



The Vitamin C, Hydrocortisone and ThiAMINE in Patients with Septic Shock Trial

The VITAMINS Trial

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1. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
ANZIC RC	Australian and New Zealand Intensive Care Research Centre
APACHE	Acute Physiology and Chronic Health Evaluation
CRF	Case report form
eCRF	Electronic case report form
GCP	Good clinical practice
HRC	Health Research Council
HREC	Human Research Ethics Committee
ICH	International conference on harmonisation
ICU	Intensive care unit
NHMRC	National Health and Medical Research Council
RCT	Randomised controlled trial
RRT	Renal replacement therapy
SAE	Serious adverse event
SAR	Serious adverse reaction
SOFA	Sequential Organ Failure Assessment
SUSAR	Suspected unexpected serious adverse reaction
TGA	Therapeutic Goods Administration

2. SYNOPSIS

Background

Sepsis has been characterised as a dysregulated host response to infection. Adjunctive therapies targeting the inflammatory cascade are being increasingly explored, although to date, have failed to demonstrate consistent benefit, and sepsis continues to manifest poor outcomes. Hospital mortality in patients with septic shock remains as high as 22% in Australia and New Zealand. From a global perspective, 31 million sepsis and 19 million severe sepsis cases are expected to be treated in hospitals all over the world per year.

To date, experimental data have reported that both high dose intravenous vitamin C and corticosteroids attenuate the acceleration of the inflammatory cascade and possibly reduce the endothelial injury characteristic of sepsis, enhance the release of endogenous catecholamines and improve vasopressor responsiveness.

The use of low dose corticosteroid therapy in septic shock has been evaluated in several clinical trials, with meta-analysis of these trials demonstrating improved outcomes in some groups and no effect in others. A recent large-scale RCT has confirmed that, compared with placebo, 200mg of hydrocortisone a day speeds up the resolution of shock, results in more rapid liberation from life support, and results in patients being discharged from the intensive care unit more quickly. In a pilot study of 24 patients with severe sepsis and septic shock, intravenous high dose vitamin C attenuated the inflammatory response. In a randomized study of 28 surgical patients with septic shock, the mean dose of norepinephrine and duration of norepinephrine administration were significantly lower in the high dose vitamin C group compared to the placebo group.

Previous research suggests that vitamin C and glucocorticoids may act synergistically to limit the pro-inflammatory response, limit the endothelial injury and improve microcirculatory function and vasopressor responsiveness in patients with sepsis and septic shock.

Thiamine has been recognised to have an essential role in cellular metabolism and the formation of ATP. Thiamine deficiency is common in septic patients and is associated with an increased risk of death. Thiamine also helps to keep oxylate concentrations low through facilitating alternative utilization of glyoxylate.

Vitamin C, Hydrocortisone and Thiamine in septic shock

In 2016, Marik and colleagues performed a sequential period retrospective before-after clinical study in 94 patients, in which they compared the outcome and clinical course of

	<p>consecutive septic patients treated with intravenous vitamin C (6g/d), hydrocortisone (200mg/d) and thiamine (200mg/d).</p> <p>The hospital mortality was 8.5% (4 of 47) in the treatment group compared to 40.4% (19 of 47) in the control group ($p < 0.001$). 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.</p> <p>We submit that these results justify the design of pragmatic pilot feasibility multicentre randomised controlled trial to confirm the findings of the retrospective single centre study.</p>
Aim	<p>The primary aim of the study is to determine whether the intravenous administration of high dose Vitamin C (6g/d), Thiamine (400mg/d) and Hydrocortisone (200mg/d) in patients with septic shock leads to a more rapid resolution of shock and shortens the duration of vasopressor dependence compared to Hydrocortisone alone.</p>
Design	<p>A pilot, feasibility, multi-centre, randomised, open-label, phase IIb clinical trial.</p>
Methods	<p>Eligible patients will be enrolled and randomly allocated in a 1:1 ratio to either the treatment group, receiving intravenous Vitamin C (1.5g every 6 hours), Thiamine (200mg every 12 hours) and Hydrocortisone (50mg every 6 hours), or to the control group, receiving Hydrocortisone (50mg every 6 hours) alone.</p> <p>As in the recent ADRENAL septic shock study (NCT01448109), treatment will continue until:</p> <ol style="list-style-type: none"> 1. Septic shock resolves 2. The patient leaves the ICU 3. Contraindications to Vitamin C, Thiamine or Hydrocortisone therapy arise 4. Death occurs 5. 10 days of treatment has been administered 6. Serious adverse events suspected to be secondary to the intervention therapy develop
Outcomes	<p>Primary outcome:</p> <ol style="list-style-type: none"> 1. Time alive and free of vasopressors at day 7 (168 hours) after randomisation. <p><i>[This is defined by the patient being alive at discontinuation of all intravenous vasopressors for at least 4 hours in the presence of a MAP > 65 mmHg or target MAP set by treating physicians for the same 4 hour period as recorded in the ICU charts and censored at 7 days.]</i></p>

	<p><i>If a patient dies while on vasopressor therapy during the initial (index) episode of septic shock, in such a patient, the time alive and vasopressor free time will be 0 – This approach accounts for the competing effect of mortality on the duration of initial (index episode) vasopressor therapy].</i></p> <p>Secondary Outcomes</p> <p>Feasibility outcomes:</p> <ul style="list-style-type: none"> - Time from meeting eligibility criteria to the first dose of the main study drugs - Monthly recruitment rate - Number of patients screened - Randomised to the screened patient ratio - Reasons for exclusion - Compliance with drug administration protocol <p>Patient-centred outcomes:</p> <ul style="list-style-type: none"> - Alive and ICU-free days calculated as the number of days alive and out of the ICU to day 28 - ICU mortality - Hospital mortality - 28-day mortality - 90-day mortality - Delta SOFA score at day 3, defined as the pre-randomisation SOFA score minus the day 3 SOFA score - Hospital length of stay - Cumulative vasopressor free hours from shock resolution to day28 post randomisation - Cumulative invasive mechanical ventilation-free hours during the 28 day period post randomisation - Length of renal replacement therapy dependency during the 28 day period post randomisation
<p>Trial Registration</p>	<p>ClinicalTrials.gov (NCT03333278)</p>

3. STUDY ADMINISTRATION STRUCTURE

3.1 Coordinating Centre

3.1.1 Responsibilities

- Overall management of the trial including assistance with human research ethics committee applications
- Contract management between parties as required
- Management of study budget and liaison with funding bodies
- Protocol and case report form (CRF) design and production
- Database design and management
- Randomisation
- Coordination and monitoring of data entry and feedback of data enquiries
- Serious adverse event notification
- Data analysis and collaboration on publications
- Respond to general queries, including information about current recruitment status

3.1.2 Staff

Prof Rinaldo Bellomo	Principal Investigator and Co-Director, ANZIC-RC
Mr Tony Trapani	Research Centre Manager, ANZIC-RC
Prof Andrew Udy	Co-Deputy Director, ANZIC-RC
Prof Michael Bailey	Senior Statistician, ANZIC-RC
Dr Glenn Eastwood	Senior Research Fellow, ANZIC-RC
Dr Nora Luethi	Adjunct Senior Research Fellow, ANZIC-RC
Dr Tomoko Fujii	Visiting Senior Research Fellow, ANZIC-RC

3.2 Management Committee

3.2.1 Responsibilities

Overseeing all aspects of the study management including:

- Liaison with coordinating centre staff and trial management centre staff
- Overseeing funding applications
- Overseeing disbursement and administration of funds
- Ensuring fiscal responsibilities are maintained
- Development and approval of final protocol and trial materials
- Development and approval of data collection tools and methods
- General trial management issues

3.2.2 Members

Dr	Rinaldo	Bellomo	Chair Professor	ANZIC-RC, Monash University Austin Health ICU
Dr	Glenn	Eastwood	Senior Research Fellow ICU Research Coordinator	ANZIC-RC, Monash University Austin Health ICU
Dr	Andrew	Udy	Professor	ANZIC-RC, Monash University
Dr	Nora	Luethi	Adjunct Senior Research Fellow	ANZIC-RC, Monash University
Dr	Adam	Deane	Associate Professor	Melbourne University Royal Melbourne Hospital
Dr	Neil	Orford	Associate Professor	ANZIC-RC, Monash University University Hospital of Geelong
Dr	Yahya	Shehabi	Professor	Monash Health
Dr	Craig	French	Associate Professor	Western Health
Dr	Paul	Young	Director	Wellington Regional Hospital, ICU
Dr	Daniel	Frei	Research Fellow	Wellington Hospital, Department of Anaesthesia
Dr	Ludhmila	Hajjar	Associate Professor	Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo
Dr	Gisele	Queiroz		Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo
Dr	Tomoko	Fujii	Visiting Senior Research Fellow	ANZIC-RC, Monash University

3.2.3 Meetings

Teleconferences as required.

3.3 Contact Details

3.3.1 Coordinating Centre

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3.4 Clinical Centres

3.4.1 Responsibilities

- Overall management of study at own site in line with the study protocol
- Patient follow up
- Data collection and data transfer

- Management of data queries
- Liaison with local HREC
- Adherence to local HREC guidelines and reporting requirements
- Respond to scientific queries

3.4.2 Site principal investigators

Professor Rinaldo Bellomo	Intensive Care Unit, Austin Hospital, Heidelberg, Victoria
Professor Andrew Udy	Intensive Care Unit, The Alfred Hospital, Prahran, Victoria
Assoc Professor Adam Deane	Intensive Care Unit, Royal Melbourne Hospital, Parkville, Victoria
Assoc Professor Neil Orford	Intensive Care Unit, Geelong University Hospital, Geelong, Victoria
Assoc Professor Craig French	Intensive Care Unit, Western Hospital, Footscray, Victoria
	Intensive Care Unit, Sunshine Hospital, St Albans, Victoria
Professor Yahya Shehabi	Intensive Care Unit, Monash Medical Centre, Clayton, Victoria
	Intensive Care Unit, Dandenong Hospital, Dandenong, Victoria
Dr Paul Young	Intensive Care Unit, Wellington Hospital, New Zealand
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4. BACKGROUND

4.1 The burden of sepsis

Sepsis has been characterised as a dysregulated host response to infection. Adjunctive therapies targeting the inflammatory cascade are being increasingly explored, although to date, have failed to demonstrate consistent benefit, and sepsis continues to manifest poor outcomes. Hospital mortality in patients with septic shock remains as high as 22% in Australia and New Zealand [1]. From a global perspective, 31 million sepsis and 19 million severe sepsis cases are expected to be treated in hospitals all over the world per year. [2].

Moreover, the mortality from sepsis and septic shock in middle-income countries is reported to be as high as 60% [3-5]. In addition to short-term mortality, septic patients suffer from numerous short- and long-term complications and are at an increased risk of death for up to five years following the acute event [6, 7]. Given that 87% of the world's population reside in low- or middle-income countries, not only are effective and safe treatment strategies urgently needed, but these should also be low-cost.

To date, experimental data have reported that both high dose intravenous vitamin C and corticosteroids attenuate the acceleration of the inflammatory cascade and possibly reduce the endothelial injury characteristic of sepsis, enhance the release of endogenous catecholamines and improve vasopressor responsiveness [8-14].

In animal models, these effects have resulted in reduced organ injury and increased survival. However, their

effects in critically ill humans are unknown.

4.2 Corticosteroids in septic shock

Corticosteroids in general and, low dose corticosteroid specifically (50 mg IV four times/day) have been evaluated in several clinical trials, with meta-analysis of these trials demonstrating improved outcomes in some groups and no effect in others [15, 16]. The recently published ADRENAL trial [17] investigated the impact of 200mg of intravenous hydrocortisone a day compared to placebo, in ICU patients undergoing mechanical ventilation, with a strong clinical suspicion of infection, and requiring at least 4 hours of vasopressors in the context of fulfilling two or more criteria of the systemic inflammatory response syndrome. Despite finding no significant difference in the primary mortality outcome, administration of intravenous hydrocortisone resulted in a reduced duration of shock and vasopressor use, and a reduction in the intensive care length of stay. These findings provide justification for the use of intravenous hydrocortisone in patients with severe sepsis, as standard care.

4.3 Vitamin C in the critically ill patient

Several studies have investigated the use of intravenous vitamin C in critically ill patients. In a study of 595 surgical ICU patients (91% trauma patients) the combination of high dose intravenous vitamin C and vitamin E was associated with a significant reduction in the incidence of multiple system organ failure ($p=0.04$) [18]. In a pilot study of 24 patients with severe sepsis and septic shock, intravenous high dose vitamin C attenuated the inflammatory response [19]. In a randomised study of 28 surgical patients with septic shock, the mean dose of norepinephrine and duration of norepinephrine administration were significantly lower in the high dose vitamin C group compared to the placebo group [20]. Moreover, 28-day mortality was significantly lower in the vitamin C group (14% vs. 64%, $p = 0.009$). However, such a dramatic effect appears clinically implausible. In 37 patients with severe burns randomised to very high dose vitamin C (about 90g/day) or placebo, patients who received vitamin C required less fluid resuscitation with a trend towards the reduced length of stay and mortality [21]. None of these studies reported adverse effects, even with very high doses of vitamin C. Patients 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 3 grams per day [19,22].

4.4 The synergistic effect of hydrocortisone and vitamin C

The combination of hydrocortisone and vitamin C may act synergistically in patients with sepsis. This hypothesis is supported by an in-vitro study where hydrocortisone together with vitamin C protects the vascular endothelium from damage by endotoxin while neither agent alone has this effect [23]. Previous

research has demonstrated that vitamin C reverses oxidation of the glucocorticoid receptor, a likely manifestation of sepsis [24]. Oxidation of the glucocorticoid receptor decreases the activity of glucocorticoids [24]. Furthermore, glucocorticoids increase the expression of the sodium vitamin C transporter-2 (SVCT-2) which is an essential transport protein necessary for vitamin C to be transported intracellularly [25]. Based on these data we suggest that the combination of hydrocortisone and vitamin C may act synergistically to limit the pro-inflammatory response, limit the endothelial injury and improve microcirculatory function and vasopressor responsiveness in patients with sepsis and septic shock.

4.5 Vitamin C administration is low-risk

Vitamin C is an essential water-soluble vitamin. At very high concentrations (greater than 25 mM), Vitamin C appears to be toxic to normal human cells [26]. Several studies have administered vitamin C in doses exceeding 100g/day as adjuvant therapy in patients with cancer with no discernible side effects [26-33]. A dose of 6g/day will achieve a steady-state serum concentration of about 240µM [22, 24] which is about 100 times less than the above-mentioned toxic dose. The package insert for intravenous vitamin C lists no contraindications or adverse effects of the drug and states that as much as “6 grams has been administered without evidence of toxicity” [34]. The only reported restriction to the use of high dose intravenous vitamin C is in patients with known glucose-6-phosphate deficiency (G-6PD) in whom haemolysis has been reported [35, 36].

4.6 Thiamine

Thiamine has been recognised to have an essential role in cellular metabolism and the formation of ATP. Non-cofactor roles in neuronal signalling, homeostasis, cholinergic activity, chloride channels activation and immune response are receiving increasing attention, whilst the discovery of thiamine adenine nucleotides indicates that there may be more to thiamine function still to be revealed. Through its role in energy production, thiamine deficiency (TD) has been associated with heart failure, neurological disorders, metabolic acidosis and refeeding syndrome. Each of these disorders is prevalent in intensive care. In a recent randomised double-blind placebo-controlled trial, thiamine at 200 mg IV was assessed as adjunctive therapy in septic shock [37]. The investigators found that 35% of patients were thiamine deficient at baseline and that, in such patients, treatment with thiamine significantly reduced lactate levels and mortality. In a subsequent post-hoc assessment of the impact of thiamine on renal function [38], thiamine was found to decrease serum creatinine levels and the need for renal replacement therapy. These observations support the role of thiamine as adjunctive therapy in sepsis.

4.7 Vitamin C and thiamine

A small percentage of high dose vitamin C is catabolized to oxalate [39]. Oxalate is normally excreted by the kidney, and serum levels will increase if the patient had renal dysfunction. In patients with renal dysfunction receiving mega-dose vitamin C, supersaturation of serum with oxalate may result in tissue deposition as well as crystallisation in the kidney. Glyoxylate, a by-product of intermediary metabolism, is either reduced to oxalate or oxidised to CO₂ by the enzyme glyoxylate aminotransferase; thiamine pyrophosphate is a co-enzyme for metabolism of glyoxylate to specific amino acids and thus redirecting it from conversion to oxylate [40]. Thiamine deficiency is common in septic patients and is associated with an increased risk of death [37], thus, giving thiamine together with vitamin C is logical.

4.8 The combination of Vitamin C, corticosteroids and thiamine

Marik and colleagues performed a sequential period retrospective before-after clinical study, in which they 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 their ICU during the preceding 7 months [41]. Based on published clinical data, vitamin C pharmacokinetic modelling as well as the package insert, the authors administered 6 gm vitamin C per day divided into 4 equal doses of 1.5 grams each [18–20,22,34,42]. This dose has been repeatedly reported to be free of any complications or side effects. Hydrocortisone was dosed according to the consensus guidelines of the American College of Critical Care Medicine at 50 mg IV every 6 hours [43]. Thiamine was included in the Vitamin C protocol at a dose of 200mg 12 hourly intravenously.

The primary outcome of the study was hospital survival. A propensity score was calculated to adjust for the confounding factors. 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 score-adjusted odds ratio of mortality in the patients treated with the vitamin C protocol was 0.13 (95% CI 0.04-0.48, $p=0.002$). The SOFA score decreased in all patients in the treatment group, and no patient in the treatment group had 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 ($p < 0.001$).

The results of this retrospective study suggest that the early use of intravenous vitamin C, together with corticosteroids and thiamine may prevent progressive organ dysfunction and may reduce mortality in patients with severe sepsis and septic shock. We submit that these results justify the design of pragmatic pilot feasibility multicentre randomised controlled trial (mcRCT) to confirm the findings of this retrospective single centre study and possibly subsequently justify a pivotal phase 3 mcRCT. Furthermore, we believe that is

essential to perform such a trial as vitamin C, hydrocortisone and thiamine are cheap and widely available, and this therapeutic approach has the potential to be used in developing countries and to save the lives of millions of patients who die from sepsis globally each year.

5. OBJECTIVES

5.1 Aim

The primary aim of the study is to determine whether the intravenous administration of high dose Vitamin C (6g/d), Thiamine (400mg/d) and Hydrocortisone (200mg/d) in patients with septic shock leads to a more rapid resolution of septic shock and shortens the duration of vasopressor dependence compared to Hydrocortisone alone.

5.2 Hypothesis

Treatment with a combination of intravenous Vitamin C, Thiamine and Hydrocortisone reduces the duration of vasopressor (measured by hours alive and vasopressor free) use censored at 7 days compared to standard care with Hydrocortisone alone.

6. STUDY OUTCOME MEASURES

6.1 Primary Outcome

Time alive and free of vasopressors at day 7 (168 hours) after randomisation for the index (initial) septic shock.

- This is defined by the patient being alive at discontinuation of all vasopressors for at least 4 hours in the presence of a MAP>65mmHg or target MAP set by clinicians for the same 4 hour period as recorded in the ICU charts and censored at 7 days. If a patient dies of the index (initial) septic shock while on vasopressor therapy, this patient will be assigned zero alive and vasopressor-free days.

This approach will account for the competing effect of mortality on the duration of vasopressor therapy. We have chosen this outcome because it has been previously used in ICU studies as an appropriate outcome of pilot research in septic shock to indicate the resolution of septic vasoplegia [24]. In this study, it is also directly relevant to the known biology and physiology of the intervention applied in the pilot study.

Clarification of choice of primary outcome: This discontinuation refers to the initial admission (index) episode of septic shock that led to randomisation. Once such discontinuation has occurred, the patient is considered "vasopressor free". If two or three days later a further episode of infection (e.g. line-related sepsis) occurs and intravenous vasopressor therapy has to be restarted, such information will be collected as part of secondary outcome assessment but is not relevant to the primary outcome. This is because trial treatment is stopped by design once the initial episode is finished and is not restarted for further episodes, making an assessment of the intervention for further episodes impossible.

6.2 Secondary Outcomes

6.2.1 Feasibility outcomes:

2. Time from meeting eligibility criteria to the first dose of the main study drugs
3. Monthly recruitment rate
4. Number of patients screened
5. Randomised to the screened patient ratio
6. Reasons for exclusion
7. Compliance with drug administration protocol

6.2.2 Patient-centred outcomes:

1. Alive and ICU-free days calculated as the number of days alive and out of the ICU to day 28
2. ICU mortality
3. Hospital mortality
4. 28-day mortality
5. 90-day mortality
6. Delta SOFA score at day 3, defined as the pre-randomisation SOFA score minus the day 3 SOFA score
7. Hospital length of stay
8. Cumulative vasopressor free hours from shock resolution to day28 post randomisation
9. Cumulative invasive mechanical ventilation-free hours during the 28 day period post randomisation
10. Length of renal replacement therapy dependency during the 28 day period post randomisation

7. OVERALL STUDY DESIGN

7.1 Study design

This is a prospective, feasibility, pilot, multi-centre, randomised, open-label controlled, phase IIb trial.

7.2 Study Population

Patients admitted to any ICU of the participating hospitals with the primary diagnosis of septic shock will be screened for inclusion into this study.

7.3 Inclusion Criteria

Diagnosis of septic shock

- All the diagnostic criteria of septic shock (based on the SEPSIS-3 consensus criteria [44]) below has to be fulfilled simultaneously within the last 24 hours, and vasopressor is infused continuously at the enrolment.

7.3.1 Definition of Sepsis:

1. Suspected or documented infection
AND
2. an acute increase of ≥ 2 SOFA points consequent to the infection [45] (a proxy of organ dysfunction)

7.3.2 Definition of Septic shock:

- Sepsis AND
 - need for vasopressor therapy to keep MAP > 65 mmHg for > 2 hours AND
 - lactate > 2 mmol/L, despite adequate fluid resuscitation [44]

7.4 Exclusion Criteria

1. Age < 18 years
2. Pregnancy
3. DNR (do not resuscitate)/DNI (do not intubate) orders
4. Death is deemed to be imminent or inevitable during this admission, and either the attending physician, patient or substitute decision-maker is not committed to active treatment
5. Patients with known HIV infection
6. Patients with known glucose-6 phosphate dehydrogenase (G-6PD) deficiency [35]
7. Patients transferred from another ICU or hospital with a diagnosis of a septic shock for > 24 hours
8. Patients with a diagnosis of a septic shock for > 24 hours
9. Patients with known or suspected
 - a. history of oxalate nephropathy or hyperoxaluria
 - b. short bowel syndrome or severe fat-malabsorption
 - c. acute beri-beri disease
 - d. acute Wernicke's encephalopathy
 - e. malaria
 - f. scurvy
 - g. Addison's disease
 - h. Cushing's disease
10. Clinician expects to prescribe systemic glucocorticoids for an indication other than septic shock (not including nebulised or inhaled corticosteroid)
11. Patient is receiving treatment for systemic fungal infection or has documented Strongyloides

infection at the time of randomisation

12. Patient with known chronic iron overload due to iron storage and other diseases
13. Patient previously enrolled in this study
14. Clinician expects to prescribe high dose vitamin C for another indication

7.5 Co-enrolment in other ICU trials

In accordance with the Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group (CTG) policy, we will seek agreement from other trials with regard to inclusion or exclusion for co-enrolment of patients participating in this study.

8. STUDY PROCEDURES

8.1 Summary of study treatment

All eligible ICU patients will be enrolled as soon as possible after fulfilling the criteria for randomisation. Patients will be allocated in a 1:1 ratio to either the treatment group, receiving intravenous Vitamin C (1.5g every 6 hours), Thiamine (200mg every 12 hours) and Hydrocortisone (50mg every 6 hours), or to control group, receiving Hydrocortisone (50mg every 6 hours) alone.

8.2 Study Drugs

This study will be an open-label study.

Given its feasibility component focusing on recruitment, compliance with administration of all 3 agents, development of power estimates for future trials, further preliminary evidence of safety, and the complexity of having to blind 3 medications and having to create 3 different placebos, we consider that this is the preferred initial approach in the investigational program.

8.2.1 Ascorbic acid (Vitamin C)

Vitamin C is provided as a 5ml vial at a concentration of 100mg/ml. 1500mg vitamin C will be diluted in a 100 mL solution of either Normal Saline (0.9%) or 5% dextrose in water and infused over 1 hour. 50mL of solution can be used in fluid restricted patients. 1500mg of Vitamin C will be administered intravenously every 6 hours for the duration of study treatment. If a patient in the Vitamin C group misses a dose or only a partial dose is administered for whatever reason, a catch-up dose will NOT be required. Intravenous vitamin C is not used in standard ICU practice in Australia or New Zealand, and Vitamin C will not be administered to patients in the control arm of the study.

8.2.2 Thiamine (Vitamin B1)

Patients in the treatment group will receive intravenous thiamine 200mg every 12 hours for the duration of study treatment [37]. Thiamine is provided as an ampoule at a concentration of 100mg/ml. 200mg Thiamine will be added to 100ml solution of either Normal Saline (0.9%) or 5% dextrose in water and infused over 30-60 minutes. 50mL of solution can be used in fluid restricted patients.

Patients in the control arm of the study can receive thiamine if clinically indicated at the discretion of the attending ICU staff specialist. If a patient in the Vitamin C group misses a dose or only a partial dose is administered for whatever reason, a catch-up dose will NOT be required.

8.2.3 Hydrocortisone

In both groups and according to international guidelines [43], patients will be treated with hydrocortisone 50mg IV every 6 hours for the duration study treatment. Hydrocortisone is provided in a 1ml vial at a concentration of 100mg/ml. Half a vial (50mg Hydrocortisone) will be administered by slow intravenous injection over at least 30 seconds. If a patient misses a dose or only a partial dose is administered for whatever reason, a catch-up dose will NOT be required. Hydrocortisone can be stopped or tapered after the completion of study treatment as the treating clinicians prefer. We recommend a taper period of 3 days (50mg IV every 8 hours on the first day of tapering, 50mg IV every 12 hours on the second day of tapering, then 50mg IV once on the third (last) day of tapering).

8.3 Screening Log

The screening log is designed to monitor patient recruitment at the participating site. A screening log will be maintained at each participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility and deemed ineligible for the study. When a patient is considered ineligible, the reason(s) will be noted on the log. The log will be used to assess patient recruitment targets.

8.4 Randomisation

ICU patients will be enrolled as soon as possible after fulfilling the criteria for randomisation. A permuted block randomisation method with variable block sizes of 2, 4 and 6 and stratified by site will be used to allocate eligible patients to either the treatment group, receiving intravenous Vitamin C (1.5g every 6 hours), Thiamine (200mg every 12 hours) and Hydrocortisone (50mg every 6 hours), or to the control group, receiving Hydrocortisone (50mg every 6 hours) alone in a 1:1 ratio. Randomisation will be performed by the randomisation module in Research Electronic Data Capture (REDCap), which is a secure web application for managing online data collection. The random allocation sequence is generated using a computer software program by coordinating investigators (NL and TF) at the coordinating centre (ANZIC-RC) and embedded

into the REDCap system. Site investigators, site research coordinators, or statisticians in coordinating centre do not have access to the allocation sequence.

8.5 Blinding

The trial will be conducted as an open-label study. All site personnel will be aware of treatment allocation.

8.6 Duration of Study Treatment

Aligned with the recent ADRENAL septic shock study (NCT01448109), treatment will continue until one of the following criteria for treatment cessation is met:

- Initial (index) Septic shock resolves
 - The resolution of septic shock is defined as the patient being alive and discontinuation of all vasopressors for 4 consecutive hours in the presence of a MAP>65 mmHg or a target MAP set by the clinician in charge of the patient's care (see definition of primary outcome above).
- For Vitamins group - 10 days of Vitamin C and Thiamine treatment has been administered
- For Control group – 7 days of Hydrocortisone treatment has been administered
- Patient is discharged from ICU
- Contraindications to Vitamin C, Thiamine or Hydrocortisone therapy arise
- Death occurs
- Serious adverse events suspected to be related to a study medication develops
- Any permanent withholding criteria occurred (see 8.7)

Patients who develop septic shock after the study treatment was stopped according to the above criteria or those who are readmitted to the ICU during the same hospital stay will not receive any further doses of study drugs.

8.7 Permanent Withholding Criteria

Study medications will be permanently withheld for any of the following reasons:

1. Development of a contraindication to any of the study drugs
2. Consent has been withdrawn or consent to continue has not been granted

8.8 Concomitant Treatment

All patients enrolled in this study will be managed by a standardised approach which will include the

following elements:

- a) They are treated empirically with broad-spectrum antibiotics which are then deescalated according to microbiological data and clinical progress [46]
- b) They receive enteral nutrition whenever feasible
- c) They receive deep venous thrombosis (DVT) prophylaxis with low-molecular-weight heparin (LMWH) or heparin (unless contraindicated) and/or sequential compression devices

The ICU team will have full and independent control of patient management, which will not be affected by participation in the study.

Patients can receive (additional) vitamin C or thiamine via nasogastric tube or oral in both groups if clinicians feel it is clinically indicated.

9. ETHICS

9.1 Guiding Principles

This study is to be performed in accordance with the ethical principles of the Declaration of Helsinki (June 1964 and amended 1975, 1983, 1989, 1996, 2000, 2008, 2013 and Note of Clarification 2002 and 2004), ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration comments, NHMRC National Statement on Ethical Conduct in Research Involving Humans 2007 (Updated 2018); the New Zealand Interim Good Clinical Research Practice Guidelines (Volume 2 1998 and Volume 3 2000); Resolution No. 466 of December 12, 2012 from the National Health Council, Ministry of Health, Brazil; and ICH GCP Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95).

9.2 Ethical Considerations.

The major ethical issues associated with this study involve the recruitment of participants who are dependent on medical care and in need of immediate intervention for the management of life-threatening haemodynamic instability.

9.2.1 Consent process

Australia

The NHMRC National Statement on the Ethical Conduct of Research in Humans 2007 (Updated 2018) acknowledges in Chapter 4.4 that research involving patients who are heavily dependent on medical care, such as the patients in this study, is necessary to assess and improve the efficacy and safety of interventions used in their treatment.

In the following section, the term legal surrogate will be used to refer to the substitute decision maker or the medical treatment decision maker.

The process for obtaining consent will be according to the following hierarchy:

1. *Informed consent from participant or substitute/medical treatment decision maker:* Where possible, and as authorised by law, which varies between jurisdictions, consent will be obtained from the participant himself or the participant's legal surrogate if the patient lacks decision-making capacity.
2. *Consent to continue:* Where it is not possible or practicable for the patient or the legal surrogate to consider the study and give consent immediately, the patient may be enrolled with a waiver of consent (or medical research procedures in an emergency in Victoria) and consent obtained from the participant's legal surrogate as soon as possible, provided the procedure is in accord with the requirements of the site's Human Research Ethics Committee and applicable legislation. When appropriate, the participant's legal surrogate, and, in turn, the participant, will be informed of the study and will be able to withdraw consent for ongoing participation at any time.
3. *Verbal/Telephone consent:* In cases where the participant's legal surrogate cannot attend the hospital to sign the consent form within the time constraints of the study, consent for patient participation in the study may be obtained over the telephone in accordance with local HREC guidelines. The telephone conversation must be documented in the patient's medical record. As soon as the participant's legal surrogate is able to attend the hospital, they will be asked to sign a consent form and note that telephone consent was already provided.

The participant's legal surrogate will be able to withdraw their consent for the patient to participate in the study at any time without any reduction in quality of care, and if they choose to withdraw the patient, permission will be asked to use the data collected up to that time. Once subjects are recovered and are able to consider the information sheet, they will be offered the opportunity to withdraw from study follow-up.

If a participant dies due to the nature of their critical illness before consent was able to be obtained from the person responsible/medical treatment decision maker, then consent will not be sought and data collected will be used. This is in line with the process followed by other similar intensive care studies conducted previously and approved by the relevant HRECs all where consent to continue has been utilised, i.e. ARISE (Austin Health HREC H2008/03166), CHEST (Austin Health HREC H2009/03734) STARRT-AKI (Austin Health HREC/15/Austin/207). However, for Australian sites under the jurisdiction of the Austin Hospital Human Research Ethics Committee (HREC) there is an obligation by the research team to reach out to the patient's family and/or medical substitute-decision maker of such patients, in a respectful and compassionate way, at the earliest opportunity, to at least inform them that the patient has been recruited into a clinical

trial. The Austin HREC considers it somewhat paternalistic not to disclose this information, respectfully and sensitively, and as soon as reasonably possible, as outlined in The National Statement on Ethical Conduct. The HREC understands that in some cases, this will be difficult, and at times not possible or even unreasonable, due to extreme distress and anxiety displayed by the patient's family.

In order to satisfy the committee that a waiver of consent should be approved, the committee will seek written documentation from research teams in patient's medical records that attempts have been adequately attempted to at least inform the patient's family and/or medical substitute-decision maker that the patient has been recruited into a clinical trial. The committee is also of the view that for those patients who have died, in many situations, is not disrespectful to attempt to reach out to the family, again in a very compassionate and respectful manner, to seek consent or, at least inform (with documentation in the patient notes). The committee understands that in these situations this is more difficult and challenging, but again, but thinks there is a clinical, moral and ethical obligation, where appropriate, to seek such consent. When difficult, challenging or inappropriate, or not possible, this again should be clearly documented in the medical records.

If the patient chooses to withdraw from the study, they will be asked for permission to use their data up to the time of withdrawal.

All interaction between research staff and potential or actual participants and their legal surrogate will take into consideration the stress or emotional factors associated with critical illness and ensure that the dependency of potential participants and their relatives on medical personnel providing treatment does not compromise the freedom of decision making to participate (as per the NHMRC National Statement on the Ethical Conduct of Research in Humans 2007 (Updated 2018) 4.4.11).

New Zealand

In New Zealand, the approach used will be consistent with section 7.4 of the Health and Disability Code which outlines the framework for providing treatment to patients who are unable to consent for themselves.

The specific approach will be:

1. To consider whether study treatment and study participation is in the best interest of each patient and,
2. As soon as it is practical and reasonable to seek the advice of persons interested in the patient's welfare to establish that study participation is consistent with the patient's wishes.

All participants who recover sufficiently will be given the opportunity to provide informed consent for ongoing study participation and the use of data collected for the study.

Brazil

In Brazil, the approach used will be consistent with local, national legislation.

9.2.2 Confidentiality of patient data

After randomisation, patients will be allocated a unique study number. The site research coordinator will compile an enrolment log including the patient's name, date of birth, hospital identification number, unique study number and date and time of randomisation.

Subsequent data will be identified by the unique study number only. The enrolment log and study data will be kept separately. Study data will be entered into a password protected REDCap database managed by Monash University and ANZIC-RC. No identifying data will be entered into the database.

Similarly, hard copies of enrolment log and study data collected on paper CRF's will be stored separately in the locked office of the principal investigator or research coordinator. Only the research team will have access to this information, and they will not disclose this information to any other person or entity.

When archiving, the study site investigators will take all appropriate measures to safeguard and prevent access to this data by any unauthorised third party.

The investigator will maintain the confidentiality of all study documentation and take measures to prevent the accidental or premature destruction of these documents. The investigator will retain the study documents at least 15 years after the completion or discontinuation of the study. The investigator must notify the study management committee prior to destroying any essential study documents following the study completion or discontinuation.

If any investigator retires, relocates, or otherwise withdraws from conducting a study, the responsibility for maintaining records may be transferred to the coordinating centre or another investigator. The coordinating centre must be notified of and agree to the change. All associated documentation must also be updated.

9.3 Ethics Committee Approval

Each participating site will submit this protocol, and any other relevant study documentation to the responsible local or national constituted Human Research and Ethics Committee (or equivalent). Approval of the protocol, plans for obtaining consent, and related documents will be obtained prior to the start of the study at each site. It is the principal investigator's responsibility to ensure that all conditions for approval of the study are met and that amendments to the protocol or serious adverse events are also reported to the HREC (or equivalent) as required by that committee.

10. DATA MANAGEMENT

10.1 Data collection methods

All data will be collected by trained staff at each study site using a case report form (CRF) worksheet developed by the coordinating centre. Data will then be entered into the REDCap web database (electronic case report form [eCRF]). Randomised patients will be followed up to death or 90 days post-randomisation whichever occurs first.

Study day 1 commences on randomisation and concludes at the expiry of the calendar day.

Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including: baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events.

10.2 Data variables collected

Data collection will include:

10.2.1 Baseline Data

- Demographics
 - Sex
 - Age
 - Weight
 - Source of admission (ICU and hospital)
 - ICU admission category (elective, emergency or non-surgical)
 - ICU admission diagnosis (as defined by the APACHE III severity of illness scoring system)

- Co-morbidities
 - Diabetes mellitus
 - Arterial hypertension
 - Congestive heart failure
 - Chronic obstructive airways disease
 - Chronic liver disease
 - Malignancy (metastatic cancer, lymphoma/leukaemia as per APACHE III definitions)
 - Chronic renal failure (known pre-ICU estimated GFR < 30 ml/min)

- Admitting diagnosis and suspected or identified site/source of infection
- Requirement for mechanical ventilation (Y/N).
- Use of intravenous vasopressor infusion (Y/N).
- APACHE III severity of illness score
- SOFA score
- The most recent creatinine measured within 3 months and more than 7 days prior to the ICU admission.

10.2.2 ICU data (Daily data - collected during the first 7 days in ICU)

- Daily serum creatinine
- Daily white cell count (WBC)
- Daily platelet count
- Daily total bilirubin
- Daily PaO₂/FiO₂ ratio
- Highest lactate level
- SOFA (Sepsis-related Organ Failure Assessment) score
- Requirement for mechanical ventilation (Y/N)
- Use of vasopressor agents (Y/N)
- Daily cumulative number of vasopressor free hours
- The six-hourly dosage of intravenous vasopressor infusion will be recorded
- Fluid input each day
- Urine output each day
- Fluid balance each day

After day 10, data will be collected daily including the use of the morning dosage of vasopressors until day 15, death or ICU discharge.

10.2.3 Study drug

- Administration of study drug
- Treatment days, amount of drug received
- Reason for missed study drug dose

10.2.4 Hospital data

- ICU admission and discharge dates
- Hospital admission and discharge dates
- Hospital discharge destination

10.2.5 Consent data

- Date and time of consent
- Date of consent withdrawal

10.2.6 Vital status at

- ICU discharge
- Hospital discharge
- 28 days post-randomisation
- 90 days post-randomisation

10.2.7 Outcome data

- Time alive and free of vasopressors at day 7 (168 hours) after randomisation
- Length of ICU and hospital stay
- Duration of intravenous vasopressor infusion (hours)
- Duration of mechanical ventilation (hours)
- Duration of CRRT (hours)
- Last day of IRRT (date and time)
- Presence of acute kidney injury (AKI). (AKI will be defined using the KDIGO criteria; namely, an increase of the Serum-Creatinine > 26.4 µmol/l (0.3 mg/dl) or a level > 1.5 times the baseline value within 48 hours [31]. If the baseline Serum- Creatinine is not known a value > 132 µmol/l (1.5 mg/dl) will be regarded as diagnostic of AKI).

10.2.8 Feasibility data

- Time from meeting the eligibility to the first dose of the main study drugs
- Monthly recruitment rate
- Number of patients screened
- Randomised to the screened patient ratio
- Reasons for exclusion
- Compliance with drug administration protocol
- Protocol deviations

10.2.9 Safety data

- Adverse events up to 90 days post randomisation
- Adverse reactions up to 90 days post randomisation
- Serious adverse events up to 90 days post randomisation
- Suspected unexpected serious adverse events up to 90 days post randomisation

10.3 Data management

Data management will be coordinated by the coordinating researcher, at the ANZIC-RC including programming (online study database design) and data management support (including data monitoring, database questions, technical issues, data queries, query resolution).

10.4 Data quality and monitoring

Several procedures to ensure data quality and protocol standardisation will help to minimise bias and to optimise data quality. These include:

- A site initiation teleconference will be conducted before site activation to ensure consistency in procedures
- A detailed data dictionary will define the data to be collected and entered into the eCRF
- The coordinating centre will perform timely validation of data entered into the eCRF, queries and corrections

The study will be monitored by quality control reviews of protocol compliance, data queries, safety reporting and protocol deviations. On-site monitoring will only be performed on a case by case basis if quality control issues are flagged by electronic review of data

Medical records, any other relevant source documents and the site investigator files must be made available to the monitoring representative for these monitoring visits during the study and at the completion of the study as needed.

10.5 Protocol Deviations

A protocol deviation is an unanticipated or unintentional departure from the expected conduct of an approved study that is not consistent with the current research protocol or consent document. A protocol deviation may be an omission, addition or change in any procedure described in the protocol.

Given that the investigator is responsible for patient safety and care he/she may implement a deviation from, or a change of the protocol to eliminate an immediate hazard to trial patients without prior HREC approval. The implemented deviation or change must be reported in a protocol deviation form. The deviation must be reported via the study website by the principal investigator. A six monthly summary of protocol deviations will be reported to the coordinating HREC; a serious breach will be reported ASAP.

11. STATISTICAL CONSIDERATIONS

11.1 Power calculation and sample size

11.1.1 The original sample size calculation

As described, Marik and colleagues [41] reported a mean (SD) duration of vasopressor dependency of 54.9 (28.4) hours in their control group and 18.3 (9.8) hours in those receiving vitamin C.

In this study, we are comparing the time alive and free of vasopressors on day 7 (168 hours after randomisation). This approach will account for the competing effect of mortality on the duration of vasopressor therapy. When choosing a conservative SD of 42 hours and an effect size about half that seen in the above-mentioned study, a sample size of 120 patients (60 per group) was required to identify a clinically relevant increase in hours alive and vasopressor free at 7 days of 22% (25 hours) (i.e. increase from 113 to 138 hours alive and vasopressor free at day 7) with a power of 90% at an alpha level of 0.05.

To account for an estimated drop-out rate of approximately 5%, 126 patients would have been included (63 patients per group).

11.1.2 Sample size re-calculation with the first half study population

We have recalculated the sample size to have 90% power (2 sided p-value of 0.05) to detect a 25-hour difference in hours alive and vasopressor free at 7 days based on the pooled standard deviation of hours alive and vasopressor free of the first 60 participants (50%) included in the interim analysis. One participant was excluded from the analysis as consent was not obtained at the moment of the calculation. The pooled standard deviation of hours alive and vasopressor free at 7 days for the 59 patients is 51.6 hours which is higher than the preliminary estimation of 42.

To account for non-normality of the primary outcome (alive & vasopressor free) and dropouts, we have added 20% (15% for non-normality and 5% for dropout) to the sample size.

To have a 90% power (2 sided p-value of 0.05) to detect a 25-hour difference based on a standard deviation of 51.6, the trial will require 180 patients (90 per group). Allowing for a 20% inflation for non-normality and dropout/withdrawal, the required total sample size is 216 (108 per group). The robustness of our sample size estimate will be further assessed after recruitment of 108 patients (50% of the sample size).

11.2 Analysis of Results

A senior statistician at Monash University Department of Epidemiology and Preventive Medicine will perform data analysis on an intention-to-treat basis. Summary statistics will be used to describe the clinical data and presented as mean \pm SD, median with interquartile range (IQR) or percentages as appropriate. Chi-squared analysis with Fisher's exact test (when appropriate), and Student's t-test (Mann Whitney U test for non-normal distributions) will be used to compare data between the active treatment group and the control group with statistical significance declared for probability values of 0.05 or less. Analysis of the outcome of excluded patients due to other trials etc. will be in accordance with the CONSORT guidelines.

12. SAFETY MONITORING AND REPORTING

12.1 Data Monitoring Committee

An independent Data Monitoring Committee (DMC), consisting of experts in intensive care, clinical research and biostatistics will be established before patient enrolment and will review all trial protocols. The role of the DMC will be to provide study oversight to ensure that the rights and safety of patients involved in the study are protected by reviewing reported Adverse Events and making recommendations to the Management Committee (MC). Intensive care patients experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. Intensive care patients will frequently develop life-threatening organ failure(s) unrelated to study interventions and despite optimal management. Therefore, consistent with established practice in academic ICU trials, events that are part of the natural history of the primary disease process or expected complications of critical illness will not be reported as serious adverse events in this study. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported. There are no planned interim analyses for this feasibility trial.

12.2 Adverse event (AE) / Adverse Reaction (AR)

An adverse event (AE) is any untoward medical occurrence in a clinical trial participant administered a medicinal product and does not necessarily have a causal relationship with this treatment. It is recognised that the patient population with critical illness will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying illness and the impact of standard therapies. These will not necessarily constitute an adverse event unless they are considered to be of concern or related to the study or the intervention in the investigator's clinical judgement. In all cases, the condition or disease underlying the symptom, sign or laboratory value should be reported, e.g. renal failure rather than hyperkalaemia, and agitation rather than self-extubation.

An adverse reaction is defined as any untoward and unintended response to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions.

12.2.1 Product information

All of the investigational drugs for this trial are approved drugs in Australia. The Australian Product Information will be used for the scientific information relevant to the safe and effective use of the study drugs across Australian sites. The information contained in the approved Australian Product

Information contains the information used by the investigators to determine what adverse reactions are to be considered expected adverse reactions and on the frequency and nature of those adverse reactions.

12.3 Serious adverse events (SAE) / Serious adverse reactions (SAR)

A serious adverse event (SAE) or serious adverse reaction is defined as any adverse event/reaction that:

- Results in death
- Is life-threatening
- Requires hospitalisation or prolongation of current hospitalisation
- Results in persistent or significant disability or incapacity

Death is an expected outcome among patients with septic shock, therefore, death will not be considered a serious adverse event. Standard care of patients with septic shock includes a host of complications that fit the definition of an SAE. Medical and scientific judgement will be exercised by the site principal investigator in deciding whether an adverse event/ reaction will be classified as serious in other situations to avoid over-reporting.

Specific serious adverse reaction:

- Suspected or confirmed anaphylactic or anaphylactoid reaction towards one of the study drugs

12.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Suspected unexpected serious adverse reaction (SUSAR) is defined as being an adverse reaction that is both serious and unexpected.

12.5 Reporting AEs, SAEs and SUSARs

Adverse events/reactions and serious adverse events/reactions will be recorded on a separate case report form. SAEs should be reported to the coordinating centre within 24 hours of study staff becoming aware of the event.

Minimum information on the report form will include:

- Patient initials and study number
- Nature of the event
- Commencement and cessation of the event
- An investigator's opinion of the relationship between study involvement and the event (unrelated, possibly, probably or definitively related).
- Whether treatment was required for the event and what treatment was administered.

The coordinating centre staff will be responsible for following-up all events to ensure all details are available. The coordinating centre is also responsible for reporting directly to HRECs and investigators, who forward any relevant information to their institution. It is the responsibility of each principal investigator to inform the local or lead HREC of all SUSAR events which occur at their hospital, in accordance with local requirements. Copies of any reporting and correspondence to and from the local HREC or RGO should also be sent to the coordinating centre. All SUSARs occurring in Australian participants will be reported to the Therapeutic Goods Administration:

- for fatal or life threatening Australian SUSARs, immediately, but no later than 7 calendar days after being made aware of the case, with any follow-up information within a further 8 calendar days
- for all other Australian SUSARs, no later than 15 calendar days after being made aware of the case.

The sponsors will provide the principal HREC with an annual safety report. Instead of submitting individual reports of AEs, SAEs and SUSARs, the outcomes of the coordinating centre's analyses of accumulating safety data will be provided to the HREC and the participating institutions.

12.6 Contact phone numbers for SAE advice

Chief Investigator (Prof Rinaldo Bellomo):	+61 3 9496 5992
ANZIC-RC (Dr Tomoko Fujii):	+61 3 9905 6642

12.7 Post-trial care

If post-trial care for compensation to those who suffer harm from trial participation will be covered by Monash University Insurance.

<https://www.intranet.monash/finance/our-services/insurance/certificateofcurrency>

13. FUNDING

This study is funded by the Intensive Care Foundation (Smiths Medical Research Project Grant 2018) and the PROADI (Institutional Development Support Program), a program of the Ministry of Health in Brazil. None of the funding organisations will contribute to the study design; collection, management, analysis and interpretation of data; writing of the report or the decision to submit the report for publication.

14. PUBLICATION POLICY

The study will be conducted in the name of the management committee. The principal publication

from the study will be in the name of the management committee with full credit assigned to all collaborating investigators, research coordinators and institutions. Where an individuals' name is required for publication it will be that of the writing committee, with the chair of the writing committee listed first and subsequent authors listed alphabetically. Funding bodies will be acknowledged in the publication.

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16. APPENDIX 1 - SCORES

Appendix 1.1 SOFA - Sequential Organ Failure Assessment

ORGAN SYSTEM	0	1	2	3	4	Missing	Organ Scores
Respiration PaO ₂ /FIO ₂ (in mmHg)	>400	301-400	<301	≤200 With Respiratory Support*	≤100 With Respiratory Support*	Variable not measured	
Coagulation Platelets(x10 ³ /mm ³)	>150	101-150	51-100	21-50	≤20	Variable not measured	
Liver Bilirubin (mg/dL) (μmol/L)	<1.2 <20	1.2 - 1.9 20 - 32	2.0 - 5.9 33 - 101	6.0 - 11.9 102 - 204	>12.0 >204	Variable not measured	
Cardiovascular Hypotension	MAP ≥70 mm Hg without vasopressors	MAP <70 mm Hg without vasopressors	Dopamine ≤5 or Dobutamine (any dose) or any dose milrinone or any dose levosimendan	Dopamine >5 or adr ≤0.1 or noradr ≤0.1 or any dose vasopressin or any dose Metaraminol or any dose phenylephrine	Dopamine >15 or adr >0.1 or noradr >0.1	Variable not measured	
All drug doses are μg/kg/min							
Central Nervous System Glasgow Coma Score (unsedated)	15	13 - 14	10 - 12	6 - 9	<6	Variable not measured	
Renal Creatinine (mg/dL) (μmol/L) or urine output	<1.2 <110	1.2 - 1.9 110 - 170	2.0 - 3.4 171 - 299	3.5 - 4.9 300 - 440 or <500 mL/day	>5.0 >440 or <200 mL/day	Variable not measured	

adr, adrenaline = epinephrine; noradr, noradrenaline = norepinephrine.

*Respiratory support is defined as any form of invasive or non-invasive ventilation including mask CPAP or CPAP delivered through a tracheostomy/tracheotomy or endotracheal or nasotracheal tube **PLEASE NOTE:** The highest score for someone **without** respiratory support is **2**.

^Collect the GCS recorded prior to administration of sedative agents and prior to intubation. Go back as far as necessary to the time at which the patient was first sedated and identify the

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GCS at the time of/just prior to sedation. If you cannot locate the GCS at the time of/just prior to sedation select "Variable not measured".

PLEASE NOTE: Except for GCS, score each system on data available within the previous 24 hours (from randomisation), document the value closest to randomisation

Appendix 1.2 APACHE III CALCULATION WORKSHEET

1. Physiological Score (1a)

Physiological Variable											
Temperature (°C)	<input type="checkbox"/>	≥40 = 4	36-39.9 = 0	35-35.9 = 2	34-34.9 = 8	33.5-33.9 = 13	33-33.4 = 16	≤32.9 = 20			
Mean Arterial Pressure	<input type="checkbox"/>	≥140 = 10	130-139 = 9	120-129 = 7	100-119 = 4	80-99 = 0	70-79 = 6	60-69 = 7	40-59 = 15	≤39 = 23	
Heart Rate	<input type="checkbox"/>	≥155 = 17	10-155 = 13	120-139 = 7	110-119 = 5	100-109 = 1	50-99 = 0	40-49 = 5	≤39 = 8		
Oxygenation*	<input type="checkbox"/>	If intubated and FiO ₂ ≥ 0.5 – use A-a gradient					FiO ₂ < 0.5 – use PaO ₂ (units in kPa in brackets)				
		≥500 = 14	350-499 = 11	250-349 = 9	100-249 = 7	<100 = 0	≥80 (10.7) = 0	70-79 (9.3-10.6) = 2	50-69 (6.7-9.2) = 5	≤49 (6.5) = 15	
Respiratory Rate# (breaths/min)	<input type="checkbox"/>	≤5 = 17	6-11* = 8	12-13 = 7	14-24 = 0	25-34 = 6	35-39 = 9	40-49 = 11	≥50 = 18		
Serum Creatinine (µmol/L)**	<input type="checkbox"/>	Without acute renal failure				With acute renal failure					
		≥172 = 7	133-171 = 4	44-132 = 0	<44 = 3	≥133 = 10	0-132 = 0				
Serum Sodium (mmol/L)	<input type="checkbox"/>	≥155 = 4	135-154 = 0	120-134 = 2	≤119 = 3						
Urine Output (ml)	<input type="checkbox"/>	≥4000 = 1	2000-3999 = 0	1500-1999 = 4	900-1499 = 5	600-899 = 7	400-599 = 8	≤399 = 15			
Albumin (g/L)	<input type="checkbox"/>	≥45 = 4	25-44 = 0	20-24 = 6	≤19 = 11						
Bilirubin (µmol/L)	<input type="checkbox"/>	≥136 = 16	86-135 = 8	52-85 = 6	35-51 = 5	<35 = 0					
Glucose (mmol/L)	<input type="checkbox"/>	≥19.4 = 5	11.2-19.3 = 3	3.4-11.1 = 0	2.2-3.3 = 9	<2.2 = 8					
Haematocrit (% ÷ 100)	<input type="checkbox"/>	≥50 = 3	41-49 = 0	<41 = 3							
White Blood Count (10 ⁹ /L)	<input type="checkbox"/>	≥25 = 5	20-24.9 = 1	3-19.9 = 0	1-2.9 = 5	<1 = 19					
Blood Urea Nitrogen (mmol/L)	<input type="checkbox"/>	≥28.6 = 12	14.4-28.5 = 11	7.2-14.3 = 7	6.2-7.1 = 2	<6.2 = 0					
pH		Scoring for pH relates to paco ₂ and is listed in the data dictionary (Table 1c)									
									TOTAL	<input type="checkbox"/>	

* The formula used for the calculation of the A-a gradient (needs units in mmHg):
A-a gradient = 713 x FiO₂ - PaO₂ - PaCO₂;
to convert kPa to mmHg multiply by 7.5

For patients on mechanical ventilation, no points are given for respiratory rates 6-12 breaths/min

** Acute renal failure is defined by:
Increase of creatinine by ≥132 µmol/L/day and/or urine output of <410 ml/day; and patient does not have chronic dialysis

2. Motor (Verbal) - Acute Physiology Scoring for Neurological Abnormalities → Eyes Open/Do Not Open (1b)

Motor / Verbal GCS	□	VERBAL GCS							
		Oriented, Converses (5)		Confused Conversation (4)		Inappropriate Words or Incomprehensible Sounds (2-3)		No Response (1)	
		Open	Closed	Open	Closed	Open	Closed	Open	Closed
Obeys Verbal Commands (6)		0	NA	3	NA	10	NA	15	16
Localizes Pain (5)		3	NA	8	NA	13	NA	15	16
Flexion withdrawal (3-4)		3	NA	13	NA	24	24	24	33
Extension withdrawal/No Response (1-2)		3	NA	13	NA	29	29	29	48

If the patient opens eyes for the GCS evaluation (GCS score for eye opening is 2-5 = opens eyes spontaneously or to voice or to painful stimulus)
 If the patient does not open eyes for the GCS evaluation (GCS score for eye opening is 1)

3. Acute Physiology Scoring for Acid-Base Disturbances (1c)

Acute Physiology (pH)	□	PaCO ₂ (kPa)	<25 (<3.3)	25 - <30 (3.3 - <4)	30 - <35 (4 - <4.7)	35 - <40 (4.7 - <5.3)	40 - <45 (5.3 - <6)	45 - <50 (6 - <6.7)	50 - <55 (6.7 - <7.3)	55 - <60 (7.3 - <8)	≥60 (≥8)	
pH < 7.15			12								4	
7.15 to < 7.20			12								4	
7.20 to < 7.25			12								4	
7.25 to < 7.30			9	6	3				2			
7.30 to < 7.35			12								4	
7.35 to < 7.40			12								4	
7.40 to < 7.45			5	0				1				
7.45 to < 7.50			0				2	12				
7.50 to < 7.55			12								4	
7.55 to < 7.60			3				12	12				
7.60 to < 7.65			3				12	12				
pH ≥ 7.65			0	3				12				

Table 1a) Total		a
Table 1b) Score	+	b
Table 1c) Score	+	c
Table 1d) Total	+	d

APACHE III Score	=	e-2999
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4. Age / Comorbidities (1d)

Age	□	≤ 44	45 - 59	60 - 64	65 - 69	70 - 74	75 - 84	≥ 85
		= 0	= 5	= 11	= 13	= 16	= 17	= 24
Comorbidities	□	AIDS = 23	Hepatic Failure = 16	Lymphoma = 13	Metastatic Cancer = 11	Leukaemia/Multiple Myeloma = 10	Immune Compromised = 10	Cirrhosis = 4

17. APPENDIX 2 - Pharmacokinetic sub-study of ascorbic acid in patients with septic shock treated in the VITAMINS protocol

Title: A sub-study to describe the pharmacokinetics of ascorbic acid in Intensive Care Unit (ICU) patients enrolled in the VITAMINS trial.

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17.3 Background

Critically ill patients often exhibit a high degree of vitamin C deficiency at ICU admission and plasma concentrations decrease even more during the first days (1, 2). In patients with septic shock increased utilisation of vitamin C and other factors (3) can lead to severe vitamin c deficiency (4) despite receiving standard nutrition in ICU (5, 6). Furthermore, subnormal plasma ascorbic acid concentrations correlate inversely with the incidence of multiple organ failure and directly with survival in septic patients (7).

Dosing data in humans suggest that high intravenous doses of vitamin c substitution are required to increase plasma concentrations to normal and supra-normal ranges (4, 6, 8, 9) and oral dosing seems not to be enough to reverse vitamin C deficiency (3).

However, the optimal dosing regime in patients with septic shock is still unclear and there is a paucity of pharmacokinetic data in this patient population.

Most pharmacokinetics studies of vitamin C have been performed in cancer patients (10-14), however only few have quantified volume of distribution, elimination half-life and clearance rate (10, 13, 14). In addition, these studies involved broadly ranging dose regimens and intervals that substantially vary from the vitamin c regimens used in septic shock or ICU patients, respectively. Furthermore, patients with advanced cancer may have normal initial vitamin C levels (11) whilst patients with septic shock tend to have significant vitamin C deficiency (5, 15), suggesting that these populations may not be comparable.

Similar to these cancer studies, pharmacokinetic studies in the septic population have used a range of different vitamin C dosages (15). The authors of the only study using the same dosing regimen to the VITAMINS trial (16) (6g/day intravenous vitamin C given 6 hourly) measured only baseline vitamin C concentrations (4). Whilst it has been important as a foundation for further study, baseline concentrations are insufficient to qualify as an in-depth pharmacokinetic study.

We believe that the variability in the protocol designs from previous pharmacokinetic studies together with the incompatible patient population investigated justifies the need for a detailed pharmacokinetic profile of vitamin C administered in the VITAMINS trial to patients with septic shock. This sub-study will help to describe the pharmacokinetics of repetitive vitamin c bolus therapy in septic patients in order to inform further studies in this field.

17.4 Aim

This sub-study aims to quantify and describe the pharmacokinetics of ascorbic acid during a dosing regimen of vitamin C (6g/d), hydrocortisone (200mg/d) and thiamine (400mg/d) in patients with septic shock.

An important parameter for dose selection with intravenous ascorbic acid is the peak plasma concentration achieved. We will test the hypothesis that this concentration can be predicted from the dose alone with the following assumptions:

- (i) ascorbic acid does not bind to plasma proteins (17);
- (ii) a large iv dose rapidly and evenly distributes throughout the extracellular volume, which comprises 20% of normal body weight (18) and
- (iii) exit from the extracellular space is mostly via urinary excretion.

17.5 Primary Outcomes

- Pharmacokinetic modelling:
 - Maximum plasma ascorbic acid level
 - Area under the curve (AUC)
 - Elimination half-life ($t_{1/2}$)
 - Volume of distribution
 - Ascorbic acid clearance (CL_{AA})

17.6 Study design and Population

Study design

Prospective, nested cohort study of a multi-centre randomised controlled trial.

Study setting

This pharmacokinetic study will be conducted at the VITAMINS trial study sites.

Participants

Patients admitted to the Intensive Care Unit with septic shock, enrolled in the VITAMINS trial treatment arm with an established arterial or central-venous line will be eligible for enrolment in this sub-study.

17.7 Sub-study procedures

The study procedures are the same as for the VITAMINS trial:

In the VITAMINS treatment group patients are receiving:

- 1500mg Ascorbic acid iv 6-hourly
- 200mg Thiamine iv 12-hourly
- 50mg Hydrocortisone iv 6-hourly

Treatment will continue until:

- Septic shock resolves (as defined in the parent trial)
- 10 days of treatment has been administered
- The patient is discharged from ICU
- Contraindications to Vitamin C, Thiamine or Hydrocortisone therapy arise
- Death occurs
- Serious adverse events suspected to be secondary to any of the intervention medications develop

Patients enrolled in this sub-study will have the following samples collected:

- Plasma at T=0 (pre-dose), 1 hour (at completion of infusion), 4-hours and 6-hours post infusion, over a single dosing interval during the study intervention
- 6-hour urine collection (T=0 (pre-dose) to 6-hours post infusion) during the same dosing interval as the plasma samples

Other than the additional blood and urine samples, patients enrolled in the pharmacokinetic study will continue to receive the treatment and standard of care as per the VITAMINS trial protocol.

17.8 Bioanalysis and Data collection

Plasma ascorbic acid levels

The blood and urine samples will be processed according to instructions suggested in previous studies (5,

19):

1. Prior to sampling, the arterial or central line will be cleared and the initial 1mL blood discarded. At each time point, blood samples taken from the arterial or central venous line will be collected into a lithium heparin vacutainer tube (6mL per tube) (20) and immediately placed on crushed ice.
2. Given vitamin C's instability and susceptibility to oxidation and breakdown, samples will remain at a temperature of less than 4°C throughout processing.
3. The blood will be centrifuged at 4000rpm for 10 mins at 4°C. 350µl of the separated plasma will then be aliquoted into a 1.7ml Eppendorf tube. 350µl fridge-cold 0.54M perchloric acid/diethylene triamine-pentaacetic acid (PCA/DTPA) solution will be added to precipitate protein and chelate metal ions. The Eppendorf tube will then be vortexed and put on ice for 2 mins, before being centrifuged at 12,000rpm for 2 mins at 4°C.
4. 150µl fridge-cold PCA supernatant solution will then be aliquoted into each of three 1.7ml Eppendorf tubes. The first tube is intended for vitamin C analysis, the second for reduction with tris(2-chloroethyl)phosphate (TCEP) if the sample is haemolysed, and the third tube as a spare.

Urine ascorbic acid excretion

1. 50mls of urine collected over 6 hours (T0-6) will be put into a urine specimen jar and K₂-EDTA added to a concentration of 100µmol/L.
2. Samples will then be diluted two-fold with fridge-cold 0.54M PCA/DTPA and subsequently centrifuged.

All the processed blood and urine samples and spare plasma will be kept in a -80°C freezer until further analysis by high performance liquid chromatography (HPLC). The tubes from each patient will be labelled with the patient's ID (unique patient study number), sample type (urine or plasma), sample number, time and date of sample collection.

6-hour Creatinine clearance

A 6-hour CL_{CR} will be the primary method of measuring renal function and calculate renal vitamin C clearance. Urine will be collected via the IDC from start of the study dose (T=0) and until 6 hours post-dose (T=6), following which urinary volume and creatinine concentration will be determined by laboratory analysis. Concurrent plasma creatinine concentrations will be obtained, following which CL_{CR} will be calculated using the standard formula. Creatinine measurement in plasma and urine will be using automated analysers

employing a modified Jaffe (alkaline picrate) technique, representing an isotope dilution mass spectrometry traceable assay. As per convention, CL_{CR} values will be subsequently normalized to a body surface area (BSA) of 1.73 m².

Data collection

In addition to the VITAMINS protocol the following parameters will be collected:

- Demographics:
 - Height (measured), BMI, body surface area (BSA)
- Nutrition:
 - Additional daily Vitamin C intake in form of enteral and parenteral nutritional (mg/day)
- Blood sampling
 - Date and time of blood sampling
 - Plasma ascorbic acid level (µmol/l)
- Urine sampling
 - Date and time of urine sampling
 - Urine ascorbic acid and creatinine concentration
 - 6h Creatinine Clearance

Data Analysis

Mean and 95% confidence interval will be used to describe the conventional pharmacokinetic parameters (C_{max} , AUC and $t_{1/2}$, renal clearance).

Summary statistics use to describe the clinical data will be presented as mean \pm SD, median with interquartile range (IQR) or percentages as appropriate.

17.9 Ethical considerations

Please refer to the VITAMINS study main protocol (ANZIC-RC/NL001) for full details of the ethical considerations of the parent trial.

The NHMRC National Statement on the Ethical Conduct of Research in Humans 2007 (Update 2018) acknowledges in Chapter 4.4 that in research studies involving patients who are heavily dependent on medical care, such as the patients in this study, it is necessary to assess the efficacy and safety of

interventions used in their treatment. The collection of ascorbic acid plasma and urine levels does not pose any risk to the patient, apart from the risk associated with a regular blood sample collection. However, to decrease the burden of repetitive blood sampling via venepuncture we are only including patients with an established arterial or central-venous line allowing for pain-free blood sampling in this sub-study. Consent to participate in this sub-study will be sought in addition to the main study consent process.

Confidentiality of data

Information on confidentiality of data for the parent trial can be seen in the VITAMINS study main protocol (ANZIC-RC/NL001). Additional data for this sub-study will be collected similar to the data collected in the main trial. The unique study identifier for the VITAMINS trial will be the same identifier for this sub-study, and will be the only identifier needed at the time of data collection and specimen analysis.

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