**Evaluating Vitamin C in Septic Shock (EviCT): A Randomized Controlled Trial of Vitamin C Monotherapy**

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**Supplemental Methods**

**Study design and setting**

This was an investigator-initiated, randomized, placebo controlled, double blinded study examining the effect of intravenous vitamin C on outcomes in patients with septic shock. The study was performed in five hospitals in the US state of Minnesota, including one tertiary academic medical center, and four non-teaching community hospitals (Table S1) with recruitment lasting from January 2018 through June 2020. Recruitment ended upon reaching enrollment goals. Patients were randomized to two parallel groups, one receiving intravenous vitamin C and the other placebo, with no mechanism for crossover between groups. Although there was no restriction on administration of vitamin C by the treatment team, use of this medication for septic shock is not common practice at any of the centers included in this study, and no subject who received study drug also received vitamin C outside of the study.

**Study population**

Adult patients (age ≥ 18 years) within 24 hours of onset of septic shock were eligible for enrollment. Our criteria for septic shock were 1) known or suspected source of infection as evidenced by an order for antimicrobials and either laboratory or imaging data confirming an infection, a procalcitonin level ≥ 2 ng/mL, or a clinical suspicion of infection documented by the treatment team, 2) evidence of sepsis response, defined as either meeting at least 2 of 4 systemic inflammatory response syndrome (SIRS) criteria or having an acute rise in SOFA score of at least 2 points (for patients with unknown baseline SOFA score, this was assumed to be 0), and 3) evidence of shock, defined as hypotension requiring vasopressor support despite resuscitation with at least 30 mL/kg ideal body weight of any type of intravenous fluid, and a serum lactate of greater than 2.0 mmol/L.

Exclusion criteria were inability to obtain written consent within 24 hours of eligibility or inability to obtain written consent because the patient or their decision-maker were non-English speaking, current pregnancy or breastfeeding, transition to comfort measures only (CMO) prior to enrollment, shock occurring immediately following cardiac arrest, cardiac surgery within the prior 48 hours, participation in another investigational drug trial in the 30 days prior to eligibility, allergy to vitamin C, history of nephrolithiasis, history of glucose-6-phosphate dehydrogenase deficiency, end stage renal disease requiring dialysis with vitamin C supplementation on home medication list, and clinical course which in the view of the study or treatment teams would preclude participation.

**Intervention**

After giving written informed consent, subjects were randomized to receive either vitamin C (Mylan Institutional, Rockford, IL, USA) administered as a 1000 mg bolus over 30 minutes followed by continuous infusion of 250 mg/hr for 96 hours, or placebo. Vitamin C was delivered as a 10 mg/mL solution in normal saline; for subjects receiving the full 96-hour protocol this amounts to 2500 mL of fluid. The placebo group received equivalent volumes of normal saline alone. Study drug arrived from the pharmacy and was maintained in a light protection bag throughout the infusion period. All other treatments for septic shock (e.g., antibiotics, fluids, corticosteroids, etc.) were at the discretion of the treatment team.

The study drug administration period ended after 96 hours, or 24 hours after weaning from all pressors, whichever came first. Although our protocols mandated that any subject suffering a serious adverse event suspected to be related to study drug be withdrawn from the study, no subject during our study met this criterion. All subjects whose goals of care became comfort measures only were removed from the drug portion of the study by the investigators (11 subjects), and one subject had to be removed from the drug portion of the study upon transfer to a hospital not actively participating in the study. These subjects were all included in the final analysis.

**Outcomes**

The primary outcome for this study was all-cause 28-day mortality. Secondary outcomes included all cause ICU mortality, time to lactate clearance, need for renal replacement therapy, change in serum creatinine, change in sequential organ failure assessment (SOFA) score, change in Acute Physiology and Chronic Health Evaluation II (APACHE II) score, total intravenous fluid administration following study drug initiation, and durations of pressor use, mechanical ventilation, and durations of ICU and hospital stay following study drug initiation. Procalcitonin clearance was also a planned secondary outcome, but too few subjects had this measured after the baseline level to allow for meaningful analysis.

**Sample size**

A prior retrospective study(1) had shown a greater than 30% mortality reduction (risk difference) with vitamin C based therapy; however, we were skeptical that prospective testing may not reproduce such a significant effect. We therefore chose to test for a lesser but still very clinically significant absolute mortality reduction of 20%. Institutional data from participating centers suggested historical mortality rates for septic shock of approximately 30%. Using this baseline incidence rate and employing a two-tailed alpha of 0.05, we calculated that 124 subjects (an average of 62 per group) would be needed to detect a 20% decrease in absolute mortality with 80% power. To allow for possible attrition, we initially set out to enroll 140 subjects; however, very few subjects left the study, and we were able reach our enrollment goal after enrolling 126 subjects.

**Randomization and blinding**

Randomization was stratified by site. Randomization tables were provided by a supporting statistician to the on-site pharmacy at each site via the M Health Fairview investigational drug pharmacy. The tables were generated using a pseudo-random number generator in SAS (version 9.3, SAS Institute Inc., Cary, NC), and employed a random permuted block design, using block sizes of 2 and 4. Blinding was performed at the level of each on-site pharmacy. Participants, their families, study staff, and treatment teams were all blinded to group allocation. Unblinding was allowed for subjects having severe adverse reactions to study drug and under circumstances in which, in the opinion of study staff, having specific knowledge of group allocation would aid in providing medical treatment or preventing future harm. No subjects met these criteria during the study.

**Data collection and statistical analysis**

Mortality data was obtained from the State of Minnesota Vital Records Office database. All other outcome data was extracted from the patient’s clinical chart by study investigators and study coordinators with specific training for this study.

For analysis purposes, subjects were assigned to groups based on intention to treat. In a planned subgroup analysis, subjects who received steroids as part of their treatment were analyzed separately from those who did not. We had also planned a subgroup analysis of patients who received thiamine as part of their treatment, but an insufficient number of patients (nine) received this therapy to allow for meaningful statistics.

For categorical values, statistical significance was assessed using a chi-squared test or, for contingency tables with any cell value of less than five, Fisher’s exact test. Correlation testing was performed using Spearman rank correlation. For continuous variables, the Shapiro-Wilk test for normality was applied and in almost all cases the data did not fit a normal distribution. A non-parametric test was therefore needed, and the median test was chosen because it tests sample populations against a null hypothesis of having come from populations with the same median value, whereas other non-parametric tests use the broader null hypothesis of the samples having come from the same larger population. We felt this distinction to be important because a therapy causing a significant change to its study population without affecting its median (e.g., by changing the width of distribution curve) would carry less clinical significance than a change affecting the median. All statistical testing was carried out using SAS software (version 9.4, SAS Institute Inc., Cary, NC).

In calculating SOFA scores, a significant number of variables were missing, particularly measurements of total bilirubin as this is not routinely measured daily in the participating ICUs. Missing variables for a given day’s SOFA score were handled by utilizing the value from the nearest day on which that variable was determined. In the case of a tie, the preceding value was used rather than the future value. If more than three variables were missing, the SOFA score was not calculated for that day. Very few variables were missing for calculation of APACHE II scores, thus scores were not calculated on days for which any variable was missing.

For renal outcomes (incidence of renal replacement therapy initiation and trends in creatinine), patients with a known history of end stage renal disease requiring renal replacement therapy prior to admission were excluded from analysis. For trends in creatinine, patients receiving any type of renal replacement therapy during the study period were also excluded. To further explore the difference in incidence of renal replacement therapy between groups, a post hoc analysis was performed comparing the time at which renal replacement therapy was planned or initiated relative to the time study drug was initiated. Renal replacement therapy was considered to be “planned” only if a note from a nephrologist in the subject’s medical record definitively stated the intention of starting renal replacement therapy that day.

In determining time to lactate clearance, subjects whose lactate level fell below 2.0 between the time of eligibility and study drug administration were excluded. For all pairwise comparisons of variables, subjects for whom only baseline values were available were excluded. All comparisons were structured such that positive values represent clinical improvement (e.g., decrease in illness severity indices, reduction of serum creatinine, etc.).

Subgroup stratifications by steroid administration (planned), presence of positive pressure ventilation at enrollment (post hoc) and presence of acute hypoxemic respiratory failure at enrollment (positive pressure ventilation and paO2/FiO2 ratio of 300 or less) (post hoc), were carried out using prospectively collected study data. Subgroup stratification by SOFA and APACHE II scores (post hoc) were performed using the score thresholds which best bisected our total study population (SOFA score of 10 or more versus 9 or less and APACHE II score of 23 or more versus 22 or less).

**Oversight and data availability**

This study was approved by the institutional review board of the University of Minnesota, as well as the institutional review boards pertaining to each individual study site (Table S1). An independent data and safety monitoring board consisting of two intensive care physicians and one statistician met at least every six months and oversaw study activities. This trial is registered at clinicaltrials.gov under identifier NCT03338569.

Requests for sharing of de-identified individual patient data including subject demographics, clinical variables and outcomes, will be honored within the constraints of the University of Minnesota IRB’s data sharing policies indefinitely; this process can be initiated by contacting the corresponding author.

**References**

1. Marik PE, Khangoora V, Rivera R, Hooper MH, et al: 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

**Table S1: Summary of study sites**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Description** | **Number of subjects** | **IRB oversight body** | **IRB Study number** |
| M Health Fairview University of Minnesota Medical Center | Adult medical ICU; Academic medical center | 34 | University of Minnesota IRB | STUDY00000220 |
| M Health Fairview Southdale Hospital | Adult general ICU; Community hospital | 68 | University of Minnesota IRB | STUDY00000220 |
| M Health Fairview Ridges Hospital | Adult general ICU; Community hospital | 13 | University of Minnesota IRB | STUDY00000220 |
| Healtheast St. Joseph’s Hospital*a* | Adult general ICU; Community hospital | 5 | HealthEast IRB | HE 18 05 001 |
| Essentia St. Mary’s Hospital | Adult general ICU; Community hospital | 5 | Essentia Health IRB | 00000635 |

*a* Following completion of subject enrollment, this site was renamed M Health Fairview St. Joseph’s Hospital

**Table S2: Additional baseline characteristics of subjects included in analysis**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **Vitamin C group**  **N=60** | **Placebo group**  **N=64** | **P-value** |
| Past Medical History, no. (%) |  |  |  |
| History of diabetes | 18 (30.0) | 12 (18.8) | 0.14 |
| History of cirrhosis | 6 (10.0) | 5 (7.8) | 0.67 |
| History of end-stage renal disease | 0 (0.0) | 4 (6.3) | 0.12 |
| History of congestive heart failure (EF<50% or diastolic dysfunction) | 7 (11.7) | 11 (17.2) | 0.38 |
| History of permanent atrial fibrillation | 7 (11.7) | 9 (14.1) | 0.69 |
| Immunocompromised | 8 (13.3) | 8 (12.5) | 0.89 |
| History of infection with multidrug-resistant organism | 7 (11.7) | 8 (12.5) | 0.89 |
| Known active or past tobacco use | 23 (38.3) | 28 (43.8) | 0.44 |
| Known to regularly drink three or more alcoholic drinks per day | 4 (6.7) | 3 (4.7) | 0.71 |
| Baseline laboratory values |  |  |  |
| WBC count (x109/L), median [IQR] | 16.3 [10.2, 27.2] | 14.2 [7.1, 21.6] | 0.21 |
| Lactate (mmol/L), median [IQR] | 2.8 [2, 4] | 2.8 [2, 4.4] | 0.88 |
| Creatinine (mg/dL), median [IQR] | 1.8 [1.2, 2.5] | 1.7 [1.2, 2.6] | 0.47 |
| Bilirubin (mg/dL), median [IQR] | 1.1 [0.6, 1.6] | 0.9 [0.6, 2] | 0.70 |
| Procalcitonin (ng/mL), median [IQR] | 20.9 [3, 41.6] | 6.8 [1.4, 54.2] | 0.72 |

**Table S3: Post hoc analysis of timing of renal replacement therapy initiation relative to study drug initiation**

|  |  |  |  |
| --- | --- | --- | --- |
| **RRT timing** | **Vitamin C group**  **N = 60** | **Placebo group**  **N = 60*a*** | **P-value** |
| Requirement for renal replacement therapy at any point during 96-hour study period, no. (%) | 10 (16.7) | 2 (3.3) | 0.02 |
| Renal replacement therapy planned or initiated prior to study drug initiation, no. (%) | 6 (10.0) | 1 (1.7) | 0.12 |
| Renal replacement therapy NOT planned or initiated prior to study drug initiation, no. (%) | 4 (6.7) | 1 (1.7) | 0.17 |

*a*Patients with dialysis dependence prior to hospital admission were excluded from this analysis.

**Table S4: Primary and secondary outcomes in subjects receiving steroids*a* (subgroup analysis)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Outcome** | **Vitamin C group**  **(N = 30)** | **Placebo group**  **(N = 42)** | **P-value** |
| **Primary Outcome:** |  |  |  |
| 28-day mortality, no. (%) | 10 (33.3) | 20 (47.6) | 0.23 |
|  |  |  |  |
| **Secondary Outcomes:** |  |  |  |
| ICU mortality, no. (%) | 8 (26.7) | 16 (38.1) | 0.31 |
| ICU length of stay following study drug initiation (days), median [IQR] | 3.7 [1.9, 10.0] | 2.6 [1.9, 5.0] | 0.34 |
| Hospital length of stay following study drug initiation (days), median [IQR] | 10.8 [4.2, 20.5] | 6.3 [2.6, 13.0] | 0.06 |
| Duration of pressors following initiation of study drug (hours), median [IQR] | 32.2 [14.4, 46.1] | 29.1 [18.5, 49.3] | 0.74 |
| Duration of mechanical ventilation following initiation of study drug (hours), median [IQR] | 42 [0, 96] | 21 [0, 48] | 0.55 |
| Organ failure scores: |  |  |  |
| Paired improvement in SOFA score*b*, median [IQR] | 3 [1.75, 7]  (N = 28*c*) | 4 [-0.25, 6.25]  (N = 40*c*) | 0.92 |
| Paired improvement in APACHE II score*b*, median [IQR] | 4.5 [2, 9.25]  (N = 28*c*) | 6.0 [-3, 10.25]  (N = 40*c*) | 0.74 |
| Renal function outcomes: |  |  |  |
| Paired improvement in creatinine*b* (mg/dL), median [IQR] | 0.5 [0.1, 0.9]  (N = 22*c,d,e*) | 0.2 [-0.3, 0.6]  (N = 37*c,d,e*) | 0.24 |
| Renal replacement therapy required during 96-hour study period, no. (%) | 7 (23.3) | 2 (4.8)  (N = 40*e*) | 0.02 |
| Change in serum lactate level over 96 hours (mmol/L), median [IQR] | 0.8 [0.3, 1.8] | 0.8 [-0.5, 2.0] | >0.99 |
| Total intravenous fluid administration (L), median [IQR]: |  |  |  |
| 6 hours after study drug Initiation | 1.19 [0.86, 1.90] | 0.84 [0.45, 1.39] | 0.58 |
| 24 hours after study drug initiation | 4.25 [2.54, 5.80] | 3.34 [2.05, 4.60] | 0.64 |
| Fluid balance (total intake minus output, L), median [IQR]: |  |  |  |
| 24 hours after study drug initiation | 2.03 [1.10, 3.95] | 2.13 [1.39, 3.95] | 0.89 |
| 96 hours after study drug initiation | 2.87 [2.16, 3.79] | 3.95 [1.27, 6.85] | 0.47 |

*a* Patients were considered to have received steroids if they received at least one dose of intravenous steroids between the times of onset of septic shock and completion of study drug.

*b* Baseline to final measured value within 96-hour study period.

*c* Patients for whom only baseline values were obtained were excluded from this analysis.

*d* Patients receiving renal replacement therapy at any point during the 96-hour study period were excluded from this analysis.

*e* Patients with dialysis dependence prior to hospital admission were excluded from this analysis.

**Table S5: Primary and secondary outcomes in subjects not receiving steroids*a* (subgroup analysis)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Outcome** | **Vitamin C group**  **(N = 30)** | **Placebo group**  **(N = 22)** | **P-value** |
| **Primary Outcome:** |  |  |  |
| 28-day mortality, no. (%) | 6 (20.0) | 6 (27.0) | 0.54 |
|  |  |  |  |
| **Secondary Outcomes:** |  |  |  |
| ICU mortality, no. (%) | 6 (20.0) | 4 (18.2) | 0.87 |
| ICU length of stay following study drug initiation (days), median [IQR] | 2.7 [1.6, 5.0] | 2.6 [1.0, 6.0] | >0.99 |
| Hospital length of stay following study drug initiation (days), median [IQR] | 7.9 [3.8, 19.7] | 6.7 [4.7, 12.1] | 0.58 |
| Duration of pressors following initiation of study drug (hours), median [IQR] | 25.0 [8.8, 55.1] | 23.6 [14.9, 37.6] | 0.57 |
| Duration of mechanical ventilation following initiation of study drug (hours), median [IQR] | 0 [0, 0] | 0 [0, 31] | 0.44 |
| Organ failure scores: |  |  |  |
| Paired improvement in SOFA score*b*, median [IQR] | 4 [1, 5.75]  (N = 30c) | 3 [2, 6]  (N = 21*c*) | 0.94 |
| Paired improvement in APACHE II score*b*, median [IQR] | 4.5 [1.25, 8.75]  (N = 30c) | 7 [0, 11]  (N = 21*c*) | 0.25 |
| Renal function outcomes: |  |  |  |
| Paired improvement in creatinine*b* (mg/dL), median [IQR] | 0.3 [-0.1, 0.7]  (N = 27*c,d,e*) | 0.4 [0.2, 0.9]  (N = 19*c,d,e*) | 0.37 |
| Renal replacement therapy required during 96-hour study period, no. (%) | 3 (10.0) | 0 (0)  (N = 20*e*) | 0.25 |
| Change in serum lactate level over 96 hours (mmol/L), median [IQR] | 1.5 [0.4, 2.2] | 1.7 [0.5, 2.3] | 0.71 |
| Total intravenous fluid administration (L), median [IQR]: |  |  |  |
| 6 hours after study drug initiation | 0.83 [0.57, 1.40] | 0.50 [0.28, 1.00] | 0.27 |
| 24 hours after study drug initiation | 3.43 [2.42, 3.96] | 3.43 [1.71, 4.91] | >0.99 |
| Fluid balance (total intake minus output, L), median [IQR]: |  |  |  |
| 24 hours after study drug initiation | 2.24 [1.15, 3.49] | 1.93 [0.83, 2.68] | 0.78 |
| 96 hours after study drug initiation | 2.99 [-0.77, 4.88] | 1.29 [0.02, 4.27] | 0.20 |

*a* Patients were considered to have received steroids if they received at least one dose of intravenous steroids between the times of onset of septic shock and completion of study drug.

*b* Baseline to final measured value within 96-hour study period.

*c* Patients for whom only baseline values were obtained were excluded from this analysis.

*d*Patients receiving renal replacement therapy at any point during the 96-hour study period were excluded from this analysis.

*e* Patients with dialysis dependence prior to hospital admission were excluded from this analysis.

**Table S6: Correlation testing between presence of positive pressure ventilation or hypoxemic respiratory failure on enrollment with elevation of severity index on enrollment**

|  |  |  |
| --- | --- | --- |
| **Subgroup ►**  **▼** | **SOFA score greater than 9 on enrollment** | **APACHE II score greater than 22 on enrollment** |
| **Positive pressure ventilation on enrollment** | Spearman coefficient: 0.48  P < 0.001 | Spearman coefficient: 0.30  P = 0.001 |
| **Hypoxemic respiratory failure on enrollment** | Spearman coefficient: 0.48  P < 0.001 | Spearman coefficient: 0.39  P < 0.001 |

**Table S7: Post hoc subgroup analysis of mortality outcomes by SOFA score at enrollment**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | **SOFA score 9 or less** | | | **SOFA score 10 or more** | | |
| Vitamin C group  (N = 30) | Placebo group  (N = 35) | P-value | Vitamin C group  (N = 30) | Placebo group  (N = 29) | P-value |
| 28-day mortality, no. (%) | 6 (20.0) | 10 (28.6) | 0.42 | 10 (33.3) | 16 (55.2) | 0.09 |
| ICU mortality, no. (%) | 5 (16.67) | 6 (17.1) | >0.99 | 9 (30.0) | 14 (48.3) | 0.15 |

**Table S8: Post hoc subgroup analysis of mortality outcomes by APACHE II score at enrollment**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | **APACHE II score 22 or less** | | | **APACHE II score 23 or more** | | |
| Vitamin C group  (N = 31) | Placebo group  (N = 28) | P-value | Vitamin C group  (N = 29) | Placebo group  (N = 36) | P-value |
| 28-day mortality, no. (%) | 5 (16.1) | 5 (17.9) | >0.99 | 11 (37.9) | 21 (58.3) | 0.10 |
| ICU mortality, no. (%) | 4 (12.9) | 4 (14.3) | >0.99 | 10 (34.5) | 16 (44.4) | 0.42 |

**Table S9: Adverse event reporting**

|  |  |  |  |
| --- | --- | --- | --- |
| **Event** | **Vitamin C group**  **(N = 60)** | **Placebo group**  **(N = 64)** | **P-value** |
| Cardiac, no. (%) |  |  |  |
| Arrhythmia | 7 (8.3) | 4 (6.3) | 0.29 |
| New or worsening heart failure | 0 (0) | 3 (4.7) | 0.24 |
| Elevated troponin without ST-elevation | 2 (3.3) | 1 (1.5) | 0.61 |
| Elevated troponin with ST-elevation | 0 (0) | 0 (0) | >0.99 |
| Hematologic, no. (%) |  |  |  |
| Pancytopenia | 1 (1.7) | 0 (0) | 0.48 |
| Thrombocytopenia | 1 (1.7) | 1 (1.5) | >0.99 |
| Bleeding event | 1 (1.7) | 1 (1.5) | >0.99 |
| Pulmonary, no. (%) |  |  |  |
| Pleural effusion | 0 (0) | 1 (1.5) | >0.99 |
| Gastrointestinal, no. (%) |  |  |  |
| Nausea | 1 (1.7) | 0 (0) | 0.48 |
| Vomiting | 0 (0) | 0 (0) | >0.99 |
| Loose stools | 0 (0) | 1 (1.5) | >0.99 |
| Dysphagia | 1 (1.7) | 0 (0) | 0.48 |
| Dermatologic, no. (%) |  |  |  |
| Maculopapular rash | 1 (1.7) | 0 (0) | 0.48 |
| Renal, no. (%) |  |  |  |
| New onset of renal stone | 0 (0) | 0 (0) | >0.99 |