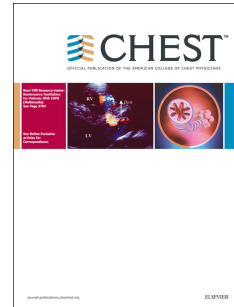# Accepted Manuscript

Hydrocortisone, Vitamin C and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study

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REVISION 1

Title: Hydrocortisone, Vitamin C and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study.

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Abstract:

Background: The global burden of sepsis is estimated as 15 to 19 million cases annually with a mortality rate approaching 60% in low income countries.

Methods: In this retrospective before-after clinical study, we compared the outcome and clinical course of consecutive septic patients treated with intravenous vitamin C, hydrocortisone and thiamine during a 7-month period (treatment group) compared to a control group treated in our ICU during the preceding 7 months. The primary outcome was hospital survival. A propensity score was generated to adjust the primary outcome.

Findings: There were 47 patients in both treatment and control groups with no significant differences in baseline characteristics between the two groups. The hospital mortality was 8.5% (4 of 47) in the treatment group compared to 40.4% (19 of 47) in the control group (p < 0.001). The propensity adjusted odds of mortality in the patients treated with the vitamin C protocol was 0.13 (95% CI 0.04-0.48, p=002). The SOFA score decreased in all patients in the treatment group with none developing progressive organ failure. Vasopressors were weaned off all patients in the treatment group, a mean of 18.3 ± 9.8 hours after starting treatment with vitamin C protocol. The mean duration of vasopressor use was 54.9 ± 28.4 hours in the control group (p<0.001).

Conclusion: Our results suggest that the early use of intravenous vitamin C, together with corticosteroids and thiamine may prove to be effective in preventing progressive organ dysfunction including acute kidney injury and reducing the mortality of patients with severe sepsis and septic shock. Additional studies are required to confirm these preliminary findings.

The global burden of sepsis is substantial with an estimated 15 to 19 million cases per year; the vast majority of these cases occur in low income countries.[1] With more timely diagnosis and improvement in supportive care the 28-day mortality from sepsis in high income countries has declined to about 25%; however, the mortality rate from septic shock remains as high as 50%. [2-5] Moreover, the mortality from sepsis and septic shock in low income countries is approximately 60%.[6-8] In addition to short term mortality, septic patients suffer from numerous short- and long-term complications with a reduced quality of life and an increased risk of death up to five years following the acute event.[9-11] Over the last 3 decades, over 100 phase II and phase III clinical trials have been performed testing various novel pharmacologic agents and therapeutic interventions in an attempt to improve the outcome of patients with severe sepsis and septic shock; all of these efforts ultimately failed to produce a novel pharmacologic agent to directly target the pathophysiologic effects of severe sepsis. [12] New therapeutic approaches to sepsis are desperately required. To impact the global burden of sepsis these interventions should be effective, cheap, safe and readily available.

We were recently confronted with three patients with fulminant sepsis who were almost certainly destined to die from overwhelming septic shock. Based on experimental and emerging clinical data, we decided to administer intravenous vitamin C to these patients as a life-saving measure.[13-17] "Moderate-dose" hydrocortisone was added for its theoretical synergistic benefit. All three of these patients made a dramatic recovery and were discharged from the ICU within days with no residual organ dysfunction. Based on this experience and the reported safety and potential benefit of this therapeutic

intervention, the combination of intravenous vitamin C and corticosteroid became routinely used as adjunctive therapy for the treatment of severe sepsis and septic shock in our ICU. Patents with sepsis predictably have very low serum vitamin C levels, which can only be corrected with intravenous vitamin C in a dose of more than 3gm per day. [16;18;19] Based on published clinical data, vitamin C pharmacokinetic modeling as well as the package insert, we decided to administer 6gm vitamin C per day divided in 4 equal doses. [16;18-23] This dosage is devoid of any reported complications or side effects. Dosages as high as 100-150gm have been safely administered to patients with burns and malignancy.[17;24;25] Hydrocortisone was dosed according to the consensus guidelines of the American College of Critical Care Medicine.[26] Intravenous thiamine (vitamin B1) was added to the vitamin C protocol (see discussion section for rationale).[27]

Methods:

This study was an Electronic Health Record (EHR) based retrospective before-after clinical study.[28] The study was approved by our Institutional Review Board (#16-08-WC-0179) and the Sentara Health System Office of Research (16-08-SRC-88). This study was conducted at Sentara Norfolk General Hospital, a tertiary care referral hospital affiliated with Eastern Virginia Medical School (EVMS) and the only tertiary care facility in the Hampton Roads area serving a population of approximately 1.8 million people. Between January 2016 and July 2016, consecutive patients admitted to the EVMS Critical Care Medicine service in the General ICU at Sentara Norfolk General Hospital with a primary diagnosis of severe sepsis or septic shock and a procalcitonin (PCT) ≥ 2ng/ml were treated with intravenous hydrocortisone, vitamin C and thiamine (vitamin C protocol) within 24 hours of ICU admission (treatment group). PCT is routinely measured in our hospital as a screening tool for sepsis and to follow the evolution of the disease. [29-32] PCT is measured in our laboratory using the VIDAS BRAHMS PCT assay, a one-step immunoassay sandwich method with final fluorescent detection (BioMerieux, Inc. Marcy l'Etoile, France); the lower limit of PCT detection was 0.05ng/ml. Septic patients with a PCT < 2ng/ml within the first 24 hours of ICU admission were not treated with the vitamin C protocol. We used a threshold PCT of 2ng/ml to increase the certainty that the patients had severe sepsis and were at risk of developing sepsis-related organ dysfunction.[32-34] Patients less than < 18 years of age, pregnant patients and patients with limitations of care were not treated with the vitamin C protocol. The control group consisted of a similar number of consecutive patients admitted to our ICU between June 2015 and December 2015 using the same

inclusion and exclusion criteria as the treatment group. During the control period, patients with sepsis did not receive intravenous vitamin C or thiamine. The diagnosis of severe sepsis and septic shock were based on the 1992 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions.[35]

We queried our EHR (EPIC, Verona, Wisconsin) to identify patients that met the inclusion criteria for the treatment and control groups. The patients' clinical and demographic data including age, sex, admitting diagnosis, co-morbidities, requirement for mechanical ventilation, use of vasopressors (and hourly dosages), daily urine output (for first 4 days), fluid balance after 24 and 72 hours, length of ICU stay (LOS) and laboratory data were abstracted from the EHR. Patients were considered immunocompromised if they were taking more than 10 mg of prednisone-equivalent per day for at least 2 weeks, were receiving cytotoxic therapy or were diagnosed with the acquired immunodeficiency syndrome. The hourly dosage of vasopressors was recorded as the norepinephrine equivalent dosage. [36;37] The serum creatinine, white cell count (WBC), platelet count, total bilirubin, PCT and lactate levels were recorded daily for the first 4 days. Acute kidney injury (AKI) was defined using the KDIGO criteria; namely, an increase of the serum creatinine > 0.3 mg/dl or a level > 1.5 times the baseline value.[38] If the baseline serum creatinine was not known a value > 1.5 mg/dl was regarded as diagnostic of AKI. The patient's admission APACHE II and APACHE IV scores including the APACHE IV predicted hospital mortality were recorded. The APACHE II score (incrementing score of 0-71) and APACHE IV score (incrementing score 0-286) are standardized measures of disease severity that are used to predict hospital mortality. [39;40] The SOFA (Sepsis-related Organ

Failure Assessment) score was calculated daily for 4 days. The SOFA score was designed to sequentially assess the severity of organ dysfunction in patients who were critically ill from sepsis (incrementing score 0-24). [41] The SOFA scores were calculated 24 hours after admission to the ICU and daily thereafter.

ICU management protocol[42]

The overall management of patients with sepsis during the control and treatment periods was similar except for the administration of the combination of vitamin C, hydrocortisone and thiamine during the treatment period. There were no known significant changes to our ICU protocols, referral patterns, or patient population during the study period. During the control period patients received hydrocortisone (50 mg q 6 hourly) per current guidelines and at the discretion of the attending physician.[26;42;43] As per standard operating procedure in our ICU, all patients with sepsis and septic shock are started empirically on broad spectrum antibiotics which are then deescalated according to microbiological data and clinical progress, [44] are managed by a conservative physiologic based fluid and vasopressor strategy, [45;46] and are ventilated with a lung protective strategy [46;47] avoiding hyperoxia[48] and with the limited use of sedative agents (dexmedetomidine preferred agent).[49] Norepinephrine is the vasopressor of first choice and titrated to a dose of 20 ug/min targeting a mean arterial pressure > 65 mmHg. In patients failing to achieve this target, fixed dose vasopressin was then added at 0.04 units/min followed by phenylephrine or epinephrine.[42;45] Patients receive enteral nutrition with a whey-based formula (Vital 1.2, Abbott, Colombia, OH) using an intermittent bolus protocol which is started 24 hours after ICU admission and once clinical stability is

achieved, [50;51] they receive deep venous thrombosis prophylaxis with both enoxaparin (or heparin in patients with a calculated creatinine clearance < 30 ml/min) and sequential compression and we allow permissive hyperglycemia.[52] Routine stress ulcer prophylaxis is not administered. [53] During the treatment period consecutive patients with a primary admitting diagnosis of severe sepsis or septic shock and a PCT > 2 ng/ml were treated with intravenous vitamin C (1.5 gm q 6 hourly for 4 days or until ICU discharge), hydrocortisone (50 mg q 6 hourly for 7 days or until ICU discharge followed by a taper over 3 days) as well as intravenous thiamine (200 mg q 12 hourly for 4 days or until ICU discharge). The vitamin C was administered as an infusion over 30 to 60 minutes and mixed in a 100ml solution of either dextrose 5% in water (D5W) or normal saline. Intravenous thiamine was given as a piggyback in 50 ml of either D5W or normal saline and was administered as a 30-minute infusion. We attempted to obtain a vitamin C level prior to the first dose of vitamin C. The vitamin C assay was performed at LabCorp (Burlington, NC) by high-pressure liquid chromatography (HPLC) with electrochemical detection. The specimens were collected in a serum separation gel tube, protected from light and transported on ice. The specimens were frozen prior transport to the local reference laboratory (LabCorp, Norfolk VA).

Data analysis:

The first three "pivotal" cases treated with the vitamin cocktail were excluded from analysis. The patients' deidentified clinical and laboratory data were recorded in an electronic spreadsheet. The primary outcome was hospital survival. Secondary outcomes included duration of vasopressor therapy, [54] requirement for renal replacement therapy (RRT) in patients with AKI, ICU LOS and the change in serum PCT and the SOFA score over

the first 72 hours. [30;55-58] The procalcitonin clearance (PCT-c) was calculated using the following formula: initial PCT minus PCT at 72 hours, divided by the initial PCT multiplied by 100. [57;59] Summary statistics were used to describe the clinical data and presented as mean ± SD, median and interquartile range (IQR) or percentages as appropriate. Chi squared analysis with Fisher's exact test (when appropriate) and Student's t test (Mann Whiney U test for non-normal distributions) were used to compare data between the treatment and control groups with statistical significance declared for probability values of 0.05 or less. Logistic discriminant analysis was performed on the entire data set to determine the independent predictors of survival. Statistical analysis was performed using NCSS 11 (Kaysville, Utah) and IBM SPSS Statistics Version 24 (IBM, Armonk, NY).

To adjust for potential baseline differences between the treatment and control groups, SPSS statistics was used to generate propensity scores for the patients' likelihood to receive the vitamin C protocol. Factors included in propensity score generation included age, weight, gender, APACHE 4 score, need for mechanical ventilation at presentation, use of vasopressor agents at presentation, WBC count at presentation, serum lactate level at presentation, procalcitonin level at presentation and serum creatinine at presentation (see e-Table 1). Binary logistic regression with propensity score adjustment was then performed to assess odds ratio for mortality by treatment group.[60] This analysis was then repeated with both age and propensity score adjustments to assess the odds ratio for mortality (see e-Table 2). The propensity score distribution for the treatment and control group is presented in e-Figure 1 while the APACHE IV distribution between the treatment and control group is presented in e-Figure 2.

Results:

There were 47 patients in each group. The baseline characteristics of the two groups are presented in Table 1; with no significant differences in baseline characteristics between the two groups. Most patients had multiple comorbidities with only 2 patients in the treatment group and one in the control group being previously "healthy". The distribution of infections was similar between the two groups with the lung being the most common site of infection. Blood cultures were positive in 13 patients (28%) in each group. *Escherichia coli* (n=6) and gram +ve organisms (n=3) were the commonest blood isolates in the treatment group while gram +ve organisms (n=6) and *E. Coli* (n=4) were the commonest isolates in the control group. Correctly timed baseline vitamin C levels were available in 22 patients in the treatment group; the mean level was 14.1 ± 11.8 umol/l (normal 40-60 umol/l) with no patient having a normal level. Twenty-two (47%) patients in each group were treated with vasopressor agents and met the criteria for septic shock. The primary and secondary outcomes are provided in Table 2. The hospital mortality was 8.5% (4 of 47 patients) in the treatment group compared to 40.4% (19 of 47 patients) in the control group (p < 0.001). The predicted and actual mortality for the treatment and control groups is illustrated in Figure 1. Logistic discriminant analysis identified three independent predictors of mortality, namely, treatment with the vitamin C protocol (F-value 17.33, p< 0.001), the APACHE IV Score (F-value 13.29, p< 0.001) and need for mechanical ventilation (F-value 3.75, p=0.05). The propensity adjusted odds of mortality in patients treated with the vitamin C protocol was 0.13 (95% CI 0.04-0.48, p=002). None of the patients in the treatment group died from complications related to sepsis. All these patients survived their ICU stay, were made "comfort care" on the hospital floor and died

from complications of their underlying disease (advanced dementia, severe heart failure, advanced sarcoidosis and severe COPD).

Twenty-eight (59.6%) patients in the control group were treated with hydrocortisone. Thirty-one patients (66%) in the treatment group met the criteria for AKI compared 30 (64%) in the control group (NS). Three patients (10%) with AKI in the treatment group required RRT compared to 11 (37%) in the control group (p=0.02). The 24-hour fluid balance was 2.1± 3.2 L in the treatment group compared to 1.9± 2.7 l in the control group (NS). Similarly, the 72- hour fluid balance was 1.9±3.7 L in the treatment group compared to 1.6 ± 3.3 L in the control group. Vasopressors were weaned off all patients in the treatment group, a mean of 18.3 ± 9.8 hours after starting treatment with vitamin C protocol. The dose of pressors was predictably weaned down between 2-4 hours after the first infusion of vitamin C. The mean duration of vasopressor use was 54.9 ± 28.4 hours in the control group (p<0.001); 9 patients in the control group had escalating doses of vasopressors and died from refractory septic shock. The mean duration of vasopressors was 61.4 ± 33.8 hours in the control patient who died compared to 38.7± 6.5 hours in those who survived. The time course of the vasopressor dose (in norepinephrine equivalents) [36;37] in the treatment group, the control patients who died and the control patients who survived is illustrated in Figure 2. The 72-hour delta SOFA score was 4.8 ± 2.4 in the treatment group compared to 0.9 ± 2.7 in the control group (p<0.001). None of the patients in the treatment group developed progressive organ failure (an increase in their SOFA score) requiring an escalation of therapy. The time course of the SOFA score in the treatment group, the control patients who died and the control patients who survived is

shown in Figure 3. The median 72 hours PCT clearance was 86.4 % (80.1-90.8) in the

treatment group compared to 33.9% (-62.4 – 64.3) in the control group (p<0.001); the time

course of the PCT over the first 4 days is illustrated in Figure 4. The median ICU LOS was 4

(3-5) days in the treatment group compared to 4 (4-10) days in the control group (NS).

Discussion:

In this observational study the combination of intravenous vitamin C, moderate-dose hydrocortisone and thiamine appeared to have a marked effect on the natural history of patients with severe sepsis and septic shock. No patient in the treatment group developed progressive organ failure and the four deaths in this group were related to the patients underlying disease; these patients did not die from sepsis related complications. Our study evaluated the use of intravenous vitamin C, hydrocortisone and thiamine in a real world setting where all eligible patients with sepsis were studied. This is important as less than 20% of eligible patient with severe sepsis and septic shock are commonly included in many of the sepsis trials limiting the applicability and generalizability of the results.[61] Furthermore, we did not test an expensive, proprietary designer molecule, but rather the combination of three cheap and readily available agents with a long safety record in clinical use since 1949. [62;63]

The findings of our study are supported by extensive experimental and clinical studies which have demonstrated the safety and potential benefit of moderate-dose hydrocortisone, intravenous vitamin C and thiamine in critically ill patients. [16;17;21;26;64] However, ours is the first study to evaluate the combination of intravenous vitamin C hydrocortisone and thiamine, a combination which we believe synergistically reverses the pathophysiologic changes of sepsis. The outcome data are supported by the time course of the PCT levels and SOFA score as well as the rapid decline in vasopressor requirements in the treatment group as compared to the control group. The time course of PCT in patients with severe sepsis and septic shock has been evaluated in a number of studies. [31;32;65] These

studies have demonstrated a linear fall in PCT reaching about 30% of the baseline value in 72 hours; a fall of greater than 30% over this time has been shown to indicate the appropriate use of antibiotics and is predictive of survival. In the treatment group, the PCT fell exponentially in all patients reaching a median of 86% of the baseline value at 72 hours. In comparison, the PCT remained relatively unchanged in the control group during this time. This observation is supported by the pilot study of Fowler et al who in a randomized controlled trial evaluated the clinical response to low dose (50 mg/kg/day) and high dose vitamin C (200 mg/kg/day) (without corticosteroids) in 24 patients with sepsis. [16] In this study, the fall of the PCT in the first 72 hours was approximately 40% in the low dose group and 80% in the high dose group whereas it increased in the control patients. In our study, the mean time to vasopressor independence after starting the vitamin C protocol was 18 hours. The mean duration of vasopressor therapy was 54 hours in the control group. The mean duration of vasopressor dependency in patients with septic shock reported in the literature varies between about 72 to 120 hrs. [37;54] In the VANISH trial, the median time to shock reversal was 45 hours (IQR 23-75hrs). [66] The mean duration of vasopressor dependency in the patients treated with vitamin C in the study by Fowler et al was 86 hrs.[16] Similarly, in the Corticosteroid Therapy of Septic Shock (CORTICUS) study, the time to vasopressor independence was 79 hrs. [54] These data support the contention that vitamin C, hydrocortisone and thiamine have synergistic effects in reversing vasoplegic shock in patients with sepsis. Shortening the duration of vasopressor treatment and preventing dose escalation likely has numerous beneficial effects, including limiting organ and limb ischemia.[66-68] Furthermore, norepinephrine exerts anti-inflammatory and

bacterial growth-promoting effects, which may potentiate the immuno-paresis of sepsis thereby increasing the susceptibility towards secondary infections.[69]

Experimental studies performed over the last 20 years have demonstrated that both vitamin C and hydrocortisone have multiple and overlapping beneficial pathophysiologic effects in sepsis. Vitamin C is a potent antioxidant which directly scavenges oxygen free radicals, restores other cellular antioxidants including tetrahydrobiopterin and α-tocopherol and is an essential co-factor for iron and copper containing enzymes. [70;71] Both drugs inhibit nuclear factor K-B (NF-KB) activation down regulating the production of pro-inflammatory mediators, increase tight junctions between endothelial and epithelial cells, preserve endothelial function and microcirculatory flow, and are required for the synthesis of catecholamines and increase vasopressor sensitivity.[13-15;26;70-75] Vitamin C plays a major role in preserving endothelial function and microcirculatory flow.[70;72] In addition, vitamin C activates the nuclear factor erythroid 2-like 2 (Nrf2)/ heme oxygenase (HO)-1 pathway which plays a critical role in anti-oxidant defences and enhances T cell and macrophage function. [76-78] The explanation as to why the combination of intravenous vitamin C, hydrocortisone and thiamine appeared to have a marked effect on the course of sepsis as compared to the myriad of designer molecules which have been evaluated in previous sepsis trials is likely related to the multiple and overlapping effects of all three agents as compared to drugs which target a single molecule or pathway.[12] Furthermore, we believe that vitamin C and corticosteroids act synergistically. [79] Oxidation of cysteine thiol groups of the glucocorticoid receptor (GR) affects ligand and DNA binding reducing the efficacy of glucocorticoids. [80;81] Vitamin C has been demonstrated to reverse these changes and

restore glucocorticoid function. [82] The transport of vitamin C into the cell is mediated by the sodium-vitamin C transporter -2 (SVCT2). [83] Proinflammatory cytokines have been demonstrated to decrease expression of SVCT2. [84] Glucocorticoids, however, have been shown to increase expression of SVCT2. [85] In an experimental model using cultured human lung vascular endothelial cells exposed to endotoxin we have demonstrated that incubation with the combination of vitamin C and hydrocortisone preserved endothelial integrity as compared to either agent alone which was no more effective than placebo. [86] This finding is in keeping with recent clinical studies which suggest that hydrocortisone and vitamin C alone have little impact on the clinical outcome of patients with sepsis. [16;54] The recently published "Hydrocortisone for Prevention of Septic Shock (HYPRESS)" study failed to demonstrate an outcome benefit from a hydrocortisone infusion in patients with severe sepsis. [87]

While the dosing strategy for corticosteroids (hydrocortisone) in patients with severe sepsis and septic shock has been well studied, [26;88] that for vitamin C is more uncertain. Critically ill patients have either very low or undetectable vitamin C levels (normal serum levels 40-60 umol/l). [18;19] Due to the saturable intestinal transporter (sodium-vitamin C transporter-1), [20;83] oral administration of doses as high as 1500 mg cannot restore normal serum levels. [20] To achieve normal serum vitamin C levels in critically ill patients, a daily dose of more than 3 gm is required. [16;18;21] Based on pharmacokinetic data and preliminary dose response data we believe that a daily dose of 6gm combined with hydrocortisone is optimal. When high dosages of vitamin C are given intravenously, metabolic conversion to oxalate increases. [19] Oxalate is normally excreted by

the kidney and serum levels will increase with renal impairment. In patients with renal impairment receiving mega-dose vitamin C, supersaturation of serum with oxalate may result in tissue deposition as well as crystallization in the kidney. [89;90] Worsening renal function is therefore a concern with mega-dose vitamin C. It is noteworthy that renal function improved in all our patients with AKI. Glyoxylate, a byproduct of intermediary metabolism, is either reduced to oxalate or oxidized to CO_2 by the enzyme glyoxylate aminotransferase; thiamine pyrophosphate is a co-enzyme required for this reaction. [91] Thiamine deficiency increases the conversion of glyoxylate to oxalate. [92;93] Thiamine deficiency is common in septic patients and associated with an increased risk of death.[64] For these reasons, thiamine was included in our vitamin C protocol.

Our study has several limitations, namely the small sample size, single center design, and the use of non-concurrent controls. Furthermore, the treatment and control periods occurred during different seasons. We used propensity score adjustment in an attempt to control for some of these factors. We believe that the data from our study is internally consistent, has valid mechanistic basis and is supported by experimental studies. In addition, the safety of hydrocortisone, vitamin C and thiamine is supported by over 50 years of clinical experience. Due to the inherent safety of the combination of hydrocortisone, vitamin C and thiamine we believe that this treatment strategy can be adopted pending the results of further clinical trials. We believe that the results of our study provide sufficient information for the design of an adequately powered, high quality pragmatic trial to confirm the findings of our study. Due to the lack of clinical equipoise and the ethics of withholding a potentially lifesaving intervention, we were unable to

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initiate a RCT in our center.   Furthermore, while our observational study suggests that a 4-day course of vitamin C is optimal additional studies are required to determine the ideal dosing strategy and the contributing role of thiamine requires further exploration.

In conclusion, the results of our study suggest that the early use of intravenous vitamin C, together with moderate-dose hydrocortisone and thiamine may prove to be effective in preventing progressive organ dysfunction including acute kidney injury and reducing the mortality of patients with severe sepsis and septic shock. This inexpensive and readily available intervention has the potential to reduce the global mortality from sepsis. Additional studies are required to confirm our preliminary findings.

Contributions:

Professor Paul Marik- Conception of study, literature review, pharmacological modeling and interpretation, study design, study execution, data collection, data analysis, data interpretation, writing of study.

Dr Vikramjit Khangoora - Literature review, study design, data analysis, data interpretation, writing of study.

Dr Racquel Rivera - Pharmacological modeling and interpretation, formulation of dosing strategy, study execution, data collection, writing of study.

Dr Michael Hooper - interpretation of data, statistical analysis, writing of study.

Professor John Catravas, study design, interpretation of data, writing of study.

Paul Marik, MD is the guarantor of and is responsible for the entire manuscript.

Table 1. Baseline characteristic of treated and control patients.

	Treated (n=47)	Control (n=47) ‡
Age	58.3 ± 14.1	62.2 ± 14.3
Sex (male)	27 (57%)	23 (49%)
Comorbidities		
None	2 (4%)	1 (2%)
Diabetes	16 (34%)	20 (42%)
Hypertension	20 (43%)	25 (53%)
Heart Failure	15 (32%)	16 (34%)
Malignancy	5 (11%)	7 (15%)
COPD	8 (17%)	7 (15%)
Cirrhosis	6 (13%)	3(6%)
CVA	8 (17%)	5 (11%)
CRF	7 (15%)	8 (17%)
Morbid Obesity	6 (13%)	8 (17%)
Immunocompromised**	6 (13%)	4 (9%)
Drug addiction	5 (11%)	5 (11%)
Primary diagnosis		
Pneumonia	18 (38%)	19 (40%)
Urosepsis	11(23%)	10 (21%)
Primary bacteremia	7 (15%)	7 (15%)
GI/ Biliary	6 (13)	6 (13%)
Other	5 (11%)	5 (11%)

Mechanical Ventilation	22 (47%)	26 (55%)
Vasopressors	22 (46%)	22 (46%)
Acute kidney injury	31 (66%)	30 (64%)
Positive Blood cultures	13 (28%)	13 (28%)
WBC x10^9†	20.6 ± 13.5	17.1 ± 13.4
Lactate (mmol/l)	2.7 ± 1.5	3.1 ± 2.8
Creatinine (mg/dl)††	1.9 ± 1.4	1.9 ± 1.1
Procalcitonin (ng/ml)	25.8 (5.8- 93.4)	15.2 (5.9 -39.0)
Day 1 SOFA	8.3 ± 2.8	8.7 ± 3.7
APACHE II	22.1 ± 6.3	22.6 ± 5.7
APACHE IV	79.5 ± 16.4	82.0 ± 27.4
Predicted mortality	39.7 ± 16.7	41.6 ± 24.2

‡ No significant difference in baseline characteristics between groups

* CVA=cerebrovascular accident; COPD=chronic obstructive lung disease; CRF =chronic

respiratory failure; GI=gastrointestinal

** HIV infection, neutropenia, post-transplant, etc.

† Excluding neutropenic patients

†† Excluding patients with chronic renal failure

Table 2. Outcome and treatment variables

	Treated (n=47)	Control (n=47)
Hospital mortality	4 (8.5%)	19 (40.4%)†
ICU LOS	4 (3-5)	4 (4-10)
Duration of vasopressors (hours)	18.3 ± 9.8	54.9 ± 28.4 †
RRT for AKI	3/31 (10%)	11/30 (33%)††
Delta SOFA (72 hr)	4.8 ± 2.4	0.9 ± 2.7 †
Procalcitonin Clearance (72 hr)	86.4% (80.1-90.8)	33.9% (-62.4 – 64.3) †

RRT= renal replacement therapy; AKI= acute kidney injury

† p < 0.001
†† p= 0.02

Legends for Figures.

Figure 1. Predicted and actual mortality in the treatment and control group. Predicted mortality was derived from the APACHE IV scoring system. p< 0.001 for comparison of treatment vs control group (see text)

Figure 2.

Time course of vasopressor dosage (in norepinephrine equivalents) in the treatment group and in the control group survivors and non-survivors. p< 0.001 for comparison of treatment vs control group (see text)

Figure 3.

Time course of the SOFA score over the four-day treatment period in the treatment group and in the control group survivors and non-survivors. p< 0.001 for comparison of treatment vs control group (see text)

Figure 4.

Time course of the PCT score over the four-day treatment period in the treatment group and in the control group survivors and non-survivors plotted on a semilog scale. p< 0.001 for comparison of treatment vs control group (see text)

Reference List

(1) Adhikari NK, Fowler RA, Bhagwanjee S et al. Critical care and the global burden of critical illness in adults. Lancet 2010; 376(9749):1339-1346.

(2) Kaukonen KM, Bailey M, Suzuki S et al. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. JAMA 2014; 311:1308-1316.

(3) Gaieski DF, Edwards JM, Kallan MJ et al. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med 2013; 41:1167-1174.

(4) Kadri SS, Rhee C, Strich JR et al. Estimating ten-year trends in septic shock incidence and morality in United States Academic Medical Centers using clinical data. Chest 2016; ePub; 10.1016/j.chest.2016.07.010

(5) Marik PE, Linde-Zwirble WT, Bittner EA et al. Fluid administration in severe sepsis and septic shock, patterns and outcomes. An analysis of a large national database. Intensive Care Med 2016;(Submitted).

(6) Silva E, de Almeida Pedro M, Beltrami Sogayar AC et al. Brazilian Sepsis Epidemiological Study (BASES study). Crit Care 2004; 8(4):R251-R260.

(7) Sales JA, David CM, Hatum R et al. Sepse Brasil: Estudo Epidemiologico da Sepse em Unidades de Terapia Intensiva Brasileiras. An epidemiological study of sepsis in Intensive Care Units. Sepsis Brazil Study. Rev Bras Ter Int 2006; 18:9-17.

(8) The10 leading causes of death by country income group 2012. WHO factsheets. World Health Organization, 2015.

(9) Karlsson S, Ruokonen E, Varpula T et al. Long-term outcome and quality-adjusted life years after severe sepsis. Crit Care Med 2009; 37(4):1268-1274.

(10) Yende S, Austin S, Rhodes A et al. Long-term qualityof life among survivors of severe sepsis: Analyses of two international trials. Crit Care Med 2016; 44:1461-1467.

(11) Ou SM, Chu H, Chao PW et al. Long-term mortality and major adverse cardiovascular events in sepsis survivors. A Nationwide population-based study. Am J Respir Crit Care Med 2016; 194:209-217.

(12) Artenstein AW, Higgins TL, Opal SM. Sepsis and scientific revolutions. Crit Care Med 2013; 41:2770-2772.

(13) Fisher BJ, Kraskauskas D, Martin EJ et al. Attenuation of sepsis-induced organ injury in mice by vitamin C. JPEN 2014; 38(7):825-839.

(14) Fisher BJ, Seropian IM, Masanori Y et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. Crit Care Med 2011; 39(6):1454-1460.

(15) Zhou G, Kamenos G, Pendem S et al. Ascorbate protects against vascular leakage in cecal ligation and puncture-induced septic peritonitis. Am J Physiol Regulatory Integrative Comp Physiol 2012; 302(4):R409-R416.

(16) Fowler AA, Syed AA, Knowlson S et al. Phase 1 safety trial of intravenous ascorbic acid in patients with severe sepsis. J Transl Med 2014; 12:32.

(17) Tanaka H, Matsuda T, Miyagantani Y et al. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: a randomized, prospective study. Arch Surg 2000; 135(3):326-331.

(18) Long CL, Maull KL, Krishman RS et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res 2003; 109(2):144-148.

(19) de Grooth HJ, Choo WP, Spoelstra-de Man AM et al. Pharmacokinetics of four high-dose regimens of intravenous Vitamin C in critically ill patients [Abstract]. Intensive Care Med 2016.

(20) Padayatty SJ, Sun H, Wang Y et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med 2004; 140(7):533-537.

(21) Nathens AB, Neff MJ, Jurkovich GJ et al. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. Ann Surg 2002; 236(6):814-822.

(22) Zabet MH, Mohammadi M, Ramezani M et al. Effect of high-dose ascorbic acid on vasopressor requirement in septic shock. J Res Pharm Pract 2016; 5:94-100.

(23) Ascorbic Acid Injection. The Torrance Company, 2015.

(24) Ohno S, Ohno Y, Suzuki N et al. High-dose vitamin C (ascorbic acid) therapy in the treatment of patients with advanced cancer. Anticancer Research 2009; 29(3):809-815.

(25) Stephenson CM, Levin RD, Spector T et al. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. Cancer Chemotherapy & Pharmacology 2013; 72(1):139-146.

(26) Marik PE, Pastores SM, Annane D et al. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: Consensus statements from an international task force by the American College of Critical Care Medicine. Crit Care Med 2008; 36:1937-1949.

(27) Marik PE. "Vitamin S' (steroids) and Vitaminc C for the treatment of severe sepsis and septic shock! Crit Care Med 2016; 44:1228-1229.

(28) Fiore LD, Lavori PW. Integrating randomized comparative effectiveness research with patient care. N Engl J Med 2016; 374:2152-2158.

(29) Wacker C, Prkno A, Brunkhorst FM et al. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013; 13:426-435.

(30) 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:R115.

(31) Charles PE, Tinel C, Barbar S et al. Procalcitonin kinetics within the first days of sepsis: relationship with the appropriateness of antibiotic therapy and outcome. Crit Care 2016; 13:R38.

(32) Reynolds SC, Shorr AF, Muscedere J et al. Longitudinal changes in procalcitonin in a heterogeneous group of critically ill patients. Crit Care Med 2012; 40:2781-2787.

(33) Arora S, Singh P, Singh PM et al. Procalcitonin levels in survivors and non survivors of sepsis: Systematic review and meta-analysis. Shock 2015; 43:212-221.

(34) Kutz A, Briel M, Christ-Crain M et al. Prognostic value of procalcitonin in respiratory tract infections across clinical settings. Crit Care 2015; 19:74.

(35) Society of Critical Care Medicine Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992; 20:864-874.

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

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

(38) Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit Care 2013; 17(1):204.

(39) Knaus WA, Draper EA, Wagner DP et al. APACHE II: A severity of disease classification system. Crit Care Med 1985; 13:818-828.

(40) Zimmerman JE, Kramer AA, McNair DS et al. Acute Physiology and Chronic Health Evaluation (APACHE) IV: hospital mortality assessment for today's critically ill patients. Crit Care Med 2006; 34(5):1297-1310.

(41) Vincent JL, Moreno R, Takala J et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996; 22(7):707-710.

(42) Marik PE. Early management of severe sepsis: Current concepts and controversies. Chest 2014; 145:1407-1418.

(43) Siemieniuk RA, Meade MP, Alonso-Coello P et al. Corticosteroid therapy for patients hospitalized with community-acquired pneumonia. A systematic review and meta-analysis. Ann Intern Med 2015.

(44) Garnacho-Montero J, Gutierrez-Pizarraya A, Escoresca-Ortega A et al. De-escalation of emperical therapy is associated with lower mortality in patients with severe sepsis and septic shock. Intensive Care Med 2014; 40(1):32-40.

(45) Marik P, Bellomo R. A rational apprach to fluid therapy in sepsis. Br J Anaesth 2016; 116:339-349.

(46) Marik PE. Fluid responsiveness and the six guiding principles of fluid resuscitation. Crit Care Med 2016; 44:1920-1922.

(47) Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med 2000; 342:1301-1308.

(48) Giradis M, Busani S, Damiani E et al. Effect of conservative vs conventional oxygen therapy on mortality among patients in a Intensive Care Unit. The oxygen-ICU randomized clinical trial. JAMA 2016; ePub:doi:10.1001/jama.2016.11993.

(49) Barr J, Fraser GL, Puntillo K et al. Clinical Practice Guidelines for the Management of Pain, Agitation and Delirium in Adult Patients in the Intensive Care Unit. Crit Care Med 2013; 41:263-306.

(50) Marik PE. Feeding critically ill patients the right "whey":thinking outside the box. A personal view. Ann Intensive Care 2015; 5:11.

(51) Marik PE. Is early starvation beneficial for the critically ill patient? Curr Opin Clin Nutr Metab Care 2016; 19:155-160.

(52) Marik PE. Tight glycemic control in acutely ill patients: low evidence of benefit, high evidence of harm! Intensive Care Med 2016; 42:1475-1477.

(53) Marik PE. Stress ulcer prophylaxis de-adoption: What the barrier? Crit Care Med 2016; 44:1939-1941.

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

(55) Karlsson S, Heikkinen M, Pettila V et al. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2010; 14(6):R205.

(56) Ferreira FL, Bota DP, Bross A et al. Serial evaluation of the SOFA score to predict outcome in critically ill patients. JAMA 2001; 286(14):1754-1758.

(57) de Azevedo JR, Torres OJ, Beraldi RA et al. Prognostic evaluation of severe sepsis and septic shock: procalcitonin clearance vs DELTA Sequential Organ Failure Assessment. J Crit Care 2015; 30(1):219-12.

(58) Guan J, Lin Z, Lue H. Dynamic change of procalcitonin, rather than concentration itself, is predictive of survival in septic shock patients when beyond 10 ng/mL. Shock 2011; 36(6):570-574.

(59) Ruiz-Rodriguez 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.

(60) D'Agostino RB. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med 1998; 17:2265-2281.

(61) Marik PE, Pastores SM, Kavanaugh BP. Selection bias negates conclusions from the CORTICUS study? N Engl J Med 2008; 358:2069-2070.

(62) Klenner FR. The treatment of poliomyelitis and other virus diseases with vitamin C. South Med Surg 1949; 111:209-214.

(63) Hench PS, Kendall EC, Slocumb CH et al. The effect of a hormone of the adreanl cortex (17-hydroxy-11-dehydrocorticosterone:compund E) and the pituitary adenocorticotrophic hormone on rheumatoid arthritis. Preliminary report. Ann Rheum DIs 1949; 8:97-104.

(64) 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; 44:360-367.

(65) Gibot S, Cravoisy A, Kolopp-Sarda MN et al. Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reative protein plasma concentrations during sepsis. Crit Care Med 2005; 33:792-796.

(66) Gordon AC, Mason AJ, Thirunavukkarasu N et al. Effect of early vasopressin vs norepinephrine on kidney failure in patients with septic shock. The VANISH randomized clinical trial. JAMA 2016; 316:509-518.

(67) Hayes MA, Yau EH, Hinds CJ et al. Symmetrical peripheral gangrene: association with noradrenaline administration. Intensive Care Med 1992; 18(7):433-436.

(68) Dunser MW, Wenzel V, Hasibeder WR. Ischemic skin lesions and microcirculatory collapse during vasopressin therapy: a possible role of the microcirculation? Acta Anaesthesiol Scand 2006; 50(5):637-638.

(69) Stolk RF, van der Poll T, Angus DC et al. Potentially inadvertant immunomodulation: norepinephrine use in sepsis. Am J Respir Crit Care Med 2016.

(70) May JM. Role of vitamin C in the function of the vascular endothelium. Antioxidants & Redox Signaling 2013; 19(17):2068-2083.

(71) Wilson JX. Evaluation of vitamin C for adjuvant sepsis therapy. Antioxidants & Redox Signaling 2013; 19(17):2129-2140.

(72) Wilson JX. Mechanism of action of vitamin C in sepsis: ascorbate modulates redox signaling in endothelium. Biofactors 2009; 35(1):5-13.

(73) Han M, Pendem S, Teh SL et al. Ascorbate protects endothelial barrier function during septic insult: Role of protein phosphatase type 2A. Free Radic Biol Med 2010; 48:128.

(74) Dillon PF, Root-Bernstein RS, Lieder CM. Antioxidant-independent ascorbate enhancement of catecholamine-induced contractions of vascular smooth muscle. Am J Physiol Heart Circ Physiol 2004; 286(6):H2353-H2360.

(75) Marik PE. Critical illness related corticoseroid insufficiency. Chest 2009; 135(1):181-193.

(76) Kalden JR. Prolonged skin allograft survival in vitamin C-deficient guinea-pigs. Preliminary communication. European Surgical Research 1972; 4(2):114-119.

(77) Kim SR. Ascorbic acid reduces HMGB1 secretion in lipopolysaccharide-activated RAW 264.7 cells and improves survival rate in septic mice by activation of Nrf2/HO-1 signals. Biochemical Pharmacology 2015; 95(4):279-289.

(78) Manning J, Mitchell B, Appadurai DA et al. Vitamin C promotes maturation of T-cells. Antioxidants & Redox Signaling 2013; 19(17):2054-2067.

(79) Fogarty A, Scrivener SL, Antoniak M et al. Corticosteroid sparing effects of vitamin C and magnesium in asthma: a randomised trial. Resp Med 2006; 100(1):174-179.

(80) Bodwell JE, Holbrook NJ, Munck A. Sulfhydryl-modifying reagents reversibly inhibit binding of glucocorticoid-receptor complexes to DNA-cellulose. Biochemistry 1984; 23(7):1392-1398.

(81) 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.

(82) 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.

(83) Burzle M, Hediger MA. Functional and physiological role of vitamin C transporters. Curr Top Membr 2012; 70:357-375.

(84) 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.

(85) Fujita I, Hirano J, Itoh N et al. Dexamethasone induces sodium-dependant vitamin C transporter in a mouse osteoblastic cell line MC3T3-E1. Br J Nutr 2001; 86(2):145-149.

(86) Khangoora V, Catravas J, Barabutis N et al. Restoration of endothelial barrier function with Vitamin C and Hydrocortisone in cells treated with lipopolysaccharide. Am J Physiol Lung Cell Mol Physiol 2016;(Submitted).

(87) Keh D, Trips E, Marx G et al. Effect of hydrocortisone on development of shock among patients with severe sepsis. The HYPRESS Randomized Clinical Trial. JAMA 2016; ePub:doi:10.1001/jama.2016.14799.

(88) Minneci PC, Deans KJ, Eichacker PQ et al. The effects of steroids during sepsis depend on dose and severity of illness: an updated meta-analysis. Clin Microbiol Infect 2009; 15(4):308-318.

(89) Massey LK. Ascorbate increases human oxaluria and kidney stone risk. Journal of Nutrition 2005; 135(7):1673-1677.

(90) Wandzilak TR. Effect of high dose vitamin C on urinary oxalate levels. Journal of Urology 1994; 151(4):834-837.

(91) Hoppe B, Beck BB, Milliner D. The primary hyperoxalurias. Kidney Int 2009; 75:1264-1271.

(92) Sidhu H, Gupta R, Thind SK et al. Oxalate metabolism in thiamine-deficient rats. Ann Nutr Metab 1987; 31(6):354-361.

(93) Ortiz-Alvarado O, Muyaoka R, Kriedberg C et al. Pyridoxine and dietary counseling for the management of idiopathic hyperoxaluria in stone-forming patients. Urology 2011; 77:1054-1058.