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Combined treatment with hydrocortisone, vitamin C, and thiamine for sepsis and septic shock (HYVCTTSSS): A randomized controlled clinical trial

Short title: hydrocortisone, vitamin C and thiamine for sepsis

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Abbreviations list

APACHE II = Acute physiology and chronic health evaluation; ICU = Intensive care unit; IQR = interquartile range; LOS = length of stay; PCT = procalcitonin; SOFA = sequential organ failure assessment

Abstract

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

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%; P = 0.47), although treatment was associated with a significant improvement of 72-h ΔSOFA score (P = 0.02). In adverse events analysis, the treatment group exhibited more incidents of hypernatremia (P = 0.005). 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 = 0.02). The study was terminated after the mid-term analysis.

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

Clinical Trial Registry: Clinicaltrials.gov; No.: NCT03258684; URL: www.clinicaltrials.gov.

Key Words: HYVCTTSSS, hydrocortisone, vitamin C, thiamine, sepsis

Introduction

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 two-fold higher.[1, 2, 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 white blood cells.[11] In a randomized, double-blind, placebo-controlled clinical study of 24 patients, vitamin C reduced sequential organ failure assessment (SOFA) score, C reactive protein level, and procalcitonin inflammatory markers. Moreover, the study confirmed the safety of a high dose (200 mg/kg/24 h) of intravenous vitamin C.[12] In another randomized, double-blind, placebo-controlled clinical trial of vitamin C for the treatment of surgical septic shock involving 28 patients, 25 mg/kg intravenous 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 recent study showed that hydrocortisone adjuvant therapy in patients with septic shock reduced time to shock relief and length of stay (LOS) in the intensive care unit (ICU LOS) but not the 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, 18, 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, 24, 25]

A recent 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 ($p < 0.001$), SOFA score and requirement for vasopressor drugs decreased in patients in the treatment group ($p < 0.001$). Considering these findings, these three affordable and readily available drugs offer a promising adjuvant treatment for sepsis. However, that was a retrospective study, and evidences from randomized controlled trials to evaluate the efficacy of the combination treatment are 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 the 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 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 (SCCM)/European Society of Intensive Care Medicine (ESICM),[29] (2) age ⩾18 years, and (3) procalcitonin (PCT) ⩾ 2 ng/mL when entering the ICU.[30] The exclusion criteria were pregnancy; limitations of care (families discontinued using treatment for sepsis); and non-infectious 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 intravenous 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 intravenous 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) 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 procalcitonin levels; SOFA score; and acute physiology and chronic health evaluation (APACHE) 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 chi-square test was used for the analysis of dichotomous variables (if it was not applicable, the Fisher's 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 $p < 0.05$. Moreover, primary outcome was examined in three prespecified subgroups, which were defined according to the following indicators that may affect mortality risk: age ≥ 65 years vs. <65 years, APACHE II score ≥ 25 vs. < 25, and the duration of sepsis at enrollment > 48 h vs. ≤ 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 half the determined size. The experiment was considered for early termination in case it reaches the O'Brien–Fleming stopping boundary[34] (i.e., $p < 0.005$ 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 (Figure 1). Of the 40 patients in the treatment group, 2 patients dropped out of the trial due to severe hypernatremia and gastrointestinal 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. 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. 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 the 80 patients. Baseline characteristics of both groups were similar (Table 1). Pulmonary infection was the most common site of infection in both treatment and control groups (31 vs. 27). 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. The number of patients requiring mechanical ventilation (30 vs. 32), patients with acute kidney injury cases (17 vs. 21), and patients requiring vasoactive drugs (22 vs. 24) did not significantly differ between the two groups. There were no significant differences in white blood cells or in lactate, creatinine, or bilirubin levels. The similar SOFA scores (9.6 ± 4.5 vs. 10.1 ± 4.0) and APACHE II scores (22.1 ± 8.4 vs. 23.8 ± 7.6) reflected similar organ function status and disease severity between the groups.

Primary outcome and secondary outcomes

Table 2 shows the results of all primary and secondary outcomes. On the 28th day after treatment, there was no difference in mortality between the treatment and control groups [relative risk (RR), 0.79; 95% confidence interval (CI), 0.41 to 1.52; p = 0.47]. Median ICU LOS was 7.5 (4–12.8) days and 7.5 (4–11.8) days 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)], 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 > 0.05]. 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-hour lactate clearance rate [21.3% (−49.7–44.2) vs. 0% (−35.1–47.7)] 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; p = 0.02). Simultaneously, the Kaplan–Meier survival curve indicated that the 28-day survival was not significant between the treatment and control groups (HR, 0.71; 95% CI, 0.32 to 1.56; p = 0.40) (Figure 2).

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 to 0.90; p = 0.02). In the post hoc analysis of secondary outcome indicators for this subgroup, PCT clearance rate was significantly higher in the treatment group than in the control group (p = 0.02; 75.6% (62.3–92.0) vs. 58.9% (16.0–79.5); e-Figure 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 > 0.05) (e-Table 1). The primary outcomes of the other subgroups were not significant between the two groups (Figure 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). Among them, 16 patients (13 in the treatment group vs. 3 in the control group; RR, 4.33; 95% CI, 1.34 to 14.1; p = 0.005) were diagnosed with severe hypernatremia (>160 mmol/L) (e-Figure 2–4, e-table3). In addition, five patients showed gastrointestinal bleeding (3 in the treatment group vs. 2 in the control group). Further, 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 (two patients).

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. In this retrospective analysis of real-world application, Litwak et al. 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.[35]

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 the 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 as well as the early reduction of inflammatory reaction in sepsis are important targets for the treatment of sepsis. Together with the pharmacological mechanisms of hydrocortisone, vitamin C, 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 to 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 ($p < 0.005$). Considering the above 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 palcebo 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 evidences 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 due to 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. In the future, there is a need to determine the optimal therapeutic dosage for this treatment.

Interpretation

In conclusion, hydrocortisone, vitamin C, and thiamine did not appear to reduce the 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, multi-center, randomized controlled trials are required to validate the effectiveness and timing of this treatment.

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Author contributions:Z.L. and P.C .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. Z. L. and P. C .were the principal investigators. Z. L. and P. C. 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 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.

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Conflicts of interest

We declare no competing interests.

Ethical approval

The study was conducted in accordance with the 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).

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Figure legends

Figure 1 Trial flow chart

There were two patients in the treatment group who discontinued the intervention due to 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 due to gastrointestinal bleeding because hydrocortisone may aggravate bleeding.

Figure 2 Kaplan–Meier estimates of survival rate distribution among patients in the treatment or control group

Log-rank (Mantel–Cox) test p = 0.42 for intergroup differences in survival rate distribution.

Hazard ratio for mortality is 0.71; 95% confidence interval (CI), 0.32 to 1.56; p = 0.40; P value was calculated using a Cox proportional-hazards model that included the randomized trial group.

Figure 3 Subgroup analysis

Subgroup analysis of mortality at 28 days. The forest map shows the grouped variables of the subgroup analysis, relative risk of mortality (RR), 95% confidence interval (95% CI) in each subgroup, number of patients (denominator), and number of deaths (numerator) in each subgroup.

Tables

Table 1. Baseline characteristics of the intention-to-treat population

Variable	Treatment (n = 40)	Control (n = 40)
Age, mean(SD), years	59.5(15.0)	63.7(12.8)
Sex, male,n (%)	22 (57.5)	21 (52.5)
Primary diagnosis, n(%)		
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, n(%)		
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, n(%)		
Mechanical ventilation	30 (75)	32 (80)

Vasopressors	22 (55)	24 (60)
Laboratory examination		
Blood culture, n (%), positive	6 (15)	9 (22.5)
WBC, median [IQR] [a], $\times 10^9$/L	13.0 [8.5–16.9]	13.1 [10.4–20.4]
Lactate, median [IQR], mmol/L	2.2 [1.6–3.2]	2.0 [1.2–3.1]
Creatinine [b], median [IQR],umol/L	112 [68.8–200.0]	136.4 [88.5–257]
Bilirubin, median [IQR], umol/L	16.4 [8.2–32.9]	18.2 [8.9–30.8]
Procalcitonin, median [IQR], ng/mL	20.6 [4.2–35.9]	14.3 [4.8–38.4]
SOFA, mean(SD)	9.6(4.5)	10.1(4.0)
APACHE II, mean(SD)	22.1(8.4)	23.8(7.6)

CHD=Coronary heart disease; SOFA = Sepsis-related organ failure assessment;

APACHEII = Acute physiology and chronic health evaluation.

[a]Excluding neutropenic patients ;

[b] Excluding patients with chronic renal failure;

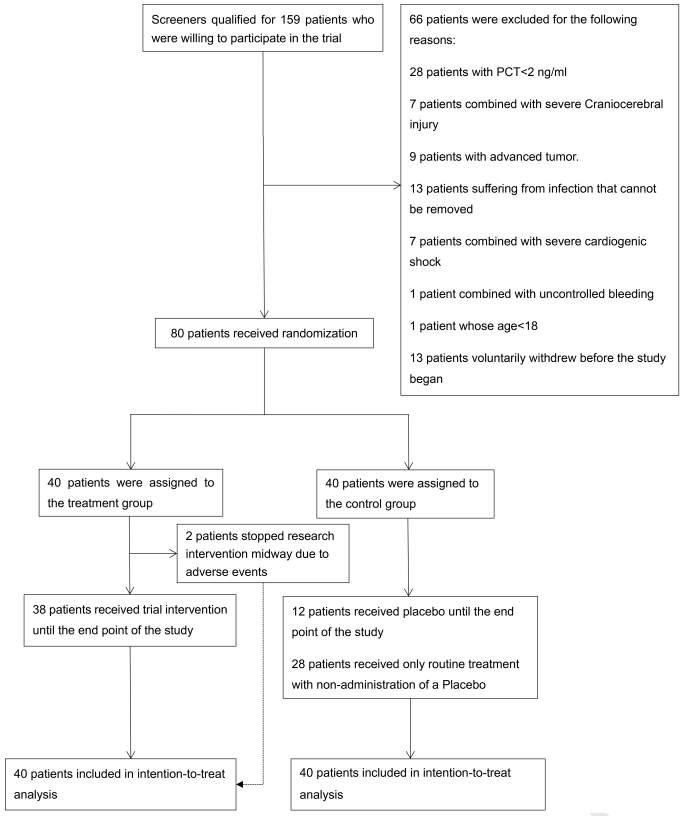
Table 2. Primary and secondary outcomes

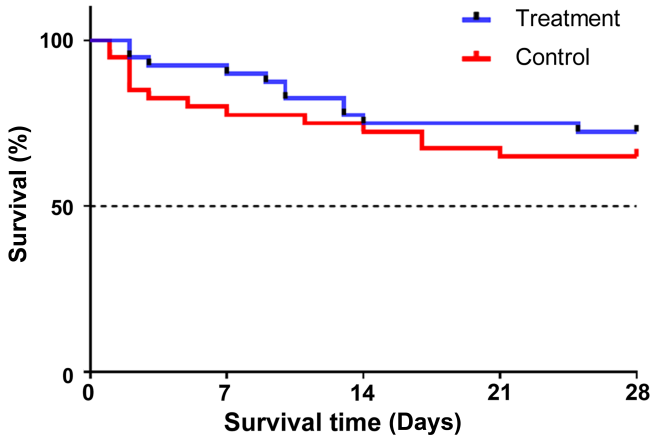
Variable	Treated (n=40)	Control (n=40)	Relative risk or difference (95% CI)	P value
28-day mortality,n (%)	11(27.5%)	14(35%)	0.79(0.41-1.52)	0.47
ICU LOS, median[IQR], days	7.5[4–12.8]	7.5[4–11.8]		0.98
Duration of vasopressors, median[IQR], h[a]	46[23.8–102.5]	58.5[28–104]		0.70
New AKI after entering ICU, n(%)	1(2.5%)	2(5%)	0.50(0.05-5.30)	1.00
△SOFA, 72h, mean(SD)	3.5(3.3)	1.8(3.0)		0.02
Procalcitonin clearance,72h, median[IQR]	75.8[62.2–86.4]	68.2[25.9–82.5]		0.07
Duration mechanical ventilation median [IQR],h[b]	126.5[63.5–239.3]	94.5[39.8–211]		0.36
Lactate clearance,72h, median[IQR],%	21.3[−49.7–44.2]	0[−35.1–47.7]		0.98

AKI = acute kidney injury; LOS = length of stay; Missing data for indicators were estimated using the last observation carry–forward (LOCF) scheme.

[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 8 patients in the control group).





No. at Risk					
Treatment	40	36	30	30	29
Control	40	31	29	26	26