Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The Effects of Ascorbic Acid Over Sepsis: Meta-analysis With Trial Sequential Analysis

Ying Wang
First Affiliated Hospital of Fujian Medical University
Hui-chang Zhuo
First Affiliated Hospital of Fujian Medical University
Jiandong Lin (1179743712@qq.com)
Department of Intensive Care Unit, the First Affiliated Hospital of Fujian Medical University, Chazhong Road, Fuzhou, Fujian Province, China.
https://orcid.org/0000-0001-8866-8117

Research

Keywords: Ascorbic Acid, Sepsis, Treatment Course, Dose, Initiation

Posted Date: October 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-95522/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background: This meta-analysis is performed to evaluate the effects of AA on the mortality over sepsis patients, focusing on the courses and initiation of treatment as well as AA doses.

Methods: Randomized controlled trials concerning sepsis patients treated with intravenous AA were included when searching the database. The meta-analysis was performed using the random (M-H heterogeneity) model to produce summary odds ratio with 95% CI. Trial sequential analysis was applied to evaluated the effect of random errors.

Results: The included 12 trials enrolled a total of 1232 patients. Intravenously administration of AA could not lower 28-day mortality over sepsis patients (OR = 0.81; 95% CI (0.54-1.23); p = 0.326). Subgroup analysis demonstrated that when administrating AA alone, in a dose ≥ 10 g/d, or within 6 h of admission, the result may turn to positive (OR = 0.36; 95% CI (0.15-0.86); p = 0.020, OR = 0.50; 95% CI (0.27-0.92); p = 0.025, OR = 0.49; 95% CI (0.27-0.89); p = 0.019, relatively). The quality of evidence is moderate.

Conclusion: IV AA may have no effects to lower mortality over sepsis patients. However, when administrating AA alone, in a dose ≥ 10 g/d, or within 6 h of admission, the result may turn to positive. Due to a moderate GRADE certainty of evidence, further studies are required to fully elaborate the effectiveness of AA during the management of the sepsis patients.

PROSPERO registration number: CRD 42020170825. 24 Feb, 2020 retrospectively registered.

Background

Ascorbic acid (AA) is a water-soluble vitamin and an essential endogenous trace element that scavenges reactive oxygen species (ROS) [1,2]. Vitro experiments show that AA influence the immune system via the following major factors, inhibition of T cell apoptosis [3] and increasing NK cytotoxic activity [4]. Previous studies have demonstrated that patients in critical condition, particularly sepsis, have low levels of AA in the plasma [5-8] which holds prognostic value owing to its inverse correlation with multiple organ failure [8]. The inability for humans to synthesize AA is due to the lack of L-gulono-γ-lactone oxidase, a key enzyme that converts glucose to AA eventually [9], which makes external intake necessary. Both dosing and bio-distribution data in humans suggest that pharmacological concentration of AA are only attainable through intravenous (IV) administration because of the saturation of intestinal transporters (sodium-vitamin C transporter-1) [10]. Results from involved animals and human beings have suggested that AA supplementation significantly improves the prognosis of critical ill patients in the following aspects, attenuating lipid peroxidation, reducing vascular permeability, improving microvascular dysfunction, stabling endothelial and microcirculatory function, promoting endogenous vasopressin synthesis, enhancing vascular sensitivity to vasopressor, maintaining hemodynamic stability, and ultimately decreasing the incidence of organ dysfunction [11-15].

Despite promising preliminary outcomes, the benefits of AA supplementation remain controversial. An observational trial via Marik and coworkers [16] stated that AA as a component of “cocktail therapy” can strikingly reduce mortality and the duration of vasopressor in severe sepsis and septic shock patients. Giladi et al. [17] reported reductions of the occurrence of abdominal compartment syndrome, postoperative infection and respiratory failure in critically ill trauma patients. Our previous meta-analysis [46] commanded an AA dose of 3-10 g/d that can significantly decreased mortality in critically ill patients. While randomized controlled trials (RCTs) published recently [18-23] failed to reach the same conclusion. Notably, the courses and the initiation of AA treatment, as well as the AA doses varied from these trials, which may account for the discrepancies.

In this meta-analysis, we recruited RCTs that AA has been intravenously administration to patients with sepsis. We aimed to identify whether the treatment course and the initiation of AA impacts mortality, furthermore, we intended to explore the other effects of AA, including length of intensive care units (ICU), duration of vasopressor requirement, acute kidney injury (AKI) and renal replacement therapy (RRT).

Materials And Methods

This study was performed and prepared according to the guidelines proposed by Cochrane Collaboration in the Cochrane Handbook for Systematic Reviews of Interventions (http://www.cochrane handbook.org) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [31,32]**Additional file 1: Fig. S1**.

Search strategy

We searched articles of all languages published from inception to September 2020 in PubMed, Embase, Ovid, Medline and Cochrane Central Register of Controlled Trials using the following keywords along with Mesh terms: “ascorbic acid” and “sepsis” (**Additional file 2**). We collected all RCTs in which AA has been intravenously administered to adult sepsis patients. We also searched the references manually to attain related articles.

Study selection criteria

Inclusion criteria

1. Performed on adults in condition of sepsis
2. IV AA vs. placebo or no-intervention groups
3. RCTs.

Exclusion criteria

1. Performed on children
2. AA administered orally, enterally or were permitted to change to an enteral dosage form once enteral access was established
3. Lack of mortality data
4. Lack of control group
5. The way of AA administration was unclear
6. Study design is observational studies including cohort studies and case-control studies.

Data extraction

Data were independently extracted by the first and the second authors. Extracted data consisted of the name of first author, year of publication, study population, number of patients, AA dose, AA course, initiation of treatment, co-interventions, clinical parameters and adverse events. We resolved disagreements through discussions until a consensus was reached. We didn't contact the authors for missing data including unreported data or additional details.

Outcome measurements and definitions

The primary outcome was 28-day mortality. Secondary outcomes included ICU and in-hospital mortality, the length of ICU stay, duration of vasopressor requirement, patients suffering from AKI or demanding RRT and the change of Sequential organ failure assessment (SOFA) score. Courses of AA treatment ≤ 3 d were defined as short term, ≥ 3 d as long term. Doses < 3 g/d were defined as low, ≥ 10 g/d as high, and 3–10 g/d as medium.

Assessment of risk of bias

We applied the Cochrane Collaboration tool to assess the risk of bias in RCTs [32,33], the remaining non-RCTs were assessed via the ROBINS-I tool [34]. We rated each domain of the trials as low risk, unclear, or high risk. Trials were considered low risk when each independent domain was rated as low risk. Any domain rated as unclear or high risk increased the overall risk score. Risk were independently rated by the first and the second authors. We resolved disagreements through discussions until a consensus was reached.

Statistical analysis

Data were analyzed using Statistics/Data Analysis 15.1. The results of dichotomous data were presented as forest plots through the odds ratios (ORs) with 95% confidence intervals (CIs). Forest plots using Weighted Mean Difference (WMD) with 95% CI were performed for the assessment of continuous data. We used random (M-H heterogeneity) model to assess the effects since clinical heterogeneity including setting, AA regimen, co-interventions was high. A begger test was conducted to assess the publication bias. Subgroup analyses were pre-specified and performed to explore the correlation between mortality and the treatment course, doses, initiation of treatment, population and combination with co-interventions. A p value ≤ 0.05 was considered statistically significant, except when otherwise specified.

Assessment of the certainty of the evidence

Grading of Recommendations Assessment, Development and Evaluate system (GRADE) was used to evaluating the quality of evidence, based on the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) [35]. The first and the second authors independently rated the quality of evidence for primary outcome. The level of evidence was classified into four categories as high, moderate, low or very low. We resolved disagreements through discussions until a consensus was reached.

Trial sequential analysis (TSA)

TSA was applied to assess the robustness of the results on RCTs, and to provide sample size needed in further trials [36]. We constructed the analysis via TSA version 0.9.5.10 Beta software with a 20% relative risk reduction. The control event rate was calculated from the control group of RCTs in this meta-analysis. The type I error rate was defined as 5%, and the type II error rate was set as 20% (a statistical power of 80%). The heterogeneity correction was based on model variance.

Results

Literature search

Through database and manual search (**Fig. 1**), 2220 records were retrieved. We screened both titles and abstracts according to the inclusion and exclusion criteria, leaving 38 records that were deemed suitable for inclusion. After reviewing the full texts, 12 records [18-30] met the inclusion criteria and were finally included (**Table 1**). Studies were excluded for the following reasons: duplication (n = 24), non-RCTs (n = 1) [37].

Study characteristics

The characteristics of each trial are listed in **Table 1**. There were 8 trials exploring the effects of AA combined with co-intervention, such as thiamine [18-24], N-acetylcysteine [28], and α-tocopherol [28]. The longest intervention course of AA lasted for 10 days [18], while the shortest was only 1 day [28]; only 1 records lack of specific data of the treatment course [29]. The articles included was published in a long span from 1997 to 2020. The sample sized ranged from 20 to 211. The included trials enrolled a total of 1232 patients of which 571 received AA administration, and 661 were controlled subjects.

Risk of bias and quality of evidence

The risk of bias was summarized in **Fig. 2**. The trial performed by Balakrishnan [24] and Jose [21] were deemed a high risk of bias as incomplete outcome data over mortality. Studies by Fujii [18], Wani [23], and Chang [19] were not performed in a double-blinded way and were rated as high risk. In total, 7 trials were defined as an unclear risk and 5 trials were deemed high risk.

Meta-analysis results

IV AA and mortality

The meta-analysis, recruited 12 RCTs, indicated that IV AA may have no influence on 28-day mortality of sepsis patients (OR = 0.81; 95% CI (0.54-1.23); p = 0.326; I2 = 44.7%, **Additional files 3: Fig. 3A**). A random (M-H heterogeneity) model was employed due to clinical heterogeneity. Subgroup analysis demonstrated that high (≥ 10 g/d) doses of AA administration was related with a lower 28-day mortality rate (OR = 0.50; 95% CI (0.27-0.92); p = 0.025; I^2 = 0.0%, **Fig. 3B**), however, the medium dose (3-10 g/d) of AA had no influence (OR = 0.92; 95% CI (0.59-1.42); p = 0.698; I^2 = 34.9%, **Fig. 3B**). Long-term course (≥ 3 d) of AA administration could not reduce the 28-day mortality of sepsis patients (OR = 0.75; 95% CI (0.48-1.18); p = 0.215; I^2 = 47.9%, **Fig. 3C**). Initiation of treatment within 6 hours may associated with a lower 28-day mortality rate (OR = 0.49; 95% CI (0.27-0.89); p = 0.019; I^2 = 0.0%, **Fig. 3D**). Additional subgroup analysis suggested that IV AA alone could decrease the 28-day mortality rate compared with co-interventions (OR = 0.36; 95% CI (0.15-0.86); p = 0.020; I^2 = 28.6% vs. OR = 1.10; 95% CI (0.79-1.54); p = 0.572; I^2 = 0.0%, **Fig. 3A**).

Upon AA application, no decreased was found over ICU or in-hospital mortality (OR = 0.95; 95% CI (0.66-1.36); p = 0.780; I^2 = 0.0% vs. OR = 1.11; 95% CI (0.79-1.56); p = 0.550; I^2 = 0.0%, **Additional file 3: Fig. S2A**).

Length of ICU stay

Three trials [21,29,30] included compared the length of ICU stays between AA and control groups. The results suggested that AA administration did not shorten the length of ICU stay in sepsis patients (WMD = -1.52; 95% CI (-5.06-2.03); p = 0.401; I^2 = 79.0%, **Additional file 3: Fig. S2B**).

Duration of vasopressor requirement

Our analysis among trials [21,23,29,30] suggested that AA administration may decline the duration of vasopressor requirement (WMD = -17.41; 95% CI (-31.16- -3.65); p = 0.013; I^2 = 89.4%, **Additional file 3: Fig. S2B**).

Change in SOFA score

Four trials [19,21,23,27] were included to assess the influence of the change in SOFA score on which AA had no impact (WMD = 0.73; 95% CI (-0.28-1.75); P = 0.158; I^2 = 60.2%, **Additional file 3: Fig. S2B**).

The incidence of AKI and RRT

From analysis among trials [20-22], AA intervention did not increase the incidence of AKI (OR = 1.28; 95% CI (0.80-2.05); p = 0.303; I^2 = 0.0%, **Additional file 3: Fig. S2A**).

No difference was observed in the incidence of RRT among trials [20,21,29] when applying AA (OR = 0.78; 95% CI (0.35-1.75); p = 0.548; I^2 = 39.0%, **Additional file 3: Fig. S2A**).

Publication bias and sensitivity analysis

A begger test was employed to assess the publication bias of the included trials, and the result suggested a minimal publication bias (p = 0.174).

Sensitivity analysis was performed through the removal of each individual trial and reanalysis the remaining trials. When excluding the trial performed by Wani [23], the impact of AA on change of SOFA score altered (WMD = 1.19; 95% CI (0.49- 1.89); p = 0.001; I^2 = 0.0%).

Overall certainty of evidence

The evidence for 28-day mortality was downgraded to moderate certainty, due to study limitations (including high or unclear risk of bias over studies, clinical heterogeneity), and serious concerns about impression (including TSA results) (**Table 2**). Future evidence is likely to change the estimated effect.

Trial sequential analysis

TSA were performed based on included RCTs using a random-effects model, and were constructed for a heterogeneity adjusted information size of 2589 patients corresponding to a relative risk reduction of 20% with an α = 0.05 and β = 0.20 (power 80%). TSA indicated lack of solid evidence for a beneficial effect on AA for mortality and further RCTs are needed to testify the effects (**Fig. 4**).

Discussion

Main findings

IV AA and in-hospital mortality

Same as other meta-analysis [38,39] which observed no significantly reduction in mortality after IV AA intervention, the major finding of this study suggested that IV AA may have no effects to lower mortality rates over sepsis patients. Notable, when administrating AA alone, in a dose ≥ 10 g/d, or within 6 h of admission, the result may turn to positive.

AA was an essential endogenous trace element and a cofactor of biosynthetic enzymes, which plays an important role in anti-oxidative stress, maintaining the function of endothelia, enhancing the effects of immune system. For patients with COVID-19, early and high IV dose of AA alone or in combination with steroids may have a benefit, especially in a dose about 12 g/d, and at a duration of at least 7 days [40]. Investigators are engaged to find out the influences of AA on COVID-19 at a dose of 24 g/d for 7 days [41].

The degradation of AA that occurs during preparation and storage, and an increased requirement may contribute to the low level of AA in critically ill patients [42]. Previous study has shown that in the post-injury period, only supraphysiologic doses (3000 mg/d) for 3 or more days could approached normal plasma levels [43]. A trial explores the optimal dose of AA demonstrated that 10 g/d dose was associated with supranormal plasma concentrations [44]. While Jackson and colleagues [45] declared that AA is insufficient to compete effectively with nitric oxide (NO) for superoxide at anything less than supraphysiologic concentrations, and a 10-100 mmol/L of AA is an efficient scavenger for preventing the interaction of NO with superoxide. According to the previous studies, we defined a dose lower than 3 g/d as low, higher than 10 g/d as high, and 3-10 g/d (inclusive of 3 and 10 g/d) as medium dose. Our analysis revealed that high doses may have an ability to reduce mortality in sepsis condition, conversely low and medium doses don't. This is different from our previous results [46] in which after IV high dose of AA, no loss of mortality was observed. In a randomized pharmacokinetic trial, de Grooth and co-works [44] emphasized that high plasma concentrations require stained therapy. In this trial, after 48 h infusion of AA, a varying decline in plasma concentrations was observed even at the dose of 10 g/d. Therefore, they indicated a supplementation of AA more than 48 h, possibly as long as patients remain critically ill. Given the factors mentioned above, we defined a treatment course less than 3 days as short-term, and the others as long-term. IV AA at a long-term course (≥ 3 d) could not lower mortality in patients with sepsis, which is inconsistent with previous studies. Micah and co-workers [47] stated that APAHCHE-adjusted ICU mortality was lowest when AA was initiated within 6 hours, which is same as our outcomes but still needs further exploration.

As an antioxidant, AA plays vital parts in assisting in the recycling of other antioxidant agents [67]. This seems to be inconsistent with what we have observed in this meta-analysis, of which AA alone rather than co-intervention has a better survival benefit. In view of the absence of relevant RCTs comparing the prognosis of AA monotherapy with combined-therapy, we hope the result can attract the further attentions.

Duration of vasopressor requirement

The result suggested that IV AA may reduce the duration of vasopressor requirement, but the quality of evidence was graded as "very low". Studies have proven that enzymes via which endogenous norepinephrine and vasopression are synthesized required AA as a cofactor for optimal activity [14]. An RCT recruited 53 patients with ST-segment elevation myocardial infarction observed an amelioration of the left ventricular ejection fraction after administration of AA [49]. Furthermore, in the trial performed by Galley and colleagues [28], AA reduced the systemic vascular resistance index. Sum together, all mentioned above could contribute to a decreased duration of vasopressor.

The incidence of AKI and RRT

AA was reported to promotes the incidence of acute renal failure [50]. A randomized, crossover and controlled design trial observed an increased urinary oxalate and Tiselius Risk Index for calcium oxalate kidney stones [51]. Contrarily, this meta-analysis observed that the incidence of AKI and RRT did not grow after AA treatments (a maximum dose of 66 mg/kg/h), indicating that IV high dose of AA in sepsis patients may be safe. However, reports over the safety of AA in the condition of critical illness are scare in the literature. Cautions should be paid when IV high doses of AA in patients with hemochromatosis, glucose-6-phosphate dehydrogenase deficiency, renal dysfunction, kidney stone, oxaluria and pediatrics [52].

Comparison with other studies

The major strength of this meta-analysis was to investigate the effects over different AA treatment course and initiation as well as doses contribute to mortality in patients with sepsis. In addition, we focused on the adverse consequences of AA, such as the incidence of AKI and RRT.

Limitation

This meta-analysis had several weaknesses that should be noted. Firstly, 5 trials are under high risks, which may lead to a bias. Secondly, except for 5 multicenter studies [18,20-22,27], 7 of the included studies were single-center background. Thirdly, the initiation of AA treatment, the treatment courses and doses, and the role of single versus co-intervention therapy still requires clarification in future studies. Lastly, the missing data handling remained major limitations for this paper.

Conclusion

Based on the current available evidence, IV AA may have no effects to lower mortality over sepsis patients, the quality of evidence was graded as moderate. However, when administrating AA alone, in a dose ≥ 10 g/d, or within 6 h of admission, the result may turn to positive. In view of the limitations of the primary literature and a moderate GRADE certainty of evidence, further studies are required to fully elaborate the effectiveness of AA during the management of sepsis patients.

Abbreviations

AA: ascorbic acid

GRADE: Grading of Recommendations, Assessment, Development and Evaluation

ROS: reactive oxygen species

IV: intravenous

ICU: Intensive care units

AKI: acute kidney injury

RRT: acute kidney injury

RCT: randomized controlled trial

OR: odds ratios

CI: confidence interval

TSA: trial sequential analysis

WMD: Weighted Mean Difference

SOFA: sequential organ failure assessment

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Author contributions

WY/ ZHC developed the search strategy and performed the literature search. WY/ZHC did the study selection and data extraction for the systematic review. WY wrote the first draft of the manuscript. All authors contributed to the interpretation of data and critical revision of the manuscript, and approved the final manuscript. All authors confirm to the accuracy or integrity of the work.

References

1. Goode HF, Webster NR. Free radicals and antioxidants in sepsis. Crit Care Med. 1993; 21(11):1770.
2. Wu F, Schuster DP, Tyml K, et al. Ascorbate inhibits NADPH oxidase subunit p47phox expression in microvascular endothelial cells. Free Radic Biol Med. 2007;42(1):124–31.
3. Pavlovic V, Cekic S, Bojanic V, Stojiljkovic N, Rankovic G. Ascorbic acid modulates spontaneous thymocyte apoptosis. Acta Medica Medianae. 2005;44: 21-23.
4. Vojdani A, Bazargan M, Vojdani E, Wright J. New evidence for antioxidant properties of Vitamin C. Cancer Detect Prev.2000; 24: 508-523.
5. Long CL, Maull KI, Krishnan RS, et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res. 2003;109(2):144–148.
6. Schorah CJ, Downing C, Piripitsi A, et al. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am J Clin Nutr. 1996;63(5):760–765.
7. Hunt C, Chakravorty NK, Annan G, et al. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. Int J Vitam Nutr Res. 1994;64(3):212–219.
8. Borrelli E, Rouxlombard P, Grau GE, et al. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. Crit Care Med. 1996; 24(3):392–397.
9. Nishikimi M, Yagi K. Molecular basis for the deficiency in humans of gulonolactone oxidase, a key enzyme for ascorbic acid biosynthesis. Am J Clin Nutr. 1991;54(6 Suppl):1203S-1028S.
10. Padayatty SJ. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004; 140(7):533-537.
11. Matsuda T, Tanaka H, Yuasa H, et al. The effects of high-dose vitamin C therapy on post burn lipid peroxidation. J Burn Care Rehabil. 1993;14(6):624–629.
12. May JM, Harrison FE. Role of vitamin C in the function of the vascular endothelium [J]. Antioxid Redox Signal. 2013;19(17):2068–2083.
13. John A, Karel T, Darcy L, et al. Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat [J]. J Appl Physiol.2001; 90(3):795–803.
14. Carr AC, Shaw GM, Fowler AA, et al. Ascorbate-dependent vasopressor synthesis: a rationale for Vitamin C administration in severe sepsis and septic shock? Crit Care. 2015;19(1):418.
15. 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(1):32.
16. Marik PE, Khangoora V, Rivera R, et al. Hydrocortisone, Vitamin C and thiamine for the treatment of severe sepsis and septic shock: a retrospective before-after study. Chest.2016; 151(6):1229–1238.
17. Giladi, Aviram M., et al. High-dose antioxidant administration is associated with a reduction in post-injury complications in critically ill trauma patients. Injury. 2011;42: 78-82.
18. Fujii, Tomoko, 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.
19. Chang P, Liao Y, Guan J et al. Combined treatment with hydrocortisone, vitamin C and thiamine for sepsis and septic shock (HYVCTTSSS): A randomized controlled clinical trial. Chest. 2020; doi: 10.1016/j.chest.2020.02.065.
20. Hwang S Y, Ryoo S M, Park J E, et al. Combination therapy of vitamin C and thiamine for septic shock: a multi-centre, double-blinded randomized, controlled study[J]. Intensive Care Medicine. 2020; doi: 10.1007/s00134-020-06191-3.
21. Iglesias J, Vassallo AV, Patel V et al. Outcomes of metabolic resuscitation using ascorbic acid, thiamine, and glucocorticoids in the early treatment of sepsis. Chest. 2020; doi: 10.1016/j.chest.2020.02.049.

22. Moskowitz A, Huang D T, Hou P C, et al. Effect of Ascorbic Acid, Corticosteroids, and Thiamine on Organ Injury in Septic Shock: The ACTS Randomized Clinical Trial[J]. JAMA The Journal of the American Medical Association. 2020; 324(7):642.
23. Wani S J, Mufti S A, Jan R A, et al. Combination of vitamin C, thiamine and hydrocortisone added to standard treatment in the management of sepsis: results from an open label randomised controlled clinical trial and a review of the literature[J]. Infectious Diseases. 2020; doi: 10.1080/23744235.2020.1718200.
24. Balakrishnan M, Gandhi H, Shah K, et al. Hydrocortisone, Vitamin C and thiamine for the treatment of sepsis and septic shock following cardiac surgery. Indian J Anaesth. 2018; 62(12):934-939.
25. Ferrón-Celma I, Mansilla A, Hassan L, Garcia-Navarro A, Comino AM, Bueno P, Ferrón JA. Effect of vitamin C administration on neutrophil apoptosis in septic patients after abdominal surgery. J Surg Res. 2009; 153(2):224-30.
26. Fowler AA 3rd, 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.
27. Fowler AA 3rd, 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.
28. Galley, Helen F., et al. The effects of intravenous antioxidants in patients with septic shock." Free Radical Biology and Medicine. 1997;23(5): 768-774.
29. Nabil HT, Ahmed I. Early Adjuvant Intravenous Vitamin C Treatment in Septic Shock may Resolve the Vasopressor Dependence [J]. Int J Microbiol Adv Immunol. 2017; 05(1): 77-81.
30. Zabet, Mohadeseh Hosseini, et al. Effect of high-dose Ascorbic acid on vasopressor's requirement in septic shock. Journal of research in pharmacy practice. 2016;5(2): 94.
31. PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses (PRISMA). https:// prism a-state ment.org. Accessed 8 Sept 2018. 26.
32. Higgins J, Green S (eds). Cochrane handbook for systematic reviews of interventions version 5.1.0. March 2011.https://handbook-5-1. cochrane. org. Accessed 8 Sept 2018.
33. Higgins JPT, Altman DG, Gotzsche PC, et al. The cochrane collaboration's tool for assessing risk of bias in randomized trials. Br Med J. 2011;343: d5928.
34. Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. Br Med J.2016; 355: i4919.
35. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. BMJ. 2004; 328:1490.
36. Thorlund K, Engstrøm J, Wetterslev J, et al. User manual for trial sequential analysis (TSA). Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, Denmark. 2011; p. 1-115. Available from www.ctu.dk/tsa.
37. Fan K, Ronaghi R, Rees J, et al. The Effect of Using Vitamin C, Hydrocortisone, and Thiamine Triple Therapy in the Treatment of Septic Shock. Chest. 2019; 156(4): A944.
38. Langlois PL, Manzanares W, et al. Vitamin C supplementation in the critically ill: a systematic review and meta-analysis. J Parenter Enter Nutr. 2018; 10:15-20.
39. Li J. Evidence is stronger than you think: a meta-analysis of vitamin C use in patients with sepsis. Critical Care (BioMed Central). 2018;22(1):258.
40. Cheng R. Z. Can early and high intravenous dose of vitamin C prevent and treat coronavirus disease 2019 (COVID-19)? Medicine in Drug Discovery. 2020; doi:10.1016/j.medidd.2020.100028.
41. Carr A. C. A new clinical trial to test high-dose vitamin C in patients with COVID-19. Critical Care. 2020; doi:10.1186/s13054-020-02851-4.
42. Wilson J X. Mechanism of action of vitamin C in sepsis: ascorbate modulates redox signaling in endothelium[J]. Biofactors, 2009;35(1): 5-13.
43. Long CL, Maull KI, Krishnan RS, et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res. 2003;109(2):144–8.
44. De Grooth HJ, Manubulu-Choo W-P, Zandvliet AS, et al. Vitamin-C pharmacokinetics in critically ill patients: a randomized trial of four intravenous regimens. Chest. 2018;153(6):1368–77.
45. Jackson TS, Xu A, Vita JA, Keaney JF. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. Circ Res. 1998;83(9): 916–22.
46. Wang Y, Lin H, Lin B W, et al. Effects of different ascorbic acid doses on the mortality of critically ill patients: a meta-analysis[J]. Annals of Intensive Care. 2019;9(1):58.
47. Long MT, Frommelt MA, Ries MP et al. Early hydrocortisone, ascorbate and thiamine therapy for severe septic shock. Crit Care Shock 2020; 23:23-34.
48. Moskowitz A, Andersen L W, Huang D T, et al. Ascorbic acid, corticosteroids, and thiamine in sepsis: a review of the biologic rationale and the present state of clinical evaluation[J]. Critical Care. 2018; 22(1): 283.

49. Nicolás Valls, Gormaz J G, Rubén Aguayo, et al. Amelioration of persistent left ventricular function impairment through increased plasma ascorbate levels following myocardial infarction[J]. Redox Report Communications in Free Radical Research. 2015; 21(2):75-83.
50. Mashour S, Turner JMR. Acute renal failure, oxalosis, and vitamin C supplementation: a case report and review of the literature. Chest.2000;118(2): 561–3.
51. Massey LK. Ascorbate increases human oxaluria and kidney stone risk. J Nutr.2005;135(7): 1673–7.
52. Khoshnam-Rad, N., & Khalili, H. Safety of vitamin C in sepsis. Current Opinion in Critical Care. 2019;25(4): 329–333.

Tables

Table 1: The main characteristics of the studies included

Author	Country	Patients	AA dose	Treatment courses	Initiation of treatment	Co-interventions	Outcomes	Adverse events
Fujii 2020 [18]	Australia	Septic shock (n = 211)	1.5g q6h	10 days	Unavailable	Thiamine and hydrocortisone	Change in SOFA score at day 3á	Hyperglycemia
Ferron-Celma 2009 [25]	Spain	Septic abdominal surgery patients (n = 20)	450 mg qd	6 days	Within 12 h	None	Caspase-3â, PARPâ, Bcl-2á	-
Wani 2020 [23]	India	Sepsis/ septic shock (n = 100)	1.5g q6h	4 days	Unavailable	Thiamine and hydrocortisone	Duration of vasopressorâ, Lactate clearance á	-
Zabet 2016 [30]	Iran	Septic shock (n = 28)	25 mg/kg q6h	3 days	Unavailable	None	Dose and duration of NE â, 28-day mortality â	None
Fowler III 2019 [27]	USA	Patients with sepsis and ARDS (n = 167)	50mg/kg q6h	4 days	Within 6 h	None	Reduction in SOFA score á, PCT and CRP â; 28-day mortality â	None
Fowler III 2014 [26]	USA	Severe sepsis (n = 24)	50 or 200 mg/kg/d	4 days	Within 6 h	None	CRPâ, PCT (only in high-dose)â	None
Galley 1997 [28]	UK	Septic shock (n = 30)	1 g/d	1 day	Unavailable	N-acetylcysteine, α-tocopherol	HRá, Clá, SVRIâ	-
Moskowitz 2020 [22]	USA	Septic shock (n = 200)	1.5g q6h	4 days	Unavailable	Thiamine and hydrocortisone	Fail to reduce mortality	Hyperglycemia, hypernatremia
Jose 2020 [21]	USA	Sepsis/ septic shock (n = 137)	1.5g q6h	4 days	Unavailable	Thiamine and hydrocortisone	Duration of vasopressorâ	None
Chang 2020 [19]	China	Sepsis/ septic shock (n = 80)	1.5g q6h	4 days	Unavailable	Thiamine and hydrocortisone	Duration of vasopressorâ	Sever hypernatremia
Hwang 2020 [20]	Korea	Septic shock (n = 111)	50mg/kg q12h	2 days	Unavailable	Thiamine	Fail to reduce mortality	None
Balakrishnan 2019 [24]	India	Septic cardiac surgery patients (n = 24)	1.5g q6h	4 days	Unavailable	Thiamine and hydrocortisone	PCT level at day 3 and 4 â,dose of vasopressor â, SOFA score â	-
Nabil 2017 [29]	Egypt	Septic shock (n = 100)	1.5g q6h	Until ICU discharge	Within 24 h	None	Duration of vasopressor â, Length of ICU stayâ	-

*AA: Ascorbic acid; RCT: randomized controlled trial; ARDS: acute respiratory distress syndrome; SOFA score: Sequential organ failure assessment score; CRP: C-reactive protein; NE: norepinephrine; PCT: procalcitonin; ICU: Intensive care unit; HR: heart rate; CI: cardiac index; SVRI: systemic

vascular resistance index.

Table 2: Quality of evidence using GRADE approach.

Outcomes	Studies (participants)	Quality assessment						Summary of findings		
		Risk of bias	Inconsistency	Indirectness	Impression	Publication bias	Overall quality of evidence	WMD or OR (95%CI)	Heterogeneity	
									I^2(%)	P value
28-day mortality	8 (918)	Serious	No serious	No serious	Serious	No serious	ÅÅOO Moderate	0.81 (0.54,1.23)	44.7	0.326
ICU mortality	6 (776)	Serious	No serious	No serious	Serious	No serious	ÅÅOO Moderate	0.95 (0.66,1.36)	0.0	0.780
In-hospital mortality	5 (758)	Very serious	No serious	No serious	Serious	No serious	ÅOOO Low	1.11 (0.79,1.56)	0.0	0.550
AKI	3 (419)	Serious	No serious	No serious	Serious	No serious	ÅÅOO Moderate	1.28 (0.80,2.05)	0.0	0.303
RRT	3 (347)	Very serious	No serious	No serious	Serious	No serious	ÅÅOO Moderate	0.78 (0.35,1.75)	39.0	0.548
Duration of VR	4 (365)	Very serious	Serious	No serious	Serious	No serious	OOOO Very low	-17.41 (-31.16, -3.65)	89.4	0.013
Change in SOFA score	4 (484)	Very serious	Serious	No serious	Serious	No serious	OOOO Very low	0.73 (-0.28, 1.75)	60.2	0.158
ICU stay	3 (265)	Very serious	Serious	No serious	Serious	No serious	OOOO Very low	-1.52 (-5.06, 2.03)	79.0	0.401

*VR: vasopressor requirement

Figures

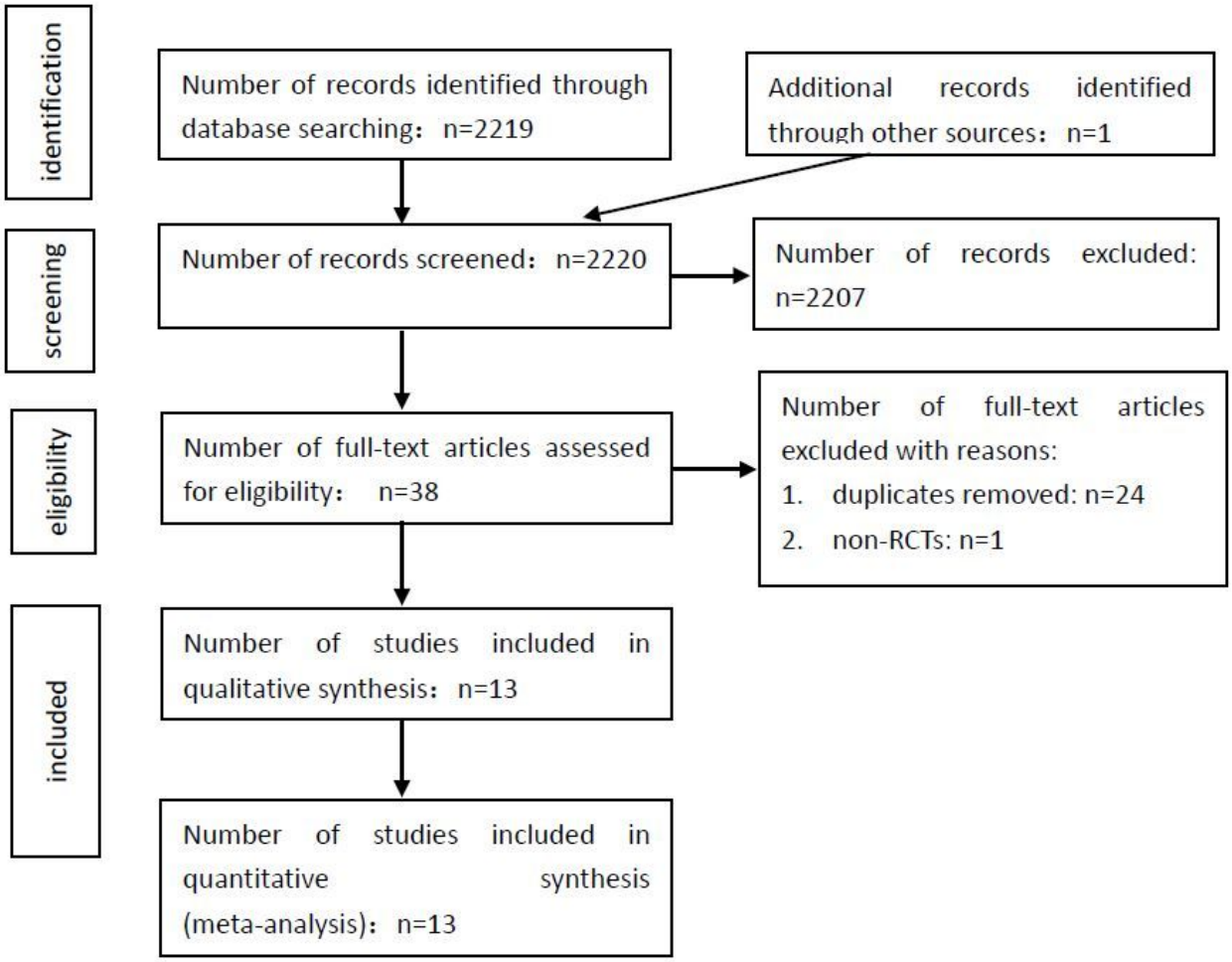

Figure 1

Study flow diagram chart

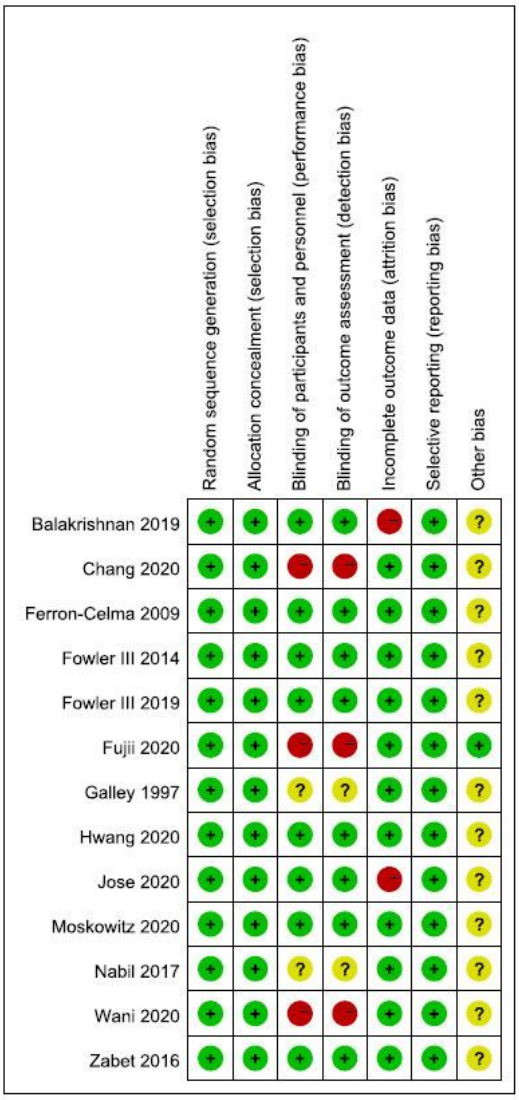

Figure 2

Risk of bias summary

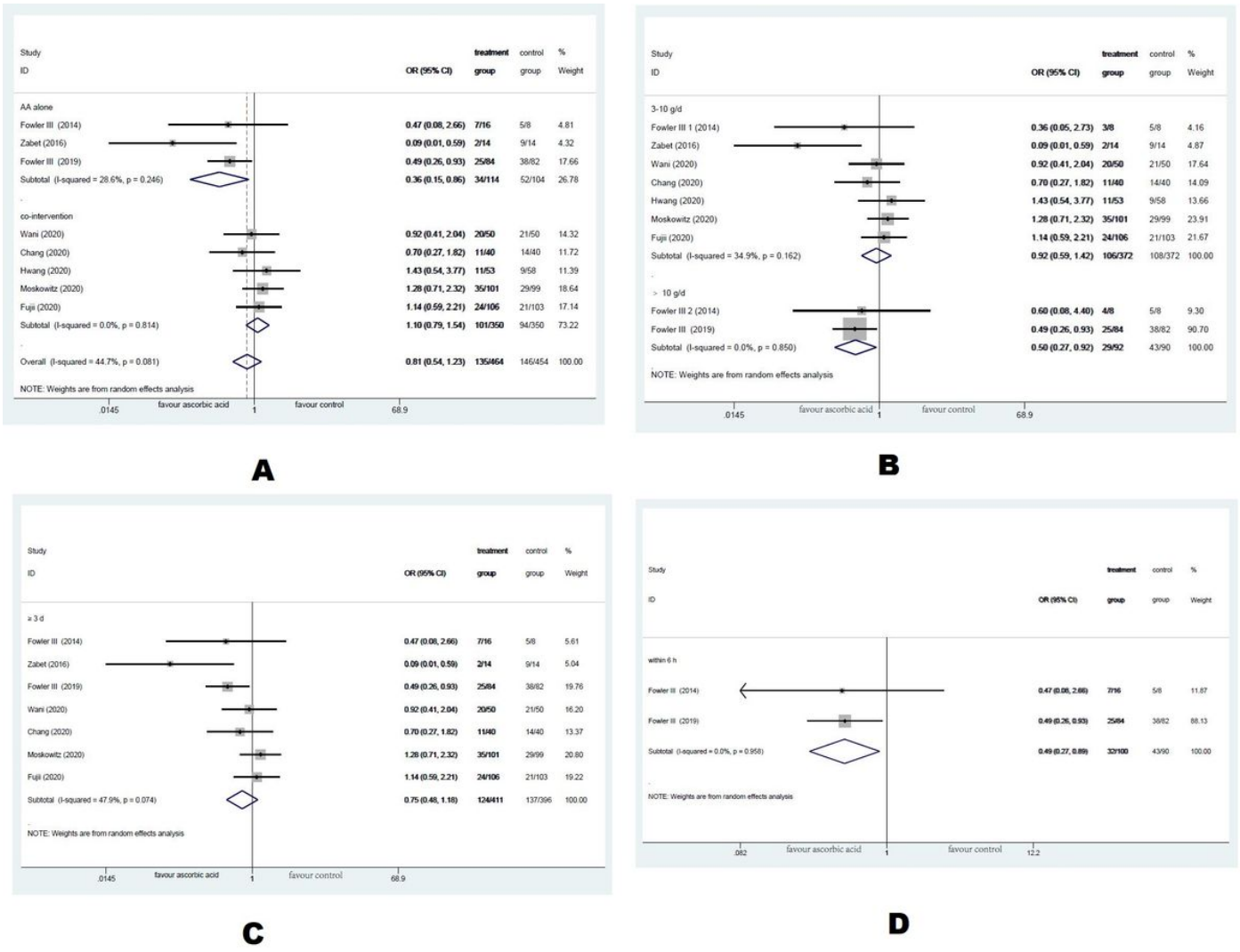

Figure 3

A: Forest plot of the effect of intravenous ascorbic acid on 28-day mortality when compared by co-intervention or AA alone. B: Forest plot of the effect of intravenous ascorbic acid on 28-day mortality when compared by AA doses. C: Forest plot of the effect of intravenous ascorbic acid on 28-day mortality when at a long-term course (≥ 3 d). D: Forest plot of the effect of intravenous ascorbic acid on 28-day mortality when AA was administrated within 6 h of admission.

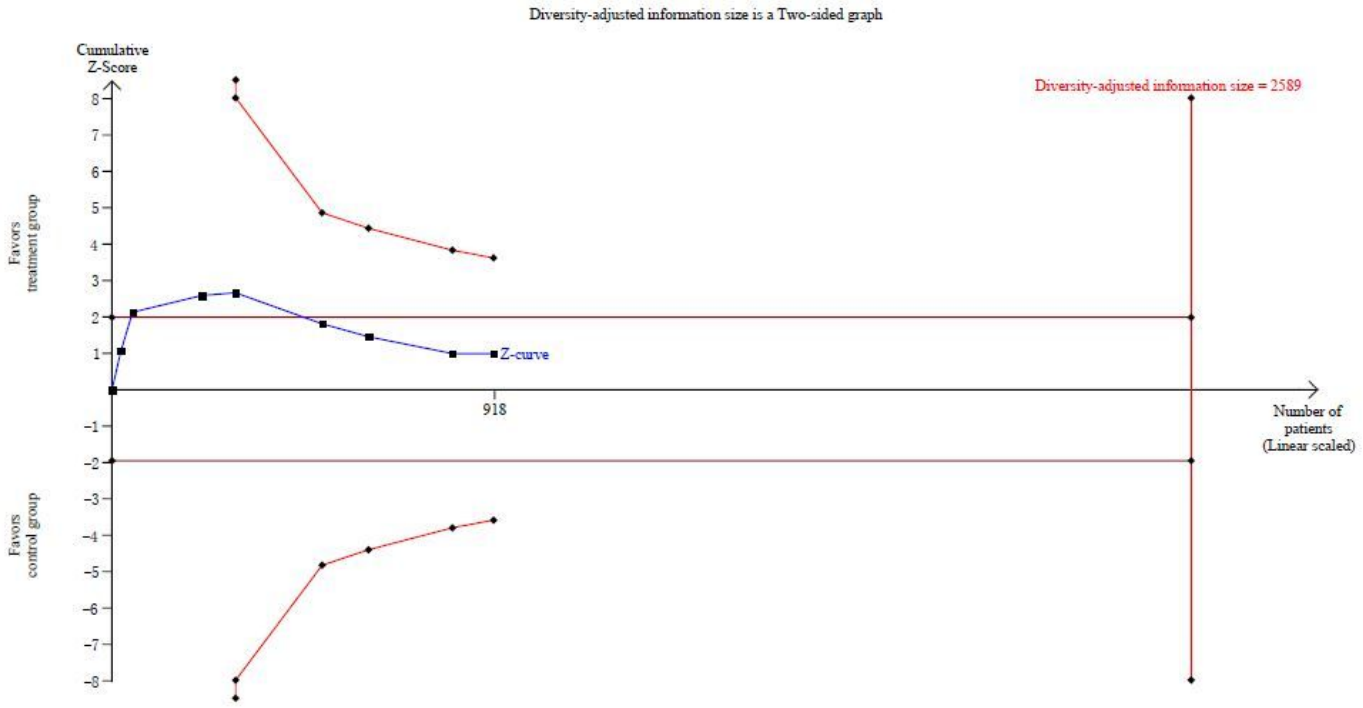

Figure 4

Trial sequential analysis of included RCTs of the effect of ascorbic acid on 28-day mortality. *The final cumulative z-curve in blue is smaller than 1.96, and does not cross the trial sequential monitoring boundary (full red line); Only 32.02% of the required information size has been reached. Thera is, however, preceding z-curve cross the conventional threshold of 1.96, illustrating that early meta-analysis may come to a false positive conclusions.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1Fig.S1.doc
- Additionalfile2.docx
- Additionalfile3Fig.S2A.pdf
- Additionalfile3Fig.S2B.pdf