosphatase type 2A. *Free Radic Biol Med*. 2010;48(1):128-135.
21. Marik PE. Critical illness-related corticosteroid insufficiency. *Chest*. 2009;135(1):181-193.
22. Abdou E, Hazell AS. Thiamine deficiency: an update of pathophysiologic mechanisms and future therapeutic considerations. *Neurochem Res*. 2015;40(2):353-361.
23. Buehner M, Pamplin J, Studer L, et al. Oxalate nephropathy after continuous infusion of high-dose vitamin C as an adjunct to burn resuscitation. *J Burn Care Res*. 2016;37(4):e374-e379.
24. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate increases human oxaluria and kidney stone risk. *J Nutr*. 2005;135(7):1673-1677.
25. Sidhu H, Gupta R, Thind SK, et al. Oxalate metabolism in thiamine-deficient rats. *Ann Nutr Metab*. 1987;31(6):354-361.
26. Marik PE. Vitamin C for the treatment of sepsis: the scientific rationale. *Pharmacol Ther*. 2018;189:63-70.
27. 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.
28. Spoelstra-de Man AME, Elbers PWG, Oudemans-Van Straaten HM. Vitamin C: Should we supplement? *Curr Opin Crit Care*. 2018;24(4):248-255.
29. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
30. Charles PE, Tinel C, Barbar S, et al. Procalcitonin kinetics within the first days of sepsis: relationship with the appropriateness of antibiotic therapy and the outcome. *Crit Care*. 2009;13(2):R38.

31. Schuetz P, Maurer P, Punjabi V, et al. Procalcitonin decrease over 72 hours in US critical care units predicts fatal outcome in sepsis patients. *Crit Care*. 2013;17(3):R115.

32. Ferreira FL, Bota DP, Bross A, Mélot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA*. 2001;286(14):1754-1758.

33. Mancl EE, Muzevich KM. Tolerability and safety of enteral nutrition in critically ill patients receiving intravenous vasopressor therapy. *JPEN J Parenter Enter Nutr*. 2013;37(5):641-651.

34. Pocock GSJ. Interim analyses in randomized clinical trials: ramifications and guidelines for practitioners. *Biometrics*. 1987;43(1):213-223.

35. Litwak J, Cho N, Nguyen H, et al. Vitamin C, hydrocortisone, and thiamine for the treatment of severe sepsis and septic shock: a retrospective analysis of real-world application. *J Clin Med*. 2019;8(4):478.

36. Ince C, Mayeux PR, Nguyen T, et al. The endothelium in sepsis. *Shock*. 2016;45(3):259-270.

37. Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. *N Engl J Med*. 2008;358(2):111-124.

38. Fowler AA, Truwit 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. *JAMA*. 2019;322(13):1261-1270.

39. Fujii T, Luethi N, Young PJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. *JAMA*. 2020;323(5):423-431.